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Désiré Collen, Biotech Pioneer

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Paul Huybrechts (1946) is a former financial journalist with the newspaper *De Morgen*, and the financial daily *De Tijd*. He later became managing director of the Publishing Company Tijd, owner of the daily, and chairman of the VFB (Vlaamse Federatie van Beleggers), the Flemish federation of retail investors. He is the author of *Hugo's Heilige Vuur*, an extensive biography of the early years of Flemish politician Hugo Schiltz, and of *SOS NMBS,* a book on the dire situation of NMBS, the Belgian railroad company. He is co-author as well of *Naar Grijsland*, a book on the financial consequences of an aging population.

Frieda Van Wijck (1950) is a former journalist and presenter for VRT – the Dutch-language public radio and television station in Belgium. She worked as a reporter and presenter for the Belgian World Service and hosted political television programs such as *Terzake* and *De Zevende Dag*. She interviewed many national and international celebrities and politicians on television for *Spraakmakers* and presented entertainment programs such as *In alle Staten*, *De Laatste Show* and *De Klas van Frieda*.

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Foreword

Everyone eventually dies. One day we all lose our battle against viruses, bacteria or cancers. Sometimes the heart falters or stops. When our heart fails, our blood no longer circulates through the organs we use to breathe, move, think, see and survive. We pass away.

Most of us want to live as long as possible. Few are resigned to dying. And as our lifespan has been increasing since the past century, we are living much longer – twice as long - and in better health. With scientific discoveries, surgical operations and medication, we have armed ourselves against deadly viruses and bacteria, and we try to ward off cancers. The blood and the heart have gradually revealed their secrets. Science made a giant leap when DNA, the script with which life is written, was deciphered. Great researchers have constantly been ensuring breakthroughs. Désiré Collen is one such scientist.

For a long time, there was disagreement about the cause and effect of heart attacks. Does blood clot because the heart fails? Or does the heart stop because the blood flow is obstructed somewhere? It had turned out that blood sometimes clots. Then the heart fails to keep the circulation going, and the patient dies. Knowing that the key question was how to keep the blood flowing so that the heart can continue pumping, Désiré Collen has significantly contributed to solving that problem and helped to save countless lives with his discovery of t-PA, tissue type plasminogen activator. With several expert teams he later discovered other medications. With his listed company ThromboGenics, he found a remedy for a retinal disorder, but that unfortunately did not live up to expectations.

Sometimes trials are unsuccessful, but the next day tens of thousands of researchers around the world go back to work in the never-ending race to defy death. In Belgium and Flanders, scientists can keep up that costly battle, partly thanks to a large share of the money that Collen earned with his thrombolytic medicine. With his own foundation and an evergreen fund, Collen continues to finance scientific research. Set against the inexhaustible need, his contribution is inevitably modest. But it helps universities like KU Leuven to fulfil their societal vocation as a beacon of entrepreneurial research, with, one hopes, many extra healthy years for all of us.

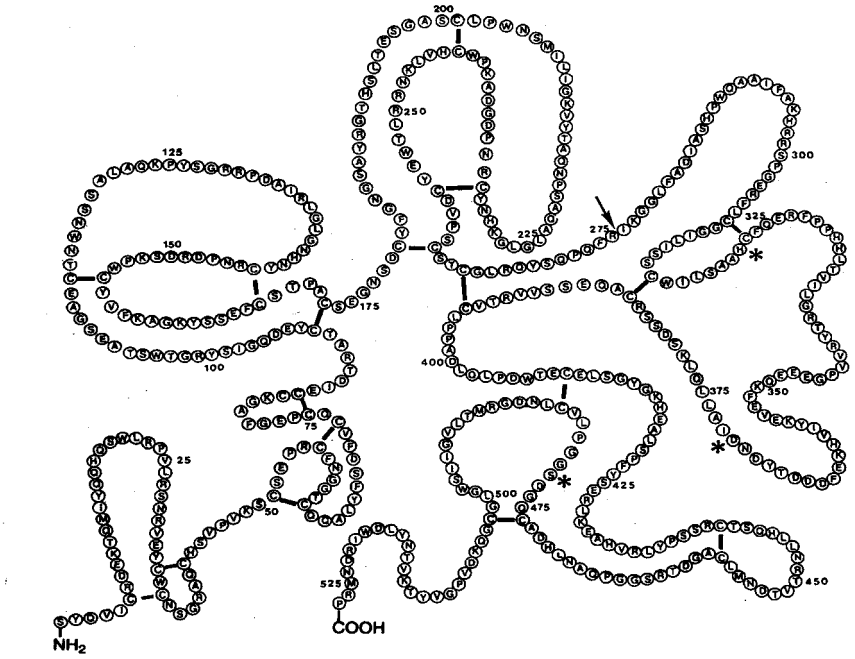
This biography has been commissioned by Désiré Collen. As journalists we had the freedom and independence without which no credible work is possible. All questions were answered. Failures are covered as comprehensively as successes, virtues as well as vices. Désiré Collen had no problems with our approach; on the contrary, he insisted upon it.

For us it was not only an honor, but a genuine professional pleasure to collaborate with Désiré Collen, and with the many academics and others who helped us, readily and often with great patience. We thank them all.

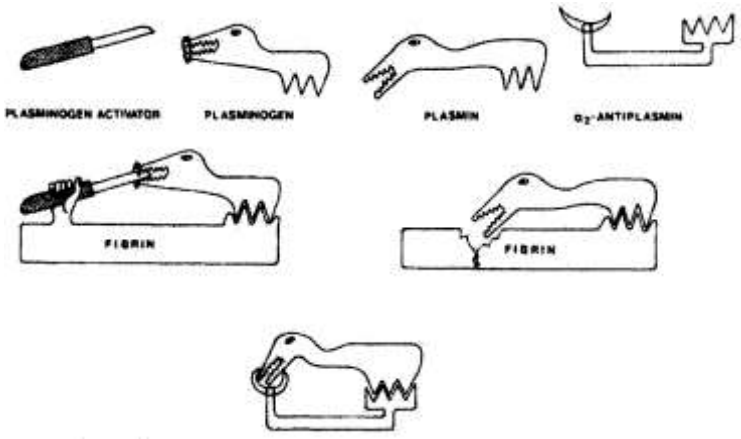
Frieda Van Wijck and Paul Huybrechts

(June 2018, updated June 2020)

PART I: FROM NATURAL T-PA TO RECOMBINANT T-PA

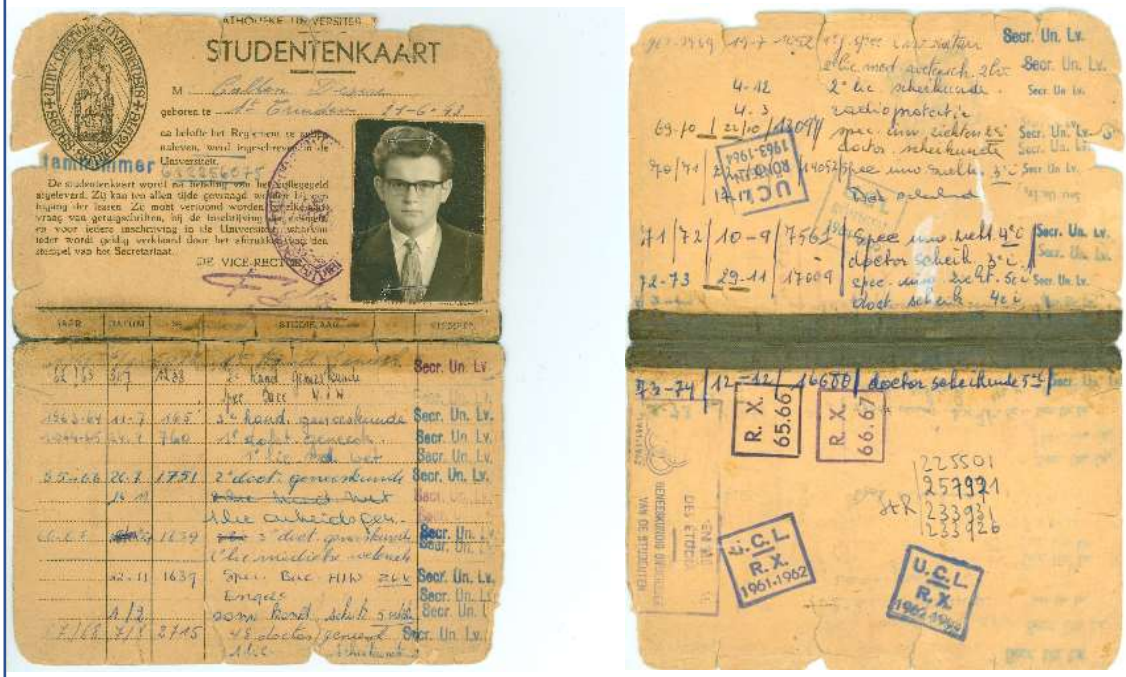


Two-dimensional representation of the t-PA molecule
(After Pennica et al, Nature 1983;301:214-21)



From: Collen D. Regulation of fibrinolysis. Plasminogen activator as a thrombolytic agent. In Pathobiology of the endothelial cell. Nossel, HL. Vogel, HJ (Editors) Acad Press NY 1982: 183-9

Chapter 1: The making of a researcher



Student registration card of Désiré Collen at KU Leuven (1961-1974)

Scientific staff of the Center for Thrombosis and Vascular Research KU Leuven (1985).

From left to right: Jos Vermylen, Marc Verstraete (head), Désiré Collen, Roger Lijnen and Raymond Verhaeghe

Identification and some properties of a new fast-reacting plasmin inhibitor in human plasma

D. Collen

Eur. J. Biochem Volume 69, pp. 209-216 1976

It is concluded that only two plasma proteins are important in the binding of plasmin generated by activation of the plasma plasminogen, namely a fast-reacting inhibitor which is different from the known plasma protease inhibitors and which we have provisionally named anti-plasmin, and α_2-macrogubin, which reacts more slowly.

Identification of the human plasma protein which inhibits fibrinolysis associated with malignant cells

D. Collen, A. Billiau, J. Edy and P. De Somer

Biochimica et Biophysica Acta Volume 499, pp. 194-201 1977

Mixed cultures of mouse fibroblasts and mouse fibroblasts transformed with Kirsten murine sarcoma virus were grown in petri dishes and overlayed with casein. The appearance of focal lysis zones required the presence of transformed cells in the culture and plasminogen in the overlay, indicating that caseinolysis was due to plasminogen activator released by the malignant cells. The culture fluid of a human melanoma cell line induced lysis of a fibrin clot. Fibrinolysis was inhibited by addition of human plasma.
Specific removal of antiplasmin, the fast-reacting plasmin inhibitor (Collen, D. (1976) Eur. J. Biochem. 69, 209), from plasma by immunoabsorption completely abolished its inhibitory activity, both in the caseinolytic and fibrinolytic assays. It is therefore concluded that antiplasmin is the only protein in human plasma capable of inhibiting the fibrinolytic activity associated with oncogenic transformation or neoplasia. Whether this effect is exclusively due to inhibition of formed plasmin or also to interference with plasminogen activation remains unsettled.

Summary: *Désiré Collen was born in Sint-Truiden in the Haspengouw region, the son of a modest family whose forbears were small farmers. At 18, he went to Leuven to study medicine and chemistry at the Catholic University. In the lab of Professor Marc Verstraete, he met Guido Tytgat, who imbued him with a passion for scientific research. He married Louisa Reniers, obtained his doctoral degrees in medicine and chemistry, and continued studying in the US and Sweden. Back in Leuven in 1973, he focused on thrombolysis, the dissolution of blood clots in heart attacks. He discovered α2-antiplasmin, the physiological inhibitor of freely circulating plasmin in the blood, protecting people against excessive bleeding. It brought him his first patent.*

An exemplary student

A war child, Désiré José Louis Collen was born in the small Limburg town of Sint-Truiden (Saint-Trond) on 21 June 1943, during the German occupation of Belgium. Sint-Truiden at the time, was the scene of regular clashes between members of the resistance and locals who collaborated with the Germans. It was the only town in Limburg province where the Belgian mayor was a member of the SS, the feared German *Schutzstaffel*.

The Germans controlled Brustem military airport, just south of Sint-Truiden, from which their Luftwaffe aimed at the English bombers on their way to German targets. The British used the nickname '*the spook of Saint-Trond'* for one particular German pilot with 121 successful nocturnal raids. But Brustem in turn was heavily bombed by the Allies.

For Collen's parents, as for most residents of Sint-Truiden, life was about survival. Haspengouw, the area around Sint-Truiden, had some of the most fertile land in Belgium, which spared the inhabitants form starvation. Small farmers in the Collen family always ensured provisions. Collen's father Frans, a civil servant, worked as a tax controller. Together with Collen's mother, Maria Hoebrechs, they had a pub near the Begijnhof, in the Slachthuisstraat. This was where livestock traders from the area sold their animals with a handshake, after which the cattle were taken to the nearby slaughterhouse. The lively and folksy neighborhood around the slaughterhouse and the Sint-Jacobskerk, accommodated sixteen such cafes or pubs. After the war Frans Collen became an independent tax advisor, and at the beginning of the 1960s, Désiré's parents took over the larger cafe Sportwereld on the Grote Markt (market square) of Sint-Truiden. The Grote Markt in those days was a busy place with much traffic, especially touring coaches and intercity buses. One building with a claim to fame is the mansion named 'The Rose', the property of the wealthy Baltus family where Aldous Huxley stayed for a short while, and where he allegedly wrote his book *Uncle Spencer* (1). In 1973 when the E40 motorway was completed, and traffic no longer needed to cross the city center, the Grote Markt became a quiet city square.

Désiré Collen's early years in primary school in Sint-Truiden passed quietly, and few anecdotes exist also about his years in secondary school. Collen says he has no recollection of noteworthy events. Schoolmates remember him as an intelligent and self-assured secondary school student, top of his class and always formal in shirt and tie. "The way he still goes around today," comments his former classmate Piet Siffert. Collen was, it seems, an exemplary boy. Like many Belgian children, he probably was affected by the aftermath of the devastating war years. Life was frugal, many goods were scarce, and there was rebuilding to be done.

Collen did his secondary school in Leuven, some 45 km from his hometown. Not that Sint-Truiden was lacking in good secondary schools, but in the 1950s parents wanted the very best for their children. For young people from the Slachthuisstraat, the obvious career until then was a job in the coalmines of Waterschei or Winterslag, or in the Liège steelworks. But by the mid-1950s, a general feeling of optimism reigned and new opportunities beckoned. The war was over, and the economy was growing by 4 to 5 percent annually, to the extent that even foreign workers had to be brought in to cope with the shortage of manpower. Owning a car was no longer the privilege of the happy few. An average Belgian could now afford his first Simca and drive to the coast on Sundays. Frans Collen wanted the best possible education for his son and that, he reasoned, would include sending him to a boarding school.

Frans himself had finished secondary school, and so had the most advanced degree in the family. Regarding his son's future, Frans Collen turned for advice to the director of the Catholic Seminary on Stenaertberg in Sint-Truiden. The director, who welcomed any new recruit in the service of the church, was glad to receive Frans Collen and inquired hopefully whether his son Désiré intended to become a priest. "I don't know," Frans Collen allegedly answered, "but I don't think so." Upon which the conversation became less cordial.

A neighborhood grocer had told Frans Collen that he had sent his son to a boarding school in Leuven. Admittedly not a Catholic school, but an Athenaeum, a state school. At the time, a certain rivalry existed between both school systems. It had started in the 19th century, when Catholic and state schools in Belgium were continuously at odds. This so-called '*school war'* lasted until both parties concluded a '*school pact'* in 1958, but some competition between the two systems remained. The Collens were certainly not antireligious, but the parents were no loyal churchgoers either. It was decided to put young Désiré in the boarding school of the Royal Athenaeum of Leuven, the '*Hogenheuvelcollege'* in Naamsestraat, one of the state schools, where he would major in Latin and maths. Désiré's sister was sent to a French-speaking Catholic boarding school run by nuns, '*Les Dames de l'Instruction Chrétienne'* in Ans, near Liège.

When asked whether he minded staying at boarding school, Désiré Collen sighs resignedly, and says that there is not much to tell about those boarding-school years: "The first years we were only allowed to go home for All Saints Day, and during the Christmas and Easter holidays, but once you were somewhat older you could go home every Saturday afternoon." On weekends they went for walks if the study master felt so inclined, and that meant 5 to 10 km hikes in the nearby woods of Heverlee. On Sundays they went to watch the soccer games of Stade Leuven or Daring in nearby Kessel-Lo, depending on which team was playing at home.

Collen never became a football fan. He concentrated on his studies and he enjoyed going to class, where maths and science fascinated him. Music did too, but he never learned to play an instrument, something he now regrets. When he was about 8 years old, there was an attempt to awaken his musical talents in the local music school of Sint-Truiden. "But that didn't work for me. During the first lesson we had to write our names on the blackboard, and for the second lesson we were taught the treble clef and then we just had to sing: do-re, do-re, do-re for the next half hour. That was where it stopped for me." End of musical career.

He says he had no specific hobbies. He was primarily a devoted student. “Désiré was very self-assured but not pretentious,” former classmate Piet Siffert remembers. “He was well aware of his capacities. But hobbies? He had to stay in boarding school from Monday morning till Saturday noon, and could only spend weekends in Sint-Truiden, where he was probably bent over his schoolbooks as well. And while the day-students had their favorite pubs in Leuven, where they would hang out or play table football after school, as a resident student Désiré had to stay indoors.”

As *primus perpetuus* of his class at the Athenaeum, it logically followed that in 1961 Désiré would enroll at Leuven university. Parents wanted their sons to opt for disciplines such as law, medicine or civil engineering, which would lead to a good job and general esteem. The local doctor along with the notary were the higher earning dignitaries, people with status in the community. However, a notary office had to be bought, so that was out of the question. Civil engineering would lead to a prosperous career, certainly in those days of reconstruction. But students who wanted to enroll in civil engineering had to take an entrance exam. For medical students, there were no such requirements, and so he opted for medicine. It was a rational choice; medicine for Désiré was not a vocation, and he never became a practicing physician. When asked about his medical experience, his laconic answer is: “I am a doctor of mice,” referring to the experimental animals in the lab.

Cristal rather than Stella

In 1961 Leuven university was still a bilingual institution, with separate curricula in French and Dutch, although the university was located in Flanders, the Dutch-speaking part of Belgium. The French-speaking authorities did not want to give up this bilingual structure because they felt that this was the only way for the university to maintain its prominent position as one of the oldest Catholic centers of thought in the world. After all French was a universal language. But from the 1960s, the dominance of French was being challenged. More and more Dutch-speaking professors were appointed, as they were often bilingual, while the majority of the French-speaking professors barely spoke Dutch. While before WW II many families in Flanders had sent their sons and daughters to the French-language section, as French was then still the *lingua franca* of the Flemish elite, in 1960 the number of students enrolled at the Dutch-language departments for the first time exceeded those enrolled at the French-language ones.

That first year in Leuven, Désiré Collen was an exemplary student. Living in a ‘*pedagogie’* or student house, (as was customary for many first-year students), was very much like staying in a boarding school. Student houses had curfews, and visits by the opposite sex were strictly monitored. Hence Désiré decided to go and live with his parents in Sint-Truiden again, while commuting daily to Leuven, about half an hour by train. That first year he attended all his classes, studied diligently and passed with honors.

He thought it more practical to rent a private student room in Leuven the second year, going home only on the weekends. Whether this was because he’d now discovered the freedom and temptations of student life, or because medicine had always been merely a rational option and not a passionate choice, Désiré Collen began to spend more and more time in the ‘*Ambiorix’*, a student cafe on the Oude Markt. The Ambiorix was a pub where student guilds from Limburg province would hang out and organize their festivities. Unusually they had

Cristal beer from the Limburg town of Alken on tap, whereas most other pubs served Stella, the local Leuven brew. Désiré Collen launched himself enthusiastically into student life and all it entailed. He became *zedenmeester*, and the following year vice-chairman. "*Zedenmeeste*r was a sort of 'beer taster'," he recalls, a task that had to be carried out at *cantuses* (singing and drinking parties) and at student initiations. This new approach to student life had its consequences. He did not fail his exams, but for the first time in his life his results were merely 'satisfactory'. There were other reasons for his disappointing results besides his involvement in student life and his visits to the Ambiorix, he argues, such as the teaching skills of certain professors. "They were in some cases below average. If you start a course of study without passion, an inspired teacher might still muster enthusiasm and interest from his students, but that was not the case in that second and third year," he contends. Exams consisted of reproducing what was in the textbooks. For chemistry, that consisted of cramming the so-called filing cards of Professor Verhulst, he recalls. If you could recite them by heart you scored an excellent 16 out of 20. So he had 16 out of 20. This outstanding result later allowed him to take up chemistry and obtain his degree without additional conditions. Chemistry was a subject that fascinated him. Medicine too, but it was pharmacology in particular that he found interesting.

Blood for Marc Verstraete

"Actually, it was quite by accident that I changed my ways, and became a devoted student again," Désiré Collen admits. Professor Marc Verstraete, then head of the Laboratory for research into bleeding disorders and vascular diseases, a laboratory which he had founded himself, needed small blood samples daily from healthy donors for his research on hemostasis and thrombolysis. Hemostasis is the mechanism the body uses to prevent hemorrhages. Thrombolysis is the dissolution of blood clots in a blood vessel. At the beginning of the 1960s Verstraete was doing research with coumarin derivatives. These are used to prevent or treat thromboses, the formation of blood clots in blood vessels. The common word is that Marcoumar, the drug that was later developed on the basis of these coumarin derivatives has been named after Marc Verstraete. The drug is still on the market.

After his class one day Professor Verstraete called for voluntary blood donors, and Collen decided to sign up. So, in 1964, he ended up, quite by coincidence, in the lab of Verstraete, where some 15 researchers were at work. The lab was housed in the Sint-Rafael Hospital on the Capucijnenvoer, in what was referred to as '*Maisin's rat cellar'*. It was the room where renowned cancer specialist Joseph Maisin (1893-1971) kept his experimental animals. Professor Maisin himself had meanwhile moved to the French-speaking department in the Sint-Pieter Hospital nearby. "A windowless cellar! That was the lab of Marc Verstraete, who at the time already was an internationally respected scientist!" Collen still marvels. Only years later did they move to more convenient premises which at least had windows, but they were still cramped for space.

> Marc Verstraete (1925-2018) graduated as a doctor in medicine, surgery and obstetrics at the Katholieke Universiteit Leuven in 1951. He further specialized in internal medicine in Basel, Oxford, and New York. His interest in blood coagulation was a direct consequence of tragic events in his family. Three of his uncle's five sons

had died of mysterious hemorrhages, which could later be attributed to Von Willebrand disease. His father, a gynecologist, had occasionally been confronted with intravascular coagulation during births, also known as the defibrination syndrome, which resulted in excessive bleeding.

Marc Verstraete's first publication dealt with the anticoagulant effect of heparin treatment in dogs. Heparin was known to prevent blood coagulation. At the time, it was extracted from the liver and later the intestines of cattle, but now it is chemically synthesised.

In 1955 Verstraete founded his one-man laboratory for hemostasis research at his Alma Mater in Leuven. He was looking for a medicine to dissolve blood clots that obstruct the arteries of the heart in acute heart attacks. He found that streptokinase, a protein extracted from the streptococcus bacteria, was doing just that, and in 1969 he published the results of his clinical study on the administration of streptokinase to patients with a heart attack, in *Acta Medica Scandinavica*. (2)

In 1971 and 1979, the *European Working Party on Streptokinase*, led by Verstraete, published additional studies on the activity of streptokinase in the *British Medical Journal* and in the *New England Journal of Medicine*. Only many years later did thrombolytic therapy, the pharmacological dissolution of blood clots, become routine treatment for heart attacks. (3, 4)

Verstraete also conducted research on hemophilia and on Von Willebrand disease, both clotting disorders caused by the lack of a certain clotting factor in the patient's blood. Furthermore, he was one of the founders of the 'Vriendenkring van hemofilielijders' (Friends of hemophilia patients) and developed a multidisciplinary center for the treatment of hemophilia and blood coagulation diseases. In addition to about 300 scientific publications, he wrote eight books devoted to his speciality. He obtained honorary doctorate degrees from the universities of Cordoba, Bologna and Bordeaux. He was an honorary member of the Royal College of Physicians of London and of Edinburgh, and of the American College of Cardiology and the American College of Physicians.

Blood clot, cause or consequence?

With the use of heparin and streptokinase to dissolve blood clots in the event of a heart attack, Marc Verstraete, Désiré Collen's mentor, adhered to the 'open artery' school of scientists. They were convinced that heart attacks are caused by a clot blocking the blood supply to the heart muscle (coronary arteries). That was also Collen's conviction and this belief determined his later research.

It may now seem strange that until late into the 20th century, scientists disagreed about the cause and effect of heart attacks and blood clots. Which occurs first, a blood clot or a failing heart muscle? Some, such as Marc Verstraete, believed that a heart attack was the consequence of a blood clot that obstructed the blood flow to the coronary arteries and so caused the heart muscle to die. However, another school of scientists was convinced that a coronary thrombosis, a clot in the coronary artery, occurred only after the heart muscle had died.

During an autopsy for his students in the mortuary of the Cook County Hospital on May 17th 1892, the American pathologist of Norwegian origin Ludvig Hektoen (1863-1951) presented the case of a 32-year old man who had died 16 hours earlier. He was a carpenter who had become unwell in the morning at work, was being taken to the hospital, but died on the way. “A well-fed muscular man,” Hektoen described the dead man on his autopsy table, while he went over everything he could observe with the naked eye. Liver, gallbladder, kidneys, stomach and so forth appeared normal, but in the aorta, just above the aortic valve, he identified a non-occlusive clot, and in the left coronary artery an occlusive clot, blocking the blood vessel completely. That clot of 4 by 3 millimeters was composed of a network of fibrin threads. And he concluded his examination with: “We have here a fine illustration of death due to a blockage of the blood flow to the heart muscle, one of the major causes of sudden death.” To Hektoen it was clear: a clot blocked the blood flow to the heart muscle which caused it to stop beating, and consequently caused the carpenter to die. In 1892, together with internists Sir William Osler (1849-1919) and George Dock (1860-1951), he described the pathophysiology of a myocardial infarction or heart attack: “*While cardiac infarction may be caused by embolism, it is caused much more frequently by thrombosis, that is usually secondary to sclerotic changes in the coronaries.”* (5)

The same theory was supported by Hektoen’s colleague and co-author, the Canadian internist Sir William Osler, co-founder of the Johns Hopkins Hospital in Baltimore, Maryland, and the renowned Johns Hopkins School of Medicine. Osler is particularly remembered for his revolutionary method of ’bedside teaching’. He went from bed to bed in the hospital with a group of students in his wake, while he lectured on the ailments and diseases of the patients he was visiting. Osler was equally convinced that blood clots led to a heart attack, and that these clots were formed by blood platelets that stuck together on sclerosed vessels. He added: *“The blocking of one of these vessels by a thrombus or an embolus leads to a condition which is known as anemic necrosis, or white infarct (*an infarct as a consequence of an absence of blood*). This is most commonly seen in the left ventricle and in the septum, in the territory of distribution of the left anterior coronary* artery.” (6)

In the 19th century and a good part of the 20th century, heart attacks were considered mostly fatal. The survival chance of patients was small. Hence little research was done on the subject. Patients, who in those days landed in hospital with a heart attack, had relatively little chance of leaving the hospital alive. As a consequence, there was hardly any useful information available on the possible symptoms associated with heart attacks.

Yet there were two researchers who gave a fairly accurate description of the pains and symptoms that preceded a non-fatal heart attack, and who even suggested possible causes: the Russian physician Vasilii Parmenovich Obraztsov (1849–1920) and his student N.D. Strazhesko (1876-1952) who published their findings in 1910. In Narodnogo Opolcheniya Street in Kiev, the Ukrainian capital where Strazhesko lived most of his life, a museum is devoted to his years of research on heart attacks. Ironically enough he himself died at the age of 74 of a heart attack. In their description of the symptoms the two scientists also refer to possible causes of the heart attack. One patient was supposed to have become unwell during a rather competitive card game, another after an unpleasant conversation and a third after he had climbed stairs. (7)

The publication of Obraztsov and Strazhesko was translated into German: *Zur Kenntnis der Thrombose der Koronararterien des Herzens.* The American doctor James B. Herrick (1861–1954) travelled to Europe and worked in Vienna and Prague. We may therefore assume that he was familiar with their medical publications. From his own experiments with animals in which he temporarily tied off their coronary artery, he had concluded that a blocked coronary artery was not always fatal. He distinguished four types of clinical outcomes that were linked to blocked coronary arteries or coronary thrombosis (the term heart attack was then not yet used): there was sudden death with no prior symptoms, there were cases with pain in the chest and shock followed several minutes later by death, there were cases with severe but atypical symptoms that did not immediately indicate heart problems but from time to time did turn out to be fatal; and fourthly, non-fatal cases with mild symptoms.(8)

Surviving patients received the advice from Herrick to stay in bed for a week, while they were treated with digitalis, a plant extract which increased the intensity of the heartbeat, and occasionally with anticoagulants such as heparin, aspirin or warfarin. We now know that bed rest can cause pulmonary embolism and digitalis can at best improve the quality of life in people with heart attacks, but there is no evidence that it extends life expectancy. In those days however, it was believed to be the best remedy. Later, patients were sometimes also administered caffeine, camphor, or morphine.

As a consequence of Herrick's assessment that there were in fact chances of survival in a 'coronary thrombosis', the search for remedies gradually intensified. It was now believed that a patient had a greater chance of survival if fast intervention unblocked the artery. This theory became the 'open artery hypothesis'.

Doubt persists

But during the 1930s two American cardiologists, Charles K. Friedberg (1905 - 1972) and Henry Horn, cast doubt again with their publication *Acute myocardial infarction not due to coronary occlusion.* (9) Friedberg was the respected head of the cardiology division of Mount Sinaï Hospital in New York and was the author of *Diseases of the Heart*, a handbook of more than 1,000 pages for cardiologists that had been translated into six languages. He was therefore a man of distinction whose opinions were valued. According to Friedberg and Horn, it was well-known that all the characteristics of a coronary thrombosis could be detected in patients in which during the autopsy, no thrombus was found. These patients had experienced a heart attack without a blood clot, they claimed, and they cited a number of physicians who had made similar observations.

And that is where the discussion began: was a blood clot the consequence of a heart attack, or rather the cause? If a thrombosis was the consequence, or if in other words a blood clot was formed after a heart attack, the research on thrombolytic drugs or medicines that could dissolve clots would be far less important.

In the following decades there were believers and non-believers, and even in the mid-1970s the influential pathologist William C. Roberts (°1932) of the National Institutes of Health - the research institute of the federal government in the United States - stated that a clot in the coronary artery was the result of the heart muscle dying rather than the cause. He maintained that heart attacks were caused by spasms and constriction of the blood vessels,

and not by clots. His conclusion was the result of autopsies on people who had died, but his interventions often took place many hours *after* the fatal heart attack.

Fortunately, at the end of the 1970s, Roberts' hypothesis was challenged by Marcus DeWood (°1948) and his colleagues from Spokane, Washington. In the journal *Medscape.com* of March 2011, Dr Lloyd Rudy (1934 – 2012) gave an account of a procedure by DeWood in 1972. Rudy and his colleagues were busy with an urgent coronary bypass operation at 2 am. Marcus DeWood was also present. It involved a 48-year-old patient in whom the left coronary artery was blocked. DeWood wanted to investigate the patient's cardiac artery with a catheter. "We have to know what that is!" he said. Doctor Rudy looked at his colleague, dumbfounded. Surely DeWood wasn't going to burden a patient on whom they were performing an urgent vein graft bypass even more by investigating what was in that blocked vessel? Not to mention the risk! What if he went searching in the vessel with a catheter and pushed the thing that blocked the vessel further up or down? That could be fatal to the patient. But DeWood could be very persuasive, and so Rudy took the catheter, blew up the balloon so that the vessel opened up, and pulled 'the thing' out slowly and very carefully. A long blood clot emerged! After which DeWood spoke the prophetic words "We are going to revolutionise the treatment of heart attack!" (10)

Marcus DeWood and his colleagues performed heart catheterization, in which a contrast medium was injected into the coronary arteries via a catheter, on 322 patients with symptoms of a heart attack. They discovered that a blood clot was found in the coronary arteries of 87 percent of the patients within less than four hours of their first symptoms. However, in patients who arrived in the hospital 12 hours after their first symptoms, only 65 percent had a clot.(11) Demonstrating that it was safe to perform coronary artery catheterisation in an acute heart attack was by itself a noteworthy achievement. But his statistics also showed that, as time passed, the patients' clots became smaller due to natural fibrinolysis, the natural capacity of the body to dissolve blood clots.

At around the same time two colleagues of DeWood, Keith A. Reimer (1945-2002) of Duke University and Robert B. Jennings (°1926) of Northwestern, both pathologists, demonstrated that the heart muscle did not die at once, but progressively. Patients in whom the blood flow to the heart could be restored within 15 minutes incurred no damage to the heart muscle at all. But after 6 hours without blood supply the heart muscle could not be saved. By the end of the 1970s there were two important insights: heart attacks can be stopped by removing the clot, but fast intervention is crucial.

Fortunately, all this time there were scientists such as Sol Sherry (1916-1993) and William S. Tillett (1892–1974) who didn't wait for DeWood's evidence and were constantly searching for clot-dissolving drugs. Brezhnev's personal physician, Yevgeniy Ivanovich Chazov (°1929), and K. Peter Rentrop (°1948) of the University of Heidelberg in Germany also administered streptokinase to patients via the coronary artery, and proved that clearing the artery restored the heart function. And last but not least, Marc Verstraete in Leuven had, at the end of the 1960s also saved the lives of heart attack patients with his streptokinase treatments. (12, 13)

In an interview with Robert M. Califf (°1951) in *Medscape* on 13 November 2014, renowned cardiologist Eugene Braunwald (° 1929), who is generally regarded as one of the most influential cardiologists, said that he too believed that Marcus DeWood's findings were of

decisive importance at the time. It was the ultimate confirmation of the theory that heart attacks were caused by blood clots and not the other way around. And that DeWood had proven that if the blood flow to the heart could be restored expeditiously, the patient could recover. The opponents of that theory had always misjudged the problem, according to Braunwald, because they based their views on post-mortem examinations. By then, the patient's natural clot-dissolving system had carried out a partial fibrinolysis (clot breakdown) after death. When Braunwald spoke, cardiologists heeded his words. He had published over 1,000 articles since 1954 and had written two of the most influential textbooks on internal medicine and cardiology: *Harrison Principles of Internal Medicine* and Braunwald's *Heart Disease Review and Assessment: Expert Consult*. Later he led important international trials with clot-dissolving drugs that were also important for Collen and t-PA: the TIMI trials. (14)

Guido Tytgat's wake-up call

Marc Verstraete had noticed that during his first visit to the laboratory Désiré Collen had been captivated by the ongoing research, so he told him that he was welcome to come back. At the time, Guido Tytgat (°1937), a young researcher, who was working in Verstraete's lab was seeking an enthusiastic student who could help him with his research. "Tytgat was a very hard worker, who opened my eyes," says Collen. They got on well from the start. Guido Tytgat was six years older than Désiré Collen and in his second year of hospital residence. It was not so much the subject Tytgat was working on which appealed to Collen but rather Tytgat's enthusiasm, his constant occupation with four or five projects simultaneously, which awoke Collen's passion for research.

Tytgat was measuring the turnover (clearance) of fibrinogen, to see how fast it disappeared from the body in healthy subjects and in patients with liver disease. For this they experimented on dogs. They let the dogs swallow carbon tetrachloride and alcohol, which caused cirrhosis of the liver. It may appal dog lovers, and these experiments are no longer practized, but medicine without experimental animals is impossible, according to Collen. Over the years the number of experiments has been reduced and methods have improved. Working with experimental animals is now strictly regulated. Researchers receive specific training and have to be certified, and there is strict monitoring by the university administration. As a consequence of increased regulations and requirements research has become much more expensive. "But there still is no alternative to research with experimental animals," Collen emphasizes. "We can make three-dimensional cell cultures that already have the looks of an organ. You can do things in vitro. But heart failure, for example, a heart muscle that no longer pumps sufficiently, that is a mechanical matter." Heart failures can't be tried out in a test tube. Rats and mice are now more frequently used than primates, but again, "there are many things that work in mice but not in humans," says Collen

It was not so surprising that Guido Tytgat and Désiré Collen hit it off, as they had quite a few things in common: both were hard workers, both passionate about research, and they had both arrived at their specialization by eliminating other options. Collen could have become an engineer, and Tytgat could have gone for a musical career. The latter obtained his medical degree in 1963 *summa cum laude*, Collen *magna cum laude* in 1968.

There was never a dull moment in Tytgat's laboratory. Research was done not just on dogs, but also on patients. When there was a minor epidemic of serum hepatitis (blood-borne

hepatitis B), an infection of the liver which in its fulminant form kills nearly all the liver cells, it was all hands on deck. A hepatitis-B patient becomes intoxicated and turns yellow. “We pulled a few of them through with ex-sanguino transfusion, a complete replacement of the patient’s blood. They received fresh blood every day, five liters were drawn out, and on the other side five liters of fresh blood went in. We had a few who made it that way,” recalls Collen.

Tytgat also studied Zollinger Ellison syndrome, by which too much gastric acid is produced. He was always running different experiments simultaneously, and Collen served as his right-hand man.

> Guido Tytgat (°Izegem 1937), just like Désiré Collen, had ended up in medicine rather by chance. He was a talented clarinet player, but when in 1957 he had to choose between a musical career or a career as a physician, his music teacher pointed out the insecurity of a musician’s life. Tytgat decided to choose medical science, and put his clarinet away. After his time in Leuven, he went for further training to the University of Washington in Seattle, where he heard of plans in Amsterdam to start with a gastroenterology department. He sent in his application and in 1971 Tytgat, his wife and three small children moved to Amsterdam to set up a completely new department. His first laboratory consisted of a small room below an auditorium in the Wilhelmina Gasthuis. Eventually the gastroenterology department in Amsterdam grew to become one of the very best in Europe. At the end of his career his laboratory counted more than 100 employees.
>
> There were enough patients from the very outset. Gastrointestinal ulcers in those days were endemic. The general theory was that ulcers were caused by stress, but Guido Tytgat contributed to the discovery that the Helicobacter pylori bacteria was responsible for the majority of stomach ulcers. In 2005 two Australians, Barry J. Marshall (°1951) and J. Robin Warren (°1937), received the Nobel Prize in Medicine after they succeeded culturing the bacteria and so definitely proved that ulcers were commonly caused by bacteria and not the consequence of stress.

After the investigation of fibrinogen in liver patients, Tytgat concentrated on the conversion of plasminogen, a non-active protein that after activation to plasmin can dissolve blood clots. “We knew virtually nothing about biochemistry at the time,” Collen acknowledges, but Professor Verstraete had brought in René De Vreker, a biochemist who worked for beer brewer Stella Artois, where he had worked on the improvement of foam formation and the cloudiness of beer. De Vreker provided them with a protein solution that dissolved blood clots after activation. “He had purified something that could be activated to plasmin. We assumed that it was pure plasminogen,” says Collen. Tytgat and Collen marked the protein with radioactive iodine and injected it intravenously, first in themselves, then in 24 healthy test subjects and in 24 patients with liver cirrhosis. Each time they determined the turnover, the speed with which the radioactive protein disappeared from the blood. But something wasn’t quite right. They saw things that did not concur with their hypotheses. “To cut a long story short, we thought that plasminogen had a half-life of 7 to 8 days, while in fact it is between 2.1 and 2.2 days. We were completely mistaken!” Ultimately the data were of no use. It turned out that they had not worked with plasminogen, but with one or more proteins that were contaminated with plasminogen. Tytgat abandoned the entire project and left for Seattle, Washington, where he continued his postdoctoral research as a gastroenterologist.

Following on from Tytgat's dissertation on clotting disorders in liver diseases, Collen continued to work on disorders of the blood coagulation system. Sometimes things in life depend on coincidences. "Had Tytgat been active in cardiology, I probably would have become a cardiologist," Collen thinks.

Donating blood in Professor Verstraete's lab was not the first or the last jab for Collen. During his career Collen drew blood from himself hundreds of times, or he injected himself as a guinea pig with trace amounts of radioiodine-labelled coagulation proteins. His right arm was eventually so scarred by punctures, that he almost failed to obtain permission to work at New York University a few years later. When in September 1971 an American nurse saw his arm during his medical exam, she threw him a suspicious look and noted 'tracks' in his file. "The friendly atmosphere changed at once. They suspected me of being a junkie!" His American employer Alan Johnson, a friend of Professor Verstraete, had to do some explaining to have his file cleared. It took a week before Collen was admitted to the US and could start to work.

Chemistry in a turbulent city

During his collaboration with Tytgat, Collen realized that biomedical research fascinated him, but that his biochemical knowledge was inadequate. Additional training would not hurt. Professor Verstraete agreed and Collen set off for the faculty of sciences and enrolled at the chemistry department.

Professor Verhulst – the teacher of the filing cards - saw his first year medical study results for chemistry and said, "Ah, very well, you seem to have some talent for chemistry. However, you must take exams for *all* the courses of the chemistry curriculum!" That meant exams for medicine in July and chemistry in September.

In the meantime, Collen had met his future wife, Louisa Reniers (°1945). She was a friend of his sister and lived in Sint-Truiden. After two years of dating they married in 1966. Their first summer holidays together consisted of Désiré studying and Louisa working in her father's factory. Louisa's father had a small margarine factory in Sint-Truiden with some ten employees, where Louisa did the bookkeeping. The couple lived on Louisa's wages and the sponsorship of Désiré's father. Frans Collen was proud of his son's results at the university and was prepared to support him a bit longer.

For his bachelor's degree in chemistry, Collen fortunately got exemptions for exams he had already passed in the medical curriculum. And in September 1967 he took all the exams for the two bachelor years of chemistry. In February and July 1968 he took his final exams in medicine and passed with high distinction, and in September 1968, he took the exams for his first year master's in chemistry, passing also with high distinction. A *tour de force,* because at the time he had not been able to attend a single class for medicine. Because he had to spend all his time in the chemistry teaching labs, for his medicine exams at the end of the seventh year he met some of his professors for the first time.

After World War II the number of students at the Katholieke Universiteit Leuven steadily increased, in particular those who enrolled at the Dutch-speaking departments. Higher education had become accessible to all social classes and 'going to university' became the ultimate aspiration of many middle and also working-class teenagers, amongst whom more and more girls. To cope with the increased number of students the university authorities sought to expand the campus. They had already opened a French-language medical campus

in Sint-Lambrechts-Woluwe near Brussels, and had acquired a plot of land near Wavre, south of Brussels, in the French-speaking part of Brabant province. This provoked Flemish fears that Leuven might eventually become a French-speaking university, absorbed in *'le très grand Bruxelles de l'avenir'* (the great Brussels of the future). When on 14 January 1968 the French-language Academic Council revealed its expansion plan, including the exclusively French-speaking branches in Sint-Lambrechts-Woluwe and Wavre, while the university in Leuven would remain bilingual, it was met with vehement Flemish opposition and riots broke out. The Belgian bishops who, in fact, were the governing body of Leuven university, sided with the French-speakers. They had already formally stated their position in 1966 with an edict, terrified as they were of a split of their centuries-old Alma Mater, founded in 1425. For some church dignitaries the mere consideration of a split was '*un peché contre l'Esprit Saint*' (a mortal sin against the Holy Spirit). But in spite of the omnipotence of the Belgian clergy, a united intellectual elite in Flanders demanded the linguistic separation of the university, with slogans such as '*Leuven Vlaams*' (Leuven Flemish) and even '*Walen buiten*' (Walloons out), which was later acknowledged by many Flemings as an unfortunate and racist slogan.

That spring in 1968, Leuven looked like a besieged city. Streets were broken up. Protestors used the cobblestones to raise barricades and smash windows. Federal police carried out charges with cudgels and water cannons, and student leaders were arrested. Classes and exams were suspended, and on 7 February 1968, the government, led by Prime Minister Paul Vanden Boeynants, fell over the issue. The separation of Leuven university into a French-language and a Dutch-language division had become inevitable and was included as such in the government declaration of the new coalition, led by the politician and Leuven economics Professor Gaston Eyskens. The French-speakers got their own catholic university, for which a new city, Louvain-la-Neuve, was built near Wavre in the French-speaking part of Brabant province. The new exclusively French-language university would be established as the Université Catholique de Louvain or UCL, while the university in Leuven would become a monolingual Dutch language institution, KU Leuven (Katholieke Universiteit Leuven).

Historians now confirm that the fight to obtain the monolingual Flemish (Dutch-speaking) nature of Leuven was a 'turning point in Belgian history'. The splitting up of the university set in motion an historical process that later heralded the end of the unitary Belgium. But also the church, then still a bastion of power in Flanders, had to give way. The secularization affected not only the Catholic university, but all Catholic schools and had its impact on society as a whole. As much as Paris, which also witnessed a student revolt that same year, Leuven definitely wrote history in 1968. At the time few would have dared to predict it, but the split into KU Leuven on one hand and Université Catholique de Louvain (-la-Neuve) on the other proved a blessing for both universities. Of course, one doesn't know how the old unified institution would have fared, but the new direction taken by the secular authority of KU Leuven towards a socially oriented and entrepreneurial university definitely ensured its future growth.

Désiré Collen was hardly aware of the revolution that was going on in the streets of Leuven, although he admits that he once participated in a demonstration. He received a couple of substantial blows from a baton. "It hurt for weeks on end!" The experience immediately cooled his enthusiasm for student protests and barricades. That year he graduated as a doctor in medicine with high distinction. The following year he continued as a research

assistant in Professor Verstraete's lab, where he prepared the last year of his master in chemistry and wrote his thesis for chemistry, under supervision of the renowned Professor Leo De Maeyer (1927-2014), head of the laboratory for Physical Chemistry. De Maeyer was known for the development of instruments measuring the initial speed of chemical reactions during the first microseconds. In 1967, the German physicist and chemist Manfred Eigen (°1927) was given the Nobel Prize for Chemistry thanks to, among other things, De Maeyer's instruments. "An intelligent fellow," says Collen, "who should have received a Nobel Prize himself. He was someone for whom I had the greatest respect."

Leo De Maeyer had little interest in blood coagulation, but still he let Collen do his research project on the polymerization of fibrin, the way in which fibrin strands are formed. Collen could purify fibrinogen and carry out the chemical research in Marc Verstraete's lab, and was allowed to use the hydrostatic pressure apparatus in the laboratory of Professor Putzeys. At the end of the year he had fantastic results, and they were judged interesting enough for a publication in *Nature.*""I was as proud as a peacock," he recalls, "because as far as I know, it is very exceptional that research for a master's dissertation leads to a publication in *Nature."* Several bottles of champagne were uncorked. "Well, it wasn't a big article, only two A4 size pages, and it was about a subject in which only a very limited group of scientists was interested. It got 50 citations at most and they were primarily from Russian scientists." Anyway, as a research assistant of the National Fund for Scientific Research (*Nationaal Fonds voor Wetenschappelijk Onderzoek*, NFWO), he felt he was now truly a researcher. (15)

Collen contracts hepatitis B

The purpose of a research assistantship from the NFWO in a clinical discipline is to combine research with formal training in a medical specialty, internal medicine in Collen's case. A research career without clinical responsibility, as he envisaged it, was highly unusual for a physician in training. The normal five year specialization programme allowed for one year of fulltime research; in the remaining four years clinical experience was to be gained. But Collen had already used up his research component during the first year. In July 1969 he started his clinical rotation in internal medicine in the Sint-Rafaël Hospital in Leuven. But he did not stand by many sickbeds that year, because three months later he contracted hepatitis.

At the time, Marc Verstraete was working on a treatment for hemophilia-A, the genetic clotting disorder that Britain's Queen Victoria (1837-1901) had passed on through her children to the son of the Russian Tsar. Patients with this clotting disorder are missing the normal gene for the protein 'Factor VIII', on their X chromosome it was assumed. Boys with a defect in the Factor VIII gene on their single X chromosome, run the risk of internal and external hemorrhages, which are often fatal. Recent findings, after DNA analysis on the bones of the last Russian Tsar family, indicated that prince Alexei Romanov, son of Tsar Nicolas II and great-grandson of Queen Victoria, indeed suffered from hemophilia, but not caused by the more common gene mutation involving Factor VIII, but instead by a mutation involving another gene, Factor IX. This discovery by Evgeny Rogaev of the University of Massachusetts medical school was published in *Science* in 2009.

"It was particularly sad seeing young boys with joint hemorrhages in their knees, contracted while playing," says Collen. It was the time when knee replacements where not yet invented.

Professor Verstraete investigated whether organ transplantation could offer a solution, and whether the spleen could be used as a source of Factor VIII, because the spleen has many blood vessels. He considered removing the spleen of a hemophilia patient and replacing it by a donor spleen that made Factor VIII, so that the patient could make at least a small quantity of Factor VIII. “Five percent can be enough, as long as there is no massive blood loss due to a serious accident for instance, or a major operation,” according to Collen.

While Collen was an assistant at internal medicine, a patient with severe hemophilia was brought to the hospital for observation. He had practically no factor VIII, less than one percent. Professor Verstraete decided to draw blood from this hemophilia patient, and by way of an experiment, let it pass through the spleen of a recently deceased patient. In the autopsy room Collen and three colleagues started removing the spleen of the deceased. “A normal spleen has the size of a fist, but this was an enormous whopper,” Collen remembers. Didn’t that raise any suspicions? “Well yes, but there we stood, all prepared, with the blood from our hemophilia patient, so we had to get on with it.” However, due to the enlargement of the donor spleen, the blood vessels were so compressed that it was practically impossible to get the hemophilic patient’s blood through. They were pumping and aspirating, it was a real *tour de force*. Eventually, the four of them were covered in blood. Shortly afterwards, it turned out that the blood of the hemophilia patient was contaminated with hepatitis B! In hindsight, that was not surprising. At the time hemophilia patients often carried the hepatitis B virus, because they needed repeated blood transfusions, and every now and then a blood donor carried the hepatitis B virus.

A week later, Collen and his colleagues became ill. “I felt tired and extremely miserable.” At first he thought he had caught influenza, but the amount of transaminases, the biomarker of liver necrosis in his blood, had shot to over 3,000 units per millilitre. It felt as if he were dying. The whole incident gave Collen definitely more comprehension for the complaints of some of his patients. “I had seen patients with liver cirrhosis complaining to me, oh doctor, I can’t do this, oh doctor I can’t do that!” He thought it was largely in their minds. But after this experience he realized their complaints were quite genuine and justified.

His illness lasted for 6 months. The first 14 days he couldn’t leave his bed. He didn’t even have the strength to hold a book in his hands. He was just lying there, staring at the ceiling. The same applied to his two Italian colleagues, Giovanni De Gaetano and Maria Benedetta Donati, and for lab technician Annie Vandenbussche who had all assisted him in the experiment.

The hemophilia patient in question had serum hepatitis in his blood, and quite a bit. But that was discovered only later when the Rega Institute, which had provided the donor blood, had developed tests to detect the virus. Afterwards this particular patient’s blood was long used as standard reference for the development of hepatitis B tests.

After two months of convalescence at home Collen felt capable of resuming work at the lab. He felt he could handle a few hours a day. But given his illness, contact with patients was out of the question. Not that he minded much, as the lab was his preferred habitat anyway. However, by March 1970, he had completely recovered from his hepatitis B infection, and could no longer escape clinical training. It meant quarterly rotations in the hospital, in the cardiology department of Professor J.V.J. Joossens, the endocrinology department of

Professor P. De Moor, the hepatology department of Professor J. De Groote, the bleeding disorder and vascular disease department of Professor M. Verstraete, the infectious disease department of Professor L. Eyckmans, and internal medicine consultation with Professor A. Amery. Even though he never became an internist, it turned out to be a useful experience in various disciplines. At the same time, he was working on his doctoral dissertation in chemistry and his aggregation thesis in medicine. Despite the heavy workload, everything was completed in 18 months; his clinical records were in order and there were no complaints, neither from his supervisors nor from the patients. But there had been no time left to attend seminars and case discussions, or to read clinical literature. It gradually became clear that he would never become a skilled clinical internist.

New York disappoints

In 1971, after his doctorate in Medicine and master's in Chemistry in Leuven, the world beckoned. Marc Verstraete arranged for Désiré to spend a year as a Fellow of the Belgian NFWO (National Fund for Scientific research) in the United States, where he could do research in the lab of Alan Johnson at New York University Medical Center in Manhattan, New York.

Verstraete and Johnson knew each other from their research on streptokinase, a protein that activates human plasminogen and which was used as a thrombolytic treatment for heart attacks and pulmonary embolism. It was W.S. Tillett, Alan Johnson's boss, who had discovered streptokinase. Johnson had then worked with streptokinase in rabbits. And Sol Sherry, a colleague of Johnson's, had treated the first patients with streptokinase. Marc Verstraete introduced streptokinase research in Europe, and did the first study demonstrating that significantly fewer people with a heart attack died after a treatment with streptokinase than in the control group. Verstraete figured that Johnson could teach Collen a thing or two.

Johnson turned out to be a very amiable man, but his laboratory was a mess. "He was already well into his sixties, and his lab was over its peak. The turnover of staff and researchers in his laboratory was very high. And there was little continuity in the research projects, while the laboratory notes of previous studies had to be consulted somewhere in the basement," Collen recalls. In those days Johnson was concentrating on the removal of the hepatitis B virus from blood derivatives. Contaminated blood was a major problem in the US. Blood donations were remunerated, which attracted donors who urgently needed small sums, such as drug addicts, often carriers of the hepatitis virus. Whoever needed a blood donation in hospital risked being infected with hepatitis, because at the time the virus was not yet routinely detectable. In Johnson's lab about 15 people were at work, most of whom were focused on the hepatitis problem, but that was a field of research which was of lesser interest to Collen. Together with two other scientists he worked on fibrinolysis, the dissolution of blood clots.

Upon his arrival in New York together with his wife and two small children, he found that accommodation had been arranged in an apartment in Brooklyn. It meant taking the subway every day, but it was best to stay in after dark. It was a rough neighbourhood, with regular street fights. Two weeks later the family found a small one-bedroom apartment on 24th Street and 2nd Avenue, on the east side of Manhattan, north of Little Italy. Johnson's lab was on 28th Street and 1st Avenue. It was only a four block' walk every day. The monthly rent

amounted to USD 259, just one dollar less than what the NFWO paid, which caused a brief panic. Fortunately, Johnson announced a few days later that as a research assistant Collen would be paid USD 12,000 from his funds of the National Institutes of Health. And when Johnson discovered two days later that Collen also had a master's in chemistry, he promoted him to associate research scientist, with a salary of USD 16,000 per year! It felt like winning the lottery, more so because six weeks later came a message from Belgium: IBM was adding an additional research grant of USD 6,000.

Alan Johnson's laboratory might have been disappointing, but Louisa Collen thoroughly enjoyed her stay in New York. "It is of course a fascinating country," Collen acknowledges. "Over the Thanksgiving weekend we flew to Florida for two days, low budget with Eastern Airlines. We visited Niagara Falls and the Space Museum in Washington. And at the end of our American year we drove through the many national parks in the Far West." Désiré Collen had to briefly return to Belgium in March 1972 when his father unexpectedly died, at the age of 60. Cause of death: heart attack.

At the time, more and more European scientists were going to the US to do an extra year of research. Anyone pursuing an academic career in Leuven preferably had some experience abroad on his resumé, usually in the United States or Great Britain. Sometimes in Germany too, but the Germans no longer played a key role in medicine then. Of the previous generation of KU Leuven scientists, many had done their doctorate at French universities, but from the 1960s, the US was the place to be.

Collen's affiliation with the US didn't end after that first year in New York. Altogether he spent about four years in the United States. At times he flew back and forth every month. He gathers he must have taken some 130 to 140 flights to the US. The places he knows best in the US are the airports!

That first year in the US was not a scientific success, however. It resulted in only one publication on the turnover of an abbreviated form of plasminogen. The article appeared two years later in a less important scientific journal. But he could use his research results from New York for his doctoral dissertation at the faculty of Sciences in Leuven. (16)

With a Viking in Sweden

Collen says he really learned his craft the following year in Sweden. "That was a fantastic year," he says recalling with great pleasure his days with Birger Blombäck, his Stockholm mentor. The Karolinska Institutet was and still is a renowned research institute. During his stay, four Nobel Prize winners were working at the institute. Blombäck's laboratory was a well-oiled machine. His lab technicians started at 6 am and did the amino acid analyses and other technical assignments for the researchers, who started later that day. Collen came in around 10 o'clock, worked until 8 pm, and then they all went to have a couple of beers. "By 11 pm we eventually went home," Collen laughs. Home was in Solna, a suburb of Stockholm where not much was going on. Louisa stayed there with, by now, three small children. She spoke no Swedish, and apart from housekeeping there was not much else to do. As much as she had enjoyed New York, she now found Sweden boring.

Collen had initially planned to go to Sweden the previous year, when Verstraete had introduced him to Blombäck. "Blombäck was glad to have me, but I had to finance my stay

myself.” At the time, NATO gave 15 grants per year to Belgians who submitted an interesting program. But Collen was already in New York when he heard that he too would receive the NATO grant. NATO was kind enough to keep it for the following year, and in September 1972 Collen ended up in the Karolinska Institutet with Birger Blombäck.

> Birger Blombäck (1926–2008) was a pioneer in research on blood coagulation. He was the first to unravel the structure of fibrinogen and the conversion of fibrinogen into fibrin. Originally he wanted to study literature, but he eventually enrolled in medicine at the Karolinska Institutet in 1949, where he met his wife Margareta Wetter. They married in 1951 and worked together in the research institute of Professor Jorpes, who had discovered heparin. To neutralize the thrombin that converted fibrinogen into fibrin, heparin needed a cofactor in plasma. To study that process Blombäck worked with pure fibrinogen. Hence the subject of his doctoral dissertation, *Studies on Fibrinogen: Purification and Conversion into Fibrin*. Blombäck and his group dominated the fibrinogen-fibrin research for the next twenty years. At the end of the 1960s Blombäck and his wife were invited by the New York Blood Center, where he later became a consultant senior researcher. During their visit to New York he got a telephone call from Dr Eberhard Mammen from Detroit, who asked his advice on a case of dysfibrinogenemia with heavy bleeding. Dysfibrinogenemia is a condition by which the fibrinogen of the patient’s blood doesn’t work properly and the patient either has a tendency to thrombosis, or to bleeding. Blombäck then discovered that the patient had a genetic mutation of an amino acid, one of the building blocks of fibrinogen. That discovery meant a breakthrough in the research on genetically inherited diseases.

Collen describes his Swedish mentor Blombäck as a real Viking, and a rather undisciplined one at that. At the time Blombäck was already spending a third of his time at the New York Blood Center. His files and his bookkeeping in Sweden were a mess. He had a strong dislike for paperwork. One of his one-liners was: “The administration exists to serve us and not the other way around!” Eventually Blombäck was given an ultimatum by the Karolinska Institutet: he had to get his unpaid bills and his administration in order at once or he would be dismissed. “He then disappeared for two weeks with a couple of accountants in the basement of his lab, cleared up everything, and was absolved. He was also divorcing at the time,” Collen recalls. Blombäck’s personal life was a bit eccentric. Several years later, in 1978, he married his Chinese wife, the choreographer and dancer Ching Chiang. She was born in Beijing in 1946 but moved to New York in 1970, where she set up her own dance company, and performed at the Metropolitan Opera. She published her memoirs in 2013.

Collen’s year in Sweden resulted in two important publications in *The Journal of Biological Chemistry*. (17, 18) Publications in scientific journals and citations are crucial in a scientist’s career. “But you need to have a good story and it has to contribute something to scientific research, it must contain new data,” says Collen. “Exceptions are review papers such as *The History of Discovery, The Tissue-Type Plasminogen Activator Story* by Désiré Collen and Roger Lijnen in 2009. That article gave an overview, but if it’s well-written, that too is cited.” (19)

In 1979, after the discovery of α2-antiplasmin (see below), Collen wrote an overview for the Kowalski Memorial Lecture in London, in which he explained in line with the prevailing

insights at the time, how fibrinolysis works. That publication has been cited more than 1,000 times. (20)

Scientists and academics sometimes complain about the relentless focus on publications as a performance indicator. Publications are necessary to receive funding, as scientists who don't publish miss out on research money. Is that fair? "Well, one's output must be quantifiable in some way or other," Collen says. "In life sciences that is not so much of a problem, but of course it is much more difficult in the social sciences or literature. Someone who studies ancient Aramaic texts probably cannot publish much and will not be cited often. Experts of Aramaic are a tiny club. While life sciences, with all those universities and research institutions, with clinical and basic research, keep a few million people busy."

The lab rather than the hospital ward

Returning to Leuven was a relief for Louisa Collen. Désiré on the other hand was facing a compulsory final year of hospital service as an assistant in internal medicine. His year in the US and that in Sweden were each counted as a year of specialization, be it without patients. His three years of clinical practice from 1968 – 1971 also did not represent much in that respect. He spent his first year in the laboratory and he was out with hepatitis for a number of months. Altogether he had barely one year and a half of experience as a physician, while for a senior resident at least three years of clinical experience were required. But Collen felt that he was absolutely not suited to do another year of internal medicine in the hospital.
"I knew too little about it, I couldn't possibly be in charge during weekends or nights with my lack of experience!" Yes, he knew how to draft reports, but if people were brought in with life-threatening conditions, he would not be able to perform adequately, he thought. So he decided to switch from internal medicine to clinical chemistry as a specialization. In those days that was still possible with his degrees in both medicine and chemistry, even though he had never had any formal training as a microbiologist or clinical chemist. It meant that he could return as a clinical biologist to the Laboratory for Bleeding Disorders and Vascular Diseases of Professor Marc Verstraete, an environment where he felt at home.

Fortunately for him, and probably also for potential patients, Collen never practised medicine outside the university. Would he have enjoyed a career as a physician, dealing with patients? "I don't think so. Especially because medicine in those days often came down to intuition and guesswork. In the meantime, significant progress has been made in medicine, but in those days? During my first year as an assistant, if someone came in with a heart attack, he had a 30 percent chance of not leaving the hospital alive! Such a patient was put in bed and told to lie there quietly! Nowadays they immediately get to work with stents and pacemakers, and mortality has dropped to less than 10 percent. But all of that didn't exist then. Professor Verstraete could sometimes pull a patient through with streptokinase. But heart attacks were often fatal at the time."

> Heart attacks or strokes arise because a clot obstructs a narrowed blood vessel, and the underlying tissue receives too little oxygen. If that occurs gradually in the coronary arteries supplying the heart muscle, we speak of angina pectoris (AP). A temporary obstruction of a blood vessel in the brain can cause a transient ischemic attack (TIA). If such a clot completely obstructs an artery, the underlying tissue no longer receives any blood and progressively dies. In the case of a clot in the coronary artery we

speak of a heart attack or an acute myocardial infarction (AMI). A clot in the blood vessels of the brain, whereby too little or no blood reaches a part of the brain, is called an ischemic stroke or a cerebrovascular accident (CVA).

In the event of a clot in the venous portion of the circulation (venous thrombosis) the outflow of blood from a body part can be hindered. The body part swells and its function becomes impaired. In that case the blood clot is often located in the deeper pelvic veins or leg veins (deep vein thrombosis). A part of the clot can break off (embolize) and travel with the bloodstream through the right heart into the lungs. The resulting obstruction is called a pulmonary embolism.

Thrombosis can also arise in the heart cavity (cardiac thrombosis). The direct cause of this is usually a heart attack, arrhythmias, or heart valve defects. In this form of thrombosis too, parts of the clot can separate and travel via the aorta to various organs (brain, kidneys, intestines, legs) and cause major problems there.

Two processes are important in the formation of a blood clot in an artery, or an arterial thrombosis. A blood clot usually forms because blood platelets (thrombocytes) stick to a vascular wall damaged by atherosclerosis and the platelets start to clot. Coagulation proteins in the blood plasma are then activated and a series of biochemical reactions ensue. These ultimately cause the blood to lose its fluidity and a soft clump is formed: the clot. In venous thrombosis on the other hand atherosclerosis does not have to play a role and the role of the blood platelets is much less important. (21)

These days most people are well informed about various risk factors that can cause narrowed blood vessels. Blood pressure, cholesterol and stress levels are regularly monitored. We are reminded to exercise, to keep our weight under control, to moderate the intake of alcohol and not to smoke. But in the 1960s and 1970s smoking and drinking were accepted social behavior. Drinking no alcohol or denying access to smokers was considered an anti-social attitude.

Collen wrote his doctoral dissertation in chemistry on '*The microheterogeneity of human plasminogen*', and his aggregation thesis in medicine, which is required to be able to teach, on the turnover of plasminogen and prothrombin in humans, '*Plasminogen and prothrombin metabolism in man'*. He defended his dissertation in chemistry in January 1974, and although it did not contain many new insights, it allowed him to introduce a number of biochemical techniques and procedures in the Laboratory for Blood Coagulation, such as polyacrylamide gel electrophoresis and immune electrophoresis, with which the identity and molecular weight of proteins could be determined. These procedures would later prove to be crucial. Six months later in July 1974 he defended his dissertation in medicine, which later led to the discovery of α2-antiplasmin, which in turn led to the development of t-PA.

Military service, but research continues

Until 1992 young men in Belgium had to do military service, and Collen also had to spend his fifteen months in the military, from September 1974 until November 1975. He considered it a great waste of time. "I once had to fire off six bullets during a training in Ghent, where I was

stationed for six weeks. And yes, I recall also running a couple of times around the Watersportbaan, a 5 km track course in Ghent." Fortunately, as a married physician with children he could benefit from a few exemptions, and after his initial training he was allowed to work at the blood transfusion service of the military hospital on the Avenue de la Couronne in Brussels. That was right up his street, and moreover it wasn't a fulltime job. He had to show up only three or four times a week for half a day.

Soldiers who volunteered to donate blood were given a day off. Professional soldiers who sometimes needed a day at home to fix up their garage or work in their vegetable gardens, were eager blood donors. However, their livers were not always in the best condition due to alcohol abuse and hence their blood was unsuitable for the blood bank. Actually, with their affected livers they never should have been allowed to give blood in the first place. But Collen turned a blind eye. As far as he was concerned, this was a win-win situation. The soldier in question got his day off and Collen used the blood for his research, because before and after his hours in the military hospital, he went to work on his own research in Leuven. "Their blood was good enough for me, I only needed the plasma to purify proteins of the blood coagulation system." In the registers of the military blood bank he justified the absence of a usable blood sample as '*insufficient quantity*" or '*hemolysis*', a condition by which the red blood cells fall apart, making the blood bag useless. Because of his previous experience in Leuven, he probably was the most experienced blood sampler that the military hospital had seen in a long time, but his track record in the registers of the military blood bank was substandard: he had contributed very little to the army's blood supply.

In November 1975, he was discharged. By now he was 32 years old and his actual career had yet to begin. He got a temporary appointment as Assistant Lecturer at Leuven University, and in October 1976 he could start as Lecturer at the Faculty of Medicine and as Adjunct Head of Clinic at the Department of Marc Verstraete in the Internal Medicine Division.

To move up the academic ladder, he had to have a teaching assignment, but due to his double degree, that was a problem. The faculty of medicine didn't want him because he was a chemist, and at the chemistry faculty they told him that as a physician, he had to apply with the medicine faculty! Both departments only had a limited amount of teaching hours to hand out. Teaching hours were a scarce commodity, over which every faculty watched anxiously and Collen would need at least five teaching hours per week to be eligible for appointment. Eventually an opportunity arose at the faculty for physical education and physical therapy, at the time a very popular curriculum with some 350 students in the first year, where someone was needed to teach biochemistry and chemistry. Oral exams were still customary at the time. It meant testing 350 to 500 students one by one in two months' time. Tiring days, but Collen had developed his own system. One of his students recalls how he arrived in the auditorium: "He came in with his book bag. We could prepare our exams, with about 50 students spread over the auditorium with a reasonable distance between us to prevent cheating. The first thing he said was 'Anyone who wants an F (failed) can hand in his paper right now!' which already thinned out the group. Students who had not studied much, but still needed proof that they had been present at the examination in order to keep their study grants, could leave without further ado."

Collen wrote his questions on the blackboard, the same for every student and whoever was ready could come up front for the oral exam. Because the whole group had the same questions, Collen could easily compare." In less than two minutes I could hear whether they knew their material or not." In his career as a lecturer, he gave about 5,000 oral exams.

He continued teaching until 1984, but from 1980, he had to fly regularly to California where he had started collaborating with Genentech Inc. Consequently, his teaching schedule needed constant adjusting and arrangements had to be made each time with one or another of his colleagues. This situation lasted until rector Roger Dillemans decided in 1984 that the situation was becoming too complicated, and colleague Roger Lijnen took over Collen's classes.

Discovery of α2-antiplasmin

When Collen started his research into fibrinolysis - the natural process by which blood clots are broken down - scientists already knew quite a bit about the formation of blood clots and the activity of plasmin, an enzyme that degrades many blood plasma proteins, including fibrin clots. Plasminogen circulates freely in the blood. When streptokinase binds to plasminogen, it forms a complex activating other plasminogen molecules to plasmin, which in turn breaks down fibrin or blood clots.

Blood clots form where tissue or a blood vessel is damaged, the tissue around the wound tightens, and blood platelets form a plug to close the wound. Prothrombin is converted into thrombin, which in turn makes fibrinogen convert into fibrin. Fibrin is a handy network of strands that neatly closes the wound, together with the red blood cells and blood platelets. When the wound has healed, the whole structure is again broken down and the blood clot is dissolved by plasmin. This natural process is called thrombolysis: the plasminogen in the blood becomes plasmin, which cuts the fibrin strands into pieces, and the job is done.

In 1933, the American physician and microbiologist William S. Tillett (1892 -1974), who worked as an assistant Professor at the Johns Hopkins School of Medicine in Baltimore, Maryland, had discovered that some strains of the streptococcus bacteria produced a protein that could dissolve fibrin clots. The streptococcus bacteria is known among other things for causing throat infections. Using bacteria to dissolve blood clots is not as surprising as it seems. Normally the human body wards off bacteria by letting the blood around the infected site clot, so as to enclose and isolate the bacteria. But bacteria in turn have developed a protein that serves as their escape mechanism. This protein sets the clot-dissolving process in motion, breaking up the fibrin strands, so that the bacteria can break free. Tillett first named this protein fibrinolysis, but later it became known as streptokinase. A decade later scientists discovered, thanks to streptokinase, how exactly fibrin fibres are broken down. (23)

In 1947 British scientist Robert Gwyn MacFarlane (1907–1987) noticed that urine also contains a substance, urokinase**,** that can dissolve blood clots. It is an enzyme that cuts plasminogen, releasing plasmin. And plasmin in turn cuts the fibrin strands into pieces. (23) That same year, Tage Astrup (1908–2006) discovered that certain animal tissues contain an enzyme with properties similar to those of streptokinase. They named it fibrinokinase, but it is now generally referred to as tissue plasminogen activator or t-PA, which later became the

essence of Collen's scientific success, but no further research was done on the subject at the time (24,25).

But science was making more and more progress. If by human intervention plasmin could be activated to dissolve blood clots, it ought to be possible to stop this process, because otherwise all the fibrin in the human body would be cut up by plasmin and people would bleed to death. In the natural event of an injury, the human body has its own safety procedure: when the wound is healed, the production of plasmin and the clot-dissolving process is stopped. But with an outside intervention, when for example streptokinase is administered, the clot-dissolving process goes into overdrive, and then it obviously needs to be stopped.
Not much was known then about means to neutralize plasmin or how it disappeared from the blood, although clearly plasmin did not remain in the body in its active form.

Collen began by focusing on plasminogen. He studied the clearance of plasminogen: how fast plasminogen is converted into plasmin and how quickly the plasmin is then cleared from the human body. In 1970 he decided to try the process out on healthy test subjects. For his experiment, he needed pure plasminogen, which he obtained from Per Wallén (1927-1999) in the Karolinska Institutet in Sweden.

Björn Wiman (°1942), who was a lab technician in Wallén's lab, did research on plasminogen. Björn Wiman and Collen were contemporaries. Wiman the lab technician eventually obtained his degree in medicine, with professor Wallén's support, and later became a clinical biologist with a doctoral degree of the new university of Umeå.
"Umeå is a pretty town by the way, but freezing cold in winter," Collen recalls. He went there on several occasions to work. "I once visited Umeå in January, when it was minus 27 degrees Celsius." He still shivers at the recollection. But the summers weren't any better: "By then the place was infested with mosquitoes."

In 1970 when Collen had gone to Stockholm to have five liters of frozen plasma purified into homogeneous plasminogen, Björn Wiman was still working as lab technician at the Karolinska Institutet. The highly purified plasminogen would be labelled with radioactive iodine, and would then be injected in volunteers. Collen would afterwards examine how fast the radioactivity and therefore also the plasminogen disappeared from the blood plasma, via the urinary passages. Collen, then a healthy male of 27 used himself as the first volunteer. The radioactivity in his blood was recorded to have halved in 2.2 days. (26)

In 1973 together with Jos Vermylen (°1937), Collen also studied the turnover of radioactively labelled plasminogen in patients who were being treated with streptokinase. "Jos and I worked well together. He is a first-class person." In 1989 Collen and Vermylen succeeded Professor Verstraete as a duo. "Jos did the clinic and I did the lab. And that succession went smooth and easy." This was not always the case in other departments, according to Collen.

At first, Collen and Vermylen were confused: streptokinase very quickly converted plasminogen into plasmin in patients, which sometimes made the blood clot disappear. But this fast conversion was very short-lived. The radioactivity on the other hand disappeared

much slower from the blood. They concluded that there had to be a substance that neutralized the plasmin, an inhibitor that was binding with plasmin. (27)

“We had to isolate this plasmin complex with its inhibitor, to find out what the inhibitor was,” says Collen. At first, they thought α1-antitrypsin or α2-macroglobulin could be involved in the neutralization of plasmin, because that was the hypothesis the textbooks suggested at the time. It could be one or the other. Maybe α1-antitrypsin was possible, but α2-macroglobulin was out of the question, because it is a much larger molecule. It eventually turned out to be a hitherto unknown protein: α2-antiplasmin! “We published our findings in *The European Journal of Biochemistry* in 1975. And our name was made, because I received a couple of scientific awards for that discovery.” Subsequently he started to study this protein and to identify its biochemical and kinetic properties together with Björn Wiman and Roger Lijnen. (28)

Among other rewards the discovery of α2-antiplasmin gained Collen, the Prix Servier Lecture in 1978 together with Björn Wiman, and the Edward Kowalski Memorial Lecture in 1979. “The latter was an extraordinary lecture. With an audience of about 3,000 people! Afterwards you were given a sort of certificate, an important document! It is still hanging on the wall in my office at Gasthuisberg.” His Kowalski lecture has been cited more than 1,000 times. The newly discovered α2-antiplasmin turned out to be an excellent inhibitor of freely circulating plasmin, but it left the plasmin bound to fibrin, (plasmin that was doing its job on a blood clot), untouched. (29)

The α2-antiplasmin was an important scientific discovery. The logical next step was therefore to apply for a patent, so that possible revenues from the commercial exploitation of α2-antiplasmin would be protected. The discovery was patented under the name ‘*Thrombosis Test’*. It tested whether the fibrinolytic system in blood was activated, that is whether the patient had experienced a thrombosis, or was having one at the time.

On 20 December 1972 the university had set up a non-profit organization for technology transfer, Leuven Research and Development (LRD), by initiative of the then Rector Pieter De Somer. It was to take care of the protection of patents and of the allocation of funds these generated. As such it was one of the first institutions of its kind in Europe.

A first version of Collen’s patent for the Thrombosis Test was submitted in the Netherlands on 19 September 1975, and, a year later, on 13 September 1976, in the United States. If a test based on the patent would generate income, then after deduction of the overhead costs, 50 percent would go to KU Leuven, part to LRD and the remainder in negotiable proportions, to the lab in question and to the inventors or the collaborators. But the board of directors of LRD could always rule otherwise.

In February 1976, shortly after filing his first patent, Collen concluded an agreement with LRD:

> ‘*Dr D. Collen and his co-workers employed at the Katholieke Universiteit in Leuven relinquish for the benefit of the non-profit organization Leuven Research and Development all legal, commercial and financial rights and the exercise thereof that*

are related to research results that they have directly or indirectly obtained in the context of their educational and research tasks at the Katholieke Universiteit Leuven….
The specific distribution modalities (of the revenues, ed.) will be determined in due course in mutual consultation between LRD and Dr D. Collen and submitted for approval to the board of directors of LRD. The board of directors of LRD can adapt its position in this matter to the circumstances at all times.'

The agreement was signed by Désiré Collen, with the approval of Marc Verstraete, the head of his department, by Jos Bouckaert, the director of LRD, and Guido Declercq, then the managing director of KU Leuven. LRD from then on took care of the further processing of the files.

Breeding rabbits

In the meantime, Collen and his family had moved from Leuven city center to one of the green suburbs. While Désiré was working in Denmark in 1976, Louisa Collen had found a house with a large living room. Not in the wooded suburbs of Heverlee or Linden, where most professors had their dwellings, but in Winksele, West of Leuven. It had a considerable garden of nearly half an acre, to which shortly afterwards a meadow was added.

As the grandson of small farmers who lived from a few chickens and dairy cows. Désiré was familiar with small livestock. Fairly quickly a respectable rabbit hutch appeared in the garden in Winksele. Rabbits were useful animals for producing antiserum, blood serum that has become immune to previously injected agents. The antibodies from antiserum are necessary to purify or identify proteins, and Collen's lab had a great need for antiserum to study the proteins involved in blood coagulation and fibrinolysis. The university animal center also had experimental animals for that purpose, but these were expensive. Researchers had to purchase the animals through the university from authorized suppliers, as the university did not breed its own. The animals would be kept in the animal center, but that also had to be paid for. It was much cheaper for a researcher to buy his own rabbits on the market and let them breed in a hutch in the garden. This is what Collen did.

Rabbits are small animals, but they provide enough antiserum. "You can collect a lot of blood from a rabbit if you know how to. A '*Flemish Giant'*, a sizeable breed can weigh up to 7 kilos, and they have long ears," Collen, recalls. In the ears runs an artery from which quite a bit of blood can be drawn. The animals in Collen's garden were injected with proteins against which they generated antibodies. A couple of weeks later, blood was drawn and the lab had its antisera. But as the need for antisera grew, the animal collection in Winksele was extended and larger animals were acquired, such as goats and a pony.

The antiserum production in Winksele had caught the attention of scientists at the Rega Institute, which was headed by Pieter De Somer. They needed large quantities of antisera to purify interferon, a protein Fons Billiau and his co-workers were studying as a possible remedy for viral infections such as hepatitis, or even for cancer.

"We too depended on the university animal center for our antisera," says Fons Billiau, "but they had no large animals, only rabbits, guinea pigs, rats and mice. We needed much more antisera than these small animals could provide." When they decided to produce and purify human interferon in the early 1970's, Pieter De Somer suggested they take on someone who

was trained in biological production techniques. An agricultural engineer from Ghent University, the future Professor Jo Van Damme (°1950), the son of azalea growers, presented himself. So, over time, a few goats were reserved at Collen's for Billiau's interferon project. It was Van Damme who regularly went to the meadow behind Collen's house to immunize the goats or draw blood for antiserum preparations. Caring for the rabbits and tending the goats was mainly Louisa Collen's responsibility, Fons Billiau remembers.

Gradually there were more labs and companies needing antisera that called on Collen's livestock business, and the antiserum production in Winksele proved a welcome source of income. The first patent for the Thrombosis Test brought in very little. On the contrary, it cost a great deal of money. "A patent is an expensive business. You have the filing charges. If you want to protect a patent in America, Australia, Canada, the European Union, Japan and South Korea, and nowadays also Russia and China, it quickly costs EUR 100,000. And when you first file it with the patent office, it is almost certainly returned with all sorts of comments. You will need a patent agent to make a rebuttal, refuting those comments, and to file the application again. You're most likely to get it back once more to modify and file it again." This process could be repeated several times, and the entire procedure can take years. Moreover, patent agents are expensive professionals, as Collen soon discovered. Luckily, his modest antiserum enterprise in Winksele had meanwhile acquired quite a reputation, and was providing a handsome income.

Chapter 2: The development of t-PA

Björn Wiman

Fons Billiau

Dick Rijken

Osamu Matsuo

Roger Lijnen

Irène Juhan-Vague

Key collaborators involved in the preclinical development of t-PA

The laboratory of Marc Verstraete in 1978. At the top second from left Marc Verstraete, fourth from left Björn Wiman, sixth from left Roger Lijnen. On the first row on the very right Désiré Collen, on the very left Jean-Marie Stassen

The laboratory of Marc Verstraete in 1980. At the top, second from left Osamu Matsuo, third from left Marc Verstraete, fourth from left Dick Rijken and at the very right Désiré Collen

***Summary**: As a treatment for heart attacks, streptokinase had limited efficacy and occasionally severe side effects. It disrupted the patient's entire blood coagulation mechanism, so the medicine sometimes turned out to be worse than the ailment. A safer remedy was needed to dissolve clots and cure heart attacks. Collen, with his colleague Fons Billiau, was at the time exploring potential new cancer drugs, for which they had cancer cells sent over from the United States. Quite by accident, they discovered t-PA (tissue type plasminogen activator) as a new substance to dissolve blood clots. Together with Dick Rijken and Osamu Matsuo, Collen succeeded in purifying human t-PA and demonstrating its thrombus-dissolving ability. They had discovered a new enzyme and became the inventors of an important patent.*

The experiments of Björn Wiman

Collen and his colleagues constantly investigated how side effects could be avoided in the thrombolytic process. With α2-antiplasmin, they had already identified the most important inhibitor of plasmin, but that didn't get them very far. They ended up with t-PA (tissue type plasminogen activator), a protease or enzyme protein that increases the breakdown rate of other proteins, in this case plasminogen. With the plasmin that is released, the thrombolytic process is set in motion; the plasmin takes care of the fibrin in the clot.

At Sweden's University of Umeå, in 1975, Björn Wiman and Per Wallén were the first to purify t-PA from the ovaries of pigs. Wiman was seeking a way to measure the activity of t-PA by having it react with plasminogen and allowing the plasmin so produced to act on casein, a protein in milk that is easy to purify. He anticipated that the cutting of plasminogen would also work in the presence of casein. But nothing happened. (1)

Wiman had been informed by colleagues of the Danish researcher Tage Astrup (1908–2006) - who had identified and partially isolated t-PA in 1952 - that they believed t-PA had an affinity for fibrin. (2) They could not prove it, because at the time they had no purified components. Therefore, Wiman tried out the t-PA on casein to which he had added purified fibrin. Surprisingly enough, the casein was quickly broken down. His conclusion was that t-PA had to be bound to fibrin to activate plasminogen and set the dissolution mechanism in motion. When, in August 1977, Björn Wiman arrived at the lab of Marc Verstraete, Collen was still very much engaged in his antiplasmin research, but Wiman's observations turned out to be crucial for further exploration into t-PA.

As previously mentioned, streptokinase was from 1958 already being administered to patients suffering from heart attacks in the United States. This was being done by Sol Sherry, the scientist who had worked with William Tillett, the discoverer of streptokinase. In Leuven, Marc Verstraete, too, had used streptokinase in patients, from the mid-1960s. (3) But the treatment had hazardous side effects: it could cause heavy bleeding. When streptokinase was administered to a patient to dissolve a blood clot in a coronary artery, the plasminogen was not only activated in the clot, but also everywhere else in the bloodstream. In other words, the patient's entire blood coagulation mechanism was disrupted. If, as Wiman had suggested, t-PA attached itself to fibrin and only did its clot-dissolving process there, that would be a better-targeted and possibly safer treatment.

Fons Billiau and the melanoma cell line

Getting hold of pure t-PA and doing research on it was no simple task and would not have been possible without the research team of Fons Billiau in the Rega Institute. A collaboration had developed between the research groups of Collen and Billiau to study the similarity between natural fibrinolytic enzymes and fibrin-splitting enzymes (proteases) secreted by cancer cells. They believed that this could be the first step towards a new cancer drug. A year earlier, an American scientist, Ed Reich of the Rockefeller Institute in New York, had observed a connection between the malignant behavior of tumors in cell cultures and the secretion of a plasminogen activator. (4) Reich demonstrated that malignant tumors frequently secreted plasminogen activator, and that the malignant phenotype of the tumors correlated with the quantity of 'malignant protease' they secreted.

"We cultured cancer cells from tumors taken from mice, but also from patients, mainly in the hypothesis that carcinogenic viruses were hidden in these cells," recalls Fons Billiau. "Cancer cells spread out – metastasise – and, in doing so, make various types of proteases. Collen was working with protease inhibitors then, and we thought that our joint efforts could lead to something." They hoped that the inhibitors would have antitumor properties. (5)

Initial tests with the mice cell lines from the Rega Institute were positive, but it took two weeks to do just one test, Collen remembers. "Fons said to me: well, if you want us to make any progress here, you have to isolate that malignant plasminogen activator so that we can do tests directly on it." Collen was told he should look out for malignant cell lines that produced large quantities of malignant plasminogen activators.

During a conference abroad in 1978, Collen one day sat next to Grant Barlow, who worked for the American pharmaceutical company Abbott. At the time, Abbott produced urokinase for thrombolysis from foetal kidney cells, and Collen told Barlow that he was looking for a cancer cell line that could produce large amounts of plasminogen activator. "Oh well," Barlow replied, "we were sent such a cancer cell line by Ed Reich in New York!" It was the cell line of a certain Mrs. Bowes who had died from melanoma. Ed Reich had suggested that Abbott use this cancer cell line instead of kidney cells to produce their plasminogen activator. The Bowes cell line would yield much more and last much longer than the kidney cell culture with which Abbott was then working. But Barlow told Collen that they had tried this, cultured those cancer cells and worked with them, but the procedure really went no better than that with the kidney cells. Furthermore, the production from kidney cells was a standardized process; it wasn't advisable to throw it out. He promised, however, to send Collen some culture medium from the particular cancer cell line. A week or two later, a jar with conditioned medium from the Bowes melanoma cell line arrived in Leuven. The liquid in which the melanoma cells had been cultured, and from which the cells had drawn nutrients to multiply, contained various substances the cells had secreted, including plasminogen activators.
Mrs Bowes had died a few years earlier from the consequences of melanoma, a skin cancer, and the cell line originated from a metastasis in her lungs. The culture medium indeed contained a fair amount of plasminogen activator activity. But Collen needed much more than the quantity that Barlow had sent him. He would need the original cell line.

During this time, Collen was contacted by Dan Rifkin (°1940), who worked at Reich's lab in New York. Rifkin had flown to Europe and was staying in the Netherlands, visiting his in-laws. He wanted to drop by in Leuven to do some work with α_2-antiplasmin and its antiserum, but when he heard that Collen was looking for the Bowes cell line for his research, he promised to get it for him.

Fons Billiau still vividly remembers the day the Bowes cell line arrived. "Désiré burst into my lab: Fons, I have a cell line here, and it makes proteases! Have a look at it!" That 'bursting in' must be taken literally. It was a characteristic feature of Collen at the time. An American researcher who worked with Collen for a while told Billiau, "Working with Collen is like trying to hold a bear by its tail!"

Unlike the Rega Institute, Collen's lab had no experience in culturing cells, but for the Rega Institute the Bowes project came right on time. Their interferon research was not leading anywhere, and Billiau was happy to collaborate on a new project. He began to culture the Bowes cell line and provided Collen with several liters of liquid. It was the beginning of a major breakthrough.

Collen took the liquid to his lab and asked his lab technician, Claire Vercruysse, to add some fibrin to it and see what would happen. To everyone's delight, it stuck! Collen was convinced it contained t-PA. The date was 9 February 1979. In an interview with the newspaper *Het Belang van Limburg* on 20 April 2013, he remembered that 9th of February as one of the happiest days of his life. All he needed now was scientific proof; the protein had to be isolated and they had to find out whether it had the same properties as natural t-PA and reacted with the same antisera.

The discovery of t-PA from the Bowes melanoma cell line turned out to be an exceptionally lucky strike, they later learned. Cancer cell lines normally only produce urokinase. Only very rarely do melanomas produce t-PA like the Bowes cell line. "It turned out that we drew the winning lottery ticket!" Collen admits. But a scientist does need luck every now and then. The gratifying factor for Collen is that he was only 35 when his lucky day arrived!

As the Bowes melanoma cell line was so productive, it had already been cultured in other labs as a source of plasminogen activator, but apparently no one had yet come up with the idea of studying what kind of plasminogen activator was involved. Still, Linette Wilson, a South African pathologist who worked with Reich, had discovered that unusual activity was indeed taking place; she became aware of this when she studied the pathology of those malignant cells. She had made extracts of the various cell lines and put them on a gel to separate them according to molecular size. "When the gel was covered with a fibrin film, she saw that there were two areas where fibrin dissolved: somewhere around molecular weight 54,000 dalton, but that is urokinase. And also around molecular weight 70,000 dalton. But at that time, she didn't know the kinetics of fibrin-specific plasminogen activation. There, we were ahead of her. She probably would have discovered it too six months later," Collen acknowledges.

"That's why you have to be the first, even if it's not by miles," he adds. "Look at Charles Darwin and Alfred Russel Wallace, who both discovered the principle of natural selection at

approximately the same time. Wallace developed his theory on the development of species through natural selection during his journeys along the Indonesian and Malaysian islands. On 9 March 1858 he sent the essay on his theory, *On the tendency of varieties to depart indefinitely from the original type*, to Darwin, who immediately recognized it coincided with his own findings. Darwin took the essay to the Linnean Society, the predecessor of the Royal Society of England, and explained that after his trip with the Beagle he had come to the same conclusions as his colleague. What now? The people at the Linnean Society decided to have both essays read at their next meeting, and to publish them together. But if Wallace hadn't sent his essay to Darwin and had published it immediately instead, Wallace would have been the first!"

Collen expects similar situations with Crispr-cas9 *(Clustered Regularly Interspaced Short Palindromic Repeats),* a revolutionary method for genetic editing. "Who will eventually get the patents on that technology or receive the Nobel Prize for it? Quite a few scientists have worked on it and discovered things at different stages."

Everyone wishes to be the parent of a discovery, but that does not prevent scientists from collaborating. At the start, culturing a few liters of Bowes cells was a small job that could be done easily on the margins of the regular work at the Rega Institute, as a kind of friendly favor. But gradually Collen required much more for his research. "Eventually he needed tens, hundreds of liters," Billiau recalls. "That was no longer possible for us. It would mean we would be constantly working for Collen in our lab, which I could not justify. However, we did have the equipment and the working space available, because we had cut down on our production of interferon, so Collen decided to pay his own lab technician, who would work on his project in our lab."

Dick Rijken and Osamu Matsuo

The first six months were difficult because the t-PA from the Bowes cell line turned out to be sticky stuff that clung to the glass and plastic lab equipment and was hard to remove. That lasted until Dutch researcher Dick (Dingeman) Rijken (°1952) arrived in Leuven. He had done his master's in biochemistry in Utrecht in 1974 and had then moved to Leiden to focus on t-PA for his doctoral dissertation. Collen and Rijken had met once before – in September 1978 at an international fibrinolysis conference in Karlovy Vary, the former Carlsbad, in Czechoslovakia. There, Collen and Wiman had received the *'Prix Servier'* for their work on α2-antiplasmin.

During a visit Rijken made to Professor Verstraete's lab in October 1978, it was suggested that he should do further research on t-PA in Leuven after finishing his doctorate in Leiden. But Rijken had some reservations; he felt that t-PA had already been identified and there was little new to discover. He had explored the functioning of t-PA in his dissertation, based on research he had done with t-PA from human uteri. Despite this, Collen was somehow able to win him over, and Rijken came to Leuven in September 1979 for his postdoctoral research. No sooner had he arrived than he solved one of the 'sticky' problems in Collen's lab.

Pending Rijken's arrival in Leuven, a system was set up to collect human uteri in various hospitals. Although in Leuven they continued culturing t-PA from the melanoma cell line, t-PA

from human uteri was needed as a reference to compare the Bowes t-PA from malignant cells with normal t-PA, and to prove that the two were identical. The Belgian Gynecological Society promised that after surgeries for fibromas – benign tumors of the uterus – the surgical specimens would be kept in the freezer for Collen. Every six weeks, one of the lab employees went around the country to collect them, and Roger Lijnen (°1952) and his collaborators then ground the tissue to extract the human t-PA. This turned out to be a tough job because, as noted previously, t-PA was sticky stuff. The minute quantities the researchers managed to produce largely stuck to the test tubes. Dick Rijken had purified small amounts of human t-PA from uteri at the Gaubius Institute in Leiden during the research for his doctoral thesis and had solved the sticking problem by adding Tween80, a detergent. “That was it! With Rijken’s Tween we had pure t-PA in six weeks’ time!” Collen recollects.

Dick Rijken was, in fact, the first to purify human t-PA. “Okay, very small quantities, some two milligrams from human uterus material, but he made the difference!” says Collen. “We later made two grams from the Bowes cell line, and by the time Genentech got involved, they eventually produced kilos and kilos of recombinant t-PA!” (6)

That same year, the first Japanese collaborator, Osamu Matsuo (°1942) from Miyazaki, a medium-sized city on the island of Kyushu, arrived in Leuven, with a one-year grant to do research on the coagulation system. In November 1979, Dick Rijken had produced pure t-PA, but further research was needed. Could t-PA dissolve blood clots in experimental animals? Matsuo had developed an in vitro experiment with circulating plasma and a clot in a test tube. But in vivo proof was also needed. Matsuo had worked out a procedure to test on rabbits, in which he injected a radioactive clot of a specific size into the neck veins of the animals. That clot went to the right heart chamber and then to the lungs. Because the pulmonary vessels (pulmonary capillaries) are small microvessels, the clot remained stuck there, causing an experimental pulmonary embolism. With a Geiger counter, Matsuo could see the exact location where the radioactive embolism had moved, and how big it was. He then administered t-PA in the hind legs of one set of rabbits, and urokinase to a control group. “After a few hours the size of the clot was measured, and it was obvious: t-PA worked better than urokinase. Bingo! An article in *Nature*!” recounts Collen. (7)

Matsuo was a disciplined man. “He started at around 9 o’clock with his rabbits, but because his tests had to be done at regular intervals, he was often busy until 3 am. The poor man gradually turned more grey-faced by the day, and I got quite concerned,” says Collen. “He was living on his own in Leuven that year, as his wife and children had stayed behind in Japan. One day I told Louisa, I’m bringing him home tonight for dinner! But because his rabbits needed constant monitoring, we took them along and put them in our bathroom. The animals didn’t budge, because they were under anesthetics. And every now and then Matsuo got up to take blood samples. After dinner we took the rabbits back to the lab for the rest of the experiment. We spent our evenings like that a couple of times.” The bottles of Old Smuggler at the Collen’s home emptied at a rapid pace, but Matsuo began to cheer up and to look much better. Consulting Matsuo’s lab notes about his rabbit experiments in Leuven later turned out to be problematic, as they were largely in Japanese. But the essential data had been analyzed, and the results had been published.

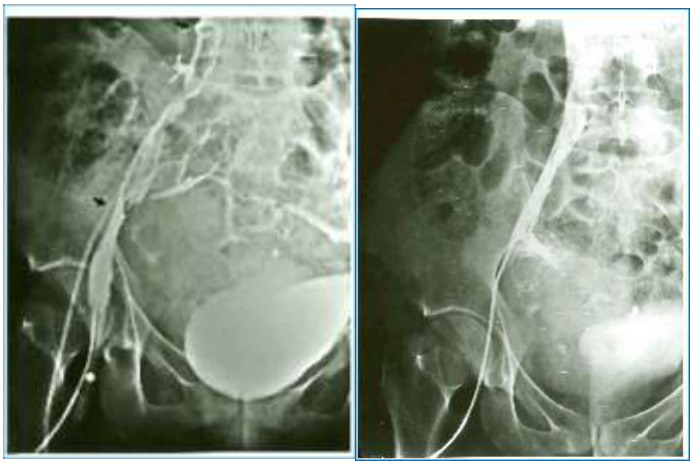
Marc Hoylaerts, meanwhile, was studying the kinetics of plasminogen activation by t-PA and the role of fibrin. “He published a fine article in the *Journal of Biological Chemistry* that was cited over 1,000 times,” says co-author Collen. (8) In 1982, Irène Juhan-Vague had started her PhD training with Collen and was studying the pathophysiological role of the just-discovered PAI-1, the main inhibitor of t-PA and urokinase. She discovered that it rose enormously in patients who had undergone a serious operation or who were gravely ill. Elevated PAI-1 levels turned out to be a risk factor for venous thrombosis. (9)

In the book *Pathobiology of the Endothelial Cell* that appeared in 1982, Collen wrote the chapter *Regulation of Fibrinolysis: Plasminogen Activator as a Thrombolytic Agent*, in which he gave an overview of the functioning and properties of the melanoma t-PA and of the results of the α2-antiplasmin research. (10)

In May 1980, when the results of Matsuo’s research were available, and a month before the patent application, Collen gave a progress report on his t-PA research at the Rega Institute because his friend Fons Billiau and the lab technicians had all been very helpful in culturing the Bowes cell line. “After my presentation at Rega, we had lunch in the Faculty Club, a fine restaurant in the Leuven beguinage, and Rector De Somer told me to sell my research to a pharmaceutical company, and he suggested Bayer. We got a research contract with Bayer for a while, and they gave some funding, but were not really interested,” Collen says. He did not yet have much experience in promoting and selling his research, and Bayer – solicited for hundreds of different projects – was not really active in his area of research. It was Collen’s first but not his last business deal.

A month later the patent application for t-PA was filed, first in the Netherlands. Europe and the US would follow. The patent was in the names of Collen, Rijken and Matsuo. “Rijken had helped with the purification, and Matsuo with the first animal tests. In retrospect I think that we should have also given Fons Billiau more credit for his work,” Collen admits. The filing was done on 11 June 1980 because, on 12 June, Collen would make the results of his t-PA research public at the *Fifth Congress on Fibrinolysis*, in Malmö, Sweden.

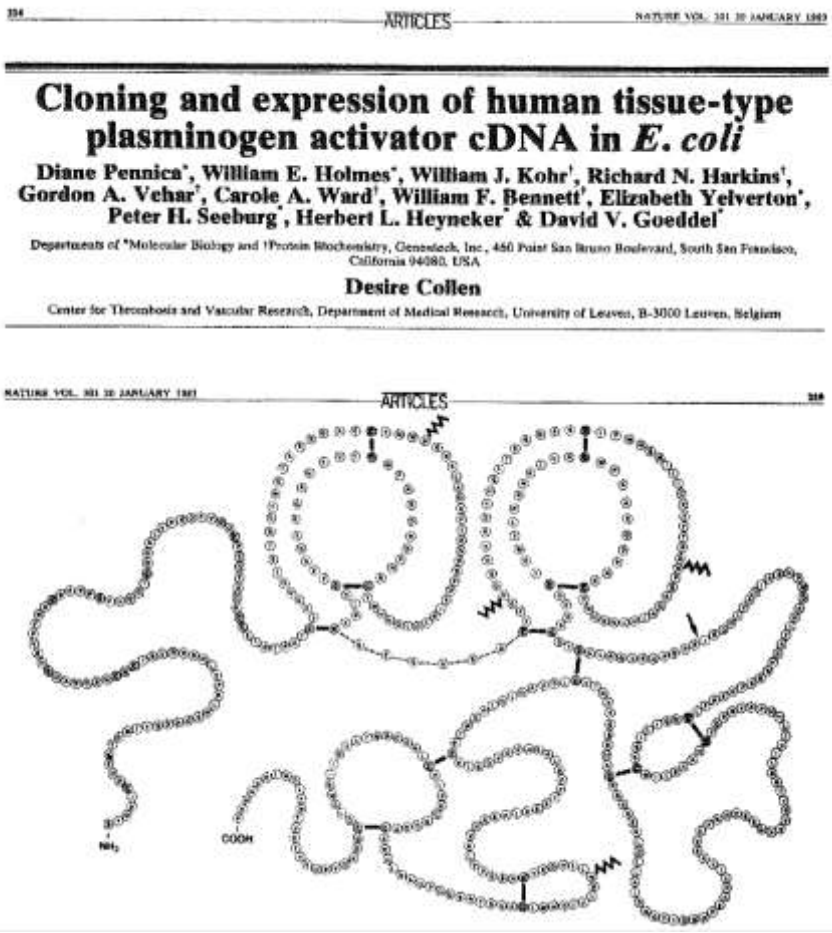
Chapter 3: Enter Genentech



Before t-PA After t-PA Diane Pennica

First renal transplant thrombosis patient

(Weimar et al. Lancet. 1981;2:1018-20)



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Cloning and expression of human tissue-type plasminogen activator cDNA in *E. coli*

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***Summary**: The American biotech company Genentech Inc, a pioneer in biotechnology and in cloning genetic material, sent young scientist Diane Pennica to a congress where Collen would present his latest research. She proposed cloning Collen's t-PA, and although he hesitated at first, he eventually concluded the most important contract of his life. When t-PA was tested and compared to streptokinase, it turned out to produce better results, and several patients were successfully treated. After months of intensive research work, Pennica succeeded in cloning t-PA, and the world's press all wanted to speak with her. When a few years later Genentech learned that other researchers in Leuven had also cloned t-PA, there was a short glitch in the relationship, but the misunderstanding was soon smoothed out.*

DNA attracts money

The day after the patent was filed, Collen left for the Fifth Congress on Fibrinolysis in Malmö. On 12 June 1980 he was at a closed meeting with scientists, listening to one another's presentations. The French pharmaceutical company Servier had organized this satellite meeting the day before the actual congress. "Among other things, we discussed stanozolol, a synthetic anabolic steroid promoting muscle formation, which was sometimes administered to horses to improve their racing performance. Servier thought in the beginning of the 80s that stanozolol could also be useful to treat reduced blood circulation in the legs," Collen says. The steroid was tried for a while but is no longer used for that condition. Moreover, in 1974 the International Olympic Committee placed it on the list of prohibited substances for athletes. Yet, years later at the Olympic Games in Seoul in 1988, the Canadian sprinter Ben Johnson was caught using stanozolol. He was disqualified, his record was removed from the books by the IAAF (the International Association of Athletic Federations), and he had to give up his gold medal. It was *the* big doping scandal of the Seoul games, although many more would follow.

During a congress, or the day before or after, it is customary for satellite meetings to be held, in which someone is invited to give additional information on the topic concerned, in this case fibrinolysis. Doctors who are less familiar with the research in that field may attend and obtain more biochemical background. Since Collen had filed his patent for t-PA a day earlier, he decided to make his t-PA story public in Malmö, and that is how he came in contact with the American company Genentech, then one of the first to work with recombinant DNA technology. It was a forerunner in experimenting with 'cloning'.

Genentech had been founded a few years earlier, in April 1976, by Herbert W. Boyer, a biochemist, and Robert A. Swanson, a young economist. Robert Swanson (1947 – 1999) was an alumnus of the Massachusetts Institute of Technology and an enterprising young man, invariably in a three-piece suit. He had a bachelor's in chemistry and a master's in business administration. After his studies in 1970, he worked as a partner at Kleiner & Perkins Venture Capital Partnership in San Francisco and as an Investment Officer at Citicorp Venture Capital Limited.

Biochemist Herbert W. Boyer (°1936) was almost his opposite; he dressed in jeans and a T-shirt, had a typical '70s moustache, and was not interested in business activities. He was a passionate researcher, based at the University of California in San Francisco. Together with Stanley N. Cohen (°1935) from Stanford University, he conducted the first successful experiments with genetic manipulation in 1973. Cohen and Boyer had developed

recombinant DNA technology. They had proven that genetically manipulated DNA molecules could be cloned in a foreign cell and had succeeded in transplanting genes from one living organism to another.

Two of Boyer's postdoc students, Francisco Bolivar and Raymond Rodriguez, had made a cloning vector, pBR 322. A cloning vector is the carrier or vehicle of the altered DNA that must be cloned and used to transfer it from one cell to another. The 'p' in pBR 322 stood for phage or bacteriophage; the 'B' and the 'R' stood for the initials of the developers Bolivar and Rodriguez.

Robert Swanson, ever the entrepreneur, was looking for promising start-ups in which to invest. He had read quite a bit about this new cloning technology and had already contacted researchers at various universities. That is how he came to ring biochemist Herbert Boyer, introducing himself as a venture capitalist who was fascinated by this new scientific development. Did Professor Boyer think that the technology could be commercialized? Boyer thought so. And could they meet? There Boyer was reluctant. He was very busy, but after much urging by Swanson, he decided to give the man ten minutes in his lab in San Francisco. An appointment was made for Friday evening, but just to be sure, Boyer briefly looked up the exact meaning of 'venture capitalist'.

Surprisingly enough, they hit it off immediately. The ten minutes in the lab became an hour, after which they decided to continue their conversation in Churchill's Bar, a neighborhood place. Another four hours later they had agreed to set up a small company for genetic manipulation. They would call it Genentech, an acronym for *'*Genetic Engineering Technology*'.* (1)

Genetic manipulation! The idea did not immediately meet with enthusiasm. In both the business and the academic world, many had reservations and did not see much prospect in this strange new technology. Nor were investors queuing up, because Boyer and Swanson had nothing in hand yet to prove that this technique would produce new medicines and could be a profitable investment. They decided to start small in order to master the technique, and began with cloning somatostatin, a small protein of 14 amino acids. At least, that is what the scientist Boyer decided, because Swanson the businessman wanted to start cloning insulin, a larger protein that was commercially more interesting. At the time there was a great need for insulin in the medical sector, but it still had to be extracted from animal pancreases. The production from animal material could hardly keep up with the demand. Some 3,600 kilograms of pancreases were needed to turn out just half a kilo of insulin. The pharmaceutical company Eli Lilly, which was a major producer of insulin, needed on average pancreases of 56 million pigs and cattle per year, to keep up with the demand for insulin in the US alone. Swanson argued that there would be an incredible market for cloned insulin, but he ultimately accepted Boyer's decision, and in April 1978 they had their first results with somatostatin, laying the foundation for a new science and industry that would become known as 'biotechnology'. (2)

In August 1978, barely a few months later, they succeeded in cloning insulin. Their cloned version even turned out to be better than the insulin from animal cells, as the cloned version caused fewer allergies. (3)

The girl in the pink sweater

When the young scientist Diane Pennica (°1951) was hired by Genentech in May 1980, the company already employed 60 people. Her assignment was to clone urokinase as a medicine against blood clots for the German pharmaceutical company Grünenthal. She had been with Genentech only for a month when she was sent to the fibrinolysis congress in Malmö. Genentech wanted to know whether something better than urokinase was in the pipelines. Diane Pennica wanted to be well prepared for her first foreign assignment and to have an idea of the place where the congress would be held, so she went to Malmö a day in advance. While checking in at her hotel, she asked the receptionist where exactly that fibrinolysis congress was to take place. When she was told that the 'meeting of doctors' had already started, Pennica rushed up to her hotel room in shock, threw her luggage in a corner and went at full speed to the congress center. Her first foreign assignment for Genentech and she would arrive too late!

A few minutes before Collen was to take the floor, the door of the conference room opened and a young girl in a pink sweater slipped in. She sat on a chair near the door and pulled out a small notebook. Heads turned, but as it goes when odd incidents with many witnesses occur, no one said anything. Everyone thought that someone else would know what was going on. "That was Diane Pennica, still very young then, and I guess that she didn't weigh 50 kilos," Collen recalls.

American researcher Diane Pennica was 29 years old at the time. "When Désiré took the floor, I immediately was all ears. He claimed to have a cell line, antibodies and pure protein! And that was exactly what Genentech had sent me to Malmö for!" Diane would later recollect.

During the break, she received some questioning looks, and someone asked whether they could help her. "I introduced myself and said that I came from Genentech, and that I was terribly sorry that I had arrived too late and had apparently missed the morning session." Oh no, they told her, this wasn't the real congress, that would only start the next day. "Apparently I sat in a preparatory session with the top people in scientific heart research. I didn't belong there at all! They let me sit there because they thought I was the daughter of one of the scientists." However, they were kind enough to invite her to dinner that evening at a castle, where she engaged in conversation with Désiré Collen, telling him that Genentech wanted to clone his t-PA. It would be the only way to make sufficient quantities of t-PA for the treatment of patients, she assured him.

Genentech had by then already cloned somatostatin, insulin and human growth hormone. But Collen hesitated; he feared Diane was slightly overconfident. T-PA is an enormous protein with a molecular weight of 70,000 (for comparison, somatostatin has a molecular weight of 1,640) and until then, to his knowledge, such large proteins had never been cloned. Diane, in her youthful enthusiasm, insisted however that it was possible and that she definitely could do it, although she later admitted that she herself had not cloned anything at all at the time.

Collen continued having reservations. "We had already discussed cloning with Rector De Somer in Leuven but had concluded that it would take years of work. Anyhow, a week later I get a telephone call from San Francisco from a Dutchman, Herb Heyneker, who worked at

Genentech: 'Hey Désiré! Diane saw you at the congress. Can we collaborate?'" Collen replied that he would have to think it over, but Heyneker insisted, and even threatened a bit, saying that they would get on board with other people if Leuven didn't collaborate. Collen eventually gave in and decided to join forces with Genentech. LRD, the Leuven non-profit organization for technology transfer, followed him.

In August 1980, Jos Bouckaert (1940-2020), the director of LRD, thus left for San Francisco and came back with a short two-page contract. Leuven would give Genentech samples from the Bowes cell line, pure t-PA and antiserum, and, in exchange, the Belgians would get recombinant t-PA for pharmacological and biochemical research, would be acknowledged in scientific publications and would receive 1 percent of the revenues from the sale of t-PA cloned with the material from Leuven. But the contract also stipulated that if LRD should get patent rights on human t-PA, Genentech would have the right of first refusal on a worldwide license. Therefore, if deals were to be concluded with other partners, Genentech had the right to take over those deals.

"One percent of the revenue is relatively standard in these cases," says Collen. "We had no proof of concept yet, as we had not yet treated a patient with t-PA and so could not guarantee its effectiveness." It was a rather naïve and small contract, he admits, but those were the early days of biotechnology. Two years later, when Genentech had succeeded in cloning t-PA, there was a genuine 18-page contract promising 3 percent of the sales until the end of the patent. The patent ended in mid-2006 and has yielded more than EUR 144 million for Leuven. According to the contract,10 percent went to the university, 7 percent to LRD, and, after cost deduction, 50 percent went to Collen's lab. The rest could be divided among the discoverers Collen, Rijken and Matsuo, and other important co-workers.

At Collen's laboratory, the scientists continued producing t-PA from the melanoma cell line, while in his garden the rabbits and goats provided the antiserum. So did the pony - something his daughters did not know about the pet on which they went riding. Genentech each year paid USD 200,000 for 200 milligrams of t-PA. "Two hundred thousand USD! I had never seen that amount of money!" Collen still marvels. But they got increasingly better at turning out t-PA and, over a four-year period, had made 2 grams in all. His lab had meanwhile expanded to 30 people.

As the interest in genetic manipulation grew, so did the expectations. When Genentech was listed on the stock market on 14 October 1980 as the very first biotech company, investors went wild. According to the *Los Angeles Times*, trade in Genentech shares that first day was "a frenzy the likes of which hasn't been seen on Wall Street since the go-go days of the 1960s." Genentech put one million shares on the market at an introductory price of USD 35, but within an hour the share price had risen to USD 88 per share! On that first trading day, Genentech closed at USD 71.25 per share. It was the beginning of a promising new science and industry. "Those who had joined Genentech from the outset, like Heyneker, Boyer and David Goeddel, all became multimillionaires," says Collen. In the early days Genentech didn't have enough money to pay its employees adequately, and everyone, including some suppliers, was paid partially in warrants, with which they could buy shares at a lower price the minute Genentech went public.
One postdoc student who worked at Genentech sold all his shares on that first trading day, pocketing one million USD at a stroke. "And now I'm going fishing," he announced, and left!

“Goeddel, in particular, did very well at Genentech,” Collen recalls, “They called him Goldfinger, because he really had an exceptional talent for cloning. In those days, you had to have feeling for the technique, it was painstaking, precision work. Nowadays, if a student doesn’t manage a straight cloning in one week, he is considered to be all fingers and thumbs!”

David Goeddel was the man who - while working day and night for months on end - had eventually managed to clone insulin. Researchers at Genentech often stayed overnight at the premises. Their only entertainment consisted of occasionally playing a sort of *pétanque* in the hall with coins. Although the corporate culture at Genentech was very rock-and-roll and student-like, compared with other traditional companies, the scientific research was nevertheless top level. The young company only differed from a university because it gave financial instead of academic incentives. The t-PA-committee of which Collen was a member in the early years consisted of seven or eight young people under the chairmanship of Chuck Hoyng.

Genentech started in a warehouse near San Francisco airport, on Point San Bruno Boulevard. The street name has meanwhile been changed to ‘DNA Way’. It is now a campus where some 12,000 people work. At the entrance a sign says: *This is where biotech was invented*. “But in the early days it was just a factory hall,” Collen recalls. “They had leased part of the building, with only two windows, one at the reception desk and one in Swanson’s office! The rest of the premises had no daylight. Diane Pennica often didn’t know whether it was day or night!”

With the company growing very fast, more buildings were added and more staff were hired. As a socializing event for the growing number of people, *‘hohos’* were organized every Friday afternoon: informal gatherings in the cafeteria, where beer, Californian wine and chips were available. And once a year a *‘powwow*’ was held. A powwow is an Indian dance event, but, in this case, dozens of cream pies were involved. “We could throw them to our heart’s content, in true slapstick style. Everyone knew when it was that time of year again and came to work in old clothes,” says Collen, who attended a couple of powwows himself. “All very entertaining, but we were expected to show up on Saturday morning to wipe the walls clean!” As Collen regularly had to spend time in the US, his lab in Leuven was kept running by the many dedicated co-workers, scientists and lab technicians. In the foreword of his 2009 memoirs he wrote: “I was fortunate enough to be able to collaborate with numerous young Belgian and foreign students, MDs and PhDs, and with a loyal group of highly skilled lab technicians.” Without the intense collaboration with other research groups from various universities and from the business sector, the Leuven t-PA story would never have been written, Collen acknowledges.

Cloning recombinant cDNA of t-PA

DNA forms the principal constituent of the chromosomes. It consists of giant molecules made up of long chains of nucleotides, components that are repeated in a specific sequence. These nucleotides sit on the sides, the strands, of what looks like a twisted rope ladder, the famous double helix of Crick (1916-2004) and Watson (°1928). (4)

The DNA in our genome encodes for proteins that constitute and perform the function of an organism. Therefore, this DNA is first transcribed into messenger RNA (mRNA) which subsequently is translated into proteins.

The coding DNA of complex proteins such as t-PA is spread in sections (exons) separated by non-coding regions (introns), and is as such not suitable for the production of recombinant proteins.

The RNA that is transcribed from this genomic DNA undergoes maturation (splicing) whereby the non-coding parts of the mRNA are removed. This mature mRNA is then converted into complementary DNA (cDNA) that comprises the joint sequence coding for the protein. It is with this cDNA that recombinant t-PA is produced.

To make identical copies of a gene or a stretch of DNA, that specific piece of DNA is isolated or cut out of its original organism and then inserted into a plasmid, which in turn is introduced into a host cell of, for example, the *E. Coli* bacterium. This results in a newly assembled DNA or recombinant DNA. With the division of the host cell, the recombinant DNA is subsequently replicated innumerable times.

Finding a specific gene or a specific piece of DNA (a sequence) is looking for a needle in a haystack. It took Diane Pennica a year to discover the first sequence that could be assigned to t-PA. Every human cell has some two meters (6.5 feet) of DNA. And even a small piece of tissue contains many kilometers (miles) of DNA. But with recombinant DNA technology it became possible to isolate one gene or a sequence of DNA and to determine its nucleotide sequence, the order in which the nucleotides sit on the DNA strands.

It all starts with cutting or cleaving, for which ‘restriction enzymes’, molecular scissors, are needed. A restriction enzyme does not cut just anywhere, it needs a recognition site, a location on the DNA sequence that it recognizes. Every restriction enzyme recognizes a different and very specific recognition site. It wraps itself around the DNA and cuts through the two strands of the DNA molecule.

Restriction enzymes do not always cut straight through the double helix. Sometimes it is unequal and a few nucleotides more are left on one strand of the helix than on the other. This overhanging piece is a ‘cohesive end’. Apart from its preferred location on the DNA, every restriction enzyme also has its favorite method of cutting, with blunt ends or with overhanging ‘sticky’ ends. Presently dozens of restriction enzymes have been identified, but in the early years of Genentech only a handful were known.

The cut-out piece of DNA is then ‘ligated’ into a plasmid. The enzymes that paste the cut-out piece of DNA into the plasmid are ligases, a sort of natural DNA glue. There are many thousands of breaks in the DNA of a human cell every day, which are repaired with ligase, such as the damage to DNA as a result of exposure to ultraviolet radiation.

The plasmid that serves as a vector of the recombinant DNA is then introduced into a host cell, usually a bacterium or yeast. That process is called transformation. During the division of the host cell the vector DNA is then replicated along with the DNA from the host cell, so that a whole stock of identical copies is made of the transformed DNA.

Bacteria play a major role in the whole process. Bacteria are versatile organisms; they can assimilate foreign DNA and replicate it, which gives them an evolutionary advantage and helps them to survive in hostile environments. The current antibiotic resistance crisis, endangering many lives because people have become resistant against antibiotics, is due to the versatility of bacteria. They can assimilate DNA that makes them resistant to antibiotics.

Other Flemish universities had meanwhile also embraced the new technology. In Ghent, two molecular biologists**,** Jozef Schell (1935–2003) and Marc Van Montagu (°1933), were working on the genetic manipulation of plants. Plant Genetic Systems, a spin-off of Ghent University, was founded in 1982 with 10 million francs from the Flemish venture capital fund GIMV (*Gewestelijke Investeringsmaatschappij voor Vlaanderen*,- Regional Investment Company for Flanders). GIMV did well out of this investment, by the way. Walter Fiers (°1931) worked with small viruses and was the first to determine the entire genome of a small RNA virus, bacteriophage MS2, in 1976, and two years later of a DNA virus, SV40. Fiers was the founder of the laboratory for molecular biology at Ghent University.

At the Rega Institute in Leuven, Rector Pieter De Somer had also set up a small research group for recombinant DNA technology. Guido Volckaert, a researcher who had come over from Ghent University in 1980, and Ghislain Opdenakker, a student who was working on his doctoral thesis, were working with purified t-PA and antibodies from Collen's lab.

At the end of 1980, shortly after Collen had signed his contract with Genentech De Somer got wind of the agreement. Collen had a contract with an American company for cloning t-PA? While the rector had wanted to develop that technology in Leuven? It caused some commotion. "The rector was furious! But our contract with Genentech had been signed a few months earlier, in September. We had assumed that De Somer would support it, because only in May, so a few months prior, he had told us that we should sell our project and he had arranged the initial meeting with Bayer!"

The parallel research at the Rega Institute would later cause more problems.

A patient in Rotterdam

In April 1981, Professor Fons Billiau of the Rega Institute drove to Rotterdam for a conference on interferon. "It was with mixed feelings," says Billiau, "because our research in Leuven on interferon was not very promising." At a reception held the evening before the conference - the customary 'welcome party' - Billiau saw internist Huub Schellekens, and the latter's colleague, the nephrologist Willem Weimar. They knew one another because they had worked together on a few tests with interferon in patients in the Dijkzigt Hospital in Rotterdam. After the exchange of pleasantries, the conversation turned to the research with interferon, and the discouraging results of the Rega Institute's tests in patients with hepatitis, cancer and MS. "But we are working on something else in Leuven," Billiau told them, "And that looks promising." Billiau then explained about Désiré's t-PA to dissolve blood clots. Weimar was immediately all ears. He had a patient who had just undergone a kidney

transplant, but she had a blood clot in a pelvic vein, which would be fatal for the transplanted kidney, and possibly for her. Could this t-PA already be administered, Weimar wanted to know. Billiau thought it could, and drove to Leuven the following morning, to see Collen and explain the situation. Collen did not hesitate and provided Billiau with 5 milligrams of t-PA, his entire supply at the time. Weimar informed his patient that he would treat her with an experimental drug, and she gave her consent. The t-PA was administered that same day, and the effect was miraculous: the blood clot disappeared! The transplanted kidney functioned perfectly again, and the patient would go on to live for several decades.

A short time later, a second kidney patient was successfully treated with t-PA. In this case, it was Dick Rijken who had driven back and forth from Leuven to Rotterdam with 7.5 milligrams. Weimar described the results of his first treatment with t-PA in an article in *The Lancet*. The case of the second patient was added as a footnote. (5)

“After that publication in *The Lancet*, Weimar treated a third patient with t-PA,” says Collen, “But that was unsuccessful. It later turned out that the dose of t-PA should have been much higher. The first two patients probably had reacted so well to the lower dose because they both had uremia. Their kidneys were not working, which meant they had a high level of urea in their blood, and urea makes blood clots fragile. But that we didn’t know then.” In any case, the success in those two patients had already caused mild euphoria at Genentech, and Collen and his coworkers now had a stronger belief that they had discovered a fantastic drug.

In his contribution to the anthology that was issued when Collen received emeritus status in 2008, Weimar wrote that thinking back to those days filled him with nostalgia: everything was so much simpler then! A great deal relied on trust between researchers and physicians. Administrative formalities consisted of the patient’s consent and filling out a few forms. Nowadays he faced a mountain of paperwork for the administration of experimental drugs: 32 different documents, 18 pages of information for the patient, three questionnaires for the hospital’s financial department, agreements with the hospital’s attorneys and subcontractors, and all sorts of other data that had to be entered in the computer. The rock-and-roll years of scientific research were definitely over; experiments were now restricted and subject to strict rules and procedures.

Tests in Saint Louis

While Diane Pennica tried to clone t-PA in San Francisco, Collen was asked by Genentech to make as much t-PA as he could from the melanoma cell line. “We think that we’re going to succeed in making recombinant t-PA. But we have to be able to compare it with natural t-PA, so set up some tests!” Genentech told him. Collen’s lab meanwhile disposed over more resources and staff: the team with Dick Rijken and Osamu Matsuo had expanded and now included, among others, Irène Juhan-Vague, Christian Korninger, Marc Hoylaerts and Roger Lijnen.

One day Collen was invited by the National Institutes of Health in the US to give a workshop on thrombolysis. After the findings of Rentrop and DeWood, more cardiologists were now convinced that heart attacks were the *consequence* of blood clots and not the cause, and that there was an urgent need for thrombolytic drugs. Just as at the fibrinolysis congress in Malmö, Collen’s talk would be on biochemistry. “I went to Washington, where I was the first

speaker that morning. Not many participants had arrived yet. Some of them probably thought: oh well, biochemistry… we'll listen to the serious work later." Collen delivered his talk about t-PA, his rabbits and his studies. An attentive listener in the audience was Eugene Braunwald (°1929), one of the most important cardiologists in the US, and also Burton Sobel (1937 -2013), then head of cardiology at Barnes Jewish Hospital in St. Louis, Missouri. "After my presentation, some questions were asked. Not many, because most physicians were not that interested in biochemistry. Except for Burton Sobel, who came up to me and said: that is very interesting! And is this t-PA fibrin-selective? Is it more efficient than urokinase in those rabbits? Yes, I answered, but we still need a model with which we can cause heart attacks in experimental animals. To which he replied: 'Oh well, we can provide you with that, we have it all.'"

Sobel had a system by which he introduced a small copper coil with a catheter into the coronary artery. The coil was highly thrombogenic and invariably caused a clot that blocked the artery. Furthermore, in St. Louis they had a PET scanner! A PET scan (Positron Emission Tomography) is an isotopic imaging system in which a weakly radioactive substance (isotope) is injected, which allows one to see the metabolism and to measure the viability of the heart muscle in the region where the infarct was, before and after treatment with t-PA. Collen was enthusiastic: "We could see which part of the heart muscle and how much of it had died. Nowadays PET scanners are everywhere, but we're talking about the early 1980s, it was a very new invention! And yes, it was a fantastic improvement."

Genentech was consulted. Their reply was: go ahead, the more research the better! Tests were set up in St. Louis with 24 dogs that received a treatment with t-PA or streptokinase. Collen and Doctor Sobel's team tested intravenous injections and administration via the coronary artery, to see which was more efficient. The second method was more cumbersome and took longer because t-PA had to be administered via an arterial catheter. This meant that later trials on patients could only be done in a hospital with a cath lab.

Steven Bergman, a postdoc student, did the actual work. Sobel, the department head, and Collen stood by and drew up the reports. First the dogs were anesthetized, then the little copper coil was introduced via a catheter in the femoral artery in the groin, into the coronary artery, where a clot was generated. The dogs were then injected with streptokinase or t-PA, and it was determined how fast the clot disappeared. They also checked whether the treatment worked better when it was administered intravenously or injected directly into the coronary artery.

T-PA turned out to be the big winner: whether it was administered intravenously or directly into the coronary, the clot disappeared after 8 minutes on average! Streptokinase, on the other hand, took 31 minutes with intracoronary administration, and a good 85 minutes with intravenous administration. Because t-PA only activates plasminogen bound to fibrin (that is, where there is a clot), fewer excessive hemorrhages also occurred elsewhere in the body, unlike with streptokinase treatments. "That first research in St. Louis took less than two years, which was very fast work. And it resulted in a fine article in *Science*!" Collen notes with satisfaction. (6)

Sobel and Collen remained good friends until the former's death in 2013. "He was a highly cultivated man - he was a jazz musician in his free time - but also fascinated by everything

happening in our sector. Each time I visited him in St. Louis, he showered me with new questions: tell me, what about the kinetics here, and explain that drawing to me, and how did you come to that conclusion?" According to Collen Sobel was more interested in basic science than most cardiologists.

From 1983 to 1988, Burton Sobel was editor-in-chief of *Circulation*, an important international scientific journal of the American Heart Association. When genetics, molecular biology and imaging were introduced in cardiology, he was one of the first to study these techniques and familiarize the readers of *Circulation* with them.

Diane's empty circles

In San Francisco, Diane Pennica had been working day and night, months on end, to clone t-PA, with many setbacks and much frustration, until in October 1981 she suddenly noticed an unusual peptide sequence of one of the clones with a strand of five amino acids: W-E-Y-C-D. She knew that this sequence, in exactly that order, was part of the t-PA gene. It was a major breakthrough: an essential piece of the puzzle had finally been found!

In May 1982 she had finished cloning t-PA. In the meantime, there had been a great deal of travelling back and forth: by Collen to the US but also by people from Genentech to Leuven. "Always low-cost with Capitol Air!" Collen remembers. Capitol had direct flights between Brussels and New York, and from there two connections: one to San Francisco and one to Puerto Rico. All Capitol transfer passengers had to wait for their connection for several hours in New York, in a separate corner of the old Pan Am building. "Those were still flower-power years, and in the corner where I waited for my connection to California, amidst many backpackers, there was a constant and distinct scent of marihuana cigarettes," he recalls.

Collen rented a small flat in San Mateo, just past South San Francisco. "I often stayed there for three to six weeks at a time. Sometimes my family accompanied me. Altogether I spent almost a year in San Mateo." In between, results were exchanged by telephone. Computers were still absurdly big things at the time, the worldwide web was far from a reality, and e-mail wouldn't come about until the mid-1990s. Genetic manipulation was still in its infancy as well.

Diane Pennica had now finished her cloning work. The structure of the recombinant complementary DNA copy of the t-PA gene was completely deciphered. She had worked for months at a time and had often stayed overnight in her lab.

Pennica had never planned to become a scientist but wanted to become a teacher. Thanks to an instructor at her college in Fredonia, New York, she switched to science and became a researcher. At the time few women were active in biotech. "Working as a researcher is a form of modern slavery!" says Collen. He admits that his wife and children did not see much of him during his career. The constant monomaniacal commitment might be even more difficult for female researchers, he believes. However, his years as a Professor taught him that women are more dedicated, more disciplined and in any event get better results than their male colleagues. But the job of a passionate researcher wears a person down. Diane Pennica decided to stop in 2010, when she was 60. "It was unbelievably hard work, 15 hours a day," she says. She barely had time to pay her bills. "And I finally wanted to live!"

In May 1982, just before Pennica left for the fibrinolysis conference in Lausanne to disclose her results, the patent application was filed. The competition had not stood still, so it was a matter of being first. Pennica was scheduled as the last speaker at the conference. She was allowed to say that she had succeeded in cloning t-PA, but the Genentech attorneys had insisted that she disclose no more than that. Still, in Lausanne the rumor went round that Pennica 'was going to reveal something sensational'. When she finally took the stage, the tension in the room was palpable. But when she eventually showed her slide with the structure of t-PA - the string of beads that represented the sequence of amino acids - viewers noted that the beads were empty! No letters revealed the identity of the amino acids. This was deliberate so as not to give the competition in Lausanne any early information. At the same time, Genentech issued a press release in the US with the announcement that they had cloned the t-PA cDNA. When Pennica finished her presentation, she got a standing ovation and was mobbed by journalists. "It felt like being at the Oscars!" Collen recalls. "There were definitely at least a dozen camera crews. It was truly Diane's moment of glory."

On 20 January 1983, *Cloning and expression of human tissue-type plasminogen activator cDNA in E.coli* was published in *Nature*, including the strand of beads *and* the letter code for the amino acids. (7)

Competitors appear

Cloning was *the* scientific revelation of the moment, and all over the world researchers and scientists were trying to clone new proteins and to refine the techniques. At the Rega Institute, Ghislain Opdenakker continued trying to clone t-PA himself. He published his first findings in the *European Journal of Biochemistry* in 1982. (8)

Collen had been co-author of Opdenakker's article, because the research had started before he had concluded his contract with Genentech, but after the agreement he refrained from further active collaboration in the Leuven project. At the Rega Institute, they could continue to have non-confidential information and materials from Collen's lab for their research, but he himself had formally ended his collaboration with the Institute, and towards the end of 1980 his colleague Roger Lijnen had taken over. With this arrangement Collen wanted to avoid problems with Genentech, although he did regret breaking off his collaboration with Fons Billiau.

The decision turned out to be a wise one, because in 1985 there was upheaval when Collen and Genentech heard that the Rega Institute had an agreement with the British biotech company Celltech. The joint publication of the Rega researchers and Celltech on how they had cloned t-PA, coincidentally also via the same sequence that Pennica had discovered in 1981, caused turmoil. (9)

"At Genentech they were furious. They thought I had been working in Leuven behind their back on my own behalf. I had to move heaven and earth to convince them of my good faith. Roger Lijnen then drafted a statement affirming that he had done the trypsin cleavage and isolated the peptides, and had passed them on to Wilfried Rombauts of biochemistry, who had determined the sequence, in which coincidentally the same piece turned up as the one Diane Pennica had discovered. I was indeed very concerned that at Genentech they might think I had double crossed them!" Ultimately everything was clarified, but the situation made

for unsettled relations between Collen and some researchers of the Rega Institute for a long time.

Chapter 4: The road to confirmation

Chip Gold

Tsunehiro Yasuda

Frans Van de Werf

Marc Verstraete

David Stump

Key collaborators involved in the clinical development of rt-PA

CORONARY THROMBOLYSIS WITH TISSUE-TYPE PLASMINOGEN ACTIVATOR IN PATIENTS WITH EVOLVING MYOCARDIAL INFARCTION

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Abstract Tissue-type plasminogen activator is a naturally occurring, clot-selective activator of fibrinolysis. We recently reported that human tissue-type plasminogen activator isolated from a Bowes-melanoma–tissue-culture supernate lysed coronary thrombi in dogs without depleting circulating fibrinogen or α_2-antiplasmin, in contrast to the case with streptokinase and urokinase. In the present study coronary thrombolysis, confirmed angiographically, was induced within 19 to 50 minutes with intravenous or intracoronary tissue-type plasminogen activator in six of seven patients with evolving myocardial infarction. Circulating fibrinogen, plasminogen, and α_2-antiplasmin were not depleted by this agent, in contrast to the case in the two patients subsequently given streptokinase. In the one patient in whom lysis was not inducible with tissue-type plasminogen activator, it was also not inducible with streptokinase.

These observations indicate that clot-selective coronary thrombolysis can be induced in patients with evolving myocardial infarction by means of tissue-type plasminogen activator, without concomitant induction of a systemic lytic state. Definition of its therapeutic benefit must await greater availability of the agent and the performance of appropriate clinical trials. (N Engl J Med 1984; 310:609-13.)

Summary: *The t-PA cloned by Genentech, recombinant t-PA or rt-PA, now went into clinical development. Endless tests and comparisons followed, primarily on dogs initially, but gradually on patients too. A first treatment with melanoma t-PA for a patient with acute myocardial infarction in Leuven failed, but the following four treatments succeeded. In St. Louis, Burton Sobel treated two patients successfully. The American regulatory body gave three hospitals permission to conduct tests with rt-PA. In a European study led by Marc Verstraete, rt-PA prevailed over treatments with a placebo and with streptokinase. The American TIMI study also turned out positive for rt-PA and through the many trials, the importance of quick intervention became apparent. In Boston, Collen met Chip Gold, who became one of his dearest friends.*

Recombinant t-PA (rt-PA) tested in Leuven and Boston

During his collaboration with Sobel, and after Pennica had finished cloning t-PA, Collen learned that a colleague in Leuven, Frans Van de Werf (°1948), had designed a similar model to cause coronary blood clots in experimental animals. He was a young staff member who worked in the experimental cardiology lab. At the end of the 1970s, Van de Werf, while at a conference in Hannover, heard Rentrop explain how he dissolved blood clots with streptokinase in patients with an acute myocardial infarction. The debate about whether blood clots were the cause or the consequence of a heart attack was still continuing. Upon his return to Leuven, Van de Werf developed a model to generate a thrombus in the coronary artery of experimental animals – dogs in this case – with a small copper coil mounted on a guide wire. His model worked well and turned out to be suitable for testing new thrombolytic agents. After a coincidental meeting at the Sint-Rafael Hospital parking lot, Collen and Van de Werf decided to collaborate.

In the first experiments rt-PA was compared to urokinase and scored better. In the nine dogs that were treated with rt-PA, blood flow was restored after an average of 13.7 minutes. The ten dogs that had received urokinase did less well; in seven, the blood clot disappeared only after 19.3 minutes, and two dogs had serious hemorrhaging; but the coagulation system was severely disrupted in all the urokinase dogs. Confidence in the cloned version of t-PA as a possible treatment for people with a heart attack increased by the day. (1)

Urokinase and streptokinase had both been in use for a while to treat heart-attack patients, but urokinase had its ups and downs. “We used urokinase for this test,” says Collen, “because streptokinase works less well in dogs. But urokinase was expensive and difficult to produce. In China they purified it from urine collected from the Chinese army, and I think that is how they still do it, but Abbott extracted its urokinase from fetal kidney cell cultures. Those kidneys came from fetuses that had been aborted in South America, mainly in Colombia. But at one point a scandal arose, followed by an investigation by the American Congress.”

According to the American consumer organization Public Citizen, many of these fetuses were obtained without the parent’s consent. Furthermore, they were not adequately checked for contagious diseases. At the end of the 1990s, the FDA as well warned that some batches of urokinase from Abbott might be contaminated. In 1999 the FDA took Abbokinase from the market as a thrombolytic agent for arterial thrombosis, but after a series of measures Abbokinase was again allowed for use in October 2002, although only for pulmonary embolism.

In Boston, an additional study was carried out that same year. Collen had now become an internationally recognized authority, and Genentech had a worldwide reputation as the first listed biotech company. Their collaboration on rt-PA was creating quite a buzz in medical circles. Genentech had connections with Massachusetts General Hospital in Boston, where Dr Chip Gold was interested in the research. His wife Dr. Barbara Nath, a gastroenterologist, had shown him an article in *The Lancet* on Weimar's kidney patients. "Chip himself did not routinely read journals, he didn't have the time," says Collen, who for years had a permanent guest room in the Gold house.

Chip Gold (1940 – 2008) was actually named Herman Kalman Gold but was generally known as Chip. It is said that he changed his name because Herman sounded too German for someone of Jewish origin. His father was a rabbi, and many of his family members had died in Auschwitz. Chip Gold was not a pure-bred researcher, but rather a passionate physician and cardiologist. He was a pioneer in translational research, in the link between academic research and patients. He closely followed new research results that could improve the treatment of his patients. As a cardiologist his curiosity was triggered by the rt-PA of Genentech and Collen. "Gold had a busy practice; as a physician he was available seven days a week, 24 hours a day. He was an icon of accessibility in patient care," says Collen, who lost one of his best friends when Chip died in March 2008.

"They were two totally different personalities, but incredibly close friends," says Dr. Barbara Nath, Chip's widow. As an interventional cardiologist, her husband was committed to his patients in the hospital, while Collen spent his days in the lab and focused on research. But at the same time, they were complementary personalities, and fascinated by each other's work. "Which is rather unusual, a scientific researcher and a physician appreciating each other's field of interest. Moreover, my husband was an extrovert and Désiré is rather reserved. But Chip recognized in Désiré a brilliant scientist *and* a good businessman! Désiré had a talent for obtaining the necessary funds and was great at concluding advantageous deals," Barbara recalls. "Whenever we had a dinner party, Désiré was the star of the evening; people hung on his every word. He could be equally entertaining about politics or economics."

Barbara even compares Collen to Thomas Jefferson (1743-1826), the third president of the United States – a versatile man, who besides being a politician was also a philosopher, a scientist, an architect, a lawyer, an inventor and a writer. This is no doubt an exaggeration, but it shows how much Collen's company was valued by the Golds. "Désiré was in any event a very dear friend to my husband," says Dr. Nath. "When Chip was diagnosed with leukemia and had to go to Seattle for a bone marrow transplant, Désiré flew to Seattle to be with him, and he brought along a whole collection of Beethoven CDs. Later he flew over from Europe for my husband's funeral. We appreciated that very much. My husband's death was definitely as great a loss for him as for us. By the way, we still refer to our guest room where Désiré stayed whenever he was in Boston as 'Désiré's room'!"

In those days, Collen occasionally gave seminars at Harvard Medical School in Boston. From the mid-1980s to the early 1990s he was regularly invited to speak in the US. "Many physicians were still relatively unfamiliar with fibrinolysis, and I explained how it worked, and how we carried out our experiments on animal models." For several years Collen was also Visiting Professor of biochemistry and medicine at the University of Vermont in Burlington in

Kenneth G. Mann's department during the summer, until his children, fed up with the computer camps and summer courses at the American campus, demanded a holiday 'like normal people'.

Could he maybe have stayed at Harvard and have a career there? "There certainly was an opportunity in Chip Gold's lab, but I would have been responsible for my own financing." And by the time the possibility arose, Collen was already involved in new research projects with staphylokinase and Thromb-X.

A third preclinical study in dogs was set up with Chip Gold and Tsunehiro Yasuda (°1942) in Boston, this time to determine the optimal dose of rt-PA to restore blood flow. Tests were done with hourly doses of 5, 10, 15 and 25 micrograms per kilogram body weight of the dogs. This required open-thorax surgery. "The man who performed the surgery was a lab technician, Luis Guerero, a self-taught Mexican who had learned his craft by watching surgeons. He didn't have a medical degree, but he knew exactly what he was doing," Collen recalls. Guerero is listed in a number of publications as a co-author. (2)

The findings showed that in all cases the clot disappeared, but the higher the dose of rt-PA, the faster the blood flow normalized. Yet a fourth test was then set up in which a number of dogs were administered rt-PA and another group was given a saline solution as a placebo. It was a randomized study, which meant that only Bob Holt, the technician, knew which dog got which treatment. The tests began in March 1983, and two to three dogs were tested per week. While Yasuda did the tests, Collen closely followed the experiment. During the celebration of Collen's emeritus status in 2008, Yasuda told the audience how Collen started his day at 7 am and continued working until late. "He documented and made tables, but in the evenings after work we had Scotch at Dr Gold's house." According to Yasuda, Collen insisted that the Scotch had a restorative effect. Collen claimed that it kept his mind fresh and gave him renewed energy the next day.

The researchers had to work fast, because Genentech now had concluded a contract with the Japanese pharmaceutical company Mitsubishi Chemical Industries, which paid for the research on rt-PA in exchange for the license rights in Japan, and the company wanted to see results before the close of the year. By the end of December 1983, the experiments were completed and the randomization code was broken; in the dogs that were treated with rt-PA, the blood clot had disappeared and the blood flow was restored; the success rate was 100 percent. In the dogs that had received a saline solution the result was zero. The exceptionally good result for rt-PA surprised them all. Reflecting on those times, what amazed Dr Yasuda was the concentration Collen displayed while recording all the data, and while doing this, that he could sit still at his desk for such a long period of time. "A quality that in the scientific world separates the extraordinarily successful people from the successful," Yasuda concluded wittily.

A first t-PA patient in Leuven

In the afternoon of 20 January 1983, a 58-year-old patient was brought into the Leuven university hospital with an acute myocardial infarction and a completely obstructed coronary blood vessel. He would be the first patient in the world to be treated for his heart condition with t-PA. Cardiologist Frans Van de Werf, who had been involved in the tests with dogs, would lead the procedure, using t-PA from the Bowes cell line of which Collen had made just enough for one treatment.

“There were no contraindications, the patient had given his consent, and we had everything ready,” says Frans Van de Werf. “Désiré was informed, just like several other people who had worked on the t-PA project, such as Roger Lijnen and Dick Rijken.” Together with the personnel needed for the procedure, at least 20 people showed up to watch. There was some tension in the air, Van de Werf admits. “It was the very first time, and the department head Professor De Geest initially had reservations because we would be administering a protein from a cancer cell line. But all had been thoroughly discussed, and also the ethics committee gave us permission.”

Wasn’t he somewhat nervous? “No, not at all,” says Van de Werf, “We had our successful animal tests and Weimar’s two successful kidney patients. Everything was under control; we had the electrocardiogram (ECG), which allowed us to observe the patient, and see the coronary arteries… It was an undertaking that could either work or would have the standard progression of an infarction evolving with a permanent occlusion, as was often the case with the standard treatment for heart attacks in those days. Reperfusion was still in its infancy.” The technique for opening up blood vessels blocked by blood clots was still very new.

The patient was given an intravenous injection of t-PA, the most logical approach because if this were to become standard procedure in the future, speed was important, and not every hospital was equipped for intracoronary treatments. However, nothing happened! The blood vessel remained blocked. In a second attempt, t-PA was given by intracoronary administration, close to the clot. Again nothing! The occlusion did not disappear. After 60 minutes the entire supply of t-PA was used. As a last attempt, streptokinase was given by intracoronary administration. But that too had no effect. The experiment was over! The patient developed a standard transmural anterior wall infarction; he survived his heart attack, although with reduced heart function. But only 20 years later did he need a pacemaker. He died in July 2008, at the age of 83. “We then also learned that after a failed reperfusion treatment for a heart attack, you can still live another 25 years,” Frans Van de Werf concluded years later in a presentation.

Immediately after the failed intervention there was great disappointment, but also concern. Van De Werf and Collen especially were worried. “It was an unbelievable setback, and a painful experience. Van de Werf and I had to go see the department head, Professor de Geest, and it was not easy to obtain permission for a second patient. It could have been the end of our experiments.” But in St. Louis doctors also stood ready to treat patients with t-PA, which created some pressure to continue in Leuven. Van de Werf treated four more patients with Bowes t-PA – fortunately with success. Sobel had started somewhat later in St. Louis, because he needed approval from the FDA, and the paperwork took time.

The failed intervention on the first patient in Leuven showed that a higher dose of t-PA was needed. In the US, Sobel treated his two patients successfully. The report from both teams was published in the *New England Journal of Medicine*. It brought Sobel and the Leuven team worldwide fame. (3)

“It gave my career a tremendous boost,” Van de Werf acknowledges. He continued his tests on dogs with the rt-PA that Genentech now had cloned. Genentech had meanwhile found a new source for its rt-PA production: Chinese hamster ovary cells (CHO). With these cells (once they had been transformed with the human t-PA cDNA), Genentech was able to turn out thousands of times more t-PA than from the Bowes melanoma cell line, and Collen’s lab could stop the production of melanoma t-PA altogether.

Thanks to the collaboration with Van de Werf, Collen developed a new interest. During a dinner at Van de Werf’s home, he happened to sit next to Urbain Boutelegier, a general practitioner from Assebroek. Boutelegier turned out to be a passionate wine connoisseur, with an interesting list of French chateau owners in his address book. “Ever since, there have been regular trips to wine chateaus, in which also former rector Mark Waer participated,” says Van de Werf. “From that time on, whenever Urbain Boutelegier was placing his wine orders, Collen said time after time: just buy the same for me, Urbain!” The fame of Désiré Collen’s wine cellar soon extended far beyond Leuven.

In Frans Van de Werf’s lab, Collen met Ik-Kyung Jang (°1954), a South Korean fellowship student who had obtained his medical degree at the Catholic Kyung Hee University in Seoul. In 1980 he arrived in Leuven for his clinical training via a collaboration agreement between the two universities. He had already studied some Dutch in Seoul, but after a couple of years in Leuven he spoke the language fluently. Jang and Collen had an immediate connection. “He was an intelligent, sensible fellow. And a hard worker,” says Collen. The respect was mutual. When Jang and his wife, both deeply religious Catholics, had their first son, they asked Collen to be the godfather. He accepted, although he warned them that where religion was concerned, they might have chosen the wrong person. Later when Jang was looking for a postdoc position in 1987, Collen introduced him to Chip Gold in Boston. They continued to collaborate for some time in the US on various t-PA studies and thrombolysis, and published a number of articles together. (4) Jang eventually became a respected cardiologist at Massachusetts General Hospital in Boston, and Collen visited him regularly. In 2010 Jang was appointed a full-time Professor at Harvard Medical School, and in 2015 he received an endowed chair. Collen flew to Boston to attend the celebration. “It’s not that we’re constantly writing each other, but there is a great mutual respect, and we do keep in touch. In Jang’s living room hangs a canvas painted by my son, and his daughter has worked in our lab for two months. I had the good fortune to work with many fine people in my life,” Collen concludes. Jang still works as an interventional cardiologist at Massachusetts General Hospital. He was one of the first to use OCT (optical coherence tomography), a method for examining the inside of blood vessels to determine the degree of atherosclerosis. His résumé still lists Dutch as a second language.

FDA allows rt-PA tests on patients

The tests on dogs had demonstrated that cloned rt-PA worked just as well as natural t-PA. But now it had to be tested on people. The American Food and Drug Administration (FDA) gave three top American hospitals permission to test rt-PA on a maximum of 50 patients. These were Washington University in St. Louis, where Sobel had already treated two patients with the melanoma t-PA; Massachusetts General Hospital in Boston with Chip Gold, which just like St. Louis had already participated in the animal tests; and, thirdly, Johns Hopkins University in Baltimore. At Johns Hopkins, which is one of the top academic hospitals in the US, Myron L. Weisfelt was head of the cardiology department. He was brought in by Genentech for this test.

It was to be a randomized, placebo-controlled study: the patient did not know what he was being treated with – rt-PA or a placebo. This was determined by chance. An endpoint was established at 90 minutes, after that the clot had to be dissolved. If not, the placebo patient was in any event given rt-PA. According to the setup, two thirds of the patients would be treated with rt-PA, one third was given the placebo.

The first patient to be treated in the study arrived at Johns Hopkins in Baltimore, where Eric Topol (°1954), a young specialist in training with Weisfelt conducted the test. The next patients eligible for the trial would be treated in St. Louis, Baltimore, or Boston. The success of rt-PA was apparent. In three-quarters of the patients who had received rt-PA the blood flow was restored within 90 minutes. Amongst the patients who had received a placebo, there was only one in whom the clot had dissolved by natural thrombolysis. The others from the placebo group did receive their rt-PA treatment after 90 minutes, and somewhat less than three-quarters of them had reperfusion.

There were also six patients who did not respond at all to the treatment with rt-PA. They subsequently received treatment with streptokinase, but that too had no effect. “Probably because their occlusion was not caused by a blood clot, but by dislodged plaque or atherosclerosis, a sort of mush obstructing the vessel on which thrombolytic drugs have no effect,” Collen suspects.

All in all, the study was a success; in 75 percent of the rt-PA patients, blood flow was restored within 90 minutes, while in the placebo group it was only 7 percent. rt-PA had passed this first test well, giving the researchers and Genentech a confidence boost, as many new medicines fail at this stage.

The test had received quite a bit of publicity in academic circles. After the successful treatment of the first patient, the public relations department of Johns Hopkins could not resist the temptation to be the first to issue a jubilant press release. This led to some resentment in the other two hospitals, where they did not appreciate the fact that Topol and Johns Hopkins seemed to take all the credit. When the trial was concluded, and a report had to be published in *Circulation*, there was again bickering over whose name should come first. After the commotion over the press release, the two other hospitals objected to Topol as the first name. Ultimately none of the three, but Collen himself headed the list of names. And then there was discussion over whose name should be last. The last name is usually reserved for the senior author. Again, they couldn’t agree on who that should be: Gold, Sobel or Weisfelt? To end the discussion, it was decided to grant the honor to Elliot Grossbard, the

Chief Medical Officer of Genentech. Collen had not been near any patient during the trial, but together with Grossbard, he had written the article, and they had drawn up the tables and processed the data together. Topol was listed second, and from there the names alternated among the three hospitals. The article eventually had 17 authors. (5)

Verstraete mobilizes European cardiologist

In the 1970s, when discussion on the cause or consequence of a thrombus in heart attacks was still going on, Marc Verstraete in Leuven had already studied, together with a number of European colleagues, the effect of streptokinase. Encouraged by the results of t-PA and rt-PA in dogs and patients, he again approached his network of cardiologists, this time for a study on the effect of rt-PA. Thirty cardiology departments of hospitals throughout Western Europe joined his European Cooperative Study Group for rt-PA.

It was to be a double-blind, placebo-controlled study with 129 patients who suffered heart attacks. rt-PA would be compared with a placebo and the endpoint was again 90 minutes. In a double-blind trial, the patient does not know whether he is treated with rt-PA or with a placebo, and neither does the cardiologist. Again, rt-PA was the winner over the placebo, with 61 percent successful procedures versus 21 percent in the placebo group. After 90 minutes the placebo patients were treated with rt-PA. (6)

In a subsequent study, rt-PA was compared to streptokinase, then the standard treatment for heart attacks. In 1982, the FDA had approved the intracoronary administration of streptokinase for heart attacks. For intravenously administered streptokinase, not enough convincing evidence of its efficacy had been provided, that came only later. In this study, 64 patients were administered rt-PA, and 65 streptokinase. It was a blind, randomized study.

A 24-hour randomization schedule was set up. “If a patient with a heart attack came into one of the hospitals, it was checked whether he or she fulfilled the criteria and did not have any other medical problems that could influence the treatment, such as diabetes or kidney problems,” Collen explains. Patients were asked to give consent to participate in the study. “Then we communicated via telephone: we have patient number such-and-such here, which vial?” The numbered vials stood ready: identically coded vials with either streptokinase or rt-PA, so the doctors ignored what they gave the patient.

rt-PA turned out to work better than streptokinase. Even before the 90 minutes were up, 75 percent of the rt-PA patients had an open artery; in the streptokinase patients the clot had disappeared in only 55 percent. But the difference was statistically not significant enough to draw definitive conclusions. To draw definite conclusions, 129 patients formed somewhat too small a group. But the study did make clear that rt-PA affected blood’s clotting function far less than streptokinase. The latter presented an increased risk of hemorrhages elsewhere in the body, while rt-PA was primarily active on the blood clot. (7)

Verstraete’s consortium then did three more studies, one to find out whether a longer treatment with rt-PA would prevent renewed obstruction of a blood vessel; another to see whether administration of heparin or aspirin after an rt-PA treatment helped to keep the blood vessel open; and a third on angioplasty, by which the blood vessel was opened with a small balloon.

rt-PA beats streptokinase

In the United States researchers were equally active. The Advisory Council of the National Heart, Lung and Blood Institute began a study of streptokinase, led by the renowned cardiologist Eugene Braunwald of Harvard University, in 1982. Thirteen hospitals in the US participated, including that of Chip Gold in Boston.

This study, *'Thrombolysis in Myocardial Infarction'* or TIMI for short, wanted to find out whether the way in which streptokinase was administered, intravenously or intracoronary made a difference, and this in comparison with patients who received a placebo. The endpoint was again the restoration of blood flow within 90 minutes. In all, 340 patients participated. But in the meantime, rt-PA had become available, and during 1983 it was decided to include rt-PA in the next TIMI trial. That was at the urging of Kenneth G. Mann, a biochemist at the Mayo Clinic in Rochester, Minnesota, and an adviser to the National Institutes of Health. Mann was responsible for the clotting analysis in the TIMI trials, and he and Collen later became personal friends. "Because they didn't have that much experience with those tests in the US, David Stump, who had worked in Leuven for two years, and I set it up together with Mann," says Collen. When in 1984 Mann became head of the biochemistry department in Burlington, Vermont, he convinced Collen to spend his summers giving seminars there, which Collen did for eight years. He even bought a condominium in Vermont for USD 110,000. "In the US you buy and sell houses the way you do cars in Europe. So, in the summer, we lived for two months in Vermont, and the rest of the year I rented out the condominium. I eventually sold the property eight years later with a USD 10,000 profit." Collen had a free summer residence for eight years and an income from the rent.

As always, patients had to be duly informed and give their consent to be included in the TIMI study. At Massachusetts General Hospital, that was the responsibility of Dr. Yasuda, a Japanese cardiologist who worked with Chip Gold in Boston. In this study, interim evaluations were conducted by the Data Monitoring and Safety Board. If it should turn out that the efficacy of one treatment was markedly superior to the other, it would not be ethical to continue administering the lesser drug. This happened on 5 February 1985 when it became clear that the rt-PA treatment was by far the better therapy. Of the 316 patients, 70 percent of the rt-PA group had restored blood flow within 90 minutes. In the streptokinase group that was only 43 percent. Although the study was not yet formally completed, the positive outcome for rt-PA was immediately published in the *New England Journal of Medicine* in April 1985. The results of a similar test in Europe by Marc Verstraete and his Study Group were published a week later in *The Lancet*. The differences between streptokinase and rt-PA were somewhat less pronounced in the European trial than in the TIMI study, but the interest in rt-PA among cardiologists was growing (8)

The trials raised new questions, however. If the treatment resulted in reperfusion, what were the patient's chances of survival afterwards? Would he or she not risk having a new occlusion after a few weeks or months? And what was the condition of the heart muscle after the treatment?

There also were ethical questions: was it justified to give patients with a life-threatening condition such as a heart attack just a placebo, in other words give these patients no treatment at all? "Indeed, that would now be inadmissible," says Van de Werf, "But at the time we did not have adequate proof that by administering streptokinase or rt-PA you improved the heart function and reduced mortality. Therefore, it was ethically justified to test versus placebo. If you can't compare with patients without reperfusion therapy, how do you prove that a treatment gives better results, that it is lifesaving? If you believe you have a

better medicine, you must compare it with the standard treatment. And that treatment was then to do nothing at all, to wait and see whether the clot was dissolved by the natural thrombolytic process."

Salim Yusuf (°1952), a young Canadian physician and researcher who in 1984 worked for the American National Institutes of Health, compiled the now-numerous published studies in which intravenously administered streptokinase was compared to placebo. He did his research together with Rory Collins and Richard Peto of the Oxford group. They concluded that mortality was 22 percent lower after a treatment with streptokinase than in the placebo group. His report appeared in the *European Heart Journal* in 1985. (9)

A number of consecutive Italian studies from the Gruppo Italiano per lo Studio della Streptochinasi nell' infarto Miocardico (or GISSI), in which most Italian coronary care units (CCUs) participated, demonstrated the importance of reperfusion and the beneficial effect of streptokinase. The GISSI 1 study of 1986 and 1987, led by G. Tognoni, was the first major study on the beneficial effect of reperfusion with streptokinase, according to Van de Werf. "The study appeared in *The Lancet*. It dealt with intravenous streptokinase versus placebo in 12,000 patients, a fairly simple study; one group of patients got streptokinase and the other group got nothing, neither aspirin nor heparin. And at the end it was counted how many patients in both groups had died after a week, after a month, and so forth. That study convincingly demonstrated that mortality was reduced by administering streptokinase." (10-12)

Marc Verstraete had actually already demonstrated the same thing with his European Cooperative Study Group. His results were published in 1979 in the *New England Journal of Medicine*, but this much smaller study was met with relatively little interest. (13)

There also were the *International Studies of Infarct Survival* or ISIS-2, a study by Oxford in 20,000 patients demonstrating that a patient's survival chances improved more if the treatment was started within four hours after the first symptoms of a heart attack. (14-15) "That was the *time is muscle* concept," says Van de Werf. "The faster a patient could be treated the less damage the heart muscle incurred. Those were relatively simple studies. Oxford was known as the academic research group that conducted large studies, but that kept it simple at the same time, by limiting the record form - the amount of registered data - and with hard endpoints: counting the number of deaths. That was a clear endpoint; no discussion could arise over that." Oxford continued to work with streptokinase and not with rt-PA because, according to Van de Werf, at Oxford they considered rt-PA much too expensive compared to streptokinase.

In Leuven, Marc Verstraete and his consortium of European hospitals then undertook a third major study with rt-PA in May 1986. The degree to which rt-PA had an effect on the recovery of the heart function and on the patient's chances of survival would be measured in comparison with a placebo group. By then it was already generally accepted that the sooner treatment was started after the infarct, the greater the effect. Hence the study focused on patients who could be treated within 4 hours after the first symptoms. They were administered rt-PA intravenously, and were given 250 milligrams of aspirin and 5,000 units of heparin along with their rt-PA treatment and during the first days after the treatment. Aspirin and heparin are both blood thinners; aspirin works on the blood platelets that have attached themselves to the vessel wall; heparin limits the formation of fibrin clots that arise on the accumulated blood platelets. The additional heparin was deemed necessary because t-PA or rt-PA dissolves the fibrin clot, but otherwise changes little in the blood coagulation system. Hence the risk that new clotting would arise was not unrealistic. A treatment with

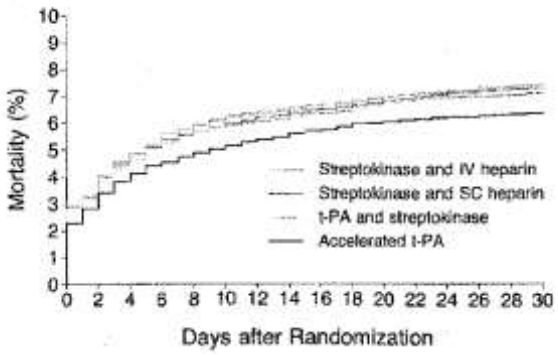
streptokinase on the other hand compromised the clotting quality of the blood throughout the body and so could result in hemorrhaging as a side effect.

Once more, the result was encouraging for rt-PA; the number of deaths in patients from the rt-PA group was 36 percent lower. And for patients who could be treated less than three hours after the onset of the first symptoms, the results were even better. In addition, the recovery of the heart function turned out to be better for this group than for the placebo group. There remained, however, an increased risk of cerebral hemorrhaging in 1.4 percent of the patients. (16)

In 1988 the ASSET study (*Anglo-Scandinavian study of Early Thrombolysis)* was carried out in the United Kingdom, Norway, Sweden and Denmark. This time 5,011 patients were involved, and the mortality was measured after one month. In the placebo group, 9.2 percent of the patients died; in the rt-PA group 7.2 percent. But in the latter group slightly more hemorrhages occurred as a complication. Several smaller studies in the US and in Australia investigated the pumping function of the heart, which was determined by the ejection fraction, the percentage of blood that could be pumped from the heart with each heartbeat.

At the beginning of 1987, the superiority of rt-PA versus streptokinase was clear and indisputable, but the results in both groups were not dramatically different. The survival rate with rt-PA outnumbered that with streptokinase with slightly more than one patient in 100, and the side effects were better under control. Not an overwhelming result, but more than acceptable, they thought at Genentech. The FDA would now have enough data in hand to recognize rt-PA as a medication, they figured. (17)

Chapter 5: The FDA approves rt-PA



Results of the GUSTO-study: thirty-day mortality rate in four treatment groups. The group with the accelerated rt-PA administration had the lowest mortality ($p<0.001$).

Activase
Cuvée Gusto 1993
Méthode Réperfusion Rapide
Château Genentech
San Francisco
Maison fondée en 1976

Founding members of the D. Collen Research Foundation, vzw.
From left to right: Rector Roger Dillemans, Karel Tavernier (general manager, KU Leuven), Désiré Collen, Lawrence Fouraker (former Dean, Harvard Business School) and Jacques Vander Eecken (chairman LRD)

***Summary**: It was a shock when in May 1987, against all expectations, the FDA did not approve rt-PA. However, when it eventually was accepted in a second session rt-PA could be sold under the brand name Activase. Genentech organized a big party! Initially a part of the flow of royalties from Genentech went to the Flemish interuniversity institution Innovi. Undeservedly, KU Leuven and Collen thought, and he decided to buy back the rights to rt-PA from Innovi. The royalties were largely placed in the Désiré Collen Research Foundation to support scientific research. In 1988 Collen had to defend his rt-PA patent in a court case of Genentech against competitors, a case which was decided in Genentech's favor. In their choice of treatment, cardiologists kept hesitating between the cheaper streptokinase and the more expensive rt-PA. The GUSTO study convincingly proved that when treated with rt-PA and heparin, a patient runs 14 percent less risk of dying within 30 days than with streptokinase and aspirin.*

rt-PA becomes Activase

At Genentech, they felt that after all the tests it was time to put rt-PA on the market. The last hurdle that had to be taken was having rt-PA approved by the American Food and Drug Administration (FDA), headquartered in Bethesda, north of Washington DC. The session at which it would take place was scheduled for 29 May 1987. On that day, the FDA would assess whether rt-PA was a suitable medication for patients suffering a heart attack, and whether streptokinase could be used intravenously as a treatment. The intracoronary use of streptokinase had already been approved in 1982. The two cases were first submitted to the Biologics Division, but then passed on to the Cardio-Renal Advisory Committee. The latter committee would transmit its recommendations to the FDA, which would base its decision on the committee's advice.

Genentech held a brainstorming session among personnel to decide on the commercial brand name of rt-PA, and the winner would receive USD 1,000. "There may have been 500 proposals sent in," says Collen. "But the winning name was 'Activase', a contraction of Activator and 'ase'. 'Ase' is a suffix indicating it is an enzyme. And no, I did not send in a suggestion myself."

On that Friday morning, 29 May, the FDA auditorium in Bethesda was packed with journalists and other interested parties. They included not only science journalists, but also the Wall Street press. Genentech had meanwhile become a sexy company and a 'glamour share' on the stock market. Shareholders would have an interesting ride when rt-PA got launched on the market.

The morning session was devoted to intravenous streptokinase; ten questions were asked, and approval granted. The case for rt-PA was scheduled for the afternoon. Here seven questions were to be answered. In his book *Ethical issues in drug testing, approval and pricing*, Baruch Brody (°1943) writes an extensive report about that day. (1)

Collen was also present: "I think there were about 200 people in the auditorium. The session proceeded like a court case, with the Advisory Committee as the jury. Elliott B. Grossbard, the Chief Medical Officer of Genentech, represented the company." Grossbard presented the results of the trials that had been conducted and which were clearly in favor of rt-PA. Everyone expected this would be a 'walk in the park'. But the committee judged the case for

rt-PA incomplete and refused to give the green light. More research was needed. No approval.

“The news hit like a bomb,” says Collen. “All the journalists rushed out at once, opened up their satellite systems - there were no mobile phones at the time - and rang their editors with their satellite phones: *It’s a no!”*

The press reactions to the FDA decision were mostly negative, especially in *The Wall Street Journal.* Major economic interests were at stake, but journalists mainly emphasized the ethical consequences. The article in the *Washington Post* was headlined: ‘*t-PA Foot-Dragging Costs 30 Lives a Day’*. The journal *Science* didn’t forego dramatic language either. According to editor-in-chief Daniel E. Koshland, the FDA had blood on its hands.

The decision had come that Friday after the stock market’s close, but on the morning of Monday, June 1st, the Genentech share tumbled from USD 48.25 to USD 36.75 on the New York Stock Exchange, immediately after the opening. With this drop of USD 928 million, Genentech saw a quarter of its share value go up in smoke!

Eugene Braunwald, the pontiff of American cardiologists, was upset as well. He had coordinated the TIMI studies, the second of which was still underway during the FDA investigation, and the results of which were not yet public.

“The whole point was that the FDA wanted a clinical endpoint for the tests that were completed, which we didn’t have formally at the time,” Collen explains. “We did have an anatomical endpoint: the artery opened, there was blood circulation again, but the FDA also wanted clinical evidence: the results on the ejection fraction, the quantity of blood that is propelled forward with every heartbeat, and the mortality figures.” The discussion about whether a blood clot was the cause or consequence of a heart attack had not yet been fully laid to rest either, and that might also have been an influencing factor. There was one other study under way, TIMI-2, but the results had not yet been published. Moreover, Genentech had modified the production of rt-PA during the course of the trial because the modification resulted in a much larger output. But this measure had also altered the molecule somewhat. The modified rt-PA was more active and caused more cerebral hemorrhages when the same dose of 150 milligrams was maintained, so the dose had to be adjusted to 100 milligrams.

In short, the conclusion of the FDA committee came down to this: we want to know the effect of a dose of 100 milligrams, and not just whether the blood vessel is open, but also the degree to which the ventricular function is improved, and whether the adjusted dose has reduced the number of cerebral hemorrhages as a side effect.

Genentech directors were ‘not amused’. At the start of the procedure they had dealt with a different committee of the FDA, the Biologics Division, which had led them to believe that everything was fine. The studies proving that there was reperfusion (blood circulation) would be sufficient for the FDA, they said, and no other endpoints would be requested. Later, however, the Cardio-Renal Advisory Committee thought otherwise. The reason that two different committees consecutively had to look into the case followed from the fact that rt-PA as a molecule was both a biologically active protein, for which the Biologics Division was the competent agency, and a drug for heart failure, which had to be assessed by the Cardio-Renal Committee.

“Well obviously, we were not drinking champagne afterwards,” says Collen. “Nevertheless Van de Werf, Sobel and I, we all knew that rt-PA had overall good scores, also with the 100 milligrams dose, but the FDA said: where is the evidence? Maybe we should have waited a bit longer before we took rt-PA to the FDA, but Genentech was eager to start selling; every month of delay meant loss of income.”

It was decided to wait for the results of the ongoing studies and then submit a new application. Over the following months, the FDA and Genentech sat together, and eventually the FDA could be persuaded that rt-PA was effective as a thrombolytic therapy and that the side effects with the 100 milligrams dosage were acceptable. Six months later, in November 1987, on a Friday the 13th, rt-PA obtained official approval. The FDA had been presented with the results of the TIMI-2 study with 2,000 patients receiving a dose of 100 milligrams. They showed no increased risk of cerebral hemorrhages. Furthermore, other studies, including the Guerci heart function study of Johns Hopkins, reassured the FDA about the improved heart function with rt-PA. (2)

In Europe, rt-PA had received French approval a few months earlier, in June 1987. The European Medicines Evaluation Agency or EMEA (the European Union agency) had not yet been set up. It was founded eight years later in 1995, and is now called the EMA or European Medicines Agency. After approval by France, most other European countries followed by means of mutual recognition. Since European countries base their approval on the same studies as the American FDA, once the green light is given by one authority, the others usually follow. In Europe rt-PA was brought to the market by Boehringer Ingelheim with the name of *Actilyse*.

Fireworks in San Francisco

The FDA approval on 13 November 1987 definitely called for a party in South San Francisco! Genentech had set up an enormous tent in the company car park; personnel walked proudly around in T-shirts with the slogan ‘Clotbuster’. There was drinking and dancing, and over the bay an enormous firework was set off, for which air traffic at San Francisco International Airport had to be temporarily halted.

For there was much at stake: The Los Angeles Times wrote the next day that the approval by the FDA was a gigantic leap forward for Genentech in its ambition to become a major pharmaceutical player. The article cited analysts who expected that the new drug Activase would easily yield 500 million USD to one billion USD per year! Kirk Raab, the president and CEO of Genentech, announced that the price would be set at USD 2,500 per dose. Rather expensive, but the research, the many studies and the production had cost the company an investment of over USD 200 million.

The PR and marketing department of Genentech pulled out all the stops. Hospitals and cardiologists were contacted, and in less than two months’ time 58 million dollars’ worth of Activase was sold. Genentech and Collen and Co. were believed to have made a ‘blockbuster’, and done so in record time, according to Collen. “I saw Diane for the first time in June 1980; in 1982 t-PA was cloned; in 1984 we already treated a first patient with rt-PA,

and in 1987 rt-PA was approved by the FDA and brought to the US market as Activase. We completed the entire procedure in seven years' time; I think that's a world record!"

Cardiologists could now choose between Activase or streptokinase for their patients with a heart attack. The first figures indicated that in over half the American hospitals they preferred Activase, although it cost ten times more than the alternative streptokinase. Genentech had eventually set the price for Activase at USD 2,200 per dose, while streptokinase was available for only USD 200. Activase was therefore mainly used in hospitals that could recover the cost of the medicine through the patient's insurance. But there also were cardiologists who did not believe in the superiority of Activase and preferred to use streptokinase.

After a first major wave of purchases in which hospitals and physicians supplied themselves with Activase, sales gradually declined. At the end of 1988 Genentech even had to halt its production of Activase for a while. In a 12 December press release Genentech stated that the sales figure for the past year 1988 would come to only USD 150 million. But the company intended to have its PR machine run overtime, put even more emphasis on the advantages of thrombolysis in heart attacks, and make full use of marketing and sales to highlight the superiority of Activase. (3)

Could Genentech have introduced Activase at a lower price? "The price of a medication is 'as high as you can and as low as you must'," says Collen. "It's not purely about the production cost, because that accounts for less than 10 percent of the sale price, but companies like Genentech have to finance their research, distribution lines, patents, studies, and so forth. You can't set the price for a medicine excessively high either; you want the authorities to approve its use, and hopefully arrange a reimbursement, so usually the final price is a compromise." Comparing the price of a treatment with streptokinase versus one with Activase was therefore a cost estimate of the advantages. How much may these advantages cost? "Look, if you treat 100 patients with streptokinase within four hours, mortality figures drop. If you treat 100 patients with Activase within four hours, then mortality drops more: out of 100, one more patient will survive with Activase than with streptokinase. The price for this is the USD 2,000 dollar difference between the price of Activase and streptokinase, and this times 100. So USD 200,000, spread over an extra life expectancy of 12 years, gives you 16,666 dollars per year. And in general, a cost of USD 30,000 to USD 50,000 per *'quality adjusted life year'* (qaly) is now accepted. Hence the extra cost of Activase was acceptable."

The failure of the Innovi adventure

Following the approval of rt-PA as a medication in the US and Europe, royalty money would be pouring in. Collen had therefore revised his original contract with Genentech. On 18 March 1983, a new agreement had been signed stipulating a higher share of the royalties for Leuven.

Renegotiating the contract actually was a task for the newly founded Innovi, a privately financed government initiative that was part of the '*Third Industrial Revolution in Flanders'* (Derde Industriële Revolutie Vlaanderen, DIRV) – a campaign of the Flemish regional government, then led by Christian democrat Gaston Geens (1931-2002). The campaign was

launched in 1982. Steel, glass, coal, shipbuilding and textiles had made the country prosperous until the middle of the 20th century, but now Flanders had to focus on the economic sectors of the future such as microelectronics and biotechnology. Taking LRD, the technology transfer office of Leuven university as an example, the Flemish government founded a similar institution, Innovi, to coordinate research valorization for *all* the Flemish universities. The Leuven LRD with its various divisions, of which Collen's Protein Research Division was the largest, would be allowed to join the new organization. Innovi would promote and foster academic research throughout Flanders.

There were many parties involved at Innovi. Almost everyone who had industrial or financial weight in Flanders became a member, from the Generale Maatschappij/Société Générale bank to Agfa-Gevaert, Bekaert, Janssen Pharmaceutica, Aveve, Bell, GIMV and several others. On 12 July 1982, LRD formally transferred its t-PA dossier to Innovi. The body would manage the t-PA contracts and patents in return for 12 percent of the revenues LRD received from t-PA sales. LRD fully relinquished its own 7 percent from the original agreement of 1976 to Innovi, and KU Leuven did the same with half of the 10 percent to which it was originally entitled. Thus KU Leuven still retained 5 percent of the revenue from rt-PA. In hindsight, this was an outstanding arrangement for Innovi but a poor deal for Leuven. Collen, however, received reassurance on the matter from Jos Bouckaert, who had negotiated his first contracts with Genentech and who would continue to do so at Innovi. Bouckaert joined Innovi to become managing director. He convinced Collen that Innovi would have more management expertise than LRD. And the clever rector Pieter De Somer apparently failed to pay attention; the pressure from the Flemish government on the universities to participate was great.

It wasn't long before Leuven regretted the deal. In reality, the agreement amounted to Innovi profiting without providing any real service in return, as soon became apparent. Collen himself had renegotiated his first contract with Genentech in 1983, giving Genentech the exclusive marketing rights to rt-PA worldwide as long as the patent of LRD was valid, until mid-2005. In exchange, Genentech raised the royalties to which Leuven was entitled: 3 percent of sales in the US and 2 percent of sales in the rest of the world. Shortly afterwards, in 1984, Genentech decided to defend the t-PA patents itself. Actually, these had all been tasks that Innovi should have carried out. But Collen and Genentech preferred to keep matters in their own hands, an arrangement which suited Innovi very well.

In one year, the deal with Leuven had yielded Innovi USD 78,000 (12 percent of USD 650,000) for which it had to do very little in return, and after two years Innovi could credit 50 million francs, or more than a million USD, to its account, all earned with rt-PA royalties. "Ultimately Innovi had to do no more than send an invoice to Genentech every three months," says Collen. "And imagine the sums when rt-PA really began to pay off, hundreds of thousands of USD per year just to carry out a marginal administrative job?" That money could better be spent on research, he thought. The 12 percent of the revenues from rt-PA that were allotted to Innovi were reduced to 10 percent in 1984; the other 2 percent went to LRD that kept doing all the administrative work. But that was still a generous compensation for Innovi, certainly in comparison to what the other institutions and universities brought in. Collen's patent was essentially Innovi's cash cow.

It had meanwhile dawned on LRD and KU Leuven that they were foregoing a lot of money for little in return, and, on 30 September 1985, Jacques Vander Eecken (1941-1995), chairman of the board of directors of LRD, let Innovi know that Leuven would terminate the agency's contract with Innovi. There was another fly in the ointment as Jos Bouckaert, the man who had persuaded Collen to join Innovi, turned his back on the organization and left for California at the end of 1985 to focus on the genetic improvement of grapes, where he became president and CEO of Vinifera, a biotech company in Petaluma, north of San Francisco.

However, the Innovi shareholders were not prepared to just let go of their goose with the golden eggs. First, there was an attempted management buyout by the CFO and CEO of Innovi. They offered 35 million francs, almost twice the original investment of the shareholders. But Collen wanted his t-PA back himself. During a personal interview with several members of Innovi's board of directors, such as Robert Stouthuysen of Janssen Pharmaceutica, Jan Hinnekens of the Boerenbond (Farmer's League) and Guido Declerq of Kredietbank, Collen tried to persuade the shareholders. Collen and LRD argued that the agreement with Innovi was a contract of agency: an assignment to manage a dossier; and that the revenues Innovi collected for this assignment were much too high. The Innovi shareholders argued that the deal involved a transfer of property and dismissed the arguments of Leuven. They maintained that Collen and LRD had to buy back their rt-PA dossier. In 1988 arbitration took place, in which two top attorneys stood opposite each other, Carl Bevernage for Innovi and Alfons Puelinckx for LRD. The verdict was in favor of Innovi. If Leuven wanted its t-PA dossier back, LRD would have to buy it from Innovi. Collen therefore had no alternative but to pay, *and* offer more than the managers in their buyout proposal.

To allow the sale to Collen, Innovi moved all the rights and obligations that related to t-PA into a separate company, the 'nv t-PA'. The value of the nv (limited company) was estimated by a statutory auditor at a little over 32 million francs, because rt-PA had meanwhile become a lucrative business. Collen first tried to persuade the board of directors of LRD to acquire the nv t-PA with central LRD resources, but chairman Jacques Vander Eecken was reluctant to do so. After which Collen and his Protein Research Division paid 38 million francs from their own resources for the nv t-PA. Quite a bit of money, but a clever investment for Collen's lab, because the nv t-PA generated its 10 percent from the royalty influx year after year, 350 million francs altogether, until the patent expired in mid-2005.

The biblical manna of the D. Collen Research Foundation

After the Innovi debacle, rector Roger Dillemans had a look at Collen's original contract with LRD from 1976, especially the distribution modalities of the royalties. The contract with Innovi also stipulated that none of the modalities could be changed without Collen's approval. This is what the distribution of the royalty looked like:

KU Leuven	5%	
LRD	2%	
Nv t-PA	10%	
Laboratories Collen	41.50%	-> 20.75%: Thrombosis and Vascular Research
		->20.75%: D. Collen Research Foundation
Remaining part	41.50%	-> 20.75%: D. Collen Research Foundation
		-> 20.75%: Inventors and collaborators

“The 41.5 percent ‘remaining part’, that is a bit much,” argued Dillemans, who meanwhile realized that enormous amounts of money were involved. It wasn’t acceptable that Collen should earn ten times more collecting royalties than with his Professor’s salary! Collen agreed. Besides, his colleagues at KU Leuven were growing increasingly envious of Collen’s division, which became richer by the day. Together with rector Roger Dillemans, Collen decided in 1988 to transfer half of the money to a non-profit organization, named the Désiré Collen Research Foundation.

Of the two portions of 41.5 percent, each time half was diverted to the Foundation, so that it had 41.50 percent of the royalty revenues. Collen became chairman, the board of directors consisted of rector Roger Dillemans (1932), Karel Tavernier (1933-2015), who was then general manager of KU Leuven, Jacques Vander Eecken (1941-1995), the chairman of LRD, and Lawrence (Larry) Fouraker (1923 –1998), who was later replaced by Chip Gold. Fouraker, former dean of Harvard Business School, was a highly regarded personality who had ended up in Chip Gold’s hospital with a heart attack, where he was treated with rt-PA. “Fouraker had been an advisor to the American president. He was already relatively old when we asked him to come on the board, but because he felt we had saved his life, he accepted. When a few years later he felt it had all become too much of a burden, we granted him an honorable discharge and he was replaced by Chip Gold,” says Collen. Of the founding group, only Collen and honorary rector Dillemans are still alive.

With the Foundation, Collen wanted to promote scientific research by financing fellowships, symposia and congresses, and by giving out research grants. The foundation could also acquire real estate if that benefitted the main goal, namely the promotion of science.

Scientific research is an international affair. Inviting foreign postdocs and sending Belgian students abroad to acquire innovative knowledge at foreign universities and research institutes enrich both the researcher and the institution. Collen knew this from his own experiences in the US and in Sweden. Grants for Belgian postdocs therefore seemed to him an ideal way to spend part of the royalties that were now coming in generously. In the beginning, the Foundation financed four fellowships per year, sending young promising scientists from KU Leuven to the US. A few years later (when Roger Dillemans had meanwhile been succeeded as rector), it turned out that the grants from Collen’s Foundation only went to students who had not been accepted anywhere else, instead of to the most deserving. Hence, Collen decided to transfer his grants to the Belgian American Educational Foundation as from 1993. For these grants, students from other universities were also

eligible. When Collen became a board member of the Francqui Foundation, he moved his fellowships to the Foundation, until 2013 when the grants ended. Altogether the D. Collen Research Foundation subsidized a postdoc year for some one hundred Belgian university graduates, primarily but not exclusively in the US.

Furthermore, the Foundation made one-off gifts, including when fellow Professors such as Maurits Sabbe (1924-2004) of the theology faculty called on Collen for financial support. Sabbe had persuaded the then-rector Pieter De Somer to build a library for his faculty, which in 1974 was inaugurated as the Sabbe Library. It is now one of the most prestigious theological libraries in the world; in addition to a number of old prints, manuscripts and incunabula, treasures such as the Anjou Bible are preserved there. The library also houses the diaries of Pope Adrian VI, who in the 16th century, before he became pope, was rector of the KU Leuven. In the early 1990s, Sabbe needed money to buy a dehumidification installation, because the precious works risked being damaged by humidity, and Collen's Foundation gave the Sabbe Library a one-off grant of half-a-million francs.

The fruit cultivation test center in Rillaar also obtained sponsoring from the Foundation to cope with financial problems. Professor Vic Goedseels, then dean of the faculty of agricultural science (now Bioengineering Sciences), was an innovative academic. According to Collen, he raised the agriculture faculty to a higher level and introduced biochemistry and biotechnology in his faculty, and his fruit cultivation center in Rillaar deserved some support.

The Foundation sponsored congresses, symposia, research and two KU Leuven chairs of 5 million francs each, mainly for research activities, first at the Center for Thrombosis and Vascular Research and later at the Center for Molecular and Vascular Biology.

The Foundation partially financed the ninth floor of the Central Services building on the Gasthuisberg campus, with Peter Carmeliet's state-of-the-art mice center, the *Marc Verstraete Specific Pathogen Free Animalium*, as it is called. The Center for Transgene Technology and Gene Therapy, later renamed the Vesalius Research Center, and now the Center for Cancer Biology, was housed there. When in 2005 the Belgian law on non-profit organizations changed, the Foundation changed its statutes and its name. It was retitled Life Sciences Research Partners (LRSP), and after the death of Chip Gold in 2008, Harvard University ceased to be a member of the Board of Directors.

In court in Delaware

Genentech's rt-PA was protected by three patents: the first one of Collen, Rijken and Matsuo, which LRD had filed for human t-PA, and the two others for Genentech's cloned rt-PA, which were in the name of David Goeddel. At least two pharmaceutical companies, Genetics Institute Inc. and Burroughs Wellcome Company, thought that they had a way to get round these patent rights, and intended to do so by making a small change here and there, to put their own version of rt-PA on the market.

This resulted in a lawsuit in Wilmington, Delaware, where Genentech's headquarters were registered: Genentech had filed a patent infringement suit against its competitors Burroughs Wellcome and Genetics Institute. Genentech argued that its patents had been infringed, but the opponents claimed their rt-PA was different. Several DNA bases of the rt-PA cDNA genes they used, differed from those of the Bowes cDNA clone with which Genentech had

made its Activase. Genentech in turn was subpoenaed by its two competitors for monopolizing the market of thrombolytic medications. And they filed an unfair competition claim against Genetics Institute and an antitrust claim against Burroughs Wellcome.

The case was heard in 1988 before the United States District Court in Delaware. “I sat on the witness stand for an entire afternoon as well as the next morning,” Collen recalls. “I was interrogated by Genentech’s attorney and by the counter-party’s attorneys.” But Genentech’s lawyers, Dennis D. Allegretti of Boston and his assistants, had prepared Collen well for the case. “They had set up a war room in the hotel, with boxes of files that covered the whole wall from top to bottom. There they grilled me as they expected the counter-parties’ lawyers would do. They gave me advice and tips. For instance, I had the tendency to answer with ‘Yes, you are right’. Just drop the ‘you are right’, the lawyers said. ‘Yes’ is more than enough.”

The jury comprised a fireman, two housewives and two preschool teachers, among others. “They first heard tutorial witnesses for four days: professors who explained in an understandable way how blood clotted, what thrombolysis was, how t-PA worked and what recombinant DNA technology was all about, but I don’t know if those people could grasp all that. And subsequently, I had to explain what I did in Leuven and in Sweden, and how I came to my results. We were requested to make nine copies of all our lab notes in Leuven, a stack of papers of over 5,000 pages! The university had allocated me an outstanding attorney, Alfons Puelinckx, the man who was later involved in the Agusta affair (a corruption case involving the Italian helicopter company Agusta and Belgian socialist parties, where Puelinkcx was the go-between). Roger Dillemans, the rector who was also an attorney, took the Concorde to the US, to testify that I was an employee in good standing, and one of the *prima donnas* of the university in Leuven. “The allegations that were fired in my direction were not mild. I was accused of being a crook, and a thief for stealing ideas. Yes, it was fairly extreme. I kept the court transcripts, about 3,000 pages, in my cellar until recently. They even tried to discredit our lab: ‘oh, that’s all routine work what you did’, they said. ‘Anyone could do that!’”

There was some nervousness in Leuven. Should Genentech lose the case, that would mean the end of the royalties! But Genentech could demonstrate that the rt-PA of Burroughs Wellcome was a clone of Genentech’s rt-PA aside from one detail, which, in addition, turned out to be an incorrect amino acid. And the rt-PA of Genetics Institute hardly differed from that of Genentech. The jury found that Wellcome and Genetics Institute infringed the three Genentech t-PA patents.

After the court’s decision in April 1990, Burroughs Wellcome decided to stop its production, while Genetics Institute continued producing a shortened version of t-PA for a while. The lawsuit cost each of the three companies more than USD one million, and because the jury found that Genentech had suffered no economic damage - the competing t-PA’s were not yet on the market - no monetary damages were awarded to Genentech.

Which is the better therapy?

Now that cardiologists were increasingly convinced of the validity of the open artery hypothesis - clots cause heart attacks and not the other way round - the question remained

which thrombolytic therapy was the best: rt-PA, streptokinase or APSAC (anistreplase), a new drug from the British company Beecham. A new bone of contention replaced the old.

Boehringer Ingelheim, which had bought the European marketing rights from Genentech, financed the Italian GISSI 2 study in 1988. GISSI 2 would examine the survival chances of some 12,000 patients in Italy. Shortly after the trial started, Frans Van de Werf was asked to add another 9,000 patients from other European countries. The trial eventually counted 20,891 patients who were treated with either rt-PA or streptokinase, combined with heparin or not. The results that were presented three years later in Florence, on 8 March 1990, cooled the enthusiasm for rt-PA. The mortality figures did not differ much, and, at 8.9 percent, were even slightly higher for rt-PA. Streptokinase had a mortality rate of 8.5 percent. The study also noted that side effects occurred in both cases; rt-PA had more strokes as a side effect (1.3% versus 1.0%), streptokinase led to a higher occurrence of hemorrhages (0.9% versus 0.6% for rt-PA). (4-5)

According to Frans Van de Werf, one could attribute the disappointing result to the manner in which rt-PA was administered. He referred to a study by Karl Neuhaus, who demonstrated that accelerated administration of rt-PA over 90 minutes (faster in the first 30 minutes and then slower over the next 60 minutes) would have ensured faster and better blood flow. In the GISSI trial the administration was spread over 3 hours. (6)

Genentech argued that a possible explanation could be the way heparin was administered in the GISSI 2/International study, namely only 12 hours *after* the rt-PA treatment. (7) The role of heparin was not yet well known when the GISSI2/International study was set up in 1987. Smaller studies had shown that although Activase (brand name of rt-PA in the US) dissolved the clot and restored the blood flow, new occlusions could occur. rt-PA works locally on the clot and then disappears, while streptokinase changes the blood coagulation system in the entire body and stays longer in the blood. This could be an additional explanation for the results of the GISSI2/International study. Repeated clotting could be drastically reduced by giving the blood thinner heparin along with rt-PA. Genentech therefore insisted on a new study.

In the meantime, another study was underway: *The International Studies of Infarct Survival* or ISIS 3, coordinated by Oxford in 41,299 patients in 1,000 hospitals. It was the largest clinical study in the world. The endpoint in this study would be mortality at 35 days, and it would compare the efficacy of streptokinase, rt-PA and anistreplase (APSAC), with aspirin or in combination with aspirin and heparin. The results were published in 1992 and again were not favorable for rt-PA; the mortality with streptokinase was 10.6%, with APSAC 10.5%, and with rt-PA, 10.3%. The differences were not significant enough to conclude that one was better than the other. Hence many cardiologists reasoned it was preferable to use the cheaper streptokinase. (8)

According to Collen, Oxford was more streptokinase-inclined in any event. “Richard Peto and Rory Collins advocated streptokinase.” But at Genentech they again argued that in the Oxford study rt-PA was not administered correctly. Half the patients were not given heparin, and the other half had heparin administered subcutaneously and not intravenously, *and* much later than was customary for treatments in the US. Frans Van de Werf added that a different type of rt-PA was used (duteplase), an experimental variant from Burroughs-

Wellcome. In its press release, Genentech quoted the ’Leuven Professor’ Désiré Collen that the doses of duteplase administered in the ISIS-3 study could not be compared with the dose of Activase, and that the results for duteplase therefore could not be extrapolated for Activase. (9) Moreover, according to Frans Van de Werf, duteplase was also not administered fast enough at the outset of the treatment; the administration was spread over four hours.

Genentech advised American cardiologists not to be guided by this study. There was yet another study of at least the same scale underway that would demonstrate the superiority of rt-PA, Kirk Raab, the CEO of Genentech, announced. This was the GUSTO study *(Global Utilization of Streptokinase and t-PA in Occluded Coronary Arteries)*, set up in 1989 and started in 1990 in more than 40,000 patients around the world. It was coordinated by cardiologist Eric Topol, the ambitious physician who had treated the first patient with rt-PA in the US at Johns Hopkins in Baltimore, Maryland. Topol had in the meantime moved to Cleveland Clinic in Ohio, where he was head of the cardiovascular department. Topol is widely credited for Cleveland Clinic's achieving its status as the leading center for heart care in the US.

The study was coordinated by cardiologist-biostatistician Robert Califf of the Duke University Medical Center in Durham, North Carolina, and by Frans Van de Werf of KU Leuven. They acted as clinical directors. Califf did the statistical analyses of the study in the US. Van de Werf worked in Leuven with his Leuven Coordinating Center (LCC), which would later carry out dozens more studies on acute coronary syndromes. He had founded the LCC at the time of the GISSI 2/International study when he was asked to extend the Italian study to patients from other European countries. In addition, there was a Data Monitoring and Safety Board and a Stroke Review Committee, while David Stump, then Director of Clinical Research at Genentech, also kept an eye on things.

> David Stump (°1951) was a specialist in hematology and oncology, but during his postdoc years he became fascinated by hemostasis and thrombosis. When he heard of the research being done in that field in Leuven, he paid an exploratory visit in 1983 and decided to collaborate with Collen. In the summer of 1984, Stump moved with his family into one of the small houses for visiting professors and postdocs in the Groot Begijnhof (the Beguinage) in Leuven and did research in Collen’s lab on, among other things, the fibrinolytic and thrombolytic properties of pro-urokinase. He enjoyed his stay so much that he even considered learning Dutch. “If you have time and energy left, you better invest it in studying biochemistry,” Collen supposedly answered. This was no doubt useful advice, because in 1986 Stump returned the US as Associate Professor of Medicine and Biochemistry at the University of Vermont, where he worked in the hemostasis lab of Ken Mann and did the blood analyses for the TIMI studies of Eugene Braunwald and the TAMI study with the then young Eric Topol. In 1989 Genentech recruited Stump to lead the department of clinical research on rt-PA. He and David Goeddel are the only two scientists who are ‘Genentech Fellows*’*. Afterwards he became vice president at Human Genome Sciences Inc.

Roche takes over Genentech

The GUSTO study was set up to test the hypothesis that to increase survival chances after a heart attack, swift intervention was crucial. The 41,021 patients who participated were divided into four groups. One group got streptokinase with subcutaneously administered heparin; a second group got streptokinase and intravenous heparin; the third group got an accelerated dose of rt-PA and intravenous heparin during the rt-PA treatment; and the fourth group received a combination of streptokinase and rt-PA. The clinical endpoint was the mortality at 30 days. The selection of the patients was also subject to stricter medical conditions than in previous studies. People who had experienced a previous heart attack, or who had been treated with streptokinase or anistreplase were excluded, as were patients who had undergone a recent trauma or a major operation, or who had uncontrollably high blood pressure.

In a subgroup of 2,431, patients the importance of the time lag between the onset of symptoms and the administration of the fibrinolytic treatment would be examined. The blood flow in the infarct related blood vessel would be evaluated in the four groups via coronary angiography. It was hoped that this GUSTO Angiographic Study could establish a connection between an open blood vessel and a lower mortality.

Bayer, CIBA-Corning, Genentech, ICI Pharmaceuticals and Sanofi Pharmaceuticals sponsored the study. All members of the steering group, the Data Monitoring and Safety Board and the Stroke Review Committee had to declare in writing that neither they nor their family members had a financial interest in any of the sponsors; because where easy money can be made, it is sometimes hard to resist temptation, judging from the following anecdote.

Genentech's CEO Kirk Raab, who apparently was not entirely certain of the outcome of the GUSTO study, in February 1990 sold 60 percent of Genentech to the Swiss family business Roche for 2.1 billion USD. In November of that year, his wife Mrs. Raab was fined more than USD 160,000 by the Securities and Exchange Commission because she allegedly had casually passed on information about the deal to her brother and his friend. The latter had made use of that information to buy option contracts before the deal was publicized, selling the next day with a nice profit of USD 127,400. This, however, turned into a loss, as the two friends received a fine of USD 197,400. (10)

Was selling to Roche really necessary? "Raab wanted to safeguard Genentech in case the GUSTO trial turned out to be negative for the company," says Collen. "Roche might have taken a risk with that deal, but the Swiss also had an option on the remaining 40 percent. That option was exercised six years later, when they bought the remaining shares and put 20 percent on the stock market again. In 2009 Genentech was completely absorbed by Roche and that is still the case. With its relisting, Roche earned more than it had paid for the entire deal. The CFO at Roche was a very talented fellow. The company made billions on Valium, among other things, but much more still on its financial transactions!" (11)

Roche had gambled and won. In 1993 the results of the GUSTO study showed that patients who had received rt-PA *with* heparin in relative numbers had 14 percent less chance of dying within 30 days, than patients who had received one of the other thrombolytic treatments. (12) The study had cost hundreds of millions of USD, but it had been worth the money. Sales of Activase increased once again, and to celebrate, Genentech distributed bottles of

champagne with a special label: *'Activase, Cuvée Gusto 1993, Château Genentech'*. Collen still has one in his cellar, but doubts whether it is still drinkable.

The sales figures for Activase rose again - at least in the US and in Western Europe, where it was sold by Boehringer Ingelheim under the name Actilyse. "In the rest of the world, in India and South America, or where they couldn't afford it, streptokinase continued to be used. In 1993, 236.3 million USD worth of Activase was sold, in 1995, 301 million USD, and we in Leuven happily watched the royalties come in."

But Collen had already turned his attention elsewhere. "We were working meanwhile on variants of t-PA, chimeras and such, to try to make it more clot-specific, more active. Ten to fifteen people were occupied with this research for years, all preclinical studies." Some fine articles resulted, of which Roger Lijnen and Collen made an overview in 1991. (13) "Every now and then, I still went to look in at Genentech, where they were occupied with mutants to make TNK- t-PA, or tenecteplase, a more stable protein. It was more fibrin-specific and could be administered right away with one injection, while Activase involved a first injection, than a rapid infusion for 30 minutes and a slower infusion for 60 minutes." From 1993 on, Collen's visits to the US grew less frequent. He had started on a new project in Leuven, his *'poor man's t-PA'*.

Oxford throws doubts on rt-PA

The GUSTO researchers published the results of their study comparing rt-PA with streptokinase in the *New England Journal of Medicine* in 1993. (12) From the study, it appeared that accelerated administration of rt-PA combined with intravenous administration of heparin was the best therapy for heart attacks. That treatment ensured the best results with regard to survival chances and the wellbeing of the patient. Fewer allergic reactions and other complications occurred. The researchers concluded modestly that this result should however not lead to complacency; ultimately the mortality in treatment was still 6.3 percent, and about 0.72 percent of the patients had a CVA or stroke. Improvement was still possible.

But there were rearguard actions too. Four years later, the Oxford scientists published a so-called meta-analysis in which they analyzed and compared all the existing research and trials regarding the treatment of heart attacks. (14) Collen found the review article in the *New England Journal of Medicine* quite upsetting. "*On the basis of all the evidence from major trials, the true difference in stroke-free survival between one fibrinolytic regimen and another is likely to be at the most only a very few events per 1,000 patients treated*," said the article. "They talk about 'events', not of mortality nor of heart attacks," Collen says. "No, they add it all up. What they do is scientifically not correct. They add up the deaths with patients who had a stroke, but one third of those people fully recover. One third of those patients die, but it is not clear whether they are double counting. And on several occasions, they manipulate the numbers to promote their theory that rt-PA was no better than streptokinase."

Frans Van de Werf adds: "They tried to downplay the favorable results of treatment with Alteplase (rt-PA) as they appeared from the GUSTO 1 study. In their review article they combine all the results from a number of different methods of administration, *and* with different forms of the rt-PA molecule, and then they come to the conclusion that treatment

with rt-PA is not significantly better than treatment with streptokinase. That is a statistically highly questionable technique."

In addition, the Oxford researchers stressed the risk of a stroke in rt-PA treatment. Their conclusion was therefore that streptokinase with aspirin was the preferable treatment of acute myocardial infarction, as streptokinase was much cheaper than rt-PA.

That led to tensions between the Oxford group of Collins, Peto, Baigent and Sleight on one side and the defenders of rt-PA, including Collen, Van de Werf, Califf and Topol, on the other - tensions that went on for quite a while. "There was some hitting below the belt," Van de Werf recalls. Peter Sleight, although a respectable and internationally reputable cardiologist with over 500 publications to his name, commented during a meeting 'that Topol needed an enema with rt-PA'.

No letters to the editor were sent regarding the review article of the Oxford group, says Van de Werf, after consulting the internet. Not even by Collen? "I could not respond to the NEJM article from Oxford at the time because as a co-beneficiary of the t-PA royalties, I had a conflict of interest," Collen says. He did respond a couple of years later, in 1999, with a presentation at the celebration of the 75th anniversary of *The Journal of Clinical Investigation*. "Before an audience of some 200 people I explained, with the graphs and statistics from the Oxford article, where they had made deliberate mistakes and where they had manipulated the numbers. One of those present was Dr Ingelfinger, editor-in-chief of the NEJM when the Oxford article appeared, but the man didn't flinch."

No further reaction came from the Oxford group. "They had moved on to other things," says Collen. "Peto was knighted in 1999 by the British Queen, Collins on 31 December 2010. For their achievements." Collen suspects that promotion of the cheaper streptokinase in British healthcare was among those achievements, given the precarious financial condition of the National Health Service. "But ultimately the Oxford group cost the NHS money, by thwarting the open artery hypothesis for a long time. While elsewhere they were already busy with angioplasty and stents, thanks to Oxford the NHS in Great Britain was at least five years behind with this therapy," Collen concludes.

The final assessment of rt-PA

What ultimately is the balance sheet for so much medical industriousness in terms of human lives? What did rt-PA contribute? Very precise statistics are not available, but to make a long story short, several hundred thousand lives have been saved with rt-PA. Approximately 8 million patients have been treated with rt-PA under the trade names Activase (Genentech-Roche) and Actilyse (Boehringer Ingelheim) since the market introduction at the end of the 1980s. This also includes 1.5 million patients who received TNK-tPA, a variant of the original rt-PA. However, patients who received and still receive rt-PA for stroke are not taken into account.

To assess what percentage of these patients survived thanks to rt-PA, we must rely on the results of the various 'megatrials', the studies with many thousands of patients that have already been extensively discussed here. A problem with this is that in the earlier studies, streptokinase (with or without aspirin) was compared with placebo, and in the subsequent

comparison of streptokinase and aspirin with rt-PA and heparin, a further comparison with placebo was no longer ethically acceptable. The mortality in the group with a placebo would have been unjustifiably much higher.

We know that in the megatrials (GISSI and ISIS-2), the mortality in the placebo groups one month after treatment was around 12.5%. With streptokinase and aspirin, the mortality fell by approximately one third (to around 8%). In the later GUSTO trial - which is the only major comparison of streptokinase and aspirin on the one hand with accelerated rt-PA and intravenous heparin on the other - there was a further drop in the 30-day mortality in the rt-PA group by one in a hundred patients (from 7.3 to 6.3%).

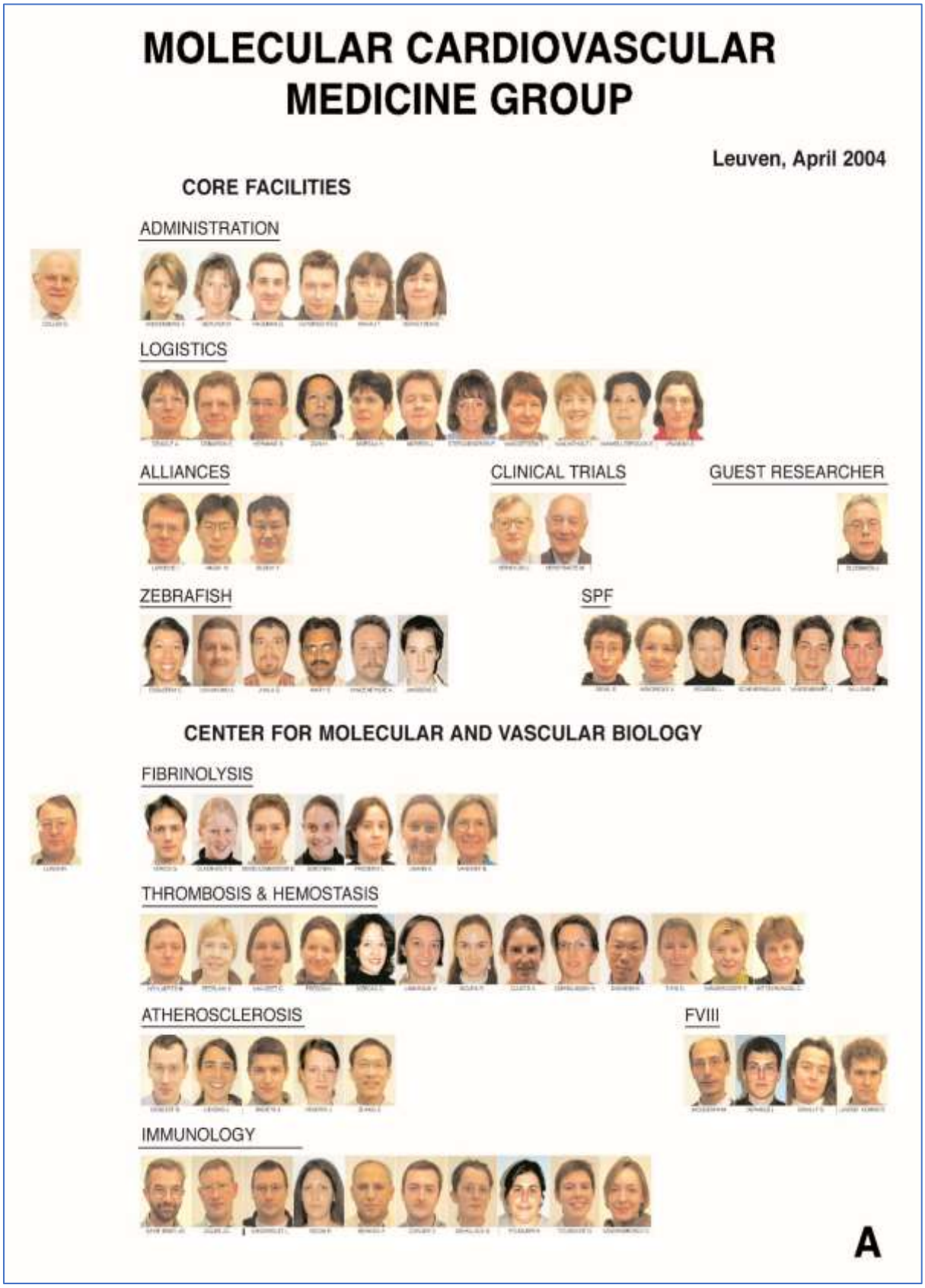
Extrapolating to the improvement with rt-PA versus placebo, there is 6.2% (12.5 minus 6.3) of 8 million, thus half a million fewer deaths. Comparing streptokinase and aspirin on the one hand and rt-PA and intravenous heparin on the other in the GUSTO megatrial, there are 80,000 additional lives saved with rt-PA (8 million times 1%). When the two studies are combined in a statistically justifiable manner, some 400,000 patients owe their lives to rt-PA. This is not considering the side effects, be it either the hemorrhages, or the improved heart function with faster recovery of the coronary blood flow with rt-PA. But physicians must of course take those aspects into account.

Was the effort worthwhile? “I think so,” says Désiré Collen. “There is scientific evidence that thanks to the procedure with rt-PA, the surviving patients could add on average 12 *qalys* or quality-adjusted life years, to their lives. If you value a qaly at USD 20,000 (presently even up to USD 50,000 is acceptable), then you arrive at USD 240,000 (20,000 times 12 years) to be compared to the additional cost of a treatment with rt-PA versus streptokinase. That additional cost amounts to USD 2,000 per treatment, which of course must be multiplied by 100, so USD 200,000, because we treat 100 patients to allow one extra to live 12 years longer; 240,000 in benefits versus 200,000 in costs. So rt-PA’s balance is quite positive. The rationale would be much simpler if that one patient (out of a hundred) who survives with rt-PA and heparin but not with streptokinase and aspirin could be identified in advance. Unfortunately, that’s not possible. It’s either one treatment regimen or the other for all patients.”

Still standard for treatment of ischemic stroke

What does rt-PA still offer in medical practice today? Angioplasty and stenting have now become the standard treatment for heart attacks, but rt-PA is still used for strokes. An article in *Stroke* from 2015 (15) states that 20 years after the publication of the study by the American NINDS (National Institute of Neurological Disorders and Stroke) (16) on the use of rt-PA for acute strokes, rt-PA is still the standard treatment for patients who arrive at hospital in time. The success of the 1995 study was based on the fact that a remarkable number of patients could be successfully treated with rt-PA within 90 minutes after the first symptoms of a stroke. The authors note that for patients with a stroke who can be treated within 4.5 hours after the first symptoms, administration of rt-PA should still be the standard treatment provided there are no contraindications.

PART II: THROMBOLYSIS FOR THE POOR



MOLECULAR CARDIOVASCULAR MEDICINE GROUP

Leuven, April 2004

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B

Chapter 1: Entrepreneurship with Thromb-X

Coronary Thrombolysis With Recombinant Staphylokinase in Patients With Evolving Myocardial Infarction

Désiré Collen, MD, PhD, and Frans Van de Werf, MD

Background. Staphylokinase (STA), a protein with known profibrinolytic properties, is produced by transduced *Staphylococcus aureus* strains. In experimental animal models, recombinant staphylokinase (STAR) is less immunogenic and more active toward platelet-rich arterial blood clots than streptokinase.

Methods and Results. In the present study, 10 mg STAR given intravenously over 30 minutes was found to induce angiographically documented coronary artery recanalization within 40 minutes in four of five patients with acute myocardial infarction. Plasma fibrinogen and α_2-antiplasmin levels were unaffected, and allergic reactions were not observed. Postinfusion disappearance of STAR antigen followed a biphasic mode with a $t_{1/2\alpha}$ of 6.3±0.6 minutes (mean±SD) and a $t_{1/2\beta}$ of 37±15 minutes, corresponding to a plasma clearance of 270±100 mL/min. Neutralizing antibodies against STAR could not be demonstrated at baseline and up to 6 days after infusion, but STAR neutralizing activity, which did not cross-react with streptokinase, was consistently demonstrable in plasma at 14–35 days.

Conclusions. STAR can induce clot-selective coronary thrombolysis in patients with evolving myocardial infarction without concomitant induction of a systemic lytic state. STAR, a small protein that can be easily produced by recombinant DNA technology, may therefore offer promise for thrombolytic therapy in patients with thromboembolic disease. (*Circulation* 1993;87:1850–1853)

Thrombosis, the blockage of blood vessels with clots, can lead to acute myocardial infarction and ischemic stroke, both leading causes of death. Other than surgical interventions to remove or by pass the blockage, or the generation of collateral vessels to provide a new blood supply, the only treatment available is the administration of thrombolytic agents to dissolve the blood clot. In this review, Désiré Collen considers the properties and characteristics of staphylokinase that make it the thrombolytic agent of choice.

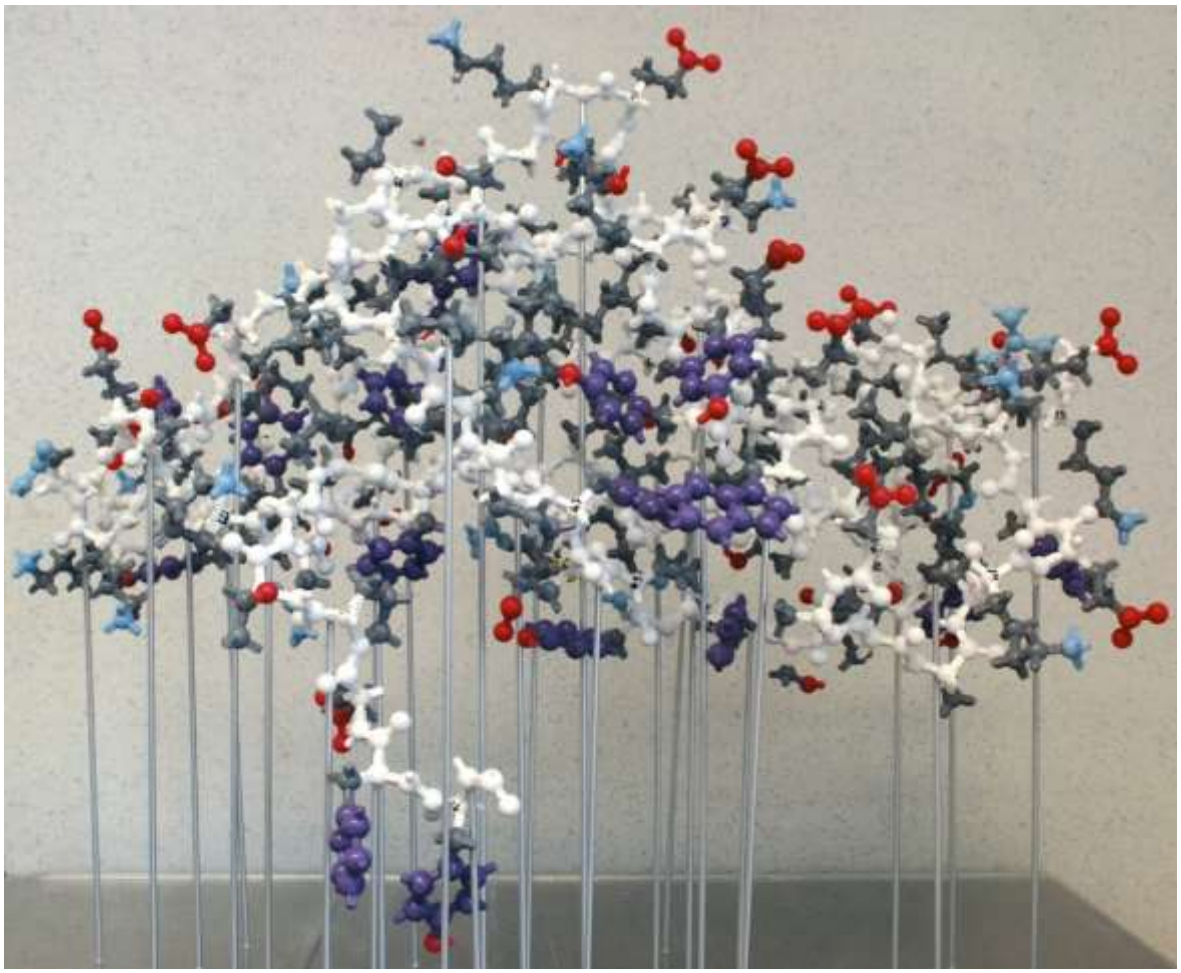
Staphylokinase: a potent, uniquely fibrin-selective thrombolytic agent

DÉSIRÉ COLLEN

Acute myocardial infarction and ischemic stroke are the two main causes of death and disability in our Western soci-

they are not all equal. Indeed, phys logical fibrinolysis is regulated by sp cific molecular interactions between

Nature Med.1998;4:279-84.



Three-dimensional model of Staphylokinase
(After: Rabijns A. et al. Nature Struct Biol. 1997; 4:357-60.)

Summary: At the end of the 1980s, Collen was in his prime, and the dollars were flowing in. But the relatively high price Genentech and Boehringer Ingelheim charged for rt-PA bothered him. Indeed, rt-PA had turned out to be an expensive drug for the privileged. Collen believed he had an inexpensive equivalent to rt-PA, staphylokinase. To finance the large-scale comparative tests with the existing thrombolytic treatments, which would require a great deal of research and money, he founded the limited company Thromb-X, and, later, in Ireland, ThromboGenics Ltd. But the Phase III study became unaffordable when the Canadian cardiologists who performed the Phase II CAPTORS trials were dissatisfied with a comparison between staphylokinase and streptokinase in the pivotal study, and instead wanted to do comparisons with the expensive rt-PA. Exit staphylokinase?

Yakult Honsha and Matsuo open up a new path

At a congress of the International Society on Thrombosis and Hemostasis in Tokyo in the summer of 1989, Collen met up with Osamu Matsuo, his former colleague who had done the rabbit tests in Leuven in 1979-1980, and with whom he shared the first patent for the discovery of t-PA as a thrombolytic drug. “Matsuo told me he was working with staphylokinase, a bacterial protein from *Staphylococcus aureus*, which he had obtained from a Japanese firm. He thought it looked like t-PA. Why don’t you send me some, I said, I’ll take a look at it. And that’s how the staphylokinase story started.”

The Japanese firm was Yakult Honsha. In 1935 the Japanese doctor Minora Shirota had discovered that intestinal bacterium *Lactobacillus casei* had interesting health benefits. He added it to a drink and called it Yakult. Yakult is fermented milk, enriched with these bacteria that are so-called probiotics, live microorganisms claiming health benefits in the gut. In 1955 Yakult Honsha Limited was founded in Tokyo to further commercialize Doctor Minora Shirota’s invention. Yakult Honsha set up a sales system, the Yakult Lady Home Delivery System, which operated along the lines of Tupperware. It turned out to be a successful business. Yakult is now sold worldwide.

Staphylokinase was a by-product isolated from fermentation at Yakult Honsha, and for which the company had no use. Matsuo and his Japanese colleagues had already done a few experiments with staphylokinase and published their results in *Blood* in 1990. They had conducted an in vitro comparison of staphylokinase and t-PA and had discovered that staphylokinase caused increased plasminogen activation and also that it was fibrin-specific. (1)

This was exactly what Collen had been seeking. With staphylokinase he could make thrombolysis accessible to everyone, even in less affluent countries! But it would turn out to be a quest that would consume almost 20 years of his life. With as much royalty revenue from rt-PA as he could muster, Collen wanted to market staphylokinase as an equivalent and much cheaper alternative to rt-PA. To realize his ambition, he founded together with the Leuven university the NV (limited company) Thromb-X.

The mission of Thromb-X was clear from the outset: to develop staphylokinase from beginning to end without outside intervention. Starting from the biochemical characterization to the clinical research, Collen would eventually launch it on the market as a new and affordable thrombolytic agent. He was full of ambition. Genentech was aware of his plans but did not consider it to be potential competition for Activase. “On the contrary, they were rather

relaxed and probably thought it would take me ten years to develop it. By then t-PA as a therapy would be outdated. And they probably also thought that I wouldn't be able to pull it off," Collen laughs. The director of LRD, Hans Claes, became the managing director of Thromb-X.

Ireland and the Cayman Islands

Some reshuffling had to be done to mobilize as much of the royalty revenues as possible for this new project. With the previous reorganization in 1988, the royalties were divided among various parties. A major portion of the money ultimately ended up in research projects carried out at Collen's lab and at his Center for Thrombosis and Vascular Research, via various channels.

After a few years, however, the tax authorities stumbled upon the transfer of royalties from the nv t-PA, a limited company, to Collen's division at LRD, a non-profit organization. "According to the tax authorities this construction was set up to dodge taxes," says Collen. In fact, all that happened in the nv t-PA was a money transfer to the non-profit organization. It served merely as a letterbox. "Fortunately, we didn't have to pay a fine, but the inspector did let us know that this construction would no longer be accepted. If a public limited company, in this case the nv t-PA, but also Genentech incorporated, pays money to a non-profit organization, a 25 percent tax was due on 85 percent of the amount received." That was money both Collen and the university thought would be better spent on scientific research than on taxes, certainly now that a great deal of money was needed to develop staphylokinase. Money from a public limited company to another public limited company would fiscally be more acceptable, but the best solution consisted of having the royalties accumulate in a public limited company with real activities. That company could then offset expenses against the royalty revenues, leaving no taxable profit and hence no taxes to be paid. This solution was rapidly implemented.

To develop staphylokinase, a new public limited company, Thromb-X, had already been founded in 1991 with two partners: Collen as a private individual, and the Protein Research Division, his department at LRD. Each partner contributed 12.5 million francs and each held half of the shares. Collen further invested an amount many times larger than the 25 million francs, using his own royalties in Thromb-X. He was the one who also came up with the name Thromb-X. The X was to indicate that thanks to t-PA (and staphylokinase) the thrombus, or blood clot, could be crossed out. He had even designed a logo for the new company, with the Greek capital letters Theta and Xi: Θ Ξ. The Theta (the eighth letter of the Greek alphabet), or Th of Thromb, and the Xi (the fourteenth letter of the Greek alphabet), created a capital X for the second part of the name. Theta is also used as a symbol for therapy. "But Randall Moreadith was not a fan," Collen admits. "He felt it sounded like the name of a fraternity." In the US fraternities or student clubs usually have names made up of two or three Greek letters. The logo was cast aside.

Collen's plans with Thromb-X were ambitious; he wanted no less than to write his own Genentech story. He would set up a business to develop his poor man's t-PA, staphylokinase, into a medicine constituting a cheap alternative for rt-PA. "I was still in my *Sturm und Drang* period then. I thought I was going to create the new Janssen Pharmaceuticals in Belgium." Collen now laughs at his former over-confidence.

To solve the tax problem, the nv t-PA was merged with Thromb-X in November 1993. From then on there was real activity with regard to 10% of the royalties. Collen gradually also bought the shares from his university partner in Thromb-X, at 3 to 6 times the original value, until he had full ownership of Thromb-X . LRD was happy with the cash it got for its shares, and Collen was satisfied to have full control of his new venture.

He now had the shares of his partner in Thromb-X, which made up 10% of the royalties, but not yet the 90% of other rights to future royalties. Taxes were still to be paid on those. That required a new transaction. He decided to buy all the royalty rights controlled by LRD and merge them into Thromb-X. Thromb-X paid the university (LRD in this case) 675 million francs, which corresponded with the value after taxes of the expected revenues from those royalties. Leuven university hit the jackpot once again. Thromb-X paid mainly with a bank loan of 400 million francs, and with 200 million in shares of the newly founded ThromboGenics Ltd in Ireland. Now Collen had 54.5% of the royalties. Broken down, this was: 41.5% from the Désiré Collen Research Foundation (the university's non-profit organization), 5% from Dick Rijken, 1% from Matsuo, 5% from KU Leuven and 2% from LRD. The rights of Rijken and Matsuo would continue to be honored by LRD from the 675 million francs Thromb-X had paid LRD for the royalties.

A second transaction followed, independent of the university. Collen relinquished the royalties he personally controlled (35.5%) to the Collen Charitable Trust, with Biggar Ltd as the executive vehicle. Biggar subsequently sold these royalties to the newly established Irish ThromboGenics Ltd for 600 million francs, which were converted into ThromboGenics shares. Biggar would later acquire the ThromboGenics shares that the university had obtained in the previous deal. And the university was again happy with the additional cash.

Above the operating company Biggar, there was the Collen Charitable Trust in the Cayman Islands, a fully discretionary trust managed by trustees in Geneva, Switzerland. To definitively and irrevocably earmark a substantial portion of the royalties for scientific research, an offshore trust was the most appropriate structure. It is a legal concept that did not exist in Belgium, but Collen decided this was no reason not to make use of it elsewhere.

Collen is not the owner of the Collen Charitable Trust, nor the director. He may submit projects, but the trustees independently assess his proposals against the mission of the Trust. Collen is only the '*enforcer*' in the Trust, with the capacity to appoint or remove trustees. If the investments in research projects are successful and yield more resources, this will not benefit Collen personally. Legally, the yield is therefore a gift, but the situation has had far-reaching consequences for the Collen family. Under Belgian law, parents cannot give away the 'reserved portion', or the fraction (at least 50%), of their legacy destined for 'compulsory heirs' - their children, and Collen's Charitable Trust was well above this percentage. He therefore took up residence in London where testators can more freely decide on the destination of their assets. In 2012, he and Louisa moved to London, and became in May 2017 permanent residents of the United Kingdom. They obtained 'settled status' under the EU Settlement Scheme of the UK in March 2019

With the Trust and Biggar Ltd, Collen founded the ThromboGenics Ltd company with several partners in Ireland in 1998. "By 1997 I had already paid over 160 million francs in personal income taxes, the university over 300 million, and we were only halfway the duration of the

patent!" Collen decided to further optimize the royalty revenues. On the eve of the introduction of the euro, Ireland was the place to be for start-ups and investors. To keep up with the stronger euro economies, the country had introduced an extremely favorable investment climate. By setting up the new ThromboGenics in Ireland, Collen hoped to benefit from the Irish policy to attract risk capital.

All seemed well; however, the acquisition of the royalty rights from the university in 1997 was for a period of only five years, agreed at the express wish of the then research coordinator of KU Leuven. Thereafter the university could buy the rights back for USD 1. This had been done against Collen's wishes. He had wanted to guarantee the compensation of any net additional proceeds after those five years. But the Leuven academic authorities turned it down. "I didn't have much choice then, I had to take it or leave it, so I agreed," says Collen. "But five years later, in 2001, the situation was quite different. We were already in Ireland then, with Ken Freeman as chief financial officer and Randall Moreadith as chief medical officer. They wanted to raise money for our staphylokinase project, but not if they didn't have the certainty that also after 2001 royalty resources would still come in. Well, Freeman said, I'm the chief financial officer here, we will go and renegotiate the contract with the university! And so they went to KU Leuven. Ken Freeman explained to the university authorities: look, the rights you have are not really worth that much anymore, because there is increasing competition for rt-PA. Angioplasty and stents were becoming more and more important. Freeman offered Leuven 150,000 shares of the now existing ThromboGenics Ltd, at EUR 5.6, which came down to EUR 840,000. Those royalties ultimately turned out to be worth much more! The university had foregone some EUR 10,000,000 to the benefit of ThromboGenics Ltd, money for which they didn't want me to be liable back then, as they refused the proposed agreement to compensate them for the additional proceeds! " Collen concludes.

Between 2000 and 2005 ThromboGenics Ltd. would acquire control of Collen's Thromb-X limited. Collen's Thromb-X shares, valued at the price at which they were acquired between 1991 and 1995, were exchanged against ThromboGenics Ltd shares valued at EUR 6.35 per share. The result of this complex but fully transparent reorganization was that Collen could now invest most of the rt-PA royalties in his project to repeat the rt-PA success with staphylokinase. Or at least he could try to do so.

Testing on baboons

Roger Lijnen, Désiré Collen's permanent associate since 1978, and his team set to work with staphylokinase in Leuven. "Pharmaceutical companies every now and then asked us to test thrombolytic agents for them," says Roger Lijnen. "Désiré believed that staphylokinase could be interesting for us. We started with the 25 milligrams of staphylokinase that Matsuo had sent, and we set to work with our standard procedures. Within a couple of months we were ready. Then Désiré went to look for staphylococci strains that produced a lot of staphylokinase in the clinical lab of the bacteriology department in the hospital. We cloned it, made it recombinant and purified it. And we began our tests."

Staphylokinase had already been tested by Jessica Lewis and John Wilson in 1964. They had experimented on dogs, but the animals got severe hemorrhages. Lewis and Wilson concluded therefore that the product was toxic, and they assumed that the effect on humans

would be the same. (2-3) “But Matsuo had discovered in his in vitro tests that it was fibrin-specific in human blood, which reassured us,” says Roger Lijnen. “In our own in vitro tests we had already discovered that the reaction to staphylokinase in dogs was completely different from that in humans.”

Staphylokinase and streptokinase work in similar ways, they activate plasminogen, but staphylokinase does so not by cutting plasminogen, but by binding with plasmin that is bound to the clot and forming a complex, cutting the plasminogen in the blood clot and only there. Staphylokinase is just like t-PA fibrin-specific, it doesn’t upset the whole blood coagulation system of a patient but only activates plasminogen trapped in the blood clots. If there are no blood clots, any staphylokinase/plasmin complex is simply neutralized by α2-antiplasmin, just like free plasmin. “In dogs the antiplasmin does not react with the complex, and hence the dogs risk bleeding to death. Lewis and Wilson had just chosen the wrong animal species for their tests,” Lijnen concludes.

Roger Lijnen’s team worked like a well-oiled machine. “We were about seven or eight people: Paul Holvoet did the cloning, Eddy Demarsin produced it in cell cultures and purified the molecule, and then it came to me for biochemical characterization and for the animal tests. Berthe Van Hoef did the kinetics, Frans De Cock did in vitro work and Jean-Marie Stassen did the animal tests. And that went wonderfully. We followed standard procedures; we had our plasma pools from a series of different animals - rats, mice, hamsters, rabbits, guinea pigs, and baboons. We made blood clots in vitro and then saw whether our product worked, after which we could already exclude a number of animal species.” In rats, for instance, it did not work. The animal models were then selected on the basis of the in vitro results. The team started with hamsters, then rabbits and finally baboons. Collen had set up a baboon colony in Leuven for that purpose.

But views on animal rights were changing, and the baboon colony was becoming controversial. Michel Vandenbosch, an animal rights activist and founder of the animal rights association Gaia (Global Action in the Interest of Animals), had been campaigning for years against animal testing, and his crusade had meanwhile captured the attention of the authorities. Collen was summoned to the Ministry of Agriculture. Michel Vandenbosch had brought along an English pathologist, and Collen was accompanied by Guy Mannaerts from the general management of the university hospital. “How can you justify using primates as experimental animals, we were asked? I defended our procedures at great length,” says Collen. “But Vandenbosch of course challenged the need for animal tests.” Then the English pathologist took the floor, her presentation angering Collen. “What are your qualifications, madam?” he asked her. “Have you ever done animal tests yourself? Have you ever done research?” The discussion definitely risked going in the wrong direction, at which time Guy Mannaerts intervened, took the irritated Collen by the sleeve and whispered: “Just be quiet and let me explain, then we’ll work it out.” The final verdict was, however, that the baboon colony had to be dismantled. Collen moved his tests on baboons to South Africa, where they were followed up by Jean-Marie Stassen, who travelled back and forth between Leuven and Bloemfontein.

It soon became clear that staphylokinase would work just as well as rt-PA and that it had additional advantages. It was relatively easy to clone, and Leuven now had enough in-house expertise for this. “It is a relatively simple protein with only 136 amino acids, without disulfide

bridges," Roger Lijnen explains. "It is also a stable protein; you can leave it in solution at room temperature for weeks and it remains active." Moreover, staphylokinase was much cheaper to produce than rt-PA because it was a bacterial protein and not, like rt-PA, a highly complex human protein. r-tPA had to be produced in mammalian cells, while staphylokinase could simply be cloned and produced in bacteria, and that made a substantial difference.

One drawback was that staphylokinase, just like streptokinase, was foreign to the body and so also immunogenic. Patients who had been treated with it once developed antibodies against staphylokinase. A subsequent treatment might have less chance of succeeding. "We tried for a while to replace the amino acids that caused the antibodies, with alanine, a neutral amino acid, but that had only partial results. Ultimately it was a lesson in humility; it's not easy to do better than nature," Roger Lijnen concludes.

Dozens of mutants were made to extend the half-life (the time in which half of the product disappears from the body), because just like rt-PA, staphylokinase has a half-life of only six minutes. After six minutes, half of the product has disappeared from the body by clearance via the liver and the kidneys. This is why it had to be administered gradually via an infusion. With a longer half-life that could be done with a single injection.

Lijnen and his team had worked many hours. Collen recalls: "We put in 50 man-years altogether. We made grams and grams of staphylokinase." The studies on, among other things, the mechanism, the biochemical properties, the genetic structure, and the interaction with plasminogen and α2-antiplasmin led to 72 publications. Over half of the existing literature on staphylokinase as a thrombolytic agent consists of publications in the names of Collen, Lijnen and collaborators. (4,15)

Envy among professors

Leuven and Collen's lab benefited significantly from the t-PA patent, and it was seen as unfair that his lab continued to apply for additional funds. According to Collen: "The idea that I was a 'rich Professor' undoubtedly damaged me and my coworkers." Indeed, it was often said that Collen and his research group had enough money and that they therefore should not compete for research funds from the university, the Flemish Community, or the federal government. "Some people even felt I should be ashamed to compete for those scarce research funds! That was the unequivocal message I got from several colleagues."

Collen disagreed with this sentiment completely, knowing that he and his coworkers needed subsidizing. But it repeatedly led to conflicts. The rectors and department heads often had to arbitrate between the argumentative types that talented people can be. Collen argued that his competitive research needed the extra funding now, because the royalties from rt-PA would end someday, as the patent protection had a time limit. But some of his colleagues thought this was a problem for the future.

Gifted researchers cost a great deal of money; Collen knew. These costs arise not so much from their salaries but from the expensive environment in which they work. The top tier of the life sciences, flourishes only in the best possible environment with experienced and trained technicians - motivated teams, with a 'critical mass' of multidisciplinary knowledge. Science had long moved past the stage of geniuses working in isolation. Collen himself was a chemist *and* physician, but that combination in one person was rare.

Obtaining research funds is not only about money. It is also a matter of recognition, a quality label with access to networks. Collen gives an example: “The Interuniversity Attraction Poles (IUAPs) that are set up by the Belgian Science Policy Office, for instance. The aim of these programs is to grant support to excellent research teams from the various language communities of our country on condition that they conduct fundamental research in a network context. Between 2007 and 2011, some 40 of those networks were supported in the fields of biology, medicine, chemistry, physics, applied sciences, historical sciences, social sciences and humanities. Suppose that ‘Blood vessel formation and vascular wall biology in pathology and medicine’ (network P6/30) had to manage without the expertise of Peter Carmeliet and his team. That he would be excluded from this network on the pretext of ‘already having enough money’. That would lead to raised eyebrows internationally and give Belgium a rather curious reputation as a research country.”

On several occasions Collen had no other option but to finance his research with his own funds. The ‘rich Professor’ then acted as sponsor, sometimes to the irritation of KU Leuven, which had little control over the independent Collen. “My stubbornness also had a downside, I realized that. Some deserving researchers from other departments of KU Leuven sometimes missed out on subsidies because of us. I therefore paid several times to the ‘IUAP solidarity fund’ of KU Leuven, out of our own resources. In one case I paid 7 million francs five times; on another occasion, 25 million francs.”

In 2004, however, a research proposal drawn up around Peter Carmeliet in the field of angiogenesis (blood vessel formation) was suddenly dropped by the Leuven university authorities even though it had received central approval in Brussels, and that was just because Collen was involved. From the research partners at the University of Liège, he had heard that Leuven – then under Rector André Oosterlinck - no longer wanted to invest in Collen ‘because he diverted public university resources to private industrial activities’. Collen still remembers the incident vividly. “I was waiting at the airport in Rome for my return flight, when the Liège participant Jean-Michel Foidart rang me and told me Leuven would not subsidize because of ‘Collen’s involvement’ in their project. I couldn’t believe my ears!” But a solution was found for the project: the team slipped another Leuven professor into the research project instead of Collen. “From then onwards I refused to be spokesman for the next IUAP projects any longer. Enough was enough,” Collen says.

“From all the above-mentioned, it may be clear that during the period 1995-2005 I lived in a sort of LAT *(living apart together)* relation with several directors of the university,” he adds, chuckling. Others might have been tempted to break with Leuven, not Collen. “After all, I was the head of the Center for Molecular and Vascular Biology and director of the VIB [Flemish Institute for Biotechnology] department of Transgene Technology and Gene Therapy (later the Vesalius Research Center and presently Center for Cancer Biology). Those were enough responsibilities – especially with regard to all my coworkers who clearly preferred that I stay, rather than leave in disappointment.”

Production by Medac

Although his team had already achieved a *tour de force*, they needed outside help to continue developing staphylokinase, Collen realized. “We ran up against the limits of our own

possibilities. In fact, our method for producing recombinant staphylokinase was not robust enough to produce sufficient protein for the clinical studies that would be necessary."

Collen looked around for an institution where they could produce large quantities of staphylokinase under GMP (Good Manufacturing Practices) conditions. A patent search led him to the Institute for Molecular Biology (I.M.B.) in Jena, in the east of Germany. Like Yakult Honsha, Jena had a patent for staphylokinase; it had been submitted in the 1980s, before the unification of Germany, and was valid for East Germany only. The Yakult patent was international *and* submitted earlier, so if ever there arose a patent conflict between Jena and Japan, Jena would have lost out, according to Collen. He contacted Jena and was told that staphylokinase was licensed out to Medac GmbH, a small pharmaceutical company in Hamburg with a base in Jena.

The East German microbiologist Detlev Behnke from Jena now worked for Medac in Hamburg. He had sold the staphylokinase license to Medac for peanuts, but with the unification of Germany he had lost his job in Jena. "Before the fall of the Berlin wall, he was working at the Institute for Molecular Biology in Jena and had excellent relations with the East German authorities. It was whispered that he had worked for the *Staatssicherheit*, the Stasi, which made me hesitate. But his former coworkers assured me that Behnke had only posed as a compliant East German researcher to obtain a visa from the authorities in order to attend conferences abroad. And I think that was true; in any event he seemed a decent fellow."

In December 1992 Collen concluded an agreement with Medac. The German company transferred all its rights on staphylokinase to Thromb-X and in exchange received 5 percent of the shares in Thromb-X and the exclusive marketing and distribution rights in Germany, plus 3 percent of the future turnover in Germany. For a while everything went smoothly. "Medac was a relatively small company that sold finished products. The production of staphylokinase took place in Jena, which I regularly visited. They had outstanding biochemists, by the way." Collen undertook quite a few studies with them, and they published several articles together. (16-20) It later turned out that Collen was being deceived by the Germans.

It works in patients

In Collen's laboratory, Jean-Marie Stassen had succeeded in demonstrating the *in vivo* thrombolytic properties of staphylokinase in various animal test models. In hamsters with a pulmonary embolism, the thrombolytic activity of staphylokinase was comparable to that of streptokinase. When blood clots enriched with blood platelets were used, staphylokinase was even five times more active. That was an additional advantage of staphylokinase, because clots that block a heart artery usually contain many blood platelets. In baboons too, Stassen could demonstrate that intravenously administered staphylokinase dissolved blood clots at least as well as streptokinase. Here again, clots rich in blood platelets were dissolved better by staphylokinase than by streptokinase. And staphylokinase was slower in generating antibodies and provoked fewer allergic reactions. Moreover, staphylokinase was much more fibrin-specific than streptokinase; it caused far fewer hemorrhages as a side effect.

The research had advanced far enough to start testing on patients. In 1992 the first patients were treated with recombinant staphylokinase. The product now also had a name: STAR or

sakSTAR. Sak is the abbreviation for staphylokinase. The staphylokinase that was made in Jena, ‘sak 42 D’, differed slightly from the Leuven product STAR, which was somewhat more stable.

On 25 June 1992, Frans Van de Werf treated a first heart attack patient with 10 mg of staphylokinase by means of an infusion that lasted 30 minutes. And shortly after that, four more patients received a treatment. After 40 minutes the blood clot was entirely or partially dissolved in four of the five patients. If, however, a second treatment with staphylokinase were needed later, it probably would be less effective, because staphylokinase is foreign to the body, which therefore reacts after a first administration by making antibodies. (21) Even people who were never treated with staphylokinase sometimes produce antibodies because they once suffered an infection with *Staphylococcus aureus,* or a staph infection in medical speak. There are many types of staph infections, which can cause a multitude of diseases. MRSA (multiple resistant staphylococcus aureus) is a dangerous superbug often found in hospitals and is resistant to multiple antibiotics.

If a heart patient has experienced such a staph infection, he or she might have an allergic reaction to a treatment with staphylokinase, or the administered staphylokinase might be neutralized. The occurrence of such antibodies is not in itself insurmountable, because the same phenomenon also occurs with streptokinase, which has been used as a thrombolytic drug to treat hundreds of thousands of patients. But in parallel with the biochemical, preclinical and clinical research, Collen also set up a line of research to examine the immunogenic nature of staphylokinase.

Collen: “We saw that antibodies against staphylokinase in general occurred less extensively than those against streptokinase. In addition, we had learned from the clinical research on 300 patients that serious allergic reactions to recombinant staphylokinase did not occur at all or at least would be very rare. We tried to suppress the immunogenic nature of staphylokinase via ‘site-directed mutagenesis’, the targeted creation of mutations in the staphylokinase gene. And we succeeded in generating several variants of SakSTAR that created antibodies less easily and yet maintained the same activity profile.”

It was time for a larger-scale patient study. Seven Belgian hospitals participated in phase II, a randomized study with staphylokinase for acute heart attacks: UZ Gasthuisberg in Leuven, AZ Middelheim in Antwerp, AZ Imelda in Bonheiden, AZ St-Jan in Ghent, AZ St- Jan in Bruges, AZ Virga Jesse in Hasselt and AZ St- Elizabeth in Ukkel.

One hundred patients were treated: 52 were given rt-PA, as the control group; 25 patients received 10 milligrams of STAR via an infusion; and 23 patients received 20 milligrams of STAR. It soon became apparent that STAR worked as well as rt-PA because the blood flow was quickly restored. And when five more patients were treated later with 40 milligrams, the results seemed to be even better. (22) Afterwards another pilot study was done in 19 patients, using 20 milligrams of staphylokinase via a ‘bolus’ injection: the staphylokinase was injected all at once, or in two halves as a double bolus, instead of gradual administration via an infusion. (23) In short, staphylokinase met all the expectations.

An unpleasant surprise

Collen: “We had a possible alternative for t-PA, three years after we had first talked about it with Matsuo! In three years’ time we had completely unraveled how staphylokinase worked, mapped out the biochemical characteristics of staphylokinase with all its binding partners – fibrin, plasminogen, plasmin, α2-antiplasmin; cloned and expressed the gene, conducted a series of preclinical studies *and* produced pure recombinant protein that could be used in clinical studies. All that in our own laboratory. It was an achievement to be proud of.”

The first studies were financed with rt-PA royalties, but for the phase III studies, more money was needed. “When we started talking money with the German pharmaceutical company Boehringer Ingelheim in March 1996, skeletons suddenly started falling out of the German closet,” Collen recalls. It turned out that there existed an agreement between the inventors in Jena and Medac about which Collen had never been informed. Medac appeared to have promised Jena royalties of 4 percent on the sales. And if the patent rights were transferred to a third party, in this case to Thromb-X, the obligation to pay 4 percent royalties also had to be honored by the new party. “We didn’t know that. It had never come up at the time, but when the staphylokinase project showed potential, they suddenly pulled out the agreement, and Jena insisted we honor it! We had already given them 5 percent of the shares in Thromb-X at the outset which were then worth 1.7 million francs, but I had meanwhile paid Medac a multiple of that!”

To save the whole staphylokinase project, Collen bought all the royalty rights and Thromb-X shares back from the Jena inventors. Although he felt they had misled and deceived him, he paid them 26 million francs, money he later tried to recover from Medac via arbitration and the courts, but he did not succeed and subsequently gave up the fight. He was 26 million francs the poorer, but Medac disappeared from the scene, albeit after another bloodletting for Thromb-X. In August 1999, Thromb-X bought back all the rights from Medac for one million USD, and the D. Collen Research Foundation bought back all their shares in Thromb-X for 14.8 million francs. The attorneys now drew up conclusive contracts. “Yes,” Collen sighs, “I’m really not a businessman, I’m an academic. I’ve been cheated here and there.”

In science too, not everyone is a saint. At a certain moment, a Chinese postdoc researcher was working in Collen’s lab. “His name was Ding. One day I came to the lab, and I saw his notes in Chinese lying on the fax machine, with the word ‘staphylokinase’ twice in recognizable Latin script,” Collen remembers. “That was odd, because he was working on something totally different. But his department head in China, Ms. Song, was working with staphylokinase. Ding apparently was keeping track of what we did; he attended our weekly lab meetings where projects and plans were discussed, so he had quite a bit of information about our research. And then one day Ms. Song came to visit, a so-called courtesy visit, but she was clearly very interested in our work with staphylokinase. I suspect Ding spied a bit in our lab on behalf of his Chinese department head. At the end of his contract he was supposed to return to China, but he left for Canada and he’s still there as far as I know!”

It happened rather often that Chinese postdoc students disappeared to the US or Canada after their year in Leuven on an EU grant. “They had to hand over half of their monthly EUR 1,000 grant to the Chinese embassy and get by with EUR 500 per month. Many of them kept the entire amount for themselves, but of course couldn’t go back to China afterwards. They

disappeared to America." Some didn't even finish their postdoc year. "A female researcher arrived here while her husband was already in the US. The first day I gave her a tour of the lab and told her to have a rest and come back the next day. The following day she phoned to say she was ill. The next day she rang with the announcement that her husband had had an accident in the US and was in a coma, and could she go visit him? I thought it a somewhat bizarre story but didn't ask for proof and told her she could go. She left and I never heard from her again. I think I even paid for her trip!" But Collen did inform the EU authorities that they could stop her grant.

Landon Clay to the rescue

Collen had to look for extra money. Ken Freeman, an American who lived in England and was a friend of Thromb-X director Hans Claes, appeared to know the way to sponsors. He wrote to several venture capitalists, companies or people who wanted to invest risk capital, but to no avail. As soon as they heard that it involved an investment in only one medicine, they turned away. Too risky they said, moreover when they learned it was a medicine that was to be the 'poor man's t-PA', they rejected it as an unprofitable investment.

Finally, in 1999, Collen found an investor in Boston: Landon Clay. According to a 2006 edition of *Boston Magazine*, Clay was number 43 on the list of the richest residents of Boston, with a personal fortune of USD 316 million. "But that has probably grown in the meantime," Collen surmises. (24)

Landon T. Clay (1926-2017) came from a respected American family. His grandfather Cassius Marcellus Clay was an ally of President Lincoln in his campaign to abolish slavery, and Lincoln later appointed him ambassador to Russia. Landon himself was an outstanding student; he graduated cum laude in literature from Harvard but began his career as a financial analyst at the Bank of New York in 1949. Later he became an investor, as the chairman of Eaton Vance, one of the oldest investment firms in Boston. When he eventually sold Eaton Vance, he founded the East Hill Management Company.

Clay had a great interest in science and donated to all sorts of organizations, from schools to the Harvard Observatory and the VLT *(Very Large Telescope)* in the Atacama Desert in Chile. He also donated a collection of pre-Columbian art to the museum in Boston. In 1999 he founded the Clay Mathematics Institute in Cambridge, Massachusetts, which finances grants and conferences on mathematics, and awards USD one million to anyone who can solve one of the seven great mathematical problems of the century. One such problem, '*the conjecture of Poincaré'*, was solved in 2010 by the Russian mathematician Grigori Perelman, who declined the prize because he felt it wouldn't be fair. He could not have solved the problem without the work other mathematicians had done before him, he said.

Ken Freeman brought Collen into contact with Landon Clay and his East Hill Management Company. Unlike all the other investors who had reacted negatively because they thought a 'poor man's t-PA' was not an interesting investment, Landon Clay was instantly charmed by the idea. "We immediately had a connection and actually became rather good friends," says Collen.

Phase III studies unaffordable

In the run-up to phase III, Collen and Van de Werf collaborated closely with several Canadian cardiologists, and that went well. Two phase II studies set up in Canada with staphylokinase versus streptokinase gave promising results. In Canada, rt-PA was not yet routinely used. In the CAPTORS study (*Collaborative Angiographic Patency Trial of Recombinant Staphylokinase*) of Frans Van de Werf and Paul W. Armstrong, a cardiologist at the University of Alberta in Edmonton, Canada, the optimal dose was determined. (25) But for phase III, the efficacy of staphylokinase had to be set off against a comparable product, and in a large number of patients. Collen was considering streptokinase as the basis for comparison, and, with Van de Werf, he tried to set up a large-scale study to compare his staphylokinase with streptokinase, again in collaboration with the Canadians: a group of six institutions around Paul Armstrong.

To have sufficient statistical power they needed 7,500 patients in each group, the treated and the control group. It meant a phase III study with 15,000 patients, and that came with a price: between USD 30 and 40 million! When the protocol was ready and the funding was finalized, Collen took his project to investor-friendly Ireland in 1998, where he founded ThromboGenics Limited. A company would make it easier to conclude agreements with clinical research companies. Landon Clay invested USD 12.8 million in ThromboGenics Ltd through his East Hill University Spinouts Fund. The offshore trust founded by Collen, with Biggar Ltd as the executive vehicle, and the Leuven non-profit organization D. Collen Research Foundation also participated.

“But then suddenly the Canadian cardiologists wanted us to compare staphylokinase with rt-PA instead of streptokinase. It meant we needed to buy 7,500 doses of rt-PA at USD 2,200 apiece which was far beyond our means, and Genentech was not going to give us its rt-PA for free, as staphylokinase was a potential competitor for their product. Our USD 30 million would never be enough. Altogether, it would cost us USD 100 million to put a drug on the market that was probably as efficient, but far cheaper than rt-PA. To earn that amount of money, we would have to sell our inexpensive alternative at a much higher price, and that was contrary to the whole intent.” Collen was faced with a Catch-22 situation. The plan fell apart. ThromboGenics Ltd was set aside as a ‘one-trick pony’ with only one asset, and also as a company that was searching for a remedy that already existed, at least for those who could afford the Activase from Genentech.

The staphylokinase undertaking was at a dead end, and as the years went by, it also became redundant in Western hospitals, who now applied alternative infarct treatments such as balloon dilatations and stents. It would never have equaled the rt-PA success story. But in less affluent regions there is still potential for staphylokinase as the cheap alternative for rt-PA.

Collen did not completely give up on staphylokinase. He would incorporate it into a broader research portfolio and then see whether there would be interest in emerging countries. Otherwise twenty years of work would have been wasted. Collen: “Around the turn of the century we realized that we had to work out a broader research portfolio for ThromboGenics. Otherwise it was the end of that story.” Fortunately, there was no lack of new challenges in the world and in science, and Collen had in the meantime become familiar with modern

genetics, with which first t-PA and then staphylokinase had been cloned. Science was taking giant leaps in genetics, as Collen, now 60, observed. He would once again follow a new avenue.

Chapter 2: The mice of Carmeliet and other happenings

Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele

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The endothelial cell-specific vascular endothelial growth factor (VEGF)[1–5] and its cellular receptors Flt-1 (refs 6,7) and Flk-1 (refs 8,9) have been implicated in the formation of the embryonic vasculature. This is suggested by their colocalized expression during embryogenesis[10,11] and the impaired vessel formation in Flk-1 (ref. 12) and Flt-1 (ref. 13) deficient embryos. However, because Flt-1 also binds placental growth factor[14,15], a VEGF homologue, the precise role of VEGF was unknown. Here we report that formation of blood vessels was abnormal, but not abolished, in heterozygous VEGF-deficient (*VEGF*$^{+/-}$) embryos, generated by aggregation of embryonic stem (ES) cells with tetraploid embryos (T-ES)[16,17], and even more impaired in homozygous VEGF-deficient (*VEGF*$^{-/-}$) T-ES embryos, resulting in death at mid-gestation. Similar phenotypes were observed in F_1-*VEGF*$^{+/-}$ embryos, generated by germline transmission. We believe that this heterozygous lethal phenotype, which differs from the homozygous lethality in VEGF-receptor-deficient embryos, is unprecedented for a targeted autosomal gene inactivation, and is indicative of a tight dose-dependent regulation of embryonic vessel development by VEGF.

Targeted inactivation of one (*VEGF*$^{+/-}$) or both (*VEGF*$^{-/-}$) alleles in ES cells was accomplished by replacement of the third common VEGF exon with the gene encoding neomycin phosphotransferase (neo), which caused a frameshift in the VEGF coding sequence[18] (unpublished observations), and deleted six of the eight essential cysteine residues[19,20] (Fig. 1*a*, *b*). Absence of exon 3 was confirmed by reverse transcription–polymerase chain reaction (RT–PCR) (Fig. 1*c*). Northern blot (Fig. 1*d*–*g*) and RT–PCR analysis (see Supplementary Information) revealed the presence of two aberrant VEGF transcripts at very low abundance (< 3% of wild type) in *VEGF*$^{-/-}$ embryos and ES cells, differentiated to cystic embryoid bodies (CEBs), which resulted from splicing into (mRNA-1) or around *neo* (mRNA-2) and contained a stop codon after 49 residues in *neo* (mRNA-1) or after 24 out-of-frame residues in *VEGF* exon 4 (mRNA-2) (Fig. 1*a* and see Supplementary Information). These mutant transcripts were similarly detected in *VEGF*$^{+/-}$ and in *riVEGF*$^{+/+}$ (containing a randomly integrated targeting vector) CEBs and embryos, and resulted from residual transcription of the randomly integrated targeting vector. Transient transfection of mRNA-2 (Fig. 1*a*) in COS cells under the control of the cytomegalovirus promoter did not reveal

(Carmeliet P et al. Nature 1996; 380:435-9)

Collen's Cessna Centurion with call sign OO-TPA

Picture presented to Désiré Collen by the collaborators of the Center for Molecular and Vascular Biology (KU Leuven) and the Center for Transgene Technology and Gene Therapy (VIB) at the occasion of this retirement from the University in 2008.

Collen's coat of arms
Motto: 'Frankness without pretence' ('rechttoe-rechtuit')

Summary: *Through Peter Carmeliet, Collen discovered the newest developments in cloning and manipulating genes. He smuggled transgenic mice from the US to Leuven and set up a specific pathogen-free facility for Peter Carmeliet. And, amidst all this, Collen became an enthusiastic pilot. He failed to become rector of KU Leuven but in hindsight, he concludes it was for the best.*

Carmeliet, a purebred researcher

At the end of the 1980s, Collen began to invite American scientists and top researchers to give lectures and seminars for the students in Leuven. This type of scientific exchange now takes place within the framework of the Francqui chairs, but that did not exist then. One of the first guests was Howard Goodman, a microbiologist from Harvard. In 1977, he had succeeded in cloning insulin, on which Genentech had continued to work. A young postdoc student, Peter Carmeliet (°1959), attended the Goodman presentation, and was one of the few who asked a question after the lecture. “Interesting lectures by foreign professors were few and far between in those days, and I had a good question,” Carmeliet recalls. Apparently, his query gained Collen’s attention and it so happened that afterwards while walking up the stairs beside each other, they started talking.

When Carmeliet suggested to Collen that maybe he could go to work with Goodman in Boston, Collen decided to give him a grant from the D. Collen Research Foundation for a postdoctoral study at Harvard Medical School. Carmeliet had just completed two years specializing in general internal medicine, but according to Collen he was a purebred researcher. “I notice such things fairly quickly,” says Collen.

The speed with which Collen took decisions had also surprised Mieke Dewerchin, a colleague of Carmeliet, when she had been hired a year earlier in 1988. Her job interview barely took 15 minutes. Collen wanted to know what her specialties were. “I told him I had worked at the Kortrijk Kulak Campus as a postdoc,” Dewerchin says, “and that I had acquired the technique to bind two proteins together through chemical reactions. I had worked with proteins involved in cancer treatment. Désiré was interested in doing something similar with proteins that could dissolve blood clots. Désiré listened and after fifteen minutes he said: you can start on Monday!” Mieke Dewerchin was even more surprised when barely a week later she was invited on a trip to the High Fens, a hilly region in Belgium. “It was March and it was snowing and Désiré had organized a bus. The lab team, Désiré included, went cross-country skiing!”

Carmeliet missed the cross-country skiing trip, as he joined Collen’s lab only a year later, when he received the grant for his postdoc year (1989-1990) with Goodman at Harvard. According to Collen, Carmeliet’s stay at Harvard didn’t meet expectations: “He got no publications out of his work there. At the end of his year in Boston, he drew my attention to Richard Mulligan, who was doing innovative work with gene targeting at the Whitehead Institute for Biological Research, a part of MIT in Massachusetts.” That seemed more interesting, and Carmeliet wondered if he could go there. A good idea, Collen thought, because gene technology was the future. So, from 1990 to 1991, Carmeliet did a second postdoc at the Whitehead Institute. It cost Collen about a million USD, because besides the

postdoc grant for Carmeliet, he also paid for operational facilities and the salaries of two lab technicians who were to assist Carmeliet with his project.

Carmeliet, however, does not consider his first year at Harvard as a wasted year. “At Goodman’s, I acquired the skills to handle RNA and DNA and learned how to clone from Steven Hyman. I learned the basics, in short, because here in Leuven little or no molecular biology was taught at the time. But I wasn’t really happy there and also felt that it would not lead to anything much. At the Whitehead Institute, on the other hand, very innovative things were going on. Professor Rudolf Jaenisch, one of the founders of the Whitehead institute, was a pioneer in transgenic science. What they were doing was more an art than a science.”

At the Whitehead institute, researchers worked with knockout mice, mice in which one gene is changed or deactivated. Although human DNA has been fully deciphered, it is not known what most genes do exactly. By deactivating a specific gene, scientists can discover what the precise function of the deactivated gene is.

Peter Carmeliet left with two lab technicians for Whitehead in Boston. “Those lab technicians were requested by Mulligan; he had argued that there had to be sufficient resources to guarantee me a productive stay in his lab. I still remember seeing him leaned back in his office chair, with his sports shoes on his desk when he proposed that deal.” Collen, a scientist, but also an entrepreneur, realized that valuable insights could be gained at Mulligan’s lab, and so Mulligan got his two extra lab technicians and a microscope on top of it! And when Carmeliet returned to Leuven a year later, colleague Mieke Dewerchin left for Whitehead, also with a grant from Collen, who wished to have a complete team to work with gene targeting in Leuven.

During his stay with Mulligan, Carmeliet succeeded in making three knockout mouse models, a t-PA mouse, a urokinase mouse and a PAI 1 mouse (PAI 1 = plasminogen activator inhibitor 1). In the PAI 1 mice, they induced a thrombosis and then checked whether the clot dissolved better in mice without the inhibitor. Carmeliet’s enthusiasm clearly appealed to Collen. He was pleasantly surprised when Carmeliet came to Belgium during the Christmas holidays and asked whether the heating could be turned on in the lab. During the Christmas holidays the heating was usually turned off because everyone collectively took time off, but Carmeliet wanted to keep working.

For Collen, it was essential to move quickly. He wanted to have knockout mice in Leuven as soon as possible, and went to get them himself. It would take too long via the official way, with too much paperwork. When he boarded the plane in Boston, he had four t-PA knockout mice in his hand luggage. “In my book bag, I had a Tupperware box with a few holes for air and some apples, so that the mice had some moisture. Scanned? Those men on the scanners were looking for metal objects; they were not thinking about mice. And Carmeliet kept waiting in the departure hall. If ever they should discover something, I would say: oh, I forgot, but there’s the man to whom these mice belong. That was our scenario.”

Creating a mice colony

The mice were not detected, and Collen passed security. He settled into business class, with the book bag under his seat. But upon his arrival in Leuven, a new problem arose. “I wanted to house these mice in the animal center, but of course I would have to follow the

regulations. The mice had to stay in quarantine and needed a health certificate, which I didn't have. So I installed the mice in our basement at home and let them breed until I had a colony." He had brought two males and two females to start with, and for four or five months he let them breed and then did tests in the lab. This went on for a while until Louisa found the stench in the basement unbearable and Collen was told to take his colony elsewhere. In the meantime, the official knockout mice had arrived from Boston, this time with a health certificate and entirely according to the rules.

When Peter Carmeliet had finished his postdoc at the Whitehead Institute, Collen saw to it that a pathogen-free mouse facility was rapidly built in Leuven. The Public Health authorities were having a new building put up at Gasthuisberg, and the plans for the Central Services building were already drawn up. When Collen learned that it would be possible to add an extra, ninth, floor on top, to accommodate his lab if he financed it, his D. Collen Research Foundation put 75 million francs on the table. For the construction of a specific pathogen-free division, another 150 million francs were needed, but he came 50 million francs short. Collen remembered then that Professor Marc Verstraete had some money stashed away. Marc Verstraete was a very frugal man who did not spend money unnecessarily. In 1986 he had organized a major international conference at the Heysel exhibition halls in Brussels. Everyone in the lab had to 'volunteer' to help with the preparations. People like Jef Arnout were unable to do anything else that year, although Collen himself was unable to cooperate. "My work at Genentech occupied all my time," he recalls. Professor Verstraete had a substantial sum left over from that conference which he had spent on his lab and on research, but Collen knew that there was still some money left. "I gathered up my courage and went to see my former mentor to try and persuade him to part with some of this money. I said: well now, Marc, we should have a plaque here that says 'Marc Verstraete Specific Pathogen-free Facility'. That won him over, and Verstraete paid 52 million francs for the animal center," says Collen with some glee.

The establishment of a mouse facility convinced Carmeliet to turn down attractive proposals from American universities and to stay in Leuven. Carmeliet himself worked out the plans for the mouse facility, but a specialist from the US was needed to assist the Leuven architects to build a state-of-the-art animal center with a perfect ventilation system. One infection could destroy the entire mouse colony, and that would be a financial and scientific drama. Marc Verstraete was acknowledged for his financial contribution, and the facility was indeed named after him as the '*Marc Verstraete Specific Pathogen-Free Facility'*. In 1992, Collen's lab moved to its new floor. The premises were inaugurated by Luc Van den Brande, then Flemish Minister-President. Collen: "We wanted to pioneer in gene technology. The very first knockouts in Belgium were made in Leuven. Peter made another eight or so knockouts here: a plasminogen knockout, an antiplasmin knockout, an antithrombin 3 knockout, a tissue factor knockout, a factor 7 knockout, a factor 10 knockout, and that is how his career got started." (1-7)

"First of all, we worked on knockouts that were useful for the t-PA research," says Carmeliet. "I remember that scientists were convinced that a mouse could not survive without t-PA protein. t-PA was clinically so important and had saved so many lives, that everyone believed that mice without the t-PA-gene would immediately have a thrombosis and die. That was almost a dogma. But the mice didn't die, and that caused quite a stir, it was big news. And even more followed, because it was also said about a related protein, u-PA (urokinase), the

little brother of t-PA, that without u-PA, survival was impossible. We started crossbreeding mice which had no t-PA or u-PA, and only then did they become seriously ill. But they still stayed alive! Our discovery turned that field of research upside down." (4)

The mouse project could count on Collen's full support and interest. When Carmeliet submitted a paper to *Nature,* he and Collen sat in an empty lab in Leuven on a holiday in May, staring impatiently at the fax machine. "A message was to come in that day to confirm whether our article was accepted," Carmeliet recalls. "It was a paper on the tissue factor knockout. Tissue factor is one of the most important elements in blood coagulation. It activates coagulation factor VII. We had established that inactivation of the Tissue Factor (TF) gene caused embryos to die because their blood vessels were underdeveloped. It had never before been demonstrated that TF played a role in the development of blood vessels. We had quite a bit of competition for this paper, so it was make or break. Outside it was a beautiful sunny day, but we stayed put at the fax machine, until suddenly the message rolled in, and the first words Collen could read were: *Dear Professor Collen, we are very sorry*… Collen gave a profound sigh of disappointment, until he saw the rest of the text: *we are very sorry… for the delay*. The article was accepted!" (5)

The formation of blood vessels

When a few years later Carmeliet concluded that the t-PA research was past its prime, he decided to focus on angiogenesis or blood vessel formation. He took his proposal to Collen and the reaction was: write down on half a page what it is exactly that you want to do. "I walked back to my office, typed out my plan on half an A4-size page, and took it to Désiré. He read it and said: that's okay, go ahead." Carmeliet had proposed to make knockouts for the *Vascular Endothelial Growth Factor*, VEGF for short. "Angiogenesis was a totally new research direction for our laboratory," Collen acknowledges, "but Carmeliet had now become an independent researcher."

Carmeliet suspected that this growth factor played an important role in the formation of the endothelial cells that constitute the inner walls of blood vessels. To determine the exact function of that gene, he tried to deactivate the gene on one of the two chromosomes in mice. But his attempts to breed mice with one VEGF gene turned out to be fruitless. In the experiments his test animals died in utero. There was nothing else to do but to remove the embryos from the pregnant mice and determine all their genotypes, to see whether the VEGF gene was properly deactivated. The gene was indeed deactivated, but the result was that the embryos had serious blood vessel deviations, which prevented them from developing further.

The conclusion was that for an embryo to survive, one VEGF gene was not enough. To get through the embryonic phase, two healthy VEGF genes were needed, one on each chromosome. This was a spectacular finding and provided new insight: no other gene is as important during the embryonic phase as the VEGF gene. "That resulted in a nice article in *Nature*, the most cited article ever for our lab at the time," Collen says proudly. (6)

At Genentech they had also been occupied with VEGF knockout mice, Collen knew. "One day in May 1995, I met Dr Simon, the head of the Research Department at Genentech who happened to be in Leuven. He told me they had the same problems with their VEGF knockout mice at Genentech: their mice also died in utero. And they had given up the project.

Ah well, I said, Carmeliet has discovered here that deactivating the VEGF gene is lethal in utero. The mouse embryos cannot form a vascular system with just one gene, they would die; I suspect Dr. Simon rang San Francisco the same day, because they started up their VEGF project again and came to the same conclusion. Carmeliet's article and that of Ferrara, who worked on VEGF at Genentech, were submitted to *Nature* a few weeks apart. The two articles were published back to back so that Carmeliet and Ferrara can both claim priority."

In the meantime, Carmeliet had become head of his own research group in the *Center for Transgene Technology and Gene Therapy*, a division of the Flemish Institute for Biotechnology at KU Leuven. "It felt a bit strange in the beginning," he recalls, "because I now was the boss of people who were much older than I. But Collen said: age, gender or degrees don't count here, only talent and excellence."

The research on blood vessel formation or angiogenesis, and the discovery of the VEGF protein that is so important for blood vessel growth, brought the center international fame. Later Carmeliet discovered that the *Placental Growth Factor* or PlGF, just like VEGF, is involved in the growth of blood vessels. And just like VEGF, PlGF also plays an important role in the formation of blood vessels in malignant tumor tissue. Inhibition of blood vessel formation with antibodies against VEGF is a strategy for fighting cancer. (7-10)

Genentech, meanwhile part of the Swiss pharmaceutical group Roche, launched Avastin, an antibody against VEGF, on the market in 2008. Avastin suppresses the production of new blood vessels and is used in cancer treatment. But while there can be significant side effects, such as thromboses, high blood pressure and hemorrhages with anti-VEGF treatments, that is less the case with anti-PlGF therapies.

New molecules

PlGF and anti-PlGF became two new molecules in the ThromboGenics product range. ThromboGenics sold the license for anti-PlGF or TB-403 to Roche for EUR 50 million in 2008. But Roche stopped the development after two years and returned its research results and material to ThromboGenics. At present, an anti-PlGF therapy is being tested by Oncurious, a spin-off of ThromboGenics and VIB, in patients with medulloblastoma, a highly dangerous brain tumor in children. Patients with diabetes show excessive blood vessel formation in their retinas, with progressive deterioration of their sight. A blood vessel inhibitor such as anti-PlGF could turn out to be a valid therapy**.** PlGF was included in the portfolio of CoBioRes, Collen's latest initiative (see below), as a possible therapy to repair the heart muscle after an acute myocardial infarction, but the research was discontinued at the end of 2019.

By the mid '90s, Collen was the director of two labs. TB-403 was a development of the VIB-KU Leuven lab of Peter Carmeliet, and the second lab, the Center for Molecular and Vascular Biology (CMVB) of KU Leuven, was directed by Roger Lijnen. There Jean-Marie Saint-Remy and Marc Jacquemin were working on TB-402, an antibody against coagulation factor VIII with a half-life in the blood of 20 days. (11) TB-402 produces a mild form of hemophilia, it doesn't cause hemorrhages, but also no thromboses, and would be an ideal drug for patients who risk deep vein thrombosis after a knee or hip operation. A deep vein thrombosis, a blood clot in a deep vein, usually in the leg, may dislodge and embolize to the

lungs. In knee and hip operations without anticoagulant treatment, the risk of deep vein thromboses can run up to 40 percent. “We did phase I studies in healthy test subjects,” says Collen, “and then phase II in knee prosthesis patients. And that study was very positive. But then we wanted to move too fast. ThromboGenics wanted a study in hip prosthesis patients, comparing our TB-402 with the champion that was then on the market, Bayer’s new antithrombotic agent. But instead of first administering a bit of heparin, and then a shot of TB-402 after 24 hours, like in the knee study, TB-402 was given a few hours after the operation.”

In Carmeliet’s lab transgenic mice were bred that produced less VEGF, and those mice showed a progressive form of motor neuron degeneration. Patients with ALS *(amyotrophic lateral sclerosis)* also produce less VEGF. A treatment with VEGF might help ALS patients. (13-14) That research was conducted together with NeuroNova, a pharmaceutical company in Stockholm, to whom the license was sold. In December 2012 NeuroNova was taken over by the Italian Newron Pharmaceuticals, which had other priorities and discontinued the clinical research. Carmeliet: “We can’t buy back the license, it would be difficult to find another partner for it now, because the patent has already been running for quite a while. So, this promising therapy will probably die a quiet death. Unfortunately.”

Concerning cancer therapy and angiogenesis, researchers are exploring new avenues from which Collen expects a great deal. The prevailing theory is that cancer cells need nutrients to grow, and therefore produce VEGF, creating more blood vessels that can supply the tumors with oxygen and sugars. The treatment then consists of starving the cancer cells by administering a VEGF inhibitor. “But the new blood vessels that are formed are not of the best quality. They leak, they are abnormal and irregular,” explains Carmeliet, “so that the sugars and oxygen barely reach the tumor. The hungry cancer cells seek a way via those blood vessels to other organs with better circulation. So, you get metastasis, spreading of cancer cells, and that is what 95 percent of cancer patients die of. We are now examining what the effect is if you were to support the formation of good quality blood vessels, so that the tumor has no reason to metastasize and will stay where it is. And also chemotherapy would reach the cancer cells much better via those first-rate blood vessels. It’s a different concept, but it’s not yet clinically validated.” (15)

The Center for Transgene Technology and Gene Therapy gradually became active in so many different areas that a new name was necessary. In 2008 the center was renamed the Vesalius Research Center, after a Leuven academic icon. “The old name no longer covered the reality,” says Carmeliet. “In the beginning the knockout technology was really new, and our group was, together with Désiré Collen, the first in Belgium and in Europe to excel in it, but in 2008 our research area had become much broader. The transgenic technology had become just a technique. And we had other interests: blood vessels and nerves. So, we thought Vesalius was an appropriate name. If you look at the anatomic illustrations in Vesalius’s books, you will see that already in the 16th century he had established the remarkable anatomical parallel between the vascular system and the nerve system. We now call that the neurovascular link. Moreover, Vesalius was a scientist from Leuven university, he was a freethinking pioneer, and he is known worldwide. He offered the ideal name for our center. By the way, it was in the vicinity of the Gasthuisberg, where our lab now stands, that Vesalius came to collect his research subjects from the gallows!”

Collen acquires wings

When asked about his hobbies, Collen would usually respond: gastronomy and oenology. But he also cherished a childhood dream. In the summer of 1992, his wife offered him a gastronomic cruise for his fiftieth birthday, albeit a year too early, but that year the Summer Olympic Games were in Barcelona, the final port of call. It was also not just a cruise, but a epicurean voyage on the Star Clipper, a luxury four-mast sailing ship. The trip went from Cadiz, via Gibraltar and an overnight stay on the party island Ibiza, to Barcelona for the opening of the Summer Games on 25 July. "At that time we were experimenting with the baboons in South Africa, and my coworker Stassen had to go there regularly for our research. Every time the ship docked at a port, I went in search of a telephone booth to find out how things were going in Bloemfontein. One day, while I was calling Stassen, there was a couple in the telephone booth next to me. The lady had heard the word t-PA a few times in my conversation. Ah, she said a bit later, I overheard you talking about t-PA? I was very surprised and answered: yes, do you know it? No, I don't, she said, but my husband does, and she calls her husband: look here, Guy, this is the t-PA man!"

The husband in question was shoe wholesaler Guy Bodson, who had had a heart attack during a trip to Hong Kong and was treated with rt-PA. The cardiologist had told him that the medicine he received was a Belgian invention. "Over the following days the Bodsons came to sit at our table, and at a certain point Mrs Bodson asked: Professor, what are your hobbies? Well, definitely not sports, so I said: gastronomy and oenology, and, well, I used to dream of becoming a pilot but that never materialized". "Well," Guy Bodson said, "you're talking to the right people: our son has a private plane at the airfield in Zwartberg in the province of Limburg, and he's coming to pick us up at Zaventem airport upon our return from Barcelona, so you can meet him!"

In Zaventem Collen was introduced to Bodson's son as the man who had saved his father's life and who wanted to fly. An appointment was made, and a week later Collen was in Zwartberg, some 70 km east of Leuven, to make a trip in Philippe Bodson's Piper. "We flew to Liège, and he showed me how to use the instrument landing system and gave me some further explanations, and we flew back. 'What do you think?' he asked. 'Well, I said, I find it fascinating.'" Collen met instructor Albert Hermans, took lessons, studied the 250 pages of flight instructions for visual flight, and took his theoretical and practical flying examination. Maurice Smets of Smets Aviation Service in Zwartberg then promised to look for an affordable plane.

Through his network, Smets discovered a bargain in Kalamazoo, Michigan: a second-hand Cessna 210 Centurion from 1982 for sale for USD 172,000. "My instructor Michel Notelaers, who was the president of the 'Limburgse Vleugels' flying club, and I took a Sabena flight to the US, with a connecting flight to Kalamazoo. We checked the Cessna, bought it and flew it to Syracuse." There they refueled, and in Bangor, Maine, had an extra fuel tank installed. The following day they flew to Goose Bay in Canada, the day afterwards to Keflavik airport about 60 km from Reykjavik, Iceland, and from there to Zwartberg in Belgium. Collen remembers very little of that crossing. "In the beginning you do have an adrenalin rush, but as the hours go by, you're just sitting there, the automatic pilot is on, and you can barely move in these small airplanes, so I dozed off."

Collen had caught the bug. He baptized his little plane OO-TPA. OO are the registration letters for Belgium, and t-PA the source of money that paid for the plane. His wife Louisa was soon a passenger. “Louisa came along when we went to eat mussels in Zeeland in the Netherlands. And she flew with me when I took part in the International Air Rally of Malta in 1997, together with Albert Degens and his wife. We finished first in the timed flight. We had to estimate how much time we would take for a trip from the flight beacon in Gozo to the landing in Malta. The cruising speed of the Cessna was 145 knots, but because there was a headwind, I had counted on 120 knots. That turned out to be correct, and so we won.”

Colleagues and other family members too could fly along every now and then. In an interview with newspaper *De Tijd* in 2015, Collen’s son Peter reminisced about flying with his father - especially on one occasion when there was severe weather over Zwartberg and Collen was advised to land at another airfield. But Collen would have none of it and stubbornly put the plane on the ground in Zwartberg. After that landing, father, son and the flight instructor had to take time to recover in the airport bar.

Altogether, Collen spent about 700 flight hours in his Cessna, but flying became a complicated activity because of new circumstances. When Landon Clay decided to invest USD 12.8 million in ThromboGenics Ltd, the successor of Thromb-X, one of his conditions was that a USD 10 million insurance policy, a key-man insurance, would be taken out for Collen as he was the key person in the enterprise. If something should happen to Collen, USD 10 million would be available to find someone of his caliber as a replacement. But to limit the premium to USD 100,000 per year, Collen was subsequently required to fly with a co-pilot. “Over time that was no longer workable. Whenever I had time to fly, I had to call Zwartberg to see whether someone was available. That way I got less than 15 flight hours in a year, and then you lose your reflexes. Flying is then really no longer responsible. I decided to get rid of the airplane and give it away.” Doctors without Borders thanked him for the gesture but declined; they didn’t have the infrastructure and the personnel for it. Virunga Park in Eastern Congo also declined the offer because the retractable landing gear was not usable in the jungle. Ultimately the Cessna was sold to a Brazilian for EUR 85,000, and Collen passed on part of the proceeds to Virunga Park, where they could buy a better suited small airplane with fixed landing gear to help track down poachers.

Farewell to the university

In 2008, Collen turned 65, and that meant mandatory retirement from the university. Peter Carmeliet took over the larger lab, the Vesalius Research Center of KUL/VIB, and Roger Lijnen took leadership of the Center for Molecular and Vascular Biology (CMVB). Carmeliet’s lab later came under Oncology, and the CMVB now falls under Cardiology. Collen had his hands full with ThromboGenics, and continued to attend lab meetings for a while, so he had no problem keeping himself occupied. He kept his office at Gasthuisberg, on the floor that he had co-financed and where LSRP (Life Sciences Research Partners, the former D. Collen Research Foundation) also rented space. In the meantime, Collen has given up his floor to the university, with the exception of three rooms that he may use until 2036. That is where he has installed his latest creation CoBioRes NV, a small company that acquired a number of research projects that were previously licensed out, but never fully developed.

For his 65th birthday, KU Leuven and the VIB organized a major symposium, '*Heart for the Future'*, in the Pieter De Somer auditorium in Leuven on 6 October 2008, with renowned international scientists. "Désiré didn't want to hear about it at first," says Jo Bury, General Director of the VIB. "'All that fuss', he said, because Désiré didn't like the spotlight." Peter Carmeliet, Rudy Dekeyser and Jo Bury then tried to persuade him during a dinner at Restaurant Arenberg, arguing that they had done the same in 1999 for Marc Van Montagu in Ghent when he was awarded his emeritus appointment. For Van Montagu, they had set up a farewell symposium with the theme 'Plants for the future', and they would do the same for Désiré under the heading 'Heart for the future'. During the first part, scientists determining the future of heart and vascular research would take the floor, and in the afternoon the t-PA story would be retold by the key figures in that research. So, the *fine fleur* of medical science and biotechnology would be on the program, such as the British-Honduran Professor Sir Salvador Moncada (°1944), husband of Belgian Princess Esmeralda. "One of our occasional associates here," says Roger Lijnen, referring to the regular visits Moncada paid to the lab in Leuven.

Another scheduled speaker was neurologist Kàri Stefànsson (°1949) from Reykjavik, the cofounder of deCODE Genetics. His company collected data on the Icelandic population and so identified genes that are involved in cardiovascular disorders, cancer and schizophrenia, among other things. The company and its database later became property of Chinese WuXi Pharma Tech. Other speakers were the Dutch geneticist Hans Clevers (°1975) from the University Medical Center in Utrecht, then director of the Hubrecht Institute for Developmental Biology and Stem Cell Research, the American cancer specialist Ronald A. DePinho (°1955) from the Dana Farber Cancer Institute in Boston, and the Polish-born Australian Marc Feldmann from Imperial College in London. When one of the speakers, Stefànsson, cancelled at the last minute, Peter Carmeliet stepped in with a presentation on the projects his lab was working on at that moment: anti-PlGF for cancer treatment, the role of oxygen sensing in tumor angiogenesis, and the role of VEGF in neurobiology and neuropathology.

Collen finally agreed and promised in return to organize a wine tasting for some colleagues and friends at his home the evening before the symposium. The tasting would be directed by his good friend and wine connoisseur Urbain Boutelegier and the French wine critic Michel Bettane (°1952), author of the *Guide Bettane et Desseauve des vins de France* and other books. About thirty people were invited: the scientists who had been decisive in the t-PA story and who were scheduled to speak the following day at the symposium, Collen's closest coworkers at ThromboGenics, and a number of friends. The French wine critic Bettane was baffled by the bottles that Collen conjured up from his wine cellar: rarely had he seen a wine enthusiast with so many consecutive vintages from the Château Haut-Bailly, Château Léoville Poyferré, Château Léoville-Las Cases, Château Pichon Comtesse de Lalande and Château Cos d'Estournel. All of them were *deuxièmes grands crus classés* which, according to connoisseur Boutelegier, are often better than the five recognized *premiers grand crus*. Jo Bury recalls that there were definitely a Château D'Yquem and a Cheval Blanc in the assortment too.

The symposium the next day received great acclaim. After the main program, coworkers came to share their part in the 't-PA story'. Roger Lijnen acted as the master of ceremonies. He and Diane De Wyngaert, Collen's secretary, had been busy for weeks trying to contact all

the former key figures. Some of them were quickly found, but were not immediately free, like Diane Pennica, who then decided to interrupt her trip to Australia to be there. “Everyone we had asked agreed,” says Lijnen. “Someone who definitely should have been there was Chip Gold, but he had then just died.” However, Dr Tsunehiro Yasuda, who had worked with Collen and Chip Gold in Boston, was present. David Stump arrived from Rockville, Maryland, in the United States, Osamu Matsuo flew over from Japan, Irene Juhan-Vague came from Marseille, Björn Wiman from Stockholm and Dick Rijken from the Netherlands. Furthermore, the main collaborators in Leuven, such as Fons Billiau, Frans Van de Werf and Marc Verstraete, were also among the speakers. Everyone recounted his specific contribution to the t-PA adventure, stories in which Collen’s determination, sense of urgency and encouragement were a common factor. The scientists praised Collen as a scientist with integrity and as a fast decision maker, under all circumstances. His mentor Marc Verstraete even concluded with the suggestion that they should just clone Collen. Dick Rijken recalled the consternation of the young Collen when they had reserved a room in a girls‘ dormitory for him on the Toronto campus on the basis of his first name. Collen had to pack his suitcases, which he regretted somewhat, but he promptly went into the city to seek other accommodations. His unique talent in writing scientific publications was also cited several times. And apparently Collen’s wine cellar was familiar to many. At the end, Jo Bury presented the professor emeritus with an enormous bottle of Château Léoville Poyferré 1999, a Médoc from the Saint-Julien vineyards.

In the evening, after the symposium had ended, there was yet another dinner for about 130 people in the Faculty Club, where scallops, quail mousse, and baby venison were on the menu, and where the wines included a Bordeaux Château Bel Air from 2007 and an Italian Tenuta de Angelis Rosso Piceno Superiore from 2005. Collen thought the food was fine, but the wines somewhat less. “They were disappointing, I forgot to taste the wine beforehand, a blunder on my part,” he says. Aside from the speakers at the symposium, he had also invited all his coworkers and friends. In the photo album that Roger Lijnen keeps of that occasion, one can spot among others European Labor law specialist Professor Roger Blanpain dining with Flemish culinary expert Herwig van Hove, or former rector Roger Dillemans and Patricia Ceysens, Economy Minister of the Flemish government at the time.

Along with the symposium, and also for Collen’s 65th birthday, an ‘Anthology of Scientific Collaborations*’*, a Festschrift, was issued, in which about 40 Belgian and international scientists wrote about their collaboration with Collen. The authors had publications with Collen with more than 100 citations. In the Festschrift they described how they came to work with Collen and what their scientific contribution had entailed, but all of them mentioned the stimulus that Collen had been for them, and they recounted memories of their time in his lab with great appreciation. Some of them wrote enjoyable anecdotes, for instance, about the way in which Collen helped to bridge cultural differences, as experienced by Japanese coworker Nobuo Nagai. Collen told him that when asked for his opinion, he should not beat around the bush Japanese-style, but answer with a clear Yes or No and stand his ground ’like a Sumo wrestler’. The not-to-be-underestimated importance of Louisa Collen was also noted by many a scientist who had been able to join the couple for dinner in Winksele throughout the years. They all regarded their collaboration with Collen as a decisive period in their careers.

Giving honor where it is due

Désiré Collen, a Sint-Truiden native of modest origins, became an outstanding scientist during his career. As one of the most-cited scientific authors in Belgium in the 1980s and 1990s, with around 650 peer-reviewed research papers and 170 review articles, Collen is not only very successful but has also been exceptionally productive as a researcher. At the beginning of the 1990s, he ranked 50th in the ISI Top 100 ranking for biomedical scientists and was the only one in the hematology subcategory. ISI stands for International Science Indexing. The organization annually measures the impact of scientific publications.

In the 1980s, Collen received a number of prizes and distinctions, including the most prestigious Belgian scientific recognition, the Francqui prize, in 1984. Two years later, he received the Louis Jeantet Prize for Medicine from the Fondation Louis Jeantet in Geneva. He gained his first *doctor honoris causa* title in 1988 from the Erasmus University in Rotterdam. He was very pleased with that first honorary doctorate: "It means that your work is appreciated. Rotterdam was participating in the rt-PA studies then, and when they offered me an honorary doctorate, I accepted at once." Then followed another honorary doctorate title from the VUB [Free University of Brussels] in 1994, and from the University of Notre Dame in Indiana, the second-largest Catholic university in the US, where he had given presentations a number of times in the middle of the t-PA period.

In 1999, he got an honorary doctorate from the University of the Mediterranean in Marseille, where Professor Irène Juhan-Vague was active. She had been in Leuven to work on her doctoral thesis in the beginning of the 1980s. "I was however never actively seeking honorary doctorates or titles," says Collen. He also downplays the fact that his name was repeatedly cited as a possible Nobel Prize winner. "I have been placed on the list several times, I know that. The first time by people from Genentech, and I was also recommended by Eugene Braunwald, the most important cardiologist in the US. He wrote me a letter then to ask whether I would agree if he put my name forward. But the Nobel Prize is like the Tour de France. There are 150 people who take part every year, but only one can win, and the difference between the first and the second is sometimes only a tyre width. The scientists listed for the Nobel Prize are often of a comparable level. I definitely would have accepted the prize, but I do not feel bypassed that it was not offered."

On the list of scientific prizes and distinctions, there are also the Prize for Medical Sciences presented every five years, awarded to Collen in 1990 by the Belgian Royal Academy of Medicine; the Bristol-Myers-Squibb Award for Cardiovascular Research, which he got together with Professor Verstraete in 1994 in New York; the Interbrew-Baillet Latour Health Prize; which he received together with Peter Carmeliet in 2005; the Harvard Leadership Prize in 2007; and the Insead Innovator Prize in 2009. In addition, he received a nomination for the European Inventor Award 2010, he obtained a Lifetime Achievement award from the European Patent Office, and, at the end of 2010, he was one of the nominees of the economic weekly *Trends* for the title of Manager of the Year. In 2013 he became an honorary citizen of his native town Sint-Truiden. That title he owed childhood friends with whom he played behind the Sint-Jacobs church in the Sint-Truiden area of Schurhoven, he says. That same year he received the Lifetime Achievement Award from the Belgian-American Chamber of Commerce in the Harvard Club in New York.

Would he have liked the office of rector? “I tried that in 1995, but that really was a mistake on my part. In hindsight, it’s good that it didn’t work out. I wanted to make the Leuven university more research-oriented. I was spending quite a bit of time at Harvard then and saw how they worked with more efficiency and professionalism. In the meantime, things have improved here as well, but I thought then that Leuven needed more focus on creative research.” To prepare himself for the election debates at the time, he got support from a limited group of colleagues. “I had a small council: Professor Mark Waer, who later became rector himself. Professor Roger Blanpain, and Professor Herman De Dijn. That was my advisory team. I invited them for dinner at home, I opened a few bottles of wine, and then we discussed and prepared my campaign.”

But his plans were not well received by the students. In the student magazine VETO of 16 January 1995, his lack of proposals for the social sector in his policy paper was criticized, and his popularity declined. When, in a debate leading up to the election, he mentioned that in Leuven there were definitely 7,000 students whose level was not high enough for university studies, his support further dwindled. KU Leuven wanted to be everyone’s university; it wanted to be democratic, but Collen’s position was that you cannot conduct excellent research in such a context. Top institutions like Harvard are not democratic, according to Collen. And at the campus in Kortrijk there was shocked reaction when he let it be known that this West Flemish satellite campus should be abolished as soon as possible. Decentralization made no sense, he said. It was a waste of scarce resources for research. His colleagues from the humanities found his idea to make the University Hospital the economic engine for the university a very one-sided vision. In short, the rector elections did not work out for Collen. Campaigning with popular slogans and empty promises to please everyone was not his style. It was engineer André Oosterlinck who won the rector elections in 1995. Collen stayed true to his ‘elitist’ views, even when he stepped on toes in his home province of Limburg. On 6 October 2008 he said in the local paper *Het Belang van Limburg*: “I am not saying that Limburg should not have a university. I am only saying that Limburgers should not study at a Limburg university.”

Collen’s motto is *‘Frankness without pretence’* (*Rechttoe Rechtuit*). That is on his coat of arms. Since July 2012 he has been a member of the hereditary nobility with the personal title of baron. “Jacques van Ypersele, the principal private secretary of King Albert, had me called one day: would I like to come by his office in the palace in Brussels? I had no idea why he would want to see me. When I arrived, he apparently had carefully gone through my entire curriculum vitae; he was better informed about everything I had done and everywhere I had been than I could remember myself. And then he literally pronounced the standard sentence: ’t has pleased His Majesty to grant you the title of baron; will you accept it?’ The Palace obviously does not want to run the risk of awarding it to someone who would then turn it down. ‘Well, what would I have to do for it?’, I asked. ‘Nothing, just accept,’ was the answer. ‘And what are my duties?’ ‘None, just be yourself.’ ‘And the rights?’ ‘Also none.’ I said, OK then.” He had a coat of arms designed, with a heart like that on playing cards, and with a letter T for thrombolysis. On top is a crown with seven pearls, a helmet and the rod of Asclepius, and he took it to the registrar of nobility. The design was made by an artist, and an accompanying text had to be drawn up in Dutch and French. All of that did turn out to cost some money. But there were no further obligations. Collen paid the EUR 750 registration fees and the costs for designing the diploma with the coat of arms. He keeps it at home, and

that's it. He now belongs to the *noblesse de mérite*. "Oh, I'm quite happy with it," he admits. "It means that outside my professional environment other people too are acknowledging: here is a man who has done something worthwhile."

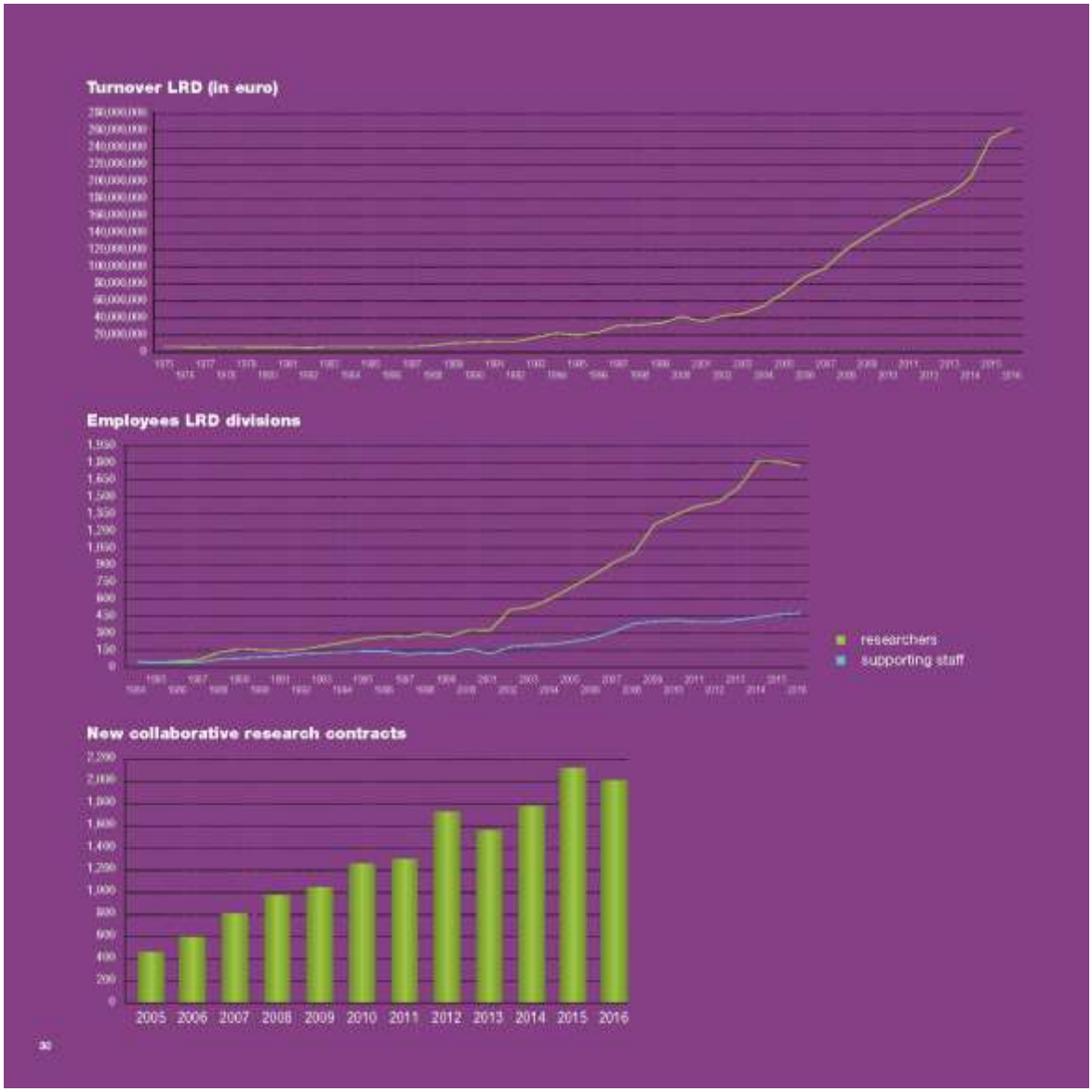
PART III: THE ENTERPRENEURIAL UNIVERSITY

REUTERS

Europe's Most Innovative Universities - 2017

The most innovative university in Europe, for the second year running, is Belgium's KU Leuven. This nearly 600-year-old institution was founded by Pope Martin V, but today it's better known for technology than theology: KU Leuven maintains one of the largest independent research and development organizations on the planet. In fiscal 2015, the university's research spending exceeded EUR 454 million, and its patent portfolio currently includes 586 active families, each one representing an invention protected in multiple countries.

Chapter 1: Our new supercharger



KU LRD: turnover, employees and research contracts
(From: KU LRD annual report 2016)

Summary: *The life and work of Désiré Collen reflect developments at the KU Leuven and at other universities over the past fifty years. This has been a period that undoubtedly saw spectacular advances in university research and development, and the valorization of academic work. Academic knowledge has been structurally transformed into economic activity, financial value and quality of life. Universities have become superchargers for the economy. With some luck and with hard work they can become an inexhaustible source of progress and wealth.*

Royalties as a bone of contention

Activase, the brand name under which rt-PA was brought onto the market by the American pharmaceutical company Genentech, was more than a drug. The large amounts in royalties that came Leuven's way from 1988 to 2006 turned Activase into a veritable bone of contention. The total amount involved was nearly EUR 145 million, or roughly 5.8 billion Belgian francs (figures were calculated in this currency until 2002). This amount did land well in the end, but not without some tribulations. Who was entitled to the royalties? Désiré Collen himself as co-inventor most probably was. But title to the patent was held by the KU Leuven, the invention's birthplace. The question was whether Leuven, with its many divisions and interested parties, was ready for commercial income streams, upon the traditional State subsidies. And, of course, the taxman was also lying in wait.

A university is an institution with a tremendous amount of knowledge, but it does not always boast the same amount of wisdom. Where money matters are concerned, things can get out of hand among academics just as much as elsewhere. In Leuven, Désiré Collen became a 'man one hates to love', particularly when it became known that he was the owner of a private plane. The envy and gossip got out of hand, to the point where, in 2009, Collen felt it necessary to explain in a book, and down to the last detail, what had happened with all the money from Genentech, and especially how well Leuven's university and the Flemish biotechnology sector had fared thanks to that manna from heaven. "Some people were claiming I had bought a business jet! That's why I included a photo of my little plane in my book," says Collen with a laugh. Many of the critics then backed off. The figures did not lie: t-PA most certainly did stimulate the bloodstream in Leuven and Flanders, and undeniably so when Collen's business child ThromboGenics was floated on the Brussels stock exchange in June 2006, and a lot of know-how and money that had ended up in Ireland and elsewhere came back to Belgium. Collen received the recognition he deserved, and, on 16 July 2012, King Albert II conferred upon him the title of baron. "In my life I have been fortunate enough to always have had far more friends than enemies," concludes Collen. "I have also always tried to ensure that each and every one is accorded his or her place in the sun."

De Somer leaves his mark

What happens when a university invents something valuable? In the 1970s this was something that had not yet been explored in Flanders. Who is the owner in this case: the inventor concerned or the university? To whom should accrue the income stemming from the commercial development of an academic idea, such as a very effective medicine or a unique material?

This academic ingenuity was for a long time (centuries in the case of Leuven) situated in a grey area. In the wake of the left-wing protest movement of May 1968, this lack of clarity was no longer accepted by society. Some even disputed whether a university could produce

anything useful ‘beyond the independent and disinterested quest for knowledge’, let alone anything lucrative by means of cooperation with the private sector. Did universities indeed have to fertilize the economy? Surely it was the universities’ vocation to transfer knowledge in their ivory towers, far from the grubby world, irrespective of the economic usefulness of that knowledge? Profit was below academic dignity! And if a profit were to be made, that ought to accrue to the government and the taxpayer, since it is that government that finances the Belgian universities.

In the turbulent 1960s and 1970s, the debate in Leuven focused on the admittedly vague *and* lucrative activities of the university’s senior officer, Professor Pieter De Somer, rector of the KU Leuven, which had become a Flemish university in 1968. That year the French-speakers had been given a ’Leuven’ university of their own, in the shape of Louvain-la-Neuve, near Leuven, just over the nearby Belgian linguistic border. After Leuven’s ‘split’, the student movement that had initially been nationalistic, came to be dominated by the extreme left wing for a few years. De Somer, the first ‘Flemish’ rector and a brilliant academic and businessman, rowed or tacked against that radical current until his death in 1985. He was a prominent professor of microbiology and immunology and laid the foundations in Leuven and Flanders of what would become the ‘entrepreneurial university’.

In *The City on the Hill* the voluminous book about the KU Leuven published by two historians in 2005, Pieter De Somer (1917-1985) is described as ‘an outstanding strategist and diplomat and an enthusiastic art lover’. In Brussels circles he was called ‘*le Florentin de Louvain’* on account of his imposing manner that evoked the *grandezza* of the Medicis.(1) As a scientist he was a pioneer in the development of penicillin that had been discovered by the Briton Alexander Fleming in 1928. This was the first antibiotic. An extract from *The City on the Hill* runs as follows: ”Reports on the use of penicillin in the allied war camp prompted De Somer to take a step further from 1944, as a researcher with the Francqui fund. Spurred on in this endeavor by the young industrialist Jacques Lannoye and with the latter’s financial help, he began to isolate penicillin in a modest pharmaceutical laboratory in Genval (near Leuven) together with his friend Christian de Duve (the later Nobel Prize winner). In 1946-47 he spent a period in Canada to learn industrial extraction methods and thereby to increase the proceeds of his work.” Together with Jacques Lannoye, De Somer developed the RIT (*Recherche et Industrie Therapeutiques)* in Genval, which he also ran. In this little company new antibiotics and antiviral vaccines were produced. In 1950 the modest lab was replaced by a modern factory. In 1954 his RIT laid the foundation for the Rega Institute (at that time still the Rega Foundation), which was housed in the Minderbroedersstraat in Leuven until 2017. For many years he paid the salaries of the academic staff. The Rega Institute in fact acted as the research center of the company in Genval.

De Somer was also behind the research center for virology. By way of a test case he decided to set up a production line for the polio vaccine Salk. Jonas Edward Salk (1914-1995) was an American doctor and inventor of the Salk vaccine that was named after him. This was one of the first successful attempts to vaccinate against a virus, and more specifically the poliomyelitis virus. After discovering this vaccine, he did not apply for a patent but gave it, as he himself said, to the people. The vaccine resulted in the near eradication of the dreaded disease of polio, an outcome that goes down as one of the great success stories in the history of medicine. That success was also apparent in Belgium, which in 1958 was one of

the first countries to meet its own vaccine requirement and to organize a major vaccination campaign.

When in 1968 De Somer became the first rector of the Dutch-speaking KU Leuven and the first lay rector, it was already apparent what he thought about universities: a university was not just an educational establishment but also an institute for scientific research. He combined the view of a business manager with that of an academic. In his mind it had to be possible for industry and science to go perfectly together, even if it was not always a smooth ride. So it was that De Somer "did not always give university colleagues credit for their scientific and commercial successes", according to the authors of *The City on the Hill*. Désiré Collen was briefly to experience that. But the Dutch-speaking KU Leuven was indeed "custom-made to De Somer's requirements", as the then-deputy rector Karel Tavernier said at Pieter De Somer's funeral ceremony in 1985.

Erik De Clercq tames HIV

As a 'genuine interdisciplinary research institute', the Rega Institute does not just still exist, but deservedly refers to itself as a 'jewel in the KU Leuven crown'. As *Division Rega*, the Rega Institute currently comes entirely under KU Leuven Research & Development (KU LRD), together with some seventy other divisions. One of the Rega Institute's stand-out successes was the discovery in the 1990s of tenofovir, an antiviral phosphonate (a compound of phosphonic acid) that was incorporated by the American pharmaceutical company Gilead in particular drugs to combat HIV *(human immunodeficiency virus*) and even Hepatitis B. From the 1970s, and thus while the Cold War was still raging, KU Leuven Professor Erik De Clercq worked from the Rega Institute for this purpose in conjunction with the Czech Professor Antonin Holy (1936-2012) and the American John C. Martin (1952) who was CEO of Gilead from 1996 till 2016. (2)
Tenofovir and the related adefovir are extremely clever medicines when it comes to combating continuously mutating viruses. The HIV virus, and the erosion of the immune system that is described as AIDS *(acquired immune deficiency syndrome*), is deadly. In the West it initially mainly affected homosexual men, and worldwide some 35 million people are estimated to have fallen victim. Twenty years after the discovery of the virus the disease is under control in the majority of infected patients, and in the case of many of them the presence of HIV in the blood is no longer even measurable. In medical circles Gilead's Truvada and Atripla drugs have been cheered as '*a miracle drug, yes: the philosopher's stone'*.

This partly Leuven-based and Belgian story does not stop here. Complera and Eviplera have been available as anti-HIV drugs since 2011. They combine tenofovir with rilpirivine, a substance that had previously been discovered by Dr. Paul Janssen, who died in 2003, and his Janssen Pharmaceutica that has been part of the American group Johnson&Johnson since 1961. The medicine was further developed by the Mechelen company Tibotec, a subsidiary of Johnson&Johnson. Overseeing this process was Rudy Pauwels, a student of Erik De Clercq.

Rudy Pauwels later set up the diagnostics company Biocartis that has been financed via the stock exchange since 2015. And even then the story continues. The Leuven Professor Erwin Blomsma incorporated tenofovir into a veterinary medicinal product that ended up in the

American company Aratana Therapeutics. And although tenofovir can be termed a genuine success story, it is just one of the many Leuven stories that, as far as visibility is concerned, have for varying reasons remained under the bushel. (3)

“Erik De Clercq no doubt earned more with his anti-HIV patents than I did with t-PA,” concludes Collen. “But the Rega Institute didn’t talk about it and the public at large didn’t know that, whereas I have never had secrets. The Rega Institute has just received an exceptionally well-equipped new lab in a high-security new construction on the Gasthuisberg campus, where from now on the most dangerous bacteria and viruses can be studied. Thanks to Erik De Clercq and his staff, they also have the money for that. And now they are using it to very good effect. The outside world can be told.” The tenofivir patent however ran out in May 2017 causing a drop in KU LRD income of more than EUR 40 million.

De Somer’s disciples

The Rega Institute’s successes may not all have been the work of Pieter De Somer, but he was the trailblazer and visionary, although his ways were not exactly orthodox. The combination of academic, rector *and* businessman became indefensible for De Somer in the 1970s. In 1977 he dissociated his Rega Institute as a non-profit institution at the KU Leuven from the original private financier RIT. The KU Leuven had since set up LRD (Leuven Research & Development) as a non-profit association under De Somer in 1972, four years ahead of its time, because it was only in April 1976 that all Belgian universities were authorized by the government to “acquire participating interests in third parties in the framework of an annual agreement”; third parties such as LRD.
The aim was to engage in technology transfer in a transparent fashion. LRD would “contribute to research in both humanities & social sciences and exact sciences, without pursuing any material profit”. To this end “inventions” had to be protected and research valorized, so that the income received could be used to finance further research work. The first chairman was the chief executive of Kredietbank, the banker Fernand Collin Sr, and of course Rector Pieter De Somer also had a seat on the board of directors. If LRD’s patents on “inventions” led to commercial products, LRD would have to determine who was entitled to what percentage of the royalties – no easy task. Day-to-day management of LRD fell to Guido Declercq, then general director of the university, and Jos Bouckaert, a man with experience in national science policy. In the 1970s, the first research park in Belgium was developed, in part by LRD, in Haasrode, near Leuven.

In 1973, LRD established its first two divisions: in one, Engineering Professor Roger Van Overstraeten (1937-1999) brought in discoveries in micro-electronics, while in the second, the metallurgy and materials engineers were given their own division within LRD. Van Overstraeten would subsequently put Leuven on the world map in the field of micro-electronics with the research institute Imec. In the faculties of medicine, Désiré Collen was the first to apply for a patent via LRD, in September 1975. On account of their historical merit, the square where Leuven’s new city hall was built was named after Professor Van Overstraeten, and the former central Field Marshal Foch Square was renamed Rector De Somer Square.

LRD (now KU LRD) has been run by the Ghent engineer Koen Debackere since 1998. “Of course, De Somer was very important as a rector,” Debackere affirms. “But he was not alone. LRD was set up in 1972 as a non-profit association at the initiative of a number of engineers: André Deruyttere, Roger Van Overstraeten, and later Jef Roos. Each and every one of them conducted research and at the same time as engineers worked in conjunction with the business world. The philosophy behind it sounded something like this: doctors have their hospital and we have our factories, to put it simply. With the hospital, doctors have a structure in which they can do their own thing. We engineers don’t have that, so we need an interface that supports us in our interactions with the industry world, as a hospital supports doctors in their patient care. Therefore, LRD came into being as the engineers’ hospital.”

Pleasures and burdens

Today LRD is no longer a non-profit association but a department – let’s say a business unit – of the KU Leuven. R&D’s contribution to the university increased spectacularly. The engineers’ initiative and the step De Somer took with the Rega Institute had a self-reinforcing effect, but it was not until 1985 that the fog around a lot of the academic private initiatives really lifted. Debackere: “There have always been a lot of non-profit associations orbiting around the university, and in terms of numbers that remains the case today – there are currently 140 in Leuven. But in the 1970s and 1980s, those non-profit associations were sometimes improperly deployed, garnering the pleasures whilst the university was left with the burdens. Some non-profit associations were using the university’s lab infrastructure for their own developments.”

“When the government gradually found that these practices *inter alia* at its State University of Ghent had gone far enough, this also had consequences for Leuven,” says Debackere. “In that context LRD was in fact discontinued as a non-profit association, and in 1985 was renamed KU LRD and incorporated into the university. But obviously a number of activities were already ongoing there at that point.” Collen’s patent with Genentech was in the LRD non-profit association, and a number of contracts were also in progress including some with materials science. “It was not evident for the university to take over those contracts, since it’s always difficult to transfer a patent with licenses to another legal entity. In such cases the licensee often makes an issue of it and that was the case with Genentech. There was a change of control and that could lead to disputes,” explains Debackere. “That was why Guido Declercq, who was general director of the university at the time, had the non-profit association LRD continued as a dormant vehicle. For that matter the non-profit association still exists under the name Leuven Innovatie vzw, although today it is an empty box. Up until 2005-2006 it contained contracts that were running out, such as Collen’s t-PA contract. In the case of those current 140 non-profit associations I would like to briefly mention that the government itself sometimes asks for certain activities to be set up as a non-profit association; the Flemish Institute for Biotechnology is a non-profit association. Like Imec, Flanders Make and the spearhead clusters are non-profit associations, as are the Alma canteens. However, the use made of these non-profit associations is different from what it was in the 1970s.”

Leuven flirts with Silicon Valley

The turbulent leftist era around 1968 did not lead to a political revolution but did bring about a flourishing symbiosis between the KU Leuven and the business world. For those nostalgic for the rebellious days of May 1968, this most probably left a sour taste, but for Leuven and Flanders it was a blessing. Debackere: “That was the time (1968-1972) when André Vlerick was Belgian Secretary of State for Economic Affairs. Vlerick had a lot of contacts in the United States and had also played a coordinating role in the post-war Marshall Plan. He saw that in the United States concepts such as *’science parks’* and ‘*tech transfer’* were all the rage. In Norway, the University of Trondheim was involved in Sintef, today one of the major independent research organizations in the Scandinavian countries.”

Accompanied by the rectors of Belgium’s universities, Vlerick travelled to the Boston area, North Carolina, and to Stanford University with what were at that time the first building blocks of Silicon Valley. “And Vlerick convinced the Belgian government to give all Belgian universities a piece of land on which they could start up research parks. This was 1976, recounts Debackere. At that time only three universities jumped at Vlerick’s proposal: Leuven with LRD, Liège with Sart-Tilman and the UCL in Louvain-la-Neuve. Ghent now has a flourishing activity in Zwijnaarde, but during my time as a student in Ghent in the early 1980s, Zwijnaarde still had to be developed as a science park. The ULB also initially did not do much with Vlerick’s proposal. Ghent only set up genuinely professionalized tech transfer in the 1990s. It long remained limited to a kind of interface of de facto one person close to the rectorship.”

Leuven was already a precursor with LRD and did not waste the opportunity Vlerick was offering. “We were fortunate enough to have had Pieter De Somer here,” says Debackere. “He knew how you had to valorize research. That was met with criticism at the time, but he knew what was at stake and made sure that Leuven seized the opportunities it was being given. This produced a degree of uncontrolled proliferation in the wake of the universities, and, in 1985, all universities were forced by the government to streamline the non-core activities. In that clean-up operation LRD was absorbed into KU LRD, a division of the university.”

Debackere stresses that Professor De Somer’s importance cannot be overestimated. “Another example: he sent all Leuven’s ‘high potentials’, the best students who had the potential to become professors, to the United States; to Stanford (as was the case with Roger Van Overstraeten) or to Berkeley (Willy Sansen is an example). That was where they picked it up! Roger Van Overstraeten went to Gaston Geens with his Imec plan, because he knew what was happening in ‘the Valley’ (now Silicon Valley). It is also on this basis that Silvar-Lisco came into being in 1981 as a collaborative partnership between Leuven and Silicon Valley. Lisco was in actual fact LRD’s first genuine spin-off. Silvar-Lisco stood for Silicon Valley Research Association – Leuven Industrial Software Company. This combination of Silvar and Lisco designed the first three-dimensional software that made CAD (Computer Aided Design) possible. This is used in the design of cars, airplanes and prostheses. So CAD is a child born of Leuven and Silicon Valley,” says Debackere, his passion for the subject evident.

And there’s more to inspire pride. “If you walk around Berkeley where the semiconductor industry originated, you’ll see that the third chip on display in the Hall of Fame was designed

by Willy Sansen from Leuven. That was the third milestone in the history of semiconductors. I don't need to tell you about the boom that industry has experienced! To cite another example, a good year ago, one of our spin-offs, Cartegenia, developed a software package to make prognoses about certain hereditary diseases or abnormalities, based on genetic information. That was a breakthrough of such proportions that Agilent Technologies, Hewlett-Packard's medical spin-off, bought the company! Herman Verrelst, Cartagenia's co-founder, became head of Agilent's medical diagnostics division and is currently head of Biocartis, a successful growth company set up by former KU Leuven student Rudi Pauwels. In a nutshell, 3D-CAD, the first semiconductors and the latest biomedical and genetic diagnostic techniques are three examples of developments in which KU LRD companies have been among the world's elite."

Leuven learns to set boundaries

Value was indeed created on a large scale in the periphery of LRD and the university – not just by Désiré Collen and sometimes on the fringe of the Leuven knowledge biotope. LMS, Leuven Measurement and Systems, was founded by engineer Urbain Vandeurzen in 1980 and taken over by the German company Siemens for EUR 640 million (for the most part added value) in 2012. Krypton and Metris, which have since been acquired by the Japanese company Nikon, are also Leuven stories for which the university provided specialist knowledge. There is nothing wrong with that, as long as the company does not use the university's facilities. But sometimes things do miscarry, as evidenced by the technology company Option and of course Collen's unfortunate brainchild ThromboGenics. The Leuven beer company AB Inbev is so large that it has outgrown any local rank, but new yeasts and flavors are being produced in its Leuven lab, Gitec (*Global Innovation and Technology Center*).

ICOS is a story with a very significant university dimension. Image Computer Systems (ICOS) was established in 1982 to develop business applications for an algorithm on which future rector André Oosterlinck took his doctor's degree. The intention was to identify chromosomes by means of automated image analysis. This research was conducted in cooperation with the Center for Human Genetics, which was then run by Professor Herman Vanden Berghe (1933-2017). ICOS started out as a 50/50 collaborative partnership between LRD and entrepreneur/engineer Paul Devrée. In 1984 the new director Jos Verjans implemented a change of direction at ICOS. Exit identification of chromosomes; ICOS Vision Systems became a specialist in inspection systems for micro-electronics. Things really gathered momentum from 1988 onwards. Several capital transactions generated capital gains, and that was very much the case when ICOS was listed on the US Nasdaq stock exchange in 1997. The share price rose from an initial bracket of USD 12 to 14, itself representing sizeable latent capital gains, to USD 32 in 2000. Some insiders hit the jackpot in those years, but that was hardly the case for LRD; on the contrary, it was actually at LRD's expense.

Désiré Collen was the only LRD director to oppose this course of events, but he was to pay for his opposition to certain dubious transactions. On 20 June 2001, Collen's Protein Research Division and the joint subsidiary Thromb-X were led to move from LRD to the Désiré Collen Research Foundation that had been set up in 1988, and Collen had to step

down from the LRD board. Collen had not anticipated this *concilium abeundi*, the measure under which recalcitrant students in Leuven are dismissed from the university, particularly as he had only pressed for good governance and respect for the university's proprietary rights, but at the same time he was very happy with this newly acquired freedom!

Collen's protest did, however, have a lasting effect, and good governance was introduced. In its decrees the Flemish government clarified that the rights to inventions belonged unambiguously to the universities, but that by the same token the inventors should be fairly recognized and remunerated. This meant LRD got a grip on the twilight zone between the university and the cash till. Things happened in those days that can no longer happen today. Whilst there are no clear rules, compliance cannot be enforced, so some insiders say. However, nobody wants to reopen these old wounds, and that goes for Désiré Collen as well. The most important thing is that what was permitted then is no longer possible now. For Collen, everything turned out well in the end. In a *living-apart-together* (LAT) relationship with the KU Leuven, he was able to go his own way from that point on, and that suited Collen's personality.

For ICOS things continued to go superbly well. ICOS Vision Systems was taken over by the American company KLA-Tencor in 2008 for USD 316.9 million or EUR 36.50 per share (quoted in EUR because ICOS Vision Systems had also been listed on Euronext Brussels since 2003). Between 1997 and 2003, ICOS floundered due to the severe technology crisis of 2001 and 2002. Results slumped from a profit of 22.4 million in 2000 to a loss of 8.7 million in 2001. Turnover took a 75% nosedive! The share price fell from USD 44 in 2000 to USD 4 in 2003, i.e. to less than half of the introduction price on the Nasdaq in 1997. Then in 2003, ICOS Vision applied for a second listing on Euronext Brussels. The initial price was EUR 6.80. The principal shareholders along with many members of staff later benefitted from the almost miraculous resurrection of their company. In 2008 ICOS Vision was taken over by the Americans for a figure almost eight times higher than the lowest prices recorded in 2003. Things can get incredibly rough in the technology sector! This success story left quite a few of the parties involved several million EUR better off, but the KU Leuven and LRD missed out. After this less edifying episode, LRD learned to say No. A repeat of a comparable financial bonanza is no longer possible today without an equivalent return for the university.

KU LRD as a business unit

"When I started out at LRD at the beginning of the 1990s," relates Koen Debackere, head of the current KU LRD, "we were rather peripheral. At that time LRD accounted for EUR 18 to 20 million – not much more than a footnote. Its successor KU LRD then experienced solid growth, to such an extent that under rector Mark Waer (rector in 2009-2013), the idea matured to give LRD the place in the university's organic framework to which it could lay claim in light of its size. KU LRD was accorded the same status in KU Leuven's organigram as the university hospitals. And that brings us back to the beginning. Just as the hospital is the business unit (I can't use that word too often) dealing with patients, so KU LRD handles the development of ideas." *(The interview with professor Debackere took place between February and April 2018. Starting September 2020 Koen Debackere became president of the Belgian bank Kredietbank, but he remains as director of KU LRD.)*

The university's various business units were then given a board and management of their own. "You could say that the KU Leuven's board of directors is today our general meeting, to which we report as managers and directors," explains Debackere. "For that matter external independent experts also sit on our KU LRD board, as is fitting in a proper board of directors. This is where the business unit's strategy is mapped out, whereupon that strategy is prepared to be implemented by the managers. All valorization of research at the university belongs in principle to KU LRD, except those that are carried out in the framework of the external non-profit association VIB (the Flemish Institute for Biotechnology)."

This arrangement led to considerable tension in the late 1990s. VIB was perceived as an 'amputation' of the KU Leuven. "That has since been absorbed and the university's other activities have experienced strong growth," says Debackere, "with the result that the VIB is no longer viewed as problematic. For example, Peter Carmeliet's angiogenesis research was in VIB. What's more, VIB is responsible for the valorization but KU Leuven remains co-owner. Only 10% of the income goes to VIB, 10% to the university involved, 40% to the university department involved and 40% to the VIB group in the university that generated the income. Anyway, the bulk of our medical research remains with KU LRD. The Rega Institute with Professor Erik De Clercq, the Leuven Cancer Institute and the diabetes research – it's all KU LRD."

The university's funds

The financial results of a successful entrepreneurial university are not to be found in the newspapers, and the KU Leuven does not divulge them. They have, however, not been secret since 1992, but if no-one asks, nothing needs to be said on the matter. *To live happily, live under the radar!* The fact of the matter is that the KU Leuven and its research groups are wealthy and 'wealth whispers'; the wealthy do not shout, and Christians avoid the sin of pride.

Koen Debackere: "At the KU Leuven we distinguish four flows of funds. For 2016 we are talking of a total operating income of around EUR 1 billion. The first flow of funds is the public basic funding for education and research. The second flow of funds is everything to do with basic research. This includes the special research funds and the FWO (Fund for Scientific Research) –i.e. genuine 'curiosity-driven' research. The third flow of funds is what we nowadays call application-oriented research. This flow of funds is also government-driven.

"From a European perspective the Flemish government is an absolute forerunner in this. It is something we do well in Flanders!" says Debackere with satisfaction. "The state reforms (the devolution granting more autonomy to the regional governments) were a blessing in this field. Look at the establishment of the IWT (Instituut voor Innovatie door Wetenschap en Technologie - Institute for innovation through Science and Technology), today the VLAIO, (Vlaams agentschap voor Innovatie en Onderneming - the Flemish Agency for Innovation and Enterprise). The period under Minister-President Luc Van den Brande, from 1992 to 1999, was a very creative and forward-looking period. This was when Flanders developed structures not only for basic research, but also for innovation and application-oriented research, which were really unequalled in Europe at the time. These were primarily activities that have been centralized with the current VLAIO that became the universities' third flow of funds, which was thus directed at applications, often with a major involvement on the part of the business world. Activities such as cooperation via Imec come under the third flow of

funds, but European valorization programs are also included and there are often private companies involved here as well. For Leuven it accounts for 13% of our operating income, i.e. around EUR 130 million per year.”

What Désiré Collen triggered….

Leuven's affluence

EUR million		**2019**	**2018**	**2016**	**2010**	**2009**	% Growth
BALANCE SHEET KU Leuven (incl. Association)							*in 10 years*
Balance sheet total		2 230,0	2 085,0	1 932,0	1 234,0	1 125,0	*98,2*
Capital and reserves		1 625,0	1 549,0	1 406,0	1 004,0	945,0	*72,0*
Fixed assets		647,0	609,0	592,0	435,0	405,0	*59,8*
Current assets		1 583,0	1 476,0	1 339,0	799,0	720,0	*119,9*
of which cash investments		1 439,0	1 341,0	1 192,0	672,0	615,0	*134,0*
INCOME STATEMENT KU Leuven							
Operating charges		1 017,0	953,0	955,0	665,0	658,0	*54,6*
Operating income		1 098,0	1 003,0	1 001,0	701,0	681,0	*61,2*
of which							
1st flow of funds		428,0	412,0	379,0	279,0	278,0	*54,0*
2nd flow of funds		156,0	131,0	124,0	106,0	112,0	*39,3*
3rd flow of funds		170,0	146,0	136,0	100,0	94,0	*80,9*
of which KU Leuven RD (around 50%)		85,0	73,0	68,0	50,0	47,0	*80,9*
4th flow of funds (KU Leuven RD)		161,0	136,0	196,0	101,0	93,0	*73,1*
of which contract research		75,0	73,3	68,0	46,0	38,8	*93,3*
of which valorization research		79,4	56,7	123,0	53,0	46,0	*72,6*
Belgian GDP (EUR billion-source NBB)		*473,1*	*459,5*	*430,2*	*363,1*	*346,5*	*36,5*

This balance sheet and income statement cannot be compared to the annual report of a private company. If income exceeds expenses there is obviously a 'profit', but this is an optical illusion because there is no discretionary scope: the profit for financial reporting purposes is already allocated to investments and research. The marked growth in some parameters has to do with the integration of the colleges (hogescholen), but mainly with the dynamic character of Leuven's research groups. The first flow of funds amounts to only 39% and the rest is in fact obtained competitively.

The UZ KU Leuven hospital is not included in these figures. It can be assumed that the turnover there is roughly of the same order, meaning that Leuven permanently stands for a financial management mandate of more than EUR 2 billion.

In 2017 the revenues of KU LRD from valorization were significantly lower than in 2016 due to the expiration of the tenofovir patent on 17 May 2017 and the lower license fees from Gilead Sciences for TAF, a derivative of tenofovir. Anyway, in the ten years starting in 2009 the research income of KU Leuven grew twice as much as the Belgian GDP. That growth was triggered many years ago by Désiré Collen and other pioneers.

Debackere: “The fourth flow of funds is pure market money flow, i.e. developments and applications in cooperation with the business world without any government subsidy, although the government may be a client. Sometimes a government agency places an order for a study and we then take part as one of the parties carrying out the assignment. In those cases, we are then in competition with other private or public companies. We have often carried out the sound measurements around the airport in Zaventem. Our acoustic lab then takes those measurements on the instructions of Brussels Airport. Researchers once conducted a study for the SMAK museum in Ghent on museum visits and on the degree to which the displays were within the grasp of the various target groups. This fourth flow of funds represents 19.6%, i.e. EUR 196 million, and is entirely within the current KU LRD. But so is part of the third flow. For example, activities with the Strategic Research Center iMinds, which merged with Imec, fall under KU LRD because they relate to research projects that are very close to applications and have a very clear valorization objective. If you count that part of the third flow of funds in the fourth flow of funds, you arrive at EUR 265 million in operating income for KU LRD. That’s 26% of the university’s income! This is reported on: there is an annual report that goes to the KU Leuven’s board, together with a budget. Since Van den Brande in the late 1990s, the Flemish government has given the universities around EUR three million a year to professionalize those valorization activities. Therefore, we also report every year to the Flemish department of education on the status of our patent portfolio, the amount of industrial income we are recording, and on how our spin-offs are faring, etc. This reporting then serves as a criterion for the apportionment of public research funds. So, there is total transparency and that has been the case since 2004-2005. Anyone who wants to follow this can do so.” KU LRD is, however, currently facing the difficult task of absorbing the expiration of the tenofovir patent.

KU LRD now cashes a ‘Collen’ every year

With EUR 196 million, KU LRD in 2016 obtained considerably more from the market than Désiré Collen did with his t-PA patent in the nearly 20 years that it was protected. The amount that KU LRD receives from valorization of research has almost doubled in the last five years *(up to 2016)* and now represents 80% of the private income of all Flemish universities added together. This gradually takes on biblical proportions although a significant part resulted from tenofovir, the patent of which expired in 2017. It will take several years to absorb this loss.

That performance is the result of critical mass and exceptional management coupled with the laws of compound interest. KU LRD indeed acts as a cross-generation capitalizing fund that does not pay out dividends but ploughs funds into new, judiciously selected research programs. In the five years up to 2016 this has resulted in a return, an internal growth, of almost 14% per annum. The challenge now is to find a new gold mine like tenofovir.

Leuven is successful in this because it has spent the past 25 years pragmatically examining what works and what doesn’t, and by moulding what does work into flexible structures. KU LRD operates centrally in respect of what works best centrally, with its staff now totalling 90, and gives its divisions considerable autonomy. In 2000 there were 32 divisions, now there are 76. The divisions do not correspond to the faculties, which continue to coordinate ‘noncommittal’ basic research. They are often collaborative partnerships between faculties. The divisions begin where research becomes application-oriented, where contracts must be established, inventions must be patented, and where flows of funds have to be apportioned. These divisions manage a large part of the income they generate via their ISLs (*industry-*

science links). They can save this income and spend it when they deem it advisable. The divisions can also acquire participating interests, of both an intellectual and financial nature, in the spin-off companies stemming from their research.
And, finally, the individual researchers also receive payment by results. There are three kinds of incentives for them. For contract research and consulting, the researchers involved are paid a supplementary wage. As far as royalties are concerned, the researchers receive a degressively determined percentage, after deduction of all costs. And as regards spin-offs, the researchers can subscribe to 40% of what are known as the IP shares, the shares corresponding to intellectual property. They can also acquire a financial stake in the new company.

KU LRD is thereby rolling out the red carpet for all sections of the university towards application-oriented research and towards the establishment of new enterprises and the recognition that goes with that. This way the KU Leuven obviously also encourages basic (albeit still noncommittal) research in the faculties and their often-numerous departments. For that basic research there is an attractive goal, a carrot on a stick. A university thus offers the best of both worlds: in addition to the permanent appointment that goes with academic professions (and the very decent pension) there is the prospect of entrepreneurial success. For their patent protection and business plans, new start-ups can count on KU LRD's expert support. The university thus becomes a kind of incubator or breeder of economic activity. KU LRD's website includes the names of 97 spin-offs, and between four and nine new ones are added every year. It's a goldmine, that is inexhaustible if well managed.

This magic spell works best, for obvious reasons, in the case of exact sciences. Of the 76 KU LRD divisions, 46 come from science and technology, 21 from biomedical sciences and nine from humanities and social sciences. Humanities and social sciences lag somewhat behind, but are probably pleased that their university has an industrial money-maker at its disposal.

Tech transfer is mainstream

Economically speaking, the entrepreneurial university and scientific knowledge really are genuine turbo chargers. Vis-à-vis the university's harvest there is private gain to the tune of a much larger amount. Economists can calculate a multiplier of public-sector expenditure for scientific research. Commissioned by the five Flemish universities, the Scottish consulting firm BIGGAR Economics calculated at the end of 2017 that the EUR 1.5 billion that the Flemish government pays the universities yields almost EUR 10 billion for the economy. (4) The League of European Research Universities, in which the KU Leuven is the only Belgian establishment represented, also asked BIGGAR to calculate the added value of the 23 universities that are members of the organization. For every euro of input there is an output of five euros, the study revealed. Standing at EUR 400 billion, the added value of the entrepreneurial universities in Europe constitutes an amount almost as large as the Belgian national product. (5) Together with labor and capital, knowledge has become a major production factor. The 'triple helix' of government, universities and industry that was devised in the 1990s has proven to be a terrific model. In what was at the time referred to as the 'European Paradox' (6), it was established that Europe had far fewer ISLs (industry-science links) than the United States. The question is whether that was ever true in Leuven's case. Leuven's TTO (technological transfer organization) was an inspiration for all the inter-university Flemish institutions (Imec, VIB and VITO) at that time, and subsequently for many other universities. Since then, thinking has turned into doing everywhere. The academic word has turned into industrial flesh.

Debackere acknowledges this. “Yes, the entire tech transfer business has gone from peripheral to mainstream – not just mainstream within this university, but mainstream in innovation policy at Flemish and European level. This has taken us into a much more complex, much more hybrid environment. Pioneers of the non-profit association LRD sometimes still have a hard time with it. In the past we negotiated with a company, came to an agreement and were able to get going. Now you’re in a European consortium of universities and you make agreements with various companies that work together. Those are difficult negotiations involving decisions as to who gets what return, if ever there is a return, of course. Some years ago people talked of the ‘triple helix’, comprising the academic world, the government and the business world. Today we refer to the quadruple helix or even the multiple helix. The fourth party is civil society, for example patients’ associations. In the USA we are seeing that these organizations often take the lead in legitimizing certain operations. So the landscape is complex and it is becoming more complex still. At Leuven university innovation and valorization have become a structural component. For example, in the doctoral schools for the preparation of our numerous doctorates, KU LRD provides a training module that poses the question: what development model could there be for my doctorate? So you see that KU LRD is currently already embedded in our educational activities,” concludes Debackere.

As one of the university’s business units, KU LRD is also involved in regional policy. Anyone driving along the Boudewijnlaan in Leuven goes past the building that houses the bio-incubator, a public limited company with twenty or so mini start-ups. This is the work of KU LRD, VIB, the former Désiré Collen Foundation (now LSRP) and of Aveve, the Farmers’ Union. “We don’t just come up with ideas that are susceptible for development, we are also involved in the infrastructure needed to make something happen with them,” explains Debackere. “One of the latest achievements is CD3, the Center for Drug Design and Discovery, which is a platform specialized in toxicity tests, preclinical trials and so forth, and on which KU LRD works in conjunction with the European Investment Fund. The investment involved runs to more than EUR 60 million and there are some thirty projects in the pipeline.”

Leuven is unique in Europe

When outsiders look at this Leuven story their eyes pop. Could it be that others do even better or is Leuven exceptional? The news agency Thomson Reuters provided the answer in May 2017. In a comprehensive survey based on ten criteria, in particular the number of publications and number of patents, Leuven came out on top among the 100 European educational and research institutions included in the study – ahead even of the Imperial College in London and the University of Cambridge! Brussels’ ULB was 38st, the University of Ghent 19th, the VUB 48th, the UC Louvain 52th and Liège 80th. The oldest Catholic university in the world is still very much alive and kicking almost 600 years after it was founded, and despite the split that occurred in 1968 – or maybe thanks to that split.

With KU Leuven being for the third time the undisputed leader in the ranking of European ‘innovative universities’ and with the Université Catholique de Louvain, UC Louvain, as a strong runner-up (going from place 61 to 52) one can state that 50 years later, the ‘split’ of the Leuven university into a Dutch and French speaking entity, was a blessing. To quote the well-known French speaking philosopher and economist Philippe Van Parijs, professor at the UCL: “To expel us from Leuven was one of the wisest decisions in the history of the country.

Had this difficult decision not been made, the province of Brabant Wallon, where Louvain-la-Neuve is located, would not have joined Vlaams-Brabant, where Leuven is located, as one of Belgium's two most prosperous provinces and the only thriving one in Wallonia. Moreover, after a difficult tense period, the universities of Leuven and Louvain survived the split quite happily. They are today the largest universities on their respective sides of the linguistic border. Thanks to what happened in 1968 they also collaborate far more smoothly than they ever did before and – certainly – than they would have done had Louvain managed to keep imposing its presence in Leuven." (See Brussels Times, April 20, 2018)

Debackere: "I would venture to say that by size we are unique in Europe with KU LRD. Some have followed similar paths, such as the Imperial College, a research university in London which according to my estimates has half a billion pounds under management, but there are few organizations that have brought everything under one roof as we have. The cooperation with industry, the patent portfolio and the spin-offs are all controlled by a single entity. Elsewhere they have various 'offices' for this, whilst spin-offs are entrusted to an external party. We have centralized that into a single body and in that respect we are unique. And if you are successful you have funds available to re-invest and chalk up fresh successes, until you reach the kind of scale we now have. And we do that with a current staff of 90 people."

The managing director of KU Leuven Research and Development is proud of the growth and development of Imec and the growth and success of its spin-off portfolio. "Imec also has no equal elsewhere in the world," says Debackere. "Imec provides a fine example of gap filling at global level: the linking of knowledge and the business world. And we are still a shareholder today in 49 of our more than 120 spin-off companies. With EUR 10 million in seed funds from KU LRD and 20 million from the Gemma Frisius Fund (also in Leuven), a spectacular investment lever has been set up and EUR 874 million in Flemish and foreign venture capital has been raised. "Those are all companies that lead to quality employment for thousands of employees," says Debackere. "And in turn they create value and growth. That's when you're really doing something for the region. The result of valorization is impact – not only economic impact but also social impact. That's why we do it. And that has to be done from a separate business unit like KU LRD, since it obviously eludes traditional academic decision making."

Leuven has only recently started actively launching out about its merits. The entrepreneurial university's support among public opinion is finally deliberately being cultivated. A new variety of apple, a prosthesis in 3D printing or a startling discovery in our DNA are given media coverage. Training tens of thousands of young people remains the main task for universities, but enabling a few dozen students to develop and grow to the point of joining the world elite in their field is just as important. University research should become what youth training is in the Premier League. That was also what Collen had in mind in 1995, when he unsuccessfully applied for the post of rector. He didn't get the job, but his program has been implemented.

"It's not something we bellow about from the rooftops," says an unassuming Debackere. "We don't brag and boast, but just keep working hard, since tomorrow things might be different. Nothing is ever taken for granted. For that matter we have very good relations with the tech transfer activities at the other Belgian universities. We have also developed and transferred a lot of know-how with VIB and the other institutes. And we also maintain a good relationship

on the Walloon side. I don't see any real problems there at all. And we'll keep things that way."

Debackere does concede that occasionally tension or friction arises that can even be fiery. "That's a slice of KU LRD's history. You know what... one of the things I remember from my nuclear physics course is that you have to strive for a '*workable cross section'*. The cross section is the probability of a certain interaction taking place between particles. We bring the different components together and create the conditions that offer the greatest chance of success. From that perspective we look at our initiatives and a law from the world of physics helps to position a university."

And fortunately that was also done at the time with Désiré Collen. "He has been a pioneer in everything he has done and he's gone all the way. He discovered a molecule, made a medicinal product from it, made sure its ownership was protected, used the proceeds from it to set up a new company, ThromboGenics, and now, with Fund^{+}, he is financing start-up companies. And never once has he lost sight of the absolute essence of it all: excellent, uncompromising and well and truly ground-breaking science. He deserves a statue, as much as anything because he's also a monolithic man."

Chapter 2: The visionary Van den Brande

Opening and dedication by Minister-President Luc Van den Brande of the new laboratories of the Center for Transgene Technology and Gene Therapy (VIB) and the Center for Molecular and Vascular Biology (KU Leuven) on 19 May 1993.

Director committee of the VIB (Flanders Institute for Biotechnology) in 2008.
Standing (left to right): Rudy Beyaert, Lode Wyns, Rudy Dekeyser, Jo Bury, Joël Vandekerckhove, Bart De Strooper
Seated (left to right): Désiré Collen, Marijke Lein, Johan Tevelein, Wim Goemaere, Dirk Inzé

Director committee of the VIB (Flanders Institute for Biotechnology) in 2018.
From left to right, from top to bottom: Rik Audenaert, Cédric Verschooten, Dieter Deforce, Bruno Lambrecht (on 28/08/2017 replaced by Eric Sleeckx), Anne De Paepe, Jean-Pierre Timmermans, Danielle Raspoet (till 18/12/17), Hilde Windels, Dirk Inzé, Gino Baron, Liliane Schoofs (on 15/12/2017 replaced by Gerard Govers), Bart De Moor, Jo Bury, Staf Van Reet, Wim Robberecht (on 18/12/2017 replaced by Chris Van Geet), Ajit Shetty, André Roef, Luc Moens, Johan Cardoen.
Not on the picture: Leen Limbourg (since 06/07/2018)

Summary: *Policy and politicians are important, often decisive. As President of the Flemish Executive at the time, Gaston Geens was the founder of Imec, the Flemish Research Center for Micro-electronics and Digital Technologies. Today Imec is a respected worldwide player in its field. In the early 1990s, Geens' successor Flemish Minister-President Luc Van den Brande launched the VITO, the Flemish Institute for Technological Research, and the VIB, the Flemish Institute for Biotechnology, two new instruments with which the government actively supports the innovation process. Désiré Collen's cooperation with LRD and its successor KU LRD was not always rainbow and butterflies, but thanks to VIB, Collen's energy and knowledge yielded good returns for Flanders. Jo Bury, CEO of VIB, was a privileged witness.*

On a study trip to the USA

Désiré Collen was one of the pioneers of the Flemish Institute for Biotechnology (VIB), an institute that made Flanders the envy of many other regions. VIB regularly appeared in the headlines whenever there were protests against plant improvements and particularly against the genetic modification of plants. In 2014, to turn the tide, VIB chief executive Jo Bury joined forces with Wim Grunewald to write an accessible and balanced book entitled '*The GMO Revolution'*. But VIB's adversaries were often more effective in securing media attention than VIB itself. Fortunately, however, politicians both in their executive and legislative context always understood the value of VIB for Flanders. In 2017 the annual government grant for the following five years was increased by EUR 15 million to EUR 59 million per year. And respect for science is also on the rise in the media.

Collen was already a successful researcher and businessman before VIB came into being. In 1987 the American FDA approved his t-PA as a thrombolytic drug, and in 1991, with the proceeds from his initial success, Collen already had set up his public limited company Thromb-X, which would later become ThromboGenics in Ireland and in Belgium. Collen became a role model. Around 1992 the idea started to take shape that Flanders needed to value and valorize its assets in life sciences to better effect, and that the work of Collen and of three other leading scientists had to be given continuity. Jo Bury ran VIB from the outset, up until 2012 together with Rudy Dekeyser. *(The interview with Jo Bury took place between February and April 2018)*

"The first person we should mention in our history is Luc Van den Brande, who was Minister-President of the Flemish Government from 1992 to 1999," says Jo Bury. "It was in this period that the idea matured. Gaston Geens, his predecessor, had launched *Flanders Technology*, but we still lacked the institutions to keep technological innovation on an adequate level in the long term." Van den Brande had reserved the Economic Affairs and Innovation portfolio for himself, while responsibility for the Budget lay with Wivina De Meester; both were on the same wavelength.

In November 1993, Van den Brande undertook a study trip to the United States, where he visited the giants in education: Harvard and MIT in Massachusetts, and Stanford in California as well as an institution in Canada. Professor Marc Van Montagu (1933), the Ghent plant geneticist, accompanied him to Stanford. On the return flight, the Leuven deputy rector Herman Vanden Berghe, then head of the Center for Human Genetics, took the opportunity to lobby, and pointed out to the Minister-President that government support for Flemish genetic research was peanuts. It was less than what Mechelen's theatre company, the

Miniature Theatre, received! Being from the small town Mechelen, that hit home with Van den Brande, who had hitherto been convinced that the existing Flemish action program for biotechnology, VLAB, adequately met the needs.

“The VLAB had been started up in 1990,” says Bury. “As a consultant of the IWT, I was director of the VLAB program. The IWT was the Agency for Innovation through Science and Technology and is now called VLAIO, the Flemish Institute for Innovation and Enterprise. Both Vanden Berghe and Collen received some support from us. But these were indeed marginal amounts, 4 or 5% of what a lab incurs in costs every year. With these limited resources we could never have a real impact. Van den Brande realized that Flanders had to start investing much more and more selectively, and to do so immediately. The available funds had to be stepped up, but above all they had to be allocated more selectively. Anyone receiving funds had to be prepared to be tested, and, if necessary, to lose that funding. This sounds self-evident, but it took years before the academic world accepted this and even came to regard it as normal.”

The Bayh and Dole Act

The opposition to supervision and monitoring did not only arise from the universities’ attachment to an almost unlimited academic freedom. There had always been private initiatives in the universities’ orbit, often in the form of non-profit associations. They welcomed subsidies and the use of the universities’ infrastructure but were not keen on having someone looking over their shoulder. LRD was built on this field of tension. In practice, most of the disputes concerned intellectual property rights. If an invention had come about with the university’s and the government’s help, to whom did it belong?

“This new approach came our way from the Anglo-Saxon world, too,” says Bury. “In 1980 the US House of Representatives approved the Bayh-Dole Act, named after senators Birch Bayh and Bob Dole. A Patents Act had already been in existence, but in the USA there had likewise not been any effective regulation governing the intellectual property of inventions that are realized with the aid of public subsidies. This Bayh-Dole Act inspired Europe and Belgium, and today this area is properly regulated. But in Flanders in 1993 we were nowhere. As it is now, the universities are the intellectual owners and can sell licenses to that property. Of inventions done in the VIB labs, VIB and the partner university are co-owners. In any publications by VIB’s labs, mention always must be made of the university and VIB. As to the proceeds from the licences, agreements are made with the inventors and the partner university involved. The rule is that 80% of the income goes to the department of the university where the intellectual property originated. The subsidizing governments are thus respected, and the universities and inventors remain motivated. We are a non-profit association, which means we have to reinvest our profit. That is best done in the department that has been successful. KU LRD in Leuven already had a lot of experience in this before we started, but VIB has since successfully taken care of this for the 540 patent applications and 18 spin-offs that it can boast, now in 2017.”

On that trans-Atlantic flight, Van den Brande realized that biotechnology needed the same kind of measures as those set in place for micro-electronics with Imec. He didn’t hesitate. “Somewhere around the end of 1993 Van den Brande called Christine Claus, director-general of the IWT,” recounts Bury. “Mrs. Claus sent me and my colleague Rudy Dekeyser to go and see Van den Brande, who announced he wanted to do more than the existing action

program. He wanted to develop top-level science in Flanders and to make targeted investments, but he insisted that, through innovation, this knowledge had to lead to employment, useful products and added value for society. We started with a blank sheet and drafted a short document with a few main points; we looked at tech transfer in the USA, at what was important and what was secondary, and so on. A couple of weeks later we went back to Van den Brande with our draft, and he promptly decided that this was what we were going to do! We still had to convince him to cast the net wider than plant genetics, beyond Marc Van Montagu's field of interest. We insisted he also include biomedical sciences. After spirited discussions between April 1994 and July 1995, the concept of an 'inter-university research institute' emerged, built on basic concepts such as excellence, selectivity, critical mass and focus on innovations and applications. There would be sufficient funds for the period between 1996 and 2000, whereupon five-yearly assessment reviews would take place."

Luc Van den Brande in the weekly *Knack*, 29 June 1994: "I am yet to hear about the slightest benefit of our funds being spread across a loose network of research groups working in dispersed battle array. I didn't want to open a new one-stop counter where anyone could just come along and collect funds with which he could do as he pleases. I wanted an extra dimension, among other things to attract the attention of companies and investors. Concentrating people and resources in a critical mass for the knowledge potential is essential, that way we become more visible in Europe and the rest of the world. Every group, including the four aces we are starting with, will have to submit a five-year project that will be assessed by a scientific board. If it turns out that the project does not produce return, it will be dropped."

Manna from heaven

The line of policy pursued by Van den Brande, with the emphasis on critical mass, the pooling of knowledge and resources, and cooperation between the universities, became even more important when politicians started to decentralize the universities at the end of the 1990s and gave every province its university. Later polytechnics were also 'academicized', with lecturers being accorded the status of affiliated researcher or associate professor. The number of professors rose dramatically, but for a world-class team you need elite players; it's the same in science as it is in football. Collen had never understood this trend of dishing out as many diplomas as possible and having 500 doctorates a year, with few of these graduates moving on into trade and industry. One of the columns he wrote on this subject for the weekly*Trends* on 4 November 2010 was jokingly entitled *'Let's all be professors!'* "Making the scarce resources available for non-university education as well, can only lead to downward levelling," wrote Collen, a view he still maintains.

However that may be, with Imec in micro-electronics, VITO in technology and VIB in bio-engineering, Flanders did take three visionary decisions in the 1990s. And Désiré Collen played an active role in the young VIB. "At that time, we had four leading scientists in Flanders who were head and shoulders above the rest," says Jo Bury. "They were the Ghent plant geneticist Marc Van Montagu and the molecular biologist Walter Fiers (1931-2019), the heredity specialist Herman Vanden Berghe from Leuven and of course Désiré Collen. We kept our four stars informed from the outset. We wanted to be sure of their willingness to participate. I remember seeing Herman Vanden Berghe first. Right at the beginning there

was also a meeting with Christine Claus, Herman Vanden Berghe, Van den Brande's deputy principal private secretary and ourselves, i.e. Rudy Dekeyser and me. I distinctly recall something that Herman said: 'This is manna from heaven!' We had clearly defined our project: it had to deal with genetic engineering, molecular biology. No clinic, no physiology, but genuine biotechnology. And the universities' effort had to be on a par with what we contributed as the subsidizing institution. A few days later we talked to Désiré Collen, and also to Walter Fiers and Marc Van Montagu."

Although the Professors were all in their fifties – at 52 Désiré was the youngest – VIB saw the possibility of high-level continuation to what they had started. After all, the alternative for Flanders would be to structurally fall behind in life sciences. Flanders would never be able to keep pace internationally if it didn't do something about its academic parcelling. VIB became the springboard for a subsequent generation of brilliant people at world level.

"So, it all began with those four, but we then extended it to nine," says Jo Bury. "The four centers were supplemented by five associated centers, meaning we had nine programs: three VIB centers in Ghent, three in Leuven, one in Antwerp and two in Brussels. During the first review, a University of Brussels department bit the dust and a new department was selected on the basis of a wide-ranging call. The following government without Van den Brande considered for a moment granting the VIB subsidies directly to the universities again, but by then our results were too impressive. You know, it wasn't until 2002, seven years later, that the two rectors of Leuven and Ghent, André Oosterlinck and André De Leenheer, conceded at our general meeting that our results would never have been achieved if the funds had gone directly to the universities. At the time Désiré Collen was spokesman for the scientific directors on the executive committee. He represented them on the board of directors. After some hesitation he became a champion of the VIB model. We now have 75 research groups, and the sole selection criterion is quality!" In 1995 Leuven saw the establishment of the Center for Transgene Technology and Gene Therapy, or VIB.3, which was run by Désiré Collen. With sponsorship from his own Foundation as well, Collen brought the promising Peter Carmeliet to Flanders. After Collen's retirement, Carmeliet took over the management of that center, which was later renamed the Vesalius Research Center.

In the mid-1990s, however, Leuven was in fact not in need of VIB. Leuven had its Rega Institute, then still the Rega Foundation, which had not even applied for recognition from the VLAB and looked down on the new initiative. But when VIB was set up, Rega did want a share of the money. As a compromise, rector Dillemans suggested that Vanden Berghe and Collen transfer part of their VIB subsidies to Rega. That plan was abandoned at the last minute, when Rega insisted on having this confirmed by means of a bank guarantee – something VIB would most probably never have accepted. Bury says that things have now been straightened out, and today various Rega projects are supported by VIB.

Leuven didn't need us

"Leuven didn't need us to know how everything had to happen," says Jo Bury. "But we coughed up a great deal of money. We started with 920 million francs, that's EUR 22 million, a year for a period of five years, so by no means an inconsiderable sum. But we still needed to work on our credibility. Don't forget that Rudy and I were civil servants who had to keep a tight rein on professors. We put together an executive committee, with Collen, Fiers, Van Montagu, Vanden Berghe, the Antwerp Alzheimer specialist Christine Van Broeckhoven, the

Brussels biologist Raymond Hamers, the Ghent biochemist Joël Vandekerckhove, the Brussels microbiologist Nicolas Glansdorff and the Leuven development biologist Danny Huylebroeck. We thus left the most important decisions to them, whilst Rudy and I would oversee their implementation. We were well aware that we only had five years in which to make good. If we didn't deliver by then, it was over. So, we devised a system whereby we sifted the wheat from the chaff every five years and only retained the very best. We widened our professional know-how in the field of technology transfer with everything that goes with it: intellectual property, licenses and start-ups. And look at where we are now, twenty years on!"

"But it wasn't easy," concedes Bury. "Sometimes we also turned the tap off. Marc Van Montagu's department promptly went 30 million francs – EUR 750,000 – into the red in the first year. That couldn't be allowed. When we gave programs a no, it wasn't accepted without further ado, but with the new generation of scientists things became easier in that respect."

Collen surprisingly did not devote the VIB funds to heart and vascular diseases, but to genetics. "Actually, it was connected with heart and vascular diseases," he says. "It had to do with the genetics of the heart and blood vessels. We had previously worked in biochemistry and physiology, but I realized that genetic engineering and gene therapy was the future. The lab was also renamed the Center for Transgene Technology and Gene Therapy. It had previously been Thrombosis and Vascular Research."

"Désiré seized the opportunity that VIB offered him," adds Bury. "By way of a new path he brought in Carmeliet's angiogenesis project, which had already been in the former VLAB. Désiré left the specialty with which he was more familiar than anyone and, with VIB's support and the royalties from Genentech, set up a development that otherwise would simply not have existed here. He was able to do it and had the resources and authority to do it. He had certainly done well with his thrombolytic, but he also gave a lot back to the university. The first specific pathogen free *animalium* in Leuven's Gasthuisberg cost Désiré a ton of money, but he also contractually saw to it that he could make use of it."

The VIB actually put into practice, at inter-university level, the dream of the entrepreneurial university with which Collen had tried to become rector of the KU Leuven in 1995 when VIB was established. Désiré Collen: "Thanks in large measure to VIB, a structural bridge has finally been built in Flanders between academic research in life sciences and the business world. The promising inventions by VIB researchers are now spotted in time and are made available to society by means of a well-considered strategy. Look at Devgen which was taken over by Syngenta, look at CropDesign which was taken over by BASF, look at Ablynx which was taken over by Sanofi Pharmaceuticals, and so on. Congratulations to the politicians who have made this possible and continue to do so."

The most important cooperation between VIB and Collen had to do with PIGF and anti-PIGF, two substances that promote or curb cell growth, projects Professor Carmeliet was working on. VIB was the intellectual owner together with Collen's non-profit association DCRF (now LSRP), but Thromb-X and later ThromboGenics became a licensee. When Roche took over the development path from ThromboGenics in 2008 and paid EUR 50 million in advance, VIB must also have hit the jackpot. "EUR 4.5 million came in at that point," Jo Bury agrees. "That was a major milestone. But a little later Roche acquired Genentech where similar research was being conducted and, in 2012, they simply gave everything back. ThromboGenics then decided to concentrate solely on its ophthalmological developments. Of course, we felt that

was a pity, since it was a very promising path. To cut a long story short, together with ThromboGenics we then set up a company, Oncurious, which investigates the relevance of anti-PIGF for an uncommon type of brain cancer in children, which may lead to an orphan medicinal product."

The top in Europe

In 2009 Désiré Collen wrote in his memoirs: "Without VIB I would not have attained my emeritus status at the KU Leuven." Jo Bury: "That applies to other people, too. I think that's also the case with Christine Van Broeckhoven. It also applies to Peter Carmeliet, Alzheimer specialist Bart De Strooper and biologist Dirk Inzé, among others. I think that with VIB we created an environment in which these people could play a part at world level, and could attract world class scientists. In their field of study, we could draw the card of excellence, far removed from the limitations that always exist at a university. And it's a competitive environment. Every five years all VIB research groups, or their PIs *(Principal Investigators)*, are reviewed and those found not fit to be among the world elite have their VIB subsidy discontinued. It's a matter of investing in thoroughbreds, no less. That is what these people want: to be competing with the likes of Harvard, Stanford and Max Planck."

To safeguard that quality, the management is controlled by a board, comprising the directors of the various research centers and four members of the management, the executive committee. "Then there's the board of directors with 13 members, six of whom are from the universities, i.e. rectors or deputy rectors, four from industry, all leading figures in biotechnology, and three directors appointed by the government," explains Bury. "They're all involved in innovation and have authority in the field. Then we have an institutional advisory board, our IAB, all of whose members are foreigners. They are some of the most prominent scientists, who often run research institutes themselves, and they're here once a year to give us advice on our strategy and therefore on the course we are pursuing. In each of the research centers we also have a scientific advisory board, which meets at least every two years and once again consists of leading figures from Harvard, Stanford, Max Planck, etc. They help us prepare the five-yearly reviews, which are also conducted by panels of experts whom we have flown in from all over the world. They pass verdict on what is world class and what is not. We are talking about eight panels of between five and ten specialists, making a total of 50 or so participants. That's certainly impressive for Flanders, but a peer review of this kind is used in all similar institutions. And credit where credit is due, it was Désiré Collen who introduced and enforced this. His point of departure was: tell me if it's not good, and I'll discontinue it myself. The board of directors then takes decisions based on this advice. Collen himself feels that the selection could be a little stricter. "The intention was that 20% should be renewed every five years, but that's not happening," he says. And that leads to frustration among some promising younger researchers, which Collen does understand. Fresh blood is not being given enough opportunities."

Can VIB's tech transfer measure up to Leuven's KU LRD? Jo Bury: "As far as size is concerned, we're still the kid brother of Leuven's KU LRD but we're now raising EUR 10 to 15 million every year from partnerships with industry, which represents 10 to 15% of our total research budget. Income from royalties so far remains limited. But we have set up around twenty companies, early stage, capital intensive, with 760 employees at the moment. These start-ups have raised EUR 930 million in private capital, which is roughly as much as KU

LRD's 120 start-ups. We're certainly proud of that. We have only two genuine failures, also because we dare to change track. We have had Devgen, also on the stock exchange and since acquired by Syngenta. That began with a pharmaceutical focus, but when it turned out that the technology concerned had more potential in the agricultural sector, they switched to that area. Ablynx, recently sold to Sanofi Pharmaceuticals, has various medicinal products in development in clinical phases I, II and III, with what was originally Brussels technology. In Antwerp we have set up the company Multiplicon, a specialist in diagnostic tests for genetic disorders, which was recently taken over for EUR 68 million by the American company Agilent. No doubt you have heard of their new non-invasive prenatal test for the detection of Down's syndrome? And there are more companies on the way."

VIB also fares well when compared to similar institutes in other countries. "In the *Times Higher Education* we came out joint first as the most innovative institute, together with the UK's *Cancer Research* and the *Scripps Research Institute* in San Diego," says Bury, proudly. "Their parameter is frequency of citation in patent applications. And when we look at tech transfer results in Europe and the creation of start-ups from basic research, I dare to say that we are among the very best in Europe! I think we're doing better than London's Imperial College and we've also left the European Molecular Biology Lab (EMBL) in Heidelberg in our wake. When we started out in 1995, we chose two benchmarks, the EMBL and, because Heidelberg doesn't work with plants, the John Innes Center in Cambridge, Europe's top establishment when it comes to plant technology. Coincidence or not, the directors of those two centers were on the assessment board that evaluated us in 2016 at the Flemish government's request. They were impressed by our results and concluded that we have kept pace with them. That was a great moment, but there's still a lot of work to do. In the main fields on which we are focusing there is still a great need: neurodegeneration, inflammatory disorders, cancer, sustainable farming, etc. Our basic research must lead to new knowledge that gives us a tool we can use to intervene. Now that we can play a part at world level, we are all the more motivated to set out a new course."

Désiré Collen is rather more cautious in his comparisons. "I live near Imperial College in London. To be able to compare ourselves with them, we still have some work to do. But, well, much has been achieved by our generation of scientists, civil servants and politicians. We can look back with pride as, on a whole, the balance is undoubtedly very positive."

(The interviews in this chapter took place between January and April 2018; the figures in the Leuven's Affluence table were updated in June 2020)

PART IV: THE ROLLER-COASTER RIDE OF THROMBOGENICS

TG Board of Directors, 2007

TG Team, 2007

ThromboGenics share price evolution since 2007

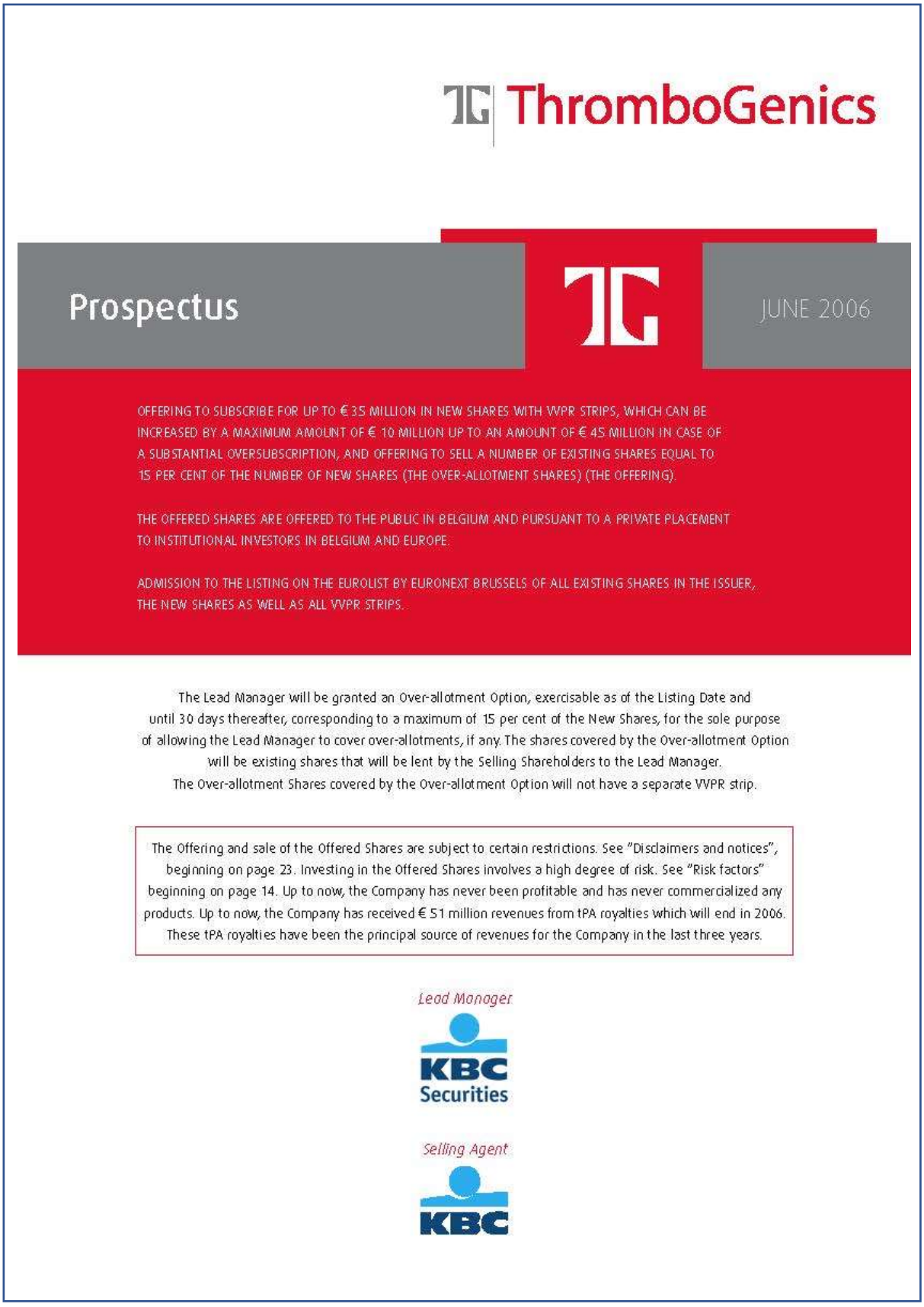
Stuart Laermer and Steve Pakola

Peter Stalmans

Peter Verhamme

Key collaborators in the development of ThromboGenics

Chapter 1: From self-confidence to overconfidence



ThromboGenics

Prospectus

JUNE 2006

OFFERING TO SUBSCRIBE FOR UP TO € 35 MILLION IN NEW SHARES WITH VVPR STRIPS, WHICH CAN BE INCREASED BY A MAXIMUM AMOUNT OF € 10 MILLION UP TO AN AMOUNT OF € 45 MILLION IN CASE OF A SUBSTANTIAL OVERSUBSCRIPTION, AND OFFERING TO SELL A NUMBER OF EXISTING SHARES EQUAL TO 15 PER CENT OF THE NUMBER OF NEW SHARES (THE OVER-ALLOTMENT SHARES) (THE OFFERING).

THE OFFERED SHARES ARE OFFERED TO THE PUBLIC IN BELGIUM AND PURSUANT TO A PRIVATE PLACEMENT TO INSTITUTIONAL INVESTORS IN BELGIUM AND EUROPE.

ADMISSION TO THE LISTING ON THE EUROLIST BY EURONEXT BRUSSELS OF ALL EXISTING SHARES IN THE ISSUER, THE NEW SHARES AS WELL AS ALL VVPR STRIPS.

The Lead Manager will be granted an Over-allotment Option, exercisable as of the Listing Date and until 30 days thereafter, corresponding to a maximum of 15 per cent of the New Shares, for the sole purpose of allowing the Lead Manager to cover over-allotments, if any. The shares covered by the Over-allotment Option will be existing shares that will be lent by the Selling Shareholders to the Lead Manager. The Over-allotment Shares covered by the Over-allotment Option will not have a separate VVPR strip.

The Offering and sale of the Offered Shares are subject to certain restrictions. See "Disclaimers and notices", beginning on page 23. Investing in the Offered Shares involves a high degree of risk. See "Risk factors" beginning on page 14. Up to now, the Company has never been profitable and has never commercialized any products. Up to now, the Company has received € 51 million revenues from tPA royalties which will end in 2006. These tPA royalties have been the principal source of revenues for the Company in the last three years.

Lead Manager

KBC Securities

Selling Agent

KBC

Summary: *When the studies with staphylokinase became prohibitively expensive, Désiré Collen's Irish ThromboGenics adventure ran aground. In 2006 he moved the company to Leuven and secured capital from the Belgian stock exchange. But the new ThromboGenics soon shifted away from thrombosis. In mid-2012, Jetrea®, a remedy for problems at the back of the eye, seemed to be a promising medicine. ThromboGenics devoted everything to this would-be blockbuster and reduced its other research projects. It was no longer about thrombosis, but ophthalmology – and profit, which led to euphoria on the stock market.*

Reservation: After reading an earlier draft of this chapter at the end of 2017, the current ThromboGenics' CEO declined to cooperate. As authors we were constructively seeking improvement and additional material, but unfortunately this was not forthcoming. The story recounted in the following pages may therefore not contain everyone's truth.

A hazardous journey

Columbus would never have sailed off, had the present-day regulators of the financial markets been around to have the final word in 1492. In the bulky 143-page prospectus with which ThromboGenics went public on the Belgian stock exchange in 2006, potential investors were alerted to every possible peril, save for perhaps a meteor strike. It was intended to be a prosperous marriage between tens of millions of EUR brought in by Flemish entrepreneurs and the knowledge, experience, network and rt-PA royalties of Désiré Collen. With those Genentech royalties, Collen had already made his way into the business world in 1991, first with Thromb-X in Belgium, and then, from 1998 onwards, in Ireland with ThromboGenics Limited. In 2006 he closed that chapter and returned to Belgium. He needed more money than he and his American colleague Landon Clay could raise, and to that end he converted the Irish company into a Belgian public limited company, and pumped capital into it via the Brussels stock exchange.

In the prospectus Collen used for investors, the brokerage house KBC Securities outlined all the conceivable risks in eight pages of small print. And the board of directors acknowledged that they were inviting investors to take part in a very hazardous journey. The numerous caveats in the prospectus were subsequently reiterated every year in the annual report. This level of caution had to do with the express intention to actively involve small investors in the initial public offering. At least 20% of the offered shares had to go to retail, and so the prospectus adds, "that percentage can be increased – yes, even by a substantial margin". There was popular capitalism behind the stock exchange flotation, an ideal that was later snowed under, but not any less noble. Should not any citizen who is thick-skinned enough, be an entrepreneur, even indirectly via the stock exchange?

Désiré Collen: "In Ireland we had a good tax ruling. Everything went well there, except that I had to fly there using Ryanair. I did that about forty times, leaving early in the morning and returning late in the evening – a two-hour flight each way from Charleroi. In those days I was on the go from 6 in the morning till 11 at night. Don't forget, I was chairman and CEO. OK, there were only five members of staff to organize the clinical tests, so that was quite manageable. But when we needed more money, we decided to raise that money in Belgium. I can't call it nostalgia, but what certainly played a part was the fact that the Flemish government had in the meantime recognized the importance of innovation and the climate here had become friendlier. Fientje Moerman and Patricia Ceysens were the Flemish ministers for Economic Affairs and Innovation around that time, and they were pleased to see

us return. And if we were then to take the step of going to the stock exchange, a Flemish Genentech was a secret dream of mine."

Collen's financial right-hand man Chris Buyse adds: "I wasn't on board yet at the time but I assume Collen also had his eye on a number of tax benefits that Belgium was starting to offer to innovative companies.I don't think those rulings were in place yet in 2006 so they can't have been an absolutely decisive factor when it came to ThromboGenics being established in Belgium. But they had already been prepared. The main benefits are the patent deduction and the payroll tax exemption for researchers. I won't go into the complexities of those measures. For patents there are currently two legislations side by side, but what it boils down to is that you only have to pay 6.8% corporation tax on patent income, instead of 34%. Only 20% of your taxable income, i.e. income after deduction of expenses, is taxed. The patent deduction does also exist in other countries, but the Belgian system is still attractive. Now, the tax ruling that ThromboGenics had on this, as the very first applicant by the way, yielded us virtually nothing, because of course you have to have taxable income."

Speaking from his own experience, Buyse says there were other advantages, too. "On top of that the federal state was also making a sizeable financial effort with the 80% payroll tax exemption for research and development executives. That was inspired by the government's aim of devoting 3% of our national product to R&D. And there were other measures that made it attractive for young enterprises such as ThromboGenics to come to Belgium around 2006 and later. The tax exemption on part of the income earned by foreign executives and researchers was also important. This amounted to EUR 30,000 a year, no less, alongside a number of other benefits. The accelerated depreciation of investments could also be attractive. And so on."

You're out to discover India…

If you apply the 26 different risk factors contained in the bulky prospectus to the seven development paths that ThromboGenics was starting out with, you arrive at least 182 threats. And even that premise is based on the proviso that the environment and the future do not harbor negative surprises that the writers of the prospectus may, in all their zeal, have overlooked. As they involuntarily failed to describe the marvels of chance or the serendipity of discovering things one was not even looking for. With his Santa Maria Columbus was looking for India, but ended up in America. In biotechnology serendipity is almost always the rule.

"Biotech companies have to dare to reinvent themselves," says Collen. "Those that are unable to do so, often do not make it. You have to be bold enough to embark on new paths. In Belgium, for example, look at Devgen which moved its research from a spastic worm to rice seeds, look at Celyad which went from the heart to cancer, look at Tigenix which was the first to bring a revolutionary but non-lucrative knee cartilage treatment onto the market, and later moved into promising work in the field of anal fistulas."

The world doesn't stand still; on the contrary, it's changing faster all the time. "A researcher constantly has to reorient himself," says Collen. "That's why my successor Peter Carmeliet is such a successful researcher. He has already changed direction three times. First it was with me, with knockout mice for the coagulation system. Then he dedicated himself to

angiogenesis with endothelial growth factors." Angiogenesis arises from the secretion of so-called angiogenic factors. These are growth factors that are produced by surrounding cells. A major pro-angiogenic factor is VEGF (Vascular Endothelial Growth Factor). The growth factors stimulate endothelial cells, the cells on the inside of the blood vessels. Some ophthalmological medicines are based on anti-VEGF factors. "Right now he's working on metabolomes," says Collen. "That's the full complement of chemical components involved in an organ's metabolism. The metabolism describes all the chemical reactions involved in maintaining the living condition of the cells and the organism. His most recent theory is that you should not shut off the blood supply to a cancer cell, but rather you should ensure a good blood supply, whereupon the cancer cells have less tendency to spread and can be more effectively eliminated with chemotherapy. If his theory is right, we have a revolutionary new insight on our hands, and then he might be bound for Stockholm to receive a Nobel Prize! Possibly only within eight years' time or so," he declares hopefully. "Peter Carmeliet uses a one-liner that I like very much*:* 'follow the phenotype'*.* If you see connections, genetic combinations in an environment, you should follow up on them. He started out as a biotechnology researcher but now works with neurologists, bio-computer scientists and cardiologists. I definitely was not mistaken about recruiting that man."

It goes without saying that with every risk, there is an opportunity. Things could go well 182 times, although that would be something of a miracle. But even if things work out well just once, that can by far outweigh 181 setbacks. Although you'd have to use a magnifying glass to look for that optimism in the prospectus, or have been imbued with it first hand in 2006 by an enthusiastic Désiré Collen himself, the Columbus at the helm of this Santa Maria. The 'precautionary principle' so deeply entrenched in Europe makes people reluctant to expose themselves to the risks run by explorers and discoverers. And were someone to lose sight of absolute safety, there are lawyers to remind them with lawsuits, and the media or politicians lying in wait to haul up culprits.

A molecule only becomes a medicine if and when it gets approved by regulatory bodies: the FDA, the Food and Drug Administration in the USA, and in the European Union the EMA, the European Medicines Agency. These approvals can take a long time, and the procedures are expensive. Even if a medicine is recognized, it is not always reimbursed by the health insurance schemes in the different countries with so many different public health authorities. As major clients, these authorities usually also want to have their say as to the price the developer charges for the medicine. In short, even if you have a miracle drug, you're still faced with an administrative calvary.

TABLE: ThromboGenics' cards

ThromboGenics appeared at the starting line in June 2006 with the components presented below. The therapeutic effect of a substance is established during the course of various phases. In the early preclinical phase, it is assumed that a substance might be worthwhile. This is followed by the preclinical phase in which the substance's chemical composition, stability, efficacy and toxicity are investigated, primarily in laboratory animals. Even at this stage, these activities are carried out under supervision and with reporting. No fewer than 700,000 laboratory animals, mostly rodents, are used for such trials in Belgium every year. During Phase I of the clinical stage, a medicine may also be tested in people to a limited degree, once approval has been obtained from an Ethical Supervisory Committee and from the Regulator. With authorizations, Phase II trials can then be conducted in a small number of patients to ascertain the optimal dose and to look into the remedy's safety. Phase III then concerns trials in large groups of patients, in which the new medicine is tested against a placebo or against an existing drug. For the sake of scientific reliability, the allocation of patients is left to chance, albeit without jeopardizing lives. The results of these tests can then be used to apply for an authorization to bring the medication onto the market. Once approved, there are regular checks and inspections of production and distribution.

The ownership structures mentioned below are more complex than is indicated by the acronyms. The non-profit association DCRF has since been renamed the non-profit association LSRP, Life Sciences Research Partners. Désiré Collen invested a large part of the Genentech royalties, including those he could recuperate from ThromboGenics, in LSRP and in the investment fund Fund+.

	NAME	*SYNDROME*	*PHASE*	*Owner*
1	*Microplasmin*	*Adhesion at the back of the eye (vitreomacular traction, diabetic retinopathy). Arterial thrombosis (peripheral or cerebral)*	*Phase II*	*TX/LRD*
2	*Staphylokinase*	*Acute myocardial infarction*	*Phase II completed*	*LRD and Collen*
3	*Anti-Factor VIII (TB-402)*	*Deep vein thrombosis*	*Phase I in 2006*	*DCRF (LSRP)*
4	*Anti-PIGF (TB-403)*	*Cancers and metastasis Age-related macular degeneration*	*Pre-clinical*	*VIB and LSRP*
5	*PIGF*	*Coronary artery disease (CAD). Heart failure*	*Pre-clinical*	*VIB and LSRP*
6	*Anti-GPIb (6B4)*	*Acute coronary syndrome (ACS)*	*Pre-clinical*	*KULRD*
7	*Anti-VPAC*	*Thrombocytopenia*	*Pre-clinical*	*DCRF (LSRP)*

Watch out for Eroom's Law

So this was how Columbus' ship was rigged up. Anyone wanting to put money into it was warned in the prospectus of what was sure enough a realistic conclusion, that many are

called but few are chosen. “The sector average,” explains Antje Witte of the Belgian pharmaceutical company UCB, “is that of every 5,000 compounds or molecules that start out in the pre-clinical phase, only one makes it to the finishing line. In Phase I, you have a 10% chance of success; in Phase II, that’s 30 to 50%; and in Phase III, it’s 70%. After the official approval, a further 10 to 15% fall by the wayside.” (1) Research is a ruthless knock-out contest, particularly concerning medicines to combat thrombosis and heart failure, for which hardly any molecules reach the pharmacies.

It’s a matter of *Eroom’s law*, which is *Moore’s law* backwards. (2,3) The latter law, named after the American computer engineer Gordon Moore (Intel Corporation), stated that the number of transistors on an integrated circuit doubles every two years. The computation capacity thus forms an exponential curve. The reverse occurs in pharmaceutical research, where the low-hanging fruit has already been picked. On average, 25% fewer medicines have been approved in the last 10 years than in the 1990s, despite the fact that R&D expenditure has doubled and 62% more substances or compounds have been tested. According to Eroom’s law, researchers face four problems. The *’better than The Beatles’* problem states that it is very difficult to design better medicines than those already in existence. There is also the ‘*cautious regulator’* problem, i.e. the caution and reserve with which the supervisory authorities act. The ‘*throw more money at it’* trend refers to the tendency to stubbornly persist with research that is proving disappointing and on which, for all kinds of reasons, nobody is pulling the plug. And with the ‘*basic research-brute force’* attitude, people take the shortest route during research, and in so doing incorporate their future failure.

According to the London Office of Health Economics, you have to think in terms of an average development cost of USD 1.2 billion per medicine. In constant prices, this is six times more than in the 1970s, and that’s an average. Vis-à-vis these costs, there is nonetheless an enormous profit potential. Take the possible profit from an effective remedy for a disease such as Alzheimer’s. According to the economic affairs monthly *Forbes,* Alzheimer costs in the USA alone now total USD 259 billion a year. (4) A medicine that were to bring these costs down significantly would immediately be worth tens of billions.

The towering R&D costs partly explain the very high unit prices of a new medicine. When a medicine does not entail savings in care costs, the high R&D costs place the pharmaceutical sector on the borderline of affordability and refundability. In this respect, one of the questions being asked is: what is the extension of a human life worth, or what might one extra year cost, irrespective of who is paying, and particularly if the public authorities and the social security system are expected to foot the bill. The British National Institute for Health and Care Excellence (NICE) uses a threshold of GBP 20,000 to 30,000 per Qaly. (5) A Qaly is the acronym denoting a quality adjusted life year. But how do you define quality? And from what age do those Qalys start to count? The limit of affordability by patients and/or insurance companies can be included as a fifth problem in Eroom’s law.

“I wasn’t familiar with this Eroom’s law,” admits Collen. “But you can certainly find many examples for those four or five handicaps. Take the corner cutting: wanting to go too fast. In my mind, that’s how we messed up our Antifactor VIII, i.e. TB-402. It looked good after knee operations, compared with heparin, with administration after 24 hours. But then we tested it

in hip operations with administration in the first few hours after the operation, and this led to hemorrhages. Actually that study saw three of Eroom's laws coincide: there was a *brute force* approach, in the form of immediate administration, aimed at being *better than The Beatles*, i.e. up against Xarelto, the best control medicine, and then a refusal to *throw more money at it*. The medical problem appeared in the meantime to have been solved with the new drugs (including Xarelto), but Bayer's medication did not work for patients with an artificial metal heart valve. Take children with rheumatoid arthritis whose heart valve fails. You can't use a biological valve, since it will have to last 50 or 60 years and a biological valve only has a useful life of 30 years. In short, if that medicine had come along, it might have been a major success. Clinical research has to be undertaken on a step-by-step basis. If you pile too many risks on top of one another, you lose perspective and move towards failure. At one point I had a similar problem with t-PA," he continues. "I thought t-PA also had to work in the case of cerebral thrombosis. The people at Genentech then urged me to exercise caution. A clot in one place is not necessarily the same as a clot elsewhere in the body. Specialists still use rt-PA now in cases of stroke, but they do record 2 to 5% brain hemorrhages. If we had started these studies at the time when rt-PA was tested in cases of myocardial infarction, rt-PA might never have come into being."

Eroom's Law therefore can be an interesting approach but must nonetheless be refined. Medical science is indeed making progress, perhaps not as exponentially as ICT, but still in the EMA zone at a reasonable rhythm of 57 new medicines or combinations in 2012, 79 in 2013, 81 in 2014, 93 in 2015, 81 in 2016 and 92 in 2017, generic drugs included. The USA's FDA presents lower figures (45 in 2015, 22 in 2016 and 46 in 2017), but that difference has to do with the European EMA's more wide-ranging field of competence. (6-9)

"Our rising life expectancy has a lot to do with hygiene," says Collen, "but much can also be attributed to advances in medicine. The mortality rate in the case of a myocardial infarction was nearby 30% when I was resident in medicine. Now it's less than 10%. Anyone refusing to acknowledge the progress made in research or only focusing on the failures is intellectually dishonest, but you do come across such views in the media! The ever-stricter supervisory bodies and the risk aversion of many parties make research much more difficult than it used to be. Everyone wants to avoid responsibility in case things miscarry. It's getting more and more expensive, too. If I were to set about doing now what I did with t-PA and staphylokinase, I'd be in for major problems. And back then we already had an ethics committee that oversaw things, and patients were well informed as well. In hopeless cases, especially where children are involved, even with the best of intentions, anyone who is bold enough to experiment (and therefore will not read the small print), has had it. This is no longer possible. I can provide examples of that. Ogeda, in which we have a participating interest through Fund^{+}, has a remedy for hot flushes (vapors). The wife of one of the members of the research team was greatly afflicted by the disorder, to the point where she could hardly leave her bed. Well, we weren't able to help that lady whilst the medicine was not approved. Compassionate treatment for non-life-threatening complaints is no longer allowed."

Farewell to a showpiece

Two of ThromboGenics' seven development paths were particularly important, as was immediately stressed in the risk factors, outlined in the prospectus. With microplasmin,

ThromboGenics hoped to be able to cure an adhesion problem in the eye, and consultation about this was already under way with the FDA in the context of a pre-IND *(investigational new drug application*). The resulting admissibility prompted Collen to move from Phase IIb to Phase III, with tests on patients. Another IND from the FDA was needed for this, but microplasmin could count on the administration's goodwill.

Staphylokinase, the thrombolysis drug for less privileged patients, was also included in the new ThromboGenics' portfolio, but the prospectus immediately pointed out that the company itself did not want to start on Phase III, because it would be unaffordable. Genentech was in no mood to have its own Activase undermined and was not prepared to make several thousand doses of rt-PA available at reduced price. So, no large-scale comparison with rt-PA could be organized. According to Collen, a study on 15,000 patients would have cost USD 100 million in 2006! It was farewell to the Irish showpiece with which it all began. The question was what that *adieu* could still yield.

From Anglo-Saxon investors, Collen had learned that his new Belgian company should not be a *'one trick pony'* and needed to have several strings to its bow. Staphylokinase and microplasmin were not enough. ThromboGenics therefore started with five other components, and the explicit intention was not to leave it at that. The prospectus promised that everything would be done to attract new substances through the Flemish Institute for Biotechnology (VIB) and the KU Leuven.

A major risk, according to the prospectus, concerned the third parties that carried out sub-assignments for ThromboGenics. A lot can go wrong there, and that can reflect on the client. Third parties were involved in the work being done with staphylokinase and microplasmin. These were CMOs (contract manufacturing organizations). For microplasmin and for PIGF, there was cooperation with Eurogentec in Liège, but ThromboGenics wanted to end this collaboration. The American company Avecia Biotechnology, now part of the Japanese Nitto, was ready to deliver. The problem did not arise in respect of anti-Factor VIII (TB 402) and anti-PIGF (TB 403), since BioInvent International was a competent partner.

ThromboGenics did not just need partners upstream, but downstream as well. Just as Collen's t-PA might never have become a medicine without Diane Pennica's cloning work and without Genentech, so it was also to be expected that ThromboGenics would have to share one or more developments with much larger parties, subject to all kinds of conditions. Such a partner might want to eliminate a potential rival and thus kill the Thrombo goose before it could lay eggs. The prospectus describes this danger in less graphic terms, but partners are not always well-intentioned. The pharmaceutical industry is not for softies.

When doctors balk at it

The statement in the prospectus that a good medicine is sometimes not accepted by the patients or doctors concerned verges on the prophetic. Lack of 'market acceptance' proved fatal for the later drug developed by ThromboGenics. The prospectus lists all kinds of reasons why doctors may oppose a better medicine. Prescribing practitioners may have doubts as to the safety, practicality, administrative activities – in short, the suitability of a new drug or remedy. What the prospectus does not say is that medical progress sometimes causes a reduction in doctors' income. This can be the case when an expensive surgical

intervention is made avoidable by a pill or an injection, whereupon a pretext is found all too easily. This becomes particularly relevant later on.

Were the ThromboGenics board at some point not to be sufficiently aware of the resistance to change that can exist among doctors, that board could itself turn into a risk. Errors of appraisal on the part of an unaware board are indeed also regarded as a risk in the prospectus. “The board shall be able to spend the collected monies as it deems fit,” reads the text. And “if the proceeds are not wisely allocated”, i.e. if things are bungled, then, well, that’s a pity for the investors. A seasoned management team is perhaps the most important asset in any company, and all the more so for a start-up. In a free market, it is essential to come up with the right product in the right place, at the right time, at the right price. The authors of the prospectus explicitly highlighted this in the small print, so that no-one could later claim they had not been warned of disillusioning business practice.

The risk that the fish might perhaps not take the bait, and doctors or patients ignore the new medicines, is linked to the uncompromising market environment in which every pharmaceutical company operates. The ailments for which ThromboGenics was seeking cures are also being targeted by other biotech companies, so the prospectus warns. The search for medicaments is highly competitive. There is nothing wrong with that. We have this competitiveness to thank for the fact that, on average, an infant today has a life expectancy twenty years longer than his grandfather had at the same age. But the road to rare success and a longer healthy life is strewn with the wrecks of companies and with investors who lost their money.

Then there are the thieves. Industrial secrets cannot always be protected. Sometimes members of staff embody crucial know-how, and the temptation may be great to move to a competitor, taking that knowledge with them. Patent law does not always provide protection, either. It is often difficult to specify exactly where one patent ends and another begins. ThromboGenics was also not sure that it was immune from all manner of accusations. Expensive lawyers readily capitalize on this, even if only to delay a development or to put a financially weak competitor to great expense and undermine its reputation. Thanks also to the experience that LRD had since built up, the intellectual title to ThromboGenics’ assets was competently described and protected. But this is never totally waterproof. Knowledge depends on other knowledge. As Isaac Newton said, a giant that looks a little further, always stands on the shoulders of previous giants.

People really are a risk!

In Phase III, a medicine also has to be tested on people. Suitable patients are often thin on the ground and are also unpredictable. They have to give their consent and are well protected by ethical supervisory bodies. Lawyers, courts and the media are standing ready should any false steps be taken or any disputes arise. At least one in 10 US biotech companies has to contend with claims for compensation (litigation). (10) This is a major concern, since the damage to a company’s reputation is done already the moment a ’scandal’ breaks out, not when a sentence has been passed.

Furthermore, what if something goes wrong not during the tests on people, but later, when the recognized medicine is being used by patients? Product liability claims are the nightmare of all small biotech companies and one of the reasons for the large size of many

pharmaceutical companies. Generally, Goliaths are more robust than Davids. The financial damage to a company can be enormous, and the damage to its reputation can also bring about its ruin. In the USA, the examples are legion. Often the companies deserve some of the blame, too. If tens of millions of USD have been invested, there is a great temptation to embellish the results somewhat. A company can try to insure itself against claims, but the premiums exceed the financial capacity of a small company. And there is no way of insuring against damage to one's reputation; in that case, it's the end of the story.

"Unlike Genentech with t-PA, ThromboGenics was spared disputes," Collen recalls with satisfaction. "We did engage in negotiations with the American *NewView Eye Care*, but there weren't any really serious disputes. What we had under license came from the university and VIB and those were professional contracts. The Spaniards of Grifols did dispute our patent for microplasmin, but they didn't have a leg to stand on. They were basing their claims on knowledge from the 1950s! It still cost us quite a lot of money all the same. In order to be able to genuinely guarantee the intellectual property rights to Jetrea® vis-à-vis Novartis, we paid them EUR 9 million. And in the event of success, that could have become much more. For the same reasons we also paid USD 10 million, without a lawsuit, to the Americans Tracey and Williams, who were the first to isolate plasmin from individual patients for intravitreal injection."

Then there was the possibility of things going amiss with the staff of ThromboGenics itself, the 40 employees and executives with whom the company started out in Belgium and the USA. What if there was disagreement among the senior managers? The resignation or departure of key figures or teams with specialist knowledge can be fatal for a small company. In the past, there had never been any problems in that area, so the prospectus tells us, but of course ThromboGenics had only just begun. Further on in the prospectus, we are told that the staff would receive warrants on shares that could only be converted into shares at a fixed price after a certain period. So, it was very much in their interest to stay on as employees or consultants and in particular to ensure that the share price exceeded the price at which they could redeem their warrants. The difference between the market price and the subscription price of those warrants is a virtually tax-free bonus. The possibility of financially entrenching staff members' loyalty by means of warrants is an important reason for a stock exchange quotation.

The eye and the heart

Imagine that ThromboGenics were to discover one or more medicines. In what kind of market would they end up? What might be the next thing to come along and in what time frame, and with what competition will that innovation have to contend? The authors of the prospectus do their best to answer these questions in 10 pages of small print. It is striking that by far the most attention is devoted to cardiovascular diseases, thrombosis in three guises (artery, vein and heart), heart attack in its manifestations, thrombosis, embolism or obstruction caused by a hardening of the walls, and to six other different syndromes for which specialists use acronyms among themselves. Fifty million people in developed countries are affected by some form of thrombosis and, in the USA alone, 2 million die of it every year. Mortality figures for heart attacks are comparable, on the understanding that a great many heart attack victims who survive are subsequently disabled. With this emphasis on cardiovascular diseases, ThromboGenics lived up to its company name in the prospectus.

Staphylokinase may already have been in the display window, but another ThromboGenics substance, microplasmin, possibly also had potential in this field. (11)

Regarding the available medication for thrombosis, the prospectus alerts readers to the trend of medication being side-lined by surgery, although this only takes up one short sentence. Thrombolytics such as t-PA, *'clot-busters'* in popular American parlance, were increasingly making way for '*dottering*' and '*stenting*'. In this process the interventional cardiologist restores the flow of blood by means of a catheter in the bloodstream, first with a little balloon or 'dotter', to open up the problematic blockage. Then the artery is strengthened with a stent, a new metal support of the artery wall that ensures the flow from that point on. The catheter and the little balloon are removed, and the stent remains. This treatment goes back to the 1950s when the American radiologist Theodore Dotter managed to locate and immediately reach the problematic arteries, but it became increasingly important from 2002 onwards. Stents are often combined with blood thinners, mainly ordinary aspirin. The ThromboGenics prospectus concedes that this development "led to a saturation of the thrombolytics market", which may have been an understatement.

Dottering and stenting came onto the scene after t-PA. "Our drug only worked in 75% of cases," says Collen. "For a time there was competition between stenting and t-PA, but a mechanical intervention had a better chance of success – provided of course you had the infrastructure to carry out this kind of operation, and that was only the case in densely populated areas with a large enough number of patients. In Leuven's Gasthuisberg hospital, they now have eight or nine catheterization rooms. So, dottering is better, that's clear." T-PA has since virtually expired as a thrombolytic to treat myocardial infarction. It is still mainly used for cerebral arterial thrombosis. "Its time came, and its time went," says Collen philosophically. "But t-PA contributed a lot to our knowledge about the thrombosis process and helped Genentech to grow into a large company. It laid the financial foundation for their growth. Now they have several medicines each with sales figures many times higher than those posted by t-PA. But it began with t-PA. Since then they've increased that income flow in spectacular fashion, and ThromboGenics might also have been able to do that."

In the prospectus, the concept of 'blockbuster' crops up once. The term comes from the film world but is used to denote a medicine with an annual sales potential in excess of 1 billion USD. One such potential jackpot is anti-PIGF (TB-403), a substance taken under license by ThromboGenics with the Flemish Institute for Biotechnology and the Désiré Collen Research Foundation, now the non-profit association LSRP. Because anti-PIGF curbs or stops the growth of vascular cells or angiogenesis, it could represent a breakthrough in the fight against cancer and also against some problems at the back of the eye: a holy grail in other words, although possibly also a pipe-dream. No wonder ThromboGenics ended up in a race with twenty or so competitors, which in fact already had some five medicines on the market.

This situation applies to a lesser degree to the other substances in the portfolio. Anti-factor VIII (TB-402) is in Phase I. Still in pre-clinical phase are PIGF, anti-GPIb (6B4), a potential orphan medicinal product and therefore with less commercial potential, and anti-VPAC, all substances that could possibly be used in iatrogenic thrombocytopenia.

Microplasmin works wonders

Microplasmin is a smaller, stable form of the proteolytic enzyme plasmin. Proteolysis can be defined as the breakdown of proteins by other proteins, called enzymes, a process that occurs on a large scale in our digestive system. Microplasmin, which would later be called ocriplasmin and still later Jetrea®, was discovered, protected and produced from 1998 onwards by the Center for Molecular and Vascular Biology, CMVB, of the KU Leuven. The production of recombinant microplasmin in yeast was developed by Thromb-X and the rights of ownership were held by the non-profit Désiré Collen Research Foundation and Thromb-X NV.

With microplasmin, it is possible to dissolve clots without the presence of plasminogen, i.e. without t-PA, the substance that turns plasminogen into plasmin, which then attacks the fibrin that makes up the clot. It is mainly useful in older blood clots and is effective up to 10 hours after the thrombosis. Moreover, all kinds of side effects of t-PA, particularly in the brain, can be avoided with microplasmin. A great deal of Phase I and II research had already been conducted with volunteers and the results were promising, especially for peripheral clots, in the leg, for example, which are referred to in medical jargon as PAOD (peripheral arterial occlusive disease) and are life-threatening disorders that affect millions of people.

From 2004 it became clear that this substance could be used to combat not only cerebral arterial thromboses, but also a common eye problem. This discovery was chiefly the work of Collen's fellow workers Nobuo Nagai (cerebral thrombosis) and Steve Pakola (vitreomacular traction). Steve Pakola joined Thromb-X and Désiré Collen as a cardiovascular specialist in May 2002. "However, I also had a year's ophthalmological training under my belt. That combination proved to be ideal when I became Chief Medical Officer (CMO) of Thromb-X," recounts Pakola. Désiré Collen was recommended to him by his teacher Randall Moreadith, a friend of Collen's.

"Collen was an inspiring leader, of the kind I was never to come across again," says Pakola. "He was the greatest leader I ever worked with. I came from large companies and the small Thromb-X consisted of Collen himself and a small complement of staff. I mostly worked in the United States and only came to Leuven every now and then, but he gave me his trust. And you know what? Trust is everything. In a climate of trust, a small team of good people with a clearly defined assignment can work wonders."

At the time, Collen was unhappy about the price Genentech was asking for rt-PA. With staphylokinase he wanted to develop a much cheaper alternative. "I could go along with that task," says Pakola. "Alongside staphylokinase, we also had microplasmin in Leuven that was tested for thrombosis as well, but medicines for cardiovascular diseases are a graveyard. You carry out tests on animals and that's a relatively homogeneous environment, but once you're dealing with people everything is much more complex." Then the Amsterdam ophthalmologist Marc de Smet asked whether microplasmin could possibly also be used in vitreomacular adhesion, adhesion of the vitreous humour. "That fell within my second area of specialization," says Pakola. "I learned that Arnd Gandorfer was also working on this in Germany and, in the literature, I found the results of tests with plasmin carried out by two American researchers Mike Tracy and George Williams. So, there was a *proof of concept*. The problem was that they had to make plasmin from each individual patient's blood, which

was expensive and laborious, whereas we had easy-to-produce recombinant microplasmin at our disposal. Collen then allowed me to put microplasmin for cardiovascular diseases on the backburner and carry on in ophthalmology. I then supervised the entire development all the way through to 2012, when I left ThromboGenics." (12)

Professor Marc de Smet was head of the ophthalmology department at the University of Amsterdam in the late 1990s. Today he runs the ophthalmological company Mios in Switzerland. "In 1998 and 1999 I had operated on a few young patients with vitreomacular adhesion," he says. "The results weren't satisfying, so I was looking for something better. During a conference in the United States I had heard about plasmin and how this was being used for babies in Detroit. Back in Amsterdam I asked the hematology department to make plasmin for me, but that wasn't possible. Fortunately, a professor told me that a group in Leuven was working on the development of a remedy that would work in the same way as plasmin. That was how I came into contact with Désiré Collen. At the time he was mainly working on staphylokinase, but that was far too toxic for my indication, and I thought that was the end of the story. A year later that same professor told me that during a visit to Leuven he had heard something about a new product: microplasmin. I got in touch again. This time Steve Pakola was there. He had had a year's ophthalmological training in Philadelphia and was therefore knowledgeable about eye problems. He understood the importance of my request, and a little later I received some microplasmin. We tested it in a pig's eye and demonstrated that the vitreous humor could become unstuck. We even discovered what the effective dose was, more or less. With Steve, and with the help of Jean-Marie Stassen and Geraldine Cahillane, the Phase IIa protocol was prepared. But then I left Amsterdam and so the cooperation came to an end." (13)

The eye diseases for which microplasmin could possibly be a remedy are explained in accessible language in the prospectus. As yet, there is no medicine to treat a problem at the back of the eye, vitreomacular traction. This involves an adhesion of the aqueous humour to the retina and not an age-related degeneration of the retina. When the problem cannot be solved by laser surgery, a vitrectomy is performed. Vitrectomy or PVD (posterior vitreous detachment) amounts to the gelatinous vitreous humor being sucked out of the eye, whereupon the retina traction is released. This is quite a complex operation involving the patient having to lie face down for several days. The prospectus states that there were an estimated 600,000 such patients worldwide in 2006, 40% of whom in the USA. A turnover of a billion USD was involved, with growth of 6 to 8% per annum. If the problem could be solved by means of an injection in the vitreous humor, this would clearly represent a major advance. But people did not know that yet; perhaps it had to be used in combination with surgery.

And perhaps microplasmin would also help to combat a diabetic adhesion of the retina. Were that to be the case, the working field in which microplasmin could be deployed would expand enormously. Diabetes is a disease of affluence that has increased by almost 400% since 1988 and now affects 26% of the over-65s. The chief executive officer of the Danish pharmaceutical company Novo Nordisk estimates that the disease could affect one in nine of the world's population, that's 736 million people, by 2045. (14) The disease also affects the patient's sight. In 2006, ThromboGenics was in Phase IIa for vitreomacular traction and at the beginning of Phase II for diabetic retinopathy. The results in several dozen patients were indeed promising.

On the brink of disaster in Ireland

Finally there was the stock exchange risk. The prospectus stresses that share prices can undergo all sorts of fluctuations for myriad reasons, including 'other external factors'. Often there is simply nothing a company can do to prevent its share price falling.

The prospectus goes on to say that this market value will for a long time consist of expectation value, i.e. redeemable hope, on top of unavoidably shrinking cash in hand. The expectation value is calculated by the market – or to be more precise, by market analysts – as the current value of hypothetical future income. In fact, this is largely guesswork, certainly in respect of ThromboGenics as it stood in mid-2006. Since the company had started up in Ireland in 1998, it had only been burning money (to the tune of around EUR 4 million a year up until 2006). That cash-burning would continue in Belgium and there was the possibility that the company would never even move above the profitability line. If it were to post a profit one year, even that might be exceptional.

Nobody knew how much longer expenses had to be made before any profit could be reaped. ThromboGenics might burn up the capital raised through the initial public offering in a few years' time. The prospectus warns that extra capital might be needed. If the existing shareholders then do not keep up or are unable to keep up because they were simply not addressed (private placement), their position will be diluted. Their stake in the company will become smaller and they will be unable to prevent that.

The scope of the already extremely cautious wording used in the prospectus is legally sealed, so that no investor or lawyer could later come up with any alleged claim based on the prospectus. Investors had to let their conscience decide, whether to agree to the proposal to become a shareholder, "including the risks and merits involved". So potentially there might be 'merits', too, in the end.

ThromboGenics' IPO was a Europe-wide financial-technical *tour de force*. The valuation technique was also particularly innovative. The Irish company ThromboGenics Limited would be absorbed by the new Belgian company ThromboGenics NV. And the valuation of the Irish shares was put on a par with the value of the new shares with which the Belgian company ThromboGenics was increasing its minimum capital. This meant that the value of the Irish shares depended on what the new shareholders were prepared to pay for the new Belgian shares. After the Belgian capital increase, ThromboGenics Ireland was incorporated into ThromboGenics Belgium, whereby the holders of the Irish shares obtained one Belgian ThromboGenics share per Irish share.

As a matter of fact, the situation in Ireland was tottering on the brink of disaster in 2006. In the prospectus, the directors of the Irish company claimed that the company still had sufficient funds to carry on working for another 12 months, but the balance sheet showed that they had carried over losses of EUR 31.6 million with only EUR 11.6 million in capital remaining. With an annual loss of more than 4 million, alarm bells should have gone off at the beginning of 2007. The issue premium on the balance sheet had been consumed in the losses, and ThromboGenics was going through its capital in the strict sense. This kind of company cannot rely on banks. Furthermore, it was most probably pointless to go on

squandering those last resources, since in any case they would not have been sufficient – neither for staphylokinase nor for microplasmin.

Fresh capital was not readily forthcoming. The prospectus clearly points out that from 2007 ThromboGenics Ireland could no longer count on the Genentech royalties, which up until then, via the Collen Trust and Biggar, had provided for around EUR 5 million every year in extra funds. Of the USD 144 million that t-PA yielded between 1988 and 2005, around USD 71 million went to ThromboGenics Ireland. That source would now dry up.

ThromboGenics preferred to get microplasmin for eye problems past Phase II under its own steam, without a partner. A partner could come on board after that. Staphylokinase and anti-PIGF were eligible for co-development with a partner. But ThromboGenics was optimistic, explaining then that "the company expects to be able to sign significant licensing and co-development deals for one or more development paths before or in the course of 2007." Staphylokinase might be equivalent to t-PA, as has actually been proven, and the cost price ought to be of the order of EUR 200 per dose, i.e. 10 times cheaper than t-PA. Developing countries would certainly be interested! But that meant the extra resources from the capital increase would not go to staphylokinase. The new capital had to be used to enable microplasmin to be brought close to Phase III studies for two indications. With an investment of EUR 8 million, Phase IIb had to be attainable in 2007 for adhesion in the eye (vitreomacular traction) and in 2008 for a thrombotic application, namely a blood clot in a peripheral limb, such as a leg, which is referred to in the jargon as PAOD.

The shareholders of the Irish company therefore wanted to press ahead. Two key figures took the decision on the matter: Désiré Collen himself in the first place, and the American Landon Clay whom Collen had brought on board as an investor in 2002. ThromboGenics NV was incorporated before notary public Liesse in Antwerp on 30 June 2006, with the minimum capital of EUR 62,000. The founders comprised Collen himself and also Biggar, the company via which Genentech royalties were gifted to science. The Désiré Collen Research Foundation was represented by the Leuven economics Professors Karel Tavernier and Raymond De Bondt. The other signatories were the Americans Landon Clay, Randall Moreadith, a Texan colleague of Collen's and co-founder of the Irish company, the American Professor Burton Sobel, the Briton Patrick J. Gaffney and three Belgians including Professor Peter Carmeliet. The directors of the transitional company were Désiré Collen and the Americans Andrew Guise, the new CFO, and Landon Clay. The aim was the absorption of the Irish ThromboGenics, which was immediately virtually established by the shareholders on the eve of the stock market flotation. Biggar held 8.4 million shares or 58.5% of the total. The Leuven non-profit association Collen Research Foundation held 8.7%, Désiré Collen as a private individual held 7.8% and seven other small shareholders held 1.3%. Landon Clay's East Hill held 23.6% with two funds. And there were also 500,000 warrants in ThromboGenics Ireland that would soon be exercised in ThromboGenics Belgium shares.

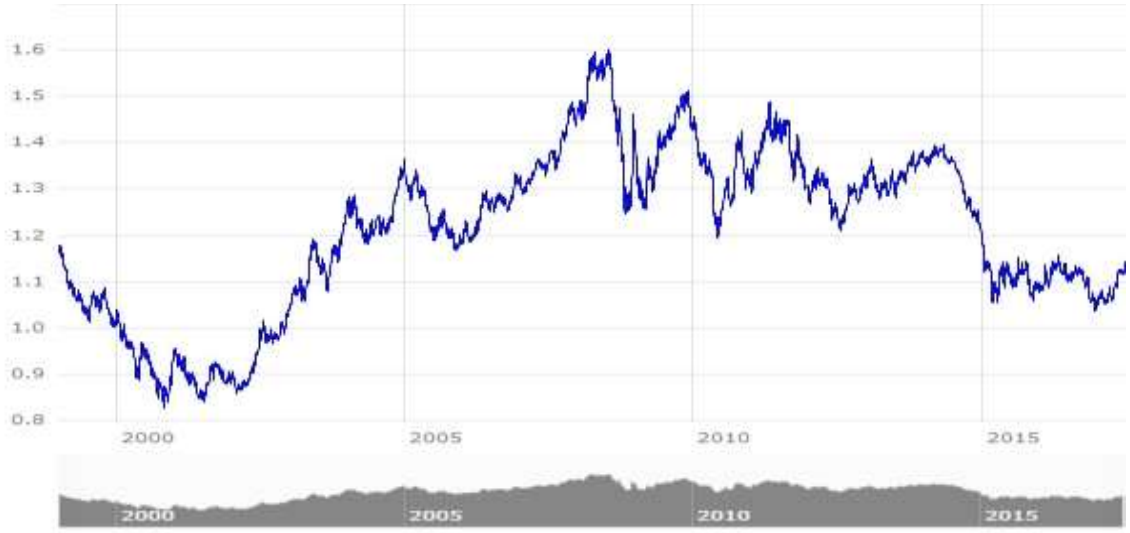
"We needed money," says Collen. "The Genentech royalties were running out. I had made my contribution, and so had Landon Clay. He had exercised the 'springing warrants' that he had stipulated when he joined. In 2002 Landon Clay had wanted to invest in a 'poor man's t-PA' but demanded a batch of extra shares at a bargain price of EUR 0.05 if we did not proceed to Phase III trials for staphylokinase within three years. We didn't manage that, so he obtained a rise in the capital with his 'springing warrants'. I then restored my position as

shareholder by exercising 540,000 warrants myself at EUR 6.35. We had to carry on together."

Collen and Clay decided to raise extra capital mainly among Belgian investors by means of a flotation in Brussels, a flotation that might also offer the possibility of making their own commitment a little lighter. For that matter Biggar and Landon Clay wanted to sell some existing shares. KBC Securities was prepared to supervise the whole process as lead manager, and KBC Bank vouched for the success of the capital increase in its role as selling agent – a particularly daring position for the Flemish bank to adopt. Usually these kinds of risks are shared by merchant banks and, nowadays, a major Anglo-Saxon bank would no doubt be involved in a placement such as this.

Investors buy Collen and Dehaene

ThromboGenics' ambitions were relatively modest in June 2006. The company hoped to raise EUR 35 million in fresh capital and expected to be able to get by with that for 20 months – at least if the exchange rates involved didn't do anything crazy. The euro's purchasing power against the dollar has fluctuated since its introduction from USD 1.2 to the euro in 2006, to USD 1.5 to the EUR in 2008 and 2009, and back to USD 1.1 at today's rate, but those quite spectacular movements obviously could not have been predicted back in 2006. And a lot of the company's costs were in dollars.



1 EUR= USD x see graph (source: ECB)

Should investor interest be so great as to see new shares subscribed to EUR 45 million, there would be enough funds for 26 months. And if 35 million was aiming too high, the board could also reconcile itself to a lower figure. The prospectus warned that in any case "the company may need additional funds which in that case may not be available."

Investors were thus buying a chance of a return in two years at best, a mission impossible. In fact, they were buying Désiré Collen, the man who had already delivered the goods once as a medical discoverer and thus deserved to be trusted. He personally embodied

ThromboGenics' "internationally recognized expertise in vascular medicine". Investors would also be buying a notable board of directors with, as an independent director, none other than Jean-Luc Dehaene, Belgian prime minister from 1992 to 1999. In his memoirs, Dehaene, who died in 2014, said that he was approached by business executive Luc Van Steenkiste, a friend of Collen. "I had already met Professor Collen a couple of times with Urbain Boutelegier, a friend with whom he shared a passion for wine," said Dehaene. "However, I mainly knew him as a young assistant and later the person who took over from my uncle Professor Marc Verstraete at the KU Leuven. My uncle was full of praise for his work. The stock market flotation was mainly based on belief in the success of Collen's scientific research." (15)

Collen also managed to enlist Staf Van Reet as director, a man with 15 years' management experience at Janssen Pharmaceutica, a Belgian subsidiary of the American company Johnson&Johnson. Professor Herman Daems was also briefly an independent director but stepped down after Gimv, the investment company he chaired, had subscribed to the ThromboGenics issue, thus rendering his position as independent director impossible. His place was taken by Luc Philips, former CFO of KBC Bank. Collen himself became Chairman and CEO until he was succeeded as CEO by Patrik De Haes in 2008.

> Staf Van Reet is a Belgian businessman with training as an applied biologist and jurist. He runs his own biotech company Viziphar Biosciences in Kasterlee (Belgium) and Bangalore (India). He held various positions at Janssen Pharmaceutica and Johnson&Johnson from 1991 to 2004. Then from 2007 onwards, he was founder and chairman of the listed company Movetis, which was acquired by the Irish company Shire in 2010. He was chairman of the board of directors of the Flemish Institute for Biotechnology, VIB.

By a narrow margin

But how much would a ThromboGenics share cost? To answer the question, the Lead Manager of the IPO, KBC Securities, asked institutional investors at what price they wished to buy shares. All investors could then subscribe at that price to the shares for which there was already a virtual institutional demand. The offering ran from 22 June to 5 July 2006.

The warnings in the prospectus did their job. The minimum EUR 35 million was attained by the narrowest of margins. "The capital increase has partially been effected" – this was how it was put in the board of directors' report. So, the aspiration of 45 million was not fulfilled, and the possibility of buying existing shares was used only to the tune of 3 million. The subscription price was EUR 4.50, lower than the unit-of-account value (EUR 5.58) of the start-up company ThromboGenics NV, and also substantially lower than the EUR 6.50 which had been used as a basis in the prospectus in two simulations. The public, comprising private individuals, but particularly institutional bodies, subscribed to 31% of the shares, once the Irish shares had been converted into Belgian shares. ThromboGenics thus started out with a capital of EUR 99,643,314.50, represented by 22,140,305 shares. Less than half was really available cash.

"Pieter Hofman and Koen Hoffman were the key figures at KBC, who did everything," Collen recalls. "They gave me, Landon Clay and our newest recruits Stuart Laermer and Steve Pakola a thorough grilling and wrote everything down in the prospectus. Then they used that

to approach all kinds of institutional investors. They concluded that if we wanted to raise 35 million, we would have to bring the price down to EUR 4.50. In addition to that, there was a 'green shoe' of 3 million, i.e. existing shares that were sold. No, that low price was not viewed as a failure, since you have to take into account that the exchange rates thwarted the valuation. And in 2006, the good times for this kind of IPO were almost over. This was the middle of 2006, and the disastrous years of 2007 to 2009 were just around the corner. A couple of months after us, Diatos attempted a flotation and failed. Diatos was a brainchild of the now deceased Professor André Trouet of the UC Louvain, a student of Nobel Prize winner Christian de Duve. We recently dug up one of his programs in our new biotech mini-company CoBioRes (see later). But in relative terms our IPO was therefore a success."

At KBC Securities, the IPO was the work of Pieter Hofman, Koen Dejonckheere (now CEO of the investment company Gimv) and Koen Hoffman. Koen Hoffman had been director and CEO of KBC Securities for 18 years and, in 2016, became CEO and co-owner of the Ghent asset management company Value Square. He remembers the ThromboGenics IPO very well and proudly displays two commemorative plaques for the IPO and for the subsequent capital increase. "We got to know Désiré Collen through the university in a period that was very difficult from a stock market point of view. The stock exchanges were still assimilating the technology bubble that marked the early years of the century. In March 2004, the Belgian phone company Belgacom had gone public and in 2006 ThromboGenics was the first new, shall we say 'technological' dossier. Initially we were going to do it in conjunction with a Japanese firm – Nomura Securities, I think it was –but they pulled out," says Koen Hoffman, who clearly held Collen in great esteem from the outset. "We had talked to Collen at length, and thereupon a factor played a part that would not normally be relevant: we felt an affinity with this man. He had something of a paternal attitude, and the history of his previous medicine was obviously impressive. We then approached Yvan Huybrechts who ran the KBC fund Pricos, asking whether there would be institutional interest in this kind of IPO. The answer was cautiously positive. Then, completely out of the blue, Nomura withdrew. I was driving home near the KBC offices on Havenlaan in Brussels when my colleague Pieter Hofman called me. Koen, he said, Nomura doesn't see it working and we're on our own. We have to decide today whether we're going to carry on." Koen Hoffman immediately made a U-turn, but the excitement and adrenalin were too much. "I crashed into the back of a car with a Moroccan driver. In that immigrant neighborhood I was immediately surrounded by a crowd of people, but we sorted things out perfectly OK. In any case, I didn't dispute the fact that I had been at fault. At KBC we then decided to press on our own. Désiré personally convinced our sales staff. 'I might also be able to secure funds in other ways,' Désiré said, 'but I want to raise that money from the public. I want to involve private investors in it. My wife tells me I 'm crazy but that is what I want to do. I don't need the money myself, but I'm also doing it because it's the right thing.' He gave an illustrative and comprehensible five-minute explanation of the problem at the back of the eye that he was going to try to find a solution for. We knew what it was about and could explain it to other people ourselves. We only had a problem with the British CFO of ThromboGenics, but we subsequently helped Désiré to solve that." (16)

ThromboGenics did have to set its sights lower in order to raise EUR 35 million. The amount was lowered, the price was lowered, and the sale of existing shares, the '*green shoe'*, remained limited. "That IPO was nonetheless actually quite successful," says Koen Hoffman,

“considering the stock exchange was really against it at the time. OK, we only raised EUR 35 million, although that is well in excess of a billion Belgian francs, and existing shares managed to be placed to the tune of only 3 million. The price was certainly on the low side, but we proved that money could also be found in Flanders for what was after all a very risky initiative: for a company that was virtually penniless and that was still a long way from having a product. By far the bulk of the shares went to Belgian investors, not so much to small private investors – in fact they tended to be rather against it – but KBC Asset Management, other funds, dozens of Flemish families and asset managers subscribed to the lion’s share of that 35 million. We looked for wealthy parties, and many invested between half a million and a million. Pieter Hofman really mobilized his staff and his network to that end, and that was that much easier with ‘grandpa’ Collen in the background. Believe me, ThromboGenics on Euronext really was an iconic transaction. It would no longer be possible today. The government decided to tax capital gains in a company, so nobody will still be prepared to take the risk of incurring capital losses.”

The IPO was celebrated in Collen’s own inimitable way. “The day after the placement, Désiré turned up at the Havenlaan in Brussels totally unexpected, carrying an icebox full of chilled champagne for the staff of KBC Securities,” recalls Hoffman. They had never experienced anything like it. And the closing dinner, the party that’s organized after every IPO, was held in the prestigious Rockox house in Antwerp. “I had had a few bottles of Léoville-las Casas, one of the best Bordeaux reds, brought up from KBC’s cellars,” Hoffman remembers. “They were standing uncorked and breathing on the table, when I suddenly saw Désiré come in with two Magnums of Chateau Clinet 1990, a Pomerol of the highest quality. All the bottles were emptied that evening, which was perhaps not entirely justified. I have experienced many IPOs and supervised many capital increases, but I have never seen a company manager coming to personally thank not just the bosses but also the staff, when it’s done. That’s what Désiré did. Chris Buyse has since picked that up from him. That relationship was maintained until around 2012, when ThromboGenics made strategic choices that many investors were doubtful about.”

Collen’s team

At the end of 2006, an authoritative board of directors was thus in place, headed by Chairman and CEO Désiré Collen with his exceptional track record, although the management and team were also outstanding. More than half of the members of staff held PhDs and master’s degrees. Four people were to report to Collen: CFO Andrew Guise, CBO (Chief Business Officer) Stuart Laermer, CMO (Chief Medical Officer) Steve Pakola and R&D Manager Jean-Marie Stassen. The latter was an old friend of Collen’s with a few joint publications on t-PA and staphylokinase.

Stassen boasted a phenomenal career path. “He was a laboratory technician, a son of a pig farmer from Sint-Truiden,” Collen explains. “He worked with us in the lab and was very skilful with laboratory animal models. He did all the surgery for us, on baboons too. But he wanted to obtain a doctorate. ‘I’m as good as all those people here who’ve got one!’ But back then someone with a laboratory technician’s diploma couldn’t pursue a doctorate in Belgium. On my recommendation, he then went to the Umeå University in Northern Sweden and proceeded to do his doctoral degree there, largely on work he had done here at the university in Leuven. So, he obtained a PhD, and became a Doctor, albeit a Swedish

Doctor." Stassen was important in the development of staphylokinase. "After that Stassen went to work at Boehringer Ingelheim for a few years, but when he wanted to come back he could immediately resume working with us," Collen adds.

An important development was the reinforcement that occurred from August 2006 onwards with Chris Buyse taking the place of Andrew Guise. In October he was co-opted director and, from May 2007 onwards, he bore the title of CFO. "Clay enlisted Andrew Guise as a financial man, in part because he also had experience with IPOs. Guise had a chemical background but had become an investment banker at Clay's East Hill venture. I went on the road with Andrew Guise," says Collen. "In Belgium and in England, anywhere where they were prepared to listen. The largest presentation took place here in Leuven at KBC in the former Cera building, which was attended by quite a throng of people. But then the misery quickly set in. Andrew Guise was not a licensed CFO in Belgium and therefore wanted to become CEO, but Stuart Laermer, our recently recruited rights specialist, and Steve Pakola, the father of our eye research, didn't see that working. We had to separate from Guise. Fortunately, Chris Buyse was free. In Ghent he had prepared the stock market listing of Crop Design, which was however sold to BASF. As he preferred not to go and work for BASF, he came along to listen to the presentation at KBC, and that's where I made his acquaintance. We then went to have a meal at the Voltaire restaurant in Leuven and a quarter of an hour later he was hired. That was one of the best recruitments I've been responsible for in my life, along with Steve Pakola as CMO and Peter Carmeliet as a scientist."

Buyse embraced the chance Collen offered him. He flawlessly carried through the complex merger of ThromboGenics Ireland, Thromb-X NV that was still in existence at that point, and the Belgian company. When he took office as CFO in 2007, he was able, thanks to KBC Securities, to immediately present a EUR 23.9 million private placement of new shares, outside the pre-emption right of the existing shareholders. The new shareholders paid EUR 10.80 per share, which was more than twice the price at which ThromboGenics had gone public ten months earlier. The company had clearly built up a certain reputation with investors in that short space of time. Those who had missed out on the IPO were evidently now keen to get on board quickly.

In 2009 Chris Buyse completed the cross-border merger with Ireland, and the company moved from Gasthuisberg to the new '*bio-incubator'* premises on Gaston Geenslaan in Leuven. In the middle of November 2009, ThromboGenics went to the market a second time. Once again it was an operation outside the pre-emption right, meaning that the existing shareholders were not addressed, and their stake was thus diluted. KBC Securities, Petercam and Jefferies International raised EUR 42.3 million (41 million after costs) at EUR 16 with 2.6 million shares. In 2010 a further EUR 53.9 million was added. And in April 2012 ThromboGenics was to refuel with capital for the last time. With a private placement at EUR 24, the company raised no less than EUR 77.8 million. After its public flotation in 2006 the company thus attracted EUR 196 million net in extra capital through four placements. ThromboGenics clearly had come into favor with investors.

Conflict of interests

A point that attracted particular attention from the outset was the position of Chairman, CEO and significant shareholder Désiré Collen. He was of course indirectly shareholder by means

of Biggar, of which he was the ‘enforcer’, not the owner. But this nuance did not prevent Collen from wearing many hats at ThromboGenics – too many to escape the suspicion of a conflict of interests. On top of that he had indicated from as early as the flotation that Biggar wanted in all respects to reduce its stake in the company. Admittedly all the ‘Irish’ shareholders had promised not to sell any shares in the first year, but this was a ‘soft lock-up’, i.e. one with loopholes.

Conflict of interests in companies is strictly regulated in Belgium by Article 523 of the Companies Code. A conflicting interest must be reported by the party concerned itself, and in the case of listed companies the director concerned is then barred from taking part in the board of directors’ deliberations. ThromboGenics’ independent directors were clearly aware that this called for close scrutiny, with the result that every step Collen and the other directors/shareholders took was legally safeguarded. Such was the case straight away in May 2007 when Biggar asked to convert registered shares into bearer shares, with a view to selling them. After advice and deliberation, it was decided to convert a maximum of 2,952,487 shares, of which 2,903,248 were held by Biggar.

When the other executive directors (Chris Buyse and the later CEO Patrik De Haes and also Désiré Collen after he had stepped down as CEO in 2008) were subsequently allotted warrants, the question of conflict of interests arose each time. The exercising of warrants in new shares leads to a dilution of the existing shareholders’ position, and that has to be justified. Warrants may not cost the company anything, on the contrary when exercised they add capital, but exercising them at a price below the share price does indirectly cost the shareholders money. So, in each case the board of directors obtained external legal advice that was presented at the shareholders’ meeting.

“Warrants are a good instrument,” reckons Collen. “I had discovered that at Genentech. That company didn’t have much money in the beginning, and they largely paid their staff with options and warrants. At ThromboGenics in Ireland I had never taken part in warrant plans until Clay demanded his ‘springing warrants’ with an exercise price of EUR 0.05 and I could only safeguard my position as shareholder by also accepting warrants. I then had to exercise them at a high price of EUR 6.35. In Belgium I didn’t take part initially either in 2006 or 2008, until 105,000 warrants were left over in 2010 from the first warrant plan. We allotted these and immediately exercised them, so that some extra cash came into the company. That was at between EUR 8 and 11. I took part in that. And in the next two warrant plans, I was allotted as many as the operational managers, but only the first one was ever exercised.”

“The tax treatment of capital gains from warrants is unclear,” Buyse concludes. “The laws are being tinkered with again now, but it was and still is an instrument with little legal certainty. For options, things are clearly regulated, but you can only allot options to existing shares that you yourself as a company have bought. So, options are only possible if you do not need capital, since options do not lead to a capital increase. But when it comes to warrants, we find ourselves in a grey area. A lot depends on whether you receive these rights as an employee or in your management company. We encountered problems primarily with the warrants in our personal companies. The length of the plans is also important, as is the moment at which you exercise the rights and particularly the moment at which you subsequently sell the shares you have acquired. If you do so within a year, you run the risk of the capital gains being considered as taxable income. These days this is interpreted on a

somewhat hit-and-miss basis in listed companies that make use of it. You have five-, seven- and even ten-year plans. You also have conditional warrants that are only allotted if you achieve certain objectives at a particular time. My advice to any company wanting to use warrants would be to arrange for a tax ruling beforehand and not to deviate from the agreed terms on any account."

"I had a lot of problems with that," sighs Collen. "Despite having made sure I had been comprehensively advised. Indeed, the 95,000 warrants that were allocated to my management company Patcobel NV in 2010 were exercised 'cashless', by selling 53,000 of my existing shares and exercising the warrants with the proceeds thereof. The remaining 42,000 shares after creation of the new shares, with a market value of EUR 20 per share, were brought in into Anpech BM, our civil society under Belgian law, in exchange for 840 Anpech shares with a value of EUR 1,000 per share, that were issued to Patcobel NV on 3 October 2010. These ThromboGenics shares were left untouched in the Anpech BM portfolio since then and reported in the annual accounts. However, an accounting error was made in that these shares should have been consolidated into the annual accounts of Patcobel NV, which was overlooked. When this was brought to my attention by our new accountant in May 2013, I immediately sought the advice of top fiscal experts in Belgium who submitted a file for 'regularization' on an anonymous basis to the Special Tax Inspection (Bijzondere Belastings Inspectie, BBI) in June 2013. While the responsible civil servant initially fixed a date for a meeting with my tax advisors, he subsequently cancelled the appointment without much explanation. My fiscal advisors were surprised and embarrassed to the extent that they even reduced their professional fees by 50%. However, a plan B had to be elaborated. A settlement statement was drafted in June and signed in December 2013 in which the 42,000 ThromboGenics shares were returned to Patcobel NV in exchange for the 840 Anpech BM shares. The ThromboGenics shares were henceforth integrated in the annual accounts of Patcobel, together with the tax due on warrants. In 2014 the BBI executed a control of the ThromboGenics warrant plans (unaware of my previous attempt at regularization or not?). The 42,000 warrants of Patcobel were reclassified as a loan from Patcobel to me personally as CEO during 2010-2013, on which I had to pay interest to Patcobel of 8 to 10 percent annually (a total of EUR 270,000 including fines and interest), and Patcobel had to pay around EUR 116,000 taxes (company tax plus fine plus interest) on that fictitious interest income.

I also made shares available to KBC three times in order to enable the employees to exercise their warrants 'cashless'. Those shares were sold and with the proceeds the new shares were created, and the employees exercised their warrants. Once the operation was over, I got back my original shares and the increase in value was pocketed by the employees. But in each case KBC sold more shares than was necessary as a buffer against share price fluctuation and paid me an amount in cash to cover the difference between the borrowed shares and the shares used to create the warrants. For the tax office, that was also unacceptable; they enforced the principle last-in-first-out; and saddled me with a tax bill of EUR 34,000 for the 2010 warrant plan. My fiscal experts advised me to accept this settlement in order to avoid a potential retroactive similar fine for the 2006 and 2008 warrant plans which would have cost me over EUR 100,000.

Because of such 'highwayman-attitudes' of the fiscal authorities in Belgium, I have come to the conclusion that it is probably wise to further reduce the activities of Patcobel NV and

Anpech BM to a bare minimum. Anyway, as I have meanwhile become a permanent resident of the UK, domiciled in London, such gimmicks will no longer be possible."

(NB: This italic section was elaborated in December 2018 by Désiré Collen)

Year	Total number (actually granted)	Patcobel nv	Sofia bvba	ViBio bvba	Exercise prices (EUR)
		Désiré Collen	Chris Buyse	Patrik De Haes	
2006	500,000 granted in 2010 (499,000)	0 35,000	50,000 35,000	42,000 35,000	4.91-11.12
2008	450,000 (380,667)	0	55,000	60,000	8.07-11.09
2010	600,000 (474,000)	60,000	60,000	60,000	15.49-19.97
2011	516,000 None exercised (516,000)	72,000	72,000	72,000	16.8-37.59

With these warrants, ThromboGenics' three key figures and the management, employees and consultants received an extra remuneration. For a period of usually three years, they could use warrants to subscribe to new shares at the average closing price over the 30 days prior to the warrants being granted. The exercising of these warrants therefore amounts to a capital increase and takes place in the framework of the authorized capital – the margin the board of directors has at its disposal to increase the capital. When the warrants are exercised, the company thus receives extra capital and the managers can redeem the capital gain, i.e. the difference between the warrant exercise price and the market price, for cash on the stock exchange. Obviously when the share price falls to below the exercise price, the warrants become worthless, which is what happened to the warrant plan of 2011. In addition to these warrants, 500,000 warrants were taken over from the Irish ThromboGenics. Their exercise prices were mostly well below the Belgian company's share price.

Of course, warrants lead to a limited dilution for shareholders. “Some shareholders simply don’t want to hear of it at all,” says Collen. “For example, biotech investor Rudi Mariën, together with Guy Ullens of the former Tienen Sugar family, rejected the last warrant plan in 2013 at the extraordinary general meeting before notary-public Liesse. In all, 75% of the votes are needed to approve a plan and we didn’t reach that. They wanted ThromboGenics to be sold as quickly as possible.”

“It has to be said that that vote was a setback for the company,” adds Buyse. “Because the existing warrant plans had become meaningless due to the fall in the share price, a new retention plan was needed if we wanted to retain our best employees. In hindsight, Mariën’s and Ullens’ action in 2013 was however a blessing, since the warrants would never have ‘come into the money’, i.e. with a share price above the exercise price, and the liberating up-front tax on these warrants would have been lost.”

Reinforcement for Collen

But the holding of three positions simultaneously by one person, even if that person is called Désiré Collen, goes against good governance, which prompted Collen to hand over his role as CEO in 2008. Landon Clay had already clipped his wings to some extent in Ireland, but now Collen had thousands of investors upstream who were represented on the board by directors and in particular by independent directors. So, there had to be professionalization downstream in the management. Immediately after the flotation, he hired Chris Buyse, an experienced and competent senior financial executive. Now all that was left for him to do was to find operational reinforcement.

For the time being, Collen retained his position as CEO but was supported from February 2007 onwards by a COO (Chief Operational Officer), a post filled by Patrik De Haes, a seasoned pharmaceutical specialist in his late forties who had international experience. They worked well together. In August 2008 Collen, who was to attain emeritus status as Professor in October that year, appointed De Haes to the post of CEO. In retrospect, Collen considers giving his unconditional trust to De Haes the biggest mistake of his whole career.

From then on, ThromboGenics was to observe the generally accepted corporate governance rule that the posts of Chairman and CEO are best not held simultaneously by the same person. De Haes’s appointment was greeted with approval in investor circles. International investors are indeed bold enough to vote against proposals at a general meeting if they disagree with the way the company is run.

> Patrik De Haes had 20 years’ experience in Switzerland and the United States when he decided to return to Belgium for family reasons, and thus ended up at ThromboGenics. Prior to this he had worked for the Swiss pharmaceutical companies Roche and Sandoz, among others. De Haes is a Doctor of Medicine of KU Leuven and obtained an MBA at St Thomas University, Minneapolis, USA. At ThromboGenics he quickly revealed himself to be a professional manager who taught the company to think in terms of projects, with budgets and schedules. The old ‘departments’ were restructured and much academic latitude restricted.

In April 2009, Patrik De Haes was confirmed by the general meeting to the post of CEO. That year Collen also reflected on his life in his book ‘A heart for research and enterprise’, in

which he writes: “ThromboGenics is well on the way to developing into one of the most promising biotech companies in Europe. The company’s strength lies in its team, which over the last few years and months has managed to record excellent progress with its clinical pipeline and conclude a major licensing agreement with Roche, and is successfully looking for new products. ThromboGenics’ success is undoubtedly based on its excellent scientific knowledge, rich academic culture and in-house expertise. All these aspects are of international stature at ThromboGenics, and constitute a source of great satisfaction for me as founder of the company.” (17)

Collen’s considerable satisfaction was justifiable. Between 2007 and 2012, ThromboGenics’ research team and management delivered some exemplary work. The management of the teams in the labs, the preparation and supervision of the applications addressed to the regulatory bodies, the increase in capital and cash reserves, this was all down to efficiency and professional skill. Via the stock exchange, the world looked on in admiration. Belgium, Flanders and its media were proud as punch. Désiré’s eventful life and works were fodder for the newspapers and magazines, long before *top doctors* became celebrities.

Seven years of plenty

ThromboGenics’ success in the first seven years was impressive. Between 2006 and 2012, a substantial profit was posted on two occasions. On balance a loss of only EUR 34 million was recorded over those six and a half years – in other words more or less the amount that had been raised with the IPO in 2006. ThromboGenics’ capital and reserves increased more than sixfold in this period, from EUR 35 million in 2006 to 228 million in 2012. Cash in hand increased fivefold to EUR 194 million. And the expectation value, i.e. the amount investors are prepared to pay over and above the book value, increased fivefold between 2006 and 2012. Investors’ belief in ThromboGenics verged on the euphoric.

In 2007, new investors were quick to acquire a shareholding: the Dutch Global Opportunities fund with a stake of 4.7% and a holding company owned by the Dutch Van Herk family with 3.92%. Collen held 2.7%, Biggar 18%, Clay 10% and KBC Asset Management 5.7%. And as promised, the company dumped staphylokinase in 2007; the technology for the production was transferred to the Indian company Bharat Biotech, and the Egyptian firm Rhein Minapharm obtained a licence. It was a matter of waiting and seeing what would come of it. There was cause for celebration with the publication of an article in the scientific journal *Cell* on the potential of a new generation of angiogenesis inhibitors (vascular cell reproduction suppressants) that Peter Carmeliet had tested in pre-clinical models. (18) Did TB-403, the anti-PlGF remedy of ThromboGenics, have the potential to be a blockbuster, as had been suggested in the prospectus?

ThromboGenics	Year	2006	2007	2008	2009	2010	2011	2012
	EUR million							
Turnover		3.20	1.50	30.40	4.20	6.18	2.48	75.11
	Real							
	Paid in advance			30.30	3.50	6.00	2.40	75.04
Profit/Loss		-10.10	-15.90	12.09	-14.00	-13.94	-21.64	30.42
Capital and reserves		35.27	48.43	62.40	93.70	138.19	118.03	227.97
	Increase (exc. warrants)		*23.90*		*41.00*	*53.90*		*77.18*
Intangible Assets and Goodwill		2.58	2.58	4.68	19.88	28.42	39.61	74.92
Cash (inc. investments)		34.18	46.81	58.87	76.64	116.10	88.05	148.23
Number of shares		22,140,305	23,935,960	25,641,020	29,059,567	32,446,757	33,346,409	36,008,391
Capital/shares (book value):		1.59	2.02	2.43	3.22	4.26	3.54	6.33
Cash/shares		1.54	1.96	2.30	2.64	3.58	2.64	4.12
Share price last week of December		10.00	8.09	8.21	16.03	22.58	18.34	46.42
Expectation value		8.41	6.07	5.78	12.81	18.32	14.80	40.09

The first annual report remarkably contains the contributions of no fewer than six Leuven Professors. Peter Carmeliet, for instance, outlines the importance of TB-403, his antiplacental growth factor (anti-PlGF) that could possibly be used to combat the uncontrolled formation of blood vessels in cases of cancer, and could therefore curb metastases. ThromboGenics developed this together with the Swedish company BioInvent (40%). Ophthalmologist Peter Stalmans and neurologist Vincent Thijs had their say about microplasmin. Marc Jaquemin and Peter Verhamme gave a brief exposé on TB-402, and Kathleen Freson pointed to a new avenue that ThromboGenics was going to explore, concerning a drug to combat the reduction in blood platelets during chemical and radiation treatment of cancers, referred to as thrombocytopenia.

This was truly the translational ThromboGenics that Collen had dreamed of. For Collen the company had to become the breeding ground into which Leuven's university and the VIB could channel promising medical ideas. Collen knew only too well that you can only find if you seek; and, if you conduct research, you very often discover things you had not been looking for. ThromboGenics had to be a result-driven company, but also had to stay close to an academic biotope where many more questions arise than for which there are answers. From a medical viewpoint, Leuven already had the Rega Institute, but there was certainly room for ThromboGenics, in cooperation with the Flemish Institute for Biotechnology, the

VIB, from which Leuven had nonetheless maintained a certain distance, at the time. A proud board of directors proclaimed at the beginning of 2008: “We are pleased to report that ThromboGenics is thriving. Both our product portfolio and our finances are in good health.”

No crisis, but a big prize

Almost no-one saw it coming, but in 2008 the financial and economic system in the Western world was shaken to its foundations, not an ideal environment for a little greenhouse plant like ThromboGenics. Yet the events of 2008 and 2009 did ThromboGenics no harm as, fortunately, the movements of listed companies do not always tally with stock market movements. In 2008 ThromboGenics’ share price held up at around EUR 8 and therefore escaped the massive crash that affected all stock markets. From August 2008 the Bel20 index lost more than 60% of its value. The malaise lasted until March 2009, from which point ThromboGenics promptly posted a greater increase than that of the Bel20 index. That year, ThromboGenics refueled EUR 41 million in capital. After a blip in 2011, the share price gradually doubled from EUR 20 to EUR 40: a success story that was to last several years.

In the 2008 annual report, CEO Patrik De Haes describes the crisis year in the followingwords: “2008 was a transformational year for ThromboGenics when we started to emerge as one of the leading biotech companies in the world.” Superlatives are not used sparingly: “Excellent scientific heritage, rich academic culture, world class in-house expertise.” The company moved from Gasthuisberg to Gaston Geenslaan. The three executive board members were paid EUR 709,153, i.e. a relatively modest figure, and the other members of the board EUR 79,000. The management additionally exercised 102,000 warrants at EUR 4.91 to EUR 11.05. In July a ‘group of Belgian private investors’ acquired an 8% shareholding in ThromboGenics.

Then ThromboGenics landed a major prize. TB-403, with Carmeliet as the expert in the background, moved into Phase I in January 2008, and in June that same year the Swiss pharmaceutical giant Roche joined in that research. For this strategic alliance, Roche paid EUR 50 million to ThromboGenics (60%) and to the Swedish firm BioInvent (40%). This led to the company’s turnover rocketing from EUR 1.5 million to EUR 30 million, and its result showing a current profit of EUR 10.6 million compared to a loss of EUR 17.4 million in 2007. Earnings per share were plus EUR 0.47 as opposed to minus EUR 0.67 the year before. Incredibly, ThromboGenics was a profitable company just two years after being incorporated!

If TB-403 were ever to lead to a medicine, Roche would pay EUR 450 million for it plus a two-figure percentage on the sales. Carmeliet: “I believe that with TB-403 we are writing a new chapter in the treatment of cancers.” The existing anti-angiogenesis drugs that break down cell formation are not selective, meaning they also curb the production of healthy cells. So, there is a need for more selective anti-angiogenesis, which is precisely what TB-403 could be able to fulfil.

ThromboGenics meanwhile was not resting on its laurels. Désiré Collen confirmed in the annual report that the company was actively looking for new molecules: “I feel confident we will be able to strengthen our research pipeline at the right time with a number of promising new paths.” Patrik De Haes added: “Within 12 months we will be reinforcing our pre-clinical pipeline with cutting-edge biotherapeutics.” Unfortunately, these were premature promises, as would be seen later.

There was further good news in 2008, too. Microplasmin for vitreomacular traction (adhesion of the aqueous humor) was tested in Phase II for six months, with a success rate of 40%, and 30% even within a week of treatment. Perhaps slightly befuddled by the success with TB-403 and Roche, the board of directors decided to take in hand the marketing and sale of the possible medicine for vitreomacular adhesion itself. There was nothing yet, but it had been figured that in the USA it would not even be necessary to visit many eye surgeons. That could be done with a small team. And should ThromboGenics be able to attract other ophthalmological products that were close to commercialization, that would be taken on as well, according to the 2008 annual report.

Focus on the retina

In those initial Phase II studies with microplasmin for problems at the back of the eye, ThromboGenics was clearly taking a very wide aim. This also had to do with a lack of knowledge of the precise nature of the problems that can arise there. Medically speaking, the back of the eye was less familiar territory. Fortunately, a new examination technique emerged around this time.

The new diagnostic technology was SD-OCT, *spectral domain optical coherence tomography*. This enables the retina to be analyzed in 10 layered and very detailed images. What is more, it is a relatively easy infrared technique without ionizing radiation. OCT works better than older techniques such as MRI or ultrasound, with a light source that is burst onto the retina. With OCT, ophthalmologists would distinguish some fifty syndromes from 2009, of which sVMA/VMT was immediately relevant for microplasmin. The acronym stands for *symptomatic vitreomacular adhesion/vitreomacular traction*. This became the ailment that ThromboGenics wanted to tackle with microplasmin in the years thereafter, along with the *macular hole*, a small hole in the yellow spot (the macula) situated in the center of the eye on the retina. ThromboGenics did not give up the other back-of-the-eye syndromes, but gradually referred them to separate paths, particularly after 2013. This was the case primarily for diabetic retinopathy.

“ThromboGenics contributed in large measure to knowledge about the back of the eye,” says Buyse. “It’s impossible to pinpoint exactly what impact the new OCT technology had or to quantify ThromboGenics’ role as a pioneer, but the interaction was certainly important and ThromboGenics can take a lot of credit for it.”

This ambition to heal much more than sVMA obviously also had to do with the market potential of the future medicine. Surgical interventions were carried out chiefly in cases of diabetes-related retinopathy. Imagine that here, too, surgery were to be rendered unnecessary in 44% of cases. Microplasmin would turn into a hit! It might even be effective against DME, also a diabetes-related eye complaint that leads to loss of sight, even in twenty-year-olds. At the end of 2009, ThromboGenics wanted to establish whether this path was worthwhile.

The company’s center of gravity thus seemed to be shifting to ophthalmology, although this was actually not yet the case. The search was on for a research partner for the testing of microplasmin in cerebral arterial thrombosis, or stroke. Apart from a presentation at the World Stroke Congress, however, not a great deal was done on this. In the meantime, there was also little news from the Indians and Egyptians who had taken over staphylokinase,

although, according to Collen himself, ThromboGenics was not particularly insistent, either. The Indians wanted supportive financing for their Phase III trial, but the board would have none of it.

A lot of attention was now being paid to TB-402 (antifactor VIII). This can prevent thrombosis after an operation and in atrial fibrillation, problems that affect millions of patients. Seven million people have to cope with atrial fibrillation in the USA, and if the blood is not circulated effectively enough, this can lead to the formation of clots. Thrombosis after a hip or knee operation is a problem of such magnitude that the number of deaths in the USA is put at 100,000 a year. Phase II of TB-402 with 300 patients had to be completed at the end of 2010. If something were to come of that, the blood thinners market would be open, and one would be looking at potential sales of USD 3.7 billion in 2006 figures. Heparin and warfarin had mainly been used until then, but both treatments caused side effects. The first still incomplete results for TB-402 showed a reduction in venous thrombosis within eight hours in 25% of patients, which was 15% better than the placebo. The annual report has little to say about anti-VPAC, which was designed to keep red blood corpuscles at the right level during radiation treatment. But one can't shout bingo on all fronts.

Into the eye, away from the heart

ThromboGenics had started out in 2006 with the following solemn mission: "The development of innovative biopharmaceutical medicines for blood circulation, in observance of the highest scientific and ethical standards and aimed at the creation of lasting value." From 2009 this mission shifted from the wide-ranging term of 'blood circulation' towards 'the back of the eye', i.e. towards ophthalmology. In January 2009, microplasmin entered into Phase III for VMA (*vitreomacular adhesion* – the adhesion of the aqueous humor to the retina). Patients were recruited and the treatment consisted of an injection in the aqueous humor – "MIVI" or *microplasmin for intravitreal injection*, without surgical intervention. MIVI-TRUST, as the test was called, concerned 652 patients in the United States and Europe.

The results were due to be announced in the middle of 2010, and ThromboGenics expected to be able to take them to the regulators FDA and EMA in the second quarter of 2011. In March, patients were also ready for Phase II in diabetic retinopathy. Meanwhile, a contract had been concluded with a producer – a contract manufacturing organization or CMO – with the first deliveries scheduled in 2010. And at the end of 2009, microplasmin was also renamed ocriplasmin, because the scientific name control services felt that was better.

The other development paths were also faring well. Roche paid a further EUR 5 million for TB-403. An initial test in 23 patients had been positive, meaning there was a possibility of Phase II getting under way in 2010. TB-402, for combating blood-clot formation after an operation, did move into Phase II. A partner was still being sought. The IWT, the Flemish Agency for Innovation through Science and Technology, granted support to the tune of EUR 3.2 million for research into the potential of VPAC1, the remedy designed to counteract the dying off of blood platelets during radiation treatment.

The management was glowing with self-confidence. "ThromboGenics is clearly one of the success stories of Europe's biotech industry. Since our IPO in 2006 we have built up a strong, well-financed company capable of valorizing our attractive pipeline with new

medicines.” Once again it was announced that the aim was to take further ophthalmological products under license, once ocriplasmin was on the market.

In 2007, ophthalmological medicines represented sales of USD 12.5 billion, and this was expected to rise by 6% per annum to 33 billion in 2023. The back of the eye was the fastest growing segment. But ThromboGenics was not the only one to have its eye on this potential. There were already related products for the back of the eye on the market. Novartis’ Lucentis was doing well in this area in terms of sales, as were Roche’s Avastin and Bayer’s and Regeneron’s upcoming Eylea. But the indications were not the same, and the efficiency still less so. With ocriplasmin, so the trade press said, ThromboGenics did indeed potentially have a unique medicine in its hands. After all, there was nothing else as yet for the specific treatment of VMA, vitreomacular adhesion. (19)

Considering the board’s decision to market ocriplasmin itself in the USA, the management worked out how many specialists would have to be lobbied there, a study that appears to stem from the decision taken a year earlier. The study concerned 1,500 to 2,000 retina specialists in the US and 1,500 in Europe, which indeed at first sight seems to be a relatively small and therefore easily reached target group of users. It would later emerge that this group became much larger around that time due to the introduction of the new diagnosis technique OCT, which non-surgical ophthalmologists could also use to diagnose VMA and therefore propose a treatment. This would hamper the commercialization of ocriplasmin in the United States.

Ocriplasmin in the starting blocks

In 2009 and 2010, the amount of funds ThromboGenics raised from the market by far exceeded the losses it sustained. Losses amounted to around EUR 14 million in each of those two years, but in 2009, EUR 43.8 million was raised via a capital increase and EUR 2.8 million through the exercising of warrants. In 2010 a refueling of EUR 53.9 million took place, with a further EUR 3.4 million coming in from warrants. ThromboGenics had around EUR 76 million in cash at its disposal at the end of 2009 and EUR 86 million at the end of 2010. The number of staff members rose to 76, with 66 of these in Leuven. CEO De Haes’ emolument was raised to more than EUR 500,000 in 2010, and would later reach EUR 1 million. As evidenced by the attendance list at the annual meeting, Clay still held over 11% of the shares, Petercam 2.6%, Baker Brothers 5% and Biggar 7.8%. So, the old shareholders appeared to have downsized their positions or had them diluted.

Of course, the tone of the 2010 annual report remains upbeat. In the second quarter of 2011, ThromboGenics planned to go to the regulators with ocriplasmin. The results of the Phase III MVI-TRUST test were published in September 2010: 652 patients had been treated for 20 months, 465 of them with ocriplasmin and 182 with a placebo. Of those 465, 26.4% were cured, with full VMA resolution occurring within 28 days. In a further 23.7% of the patients, their eyesight improved within six months. In contrast, there was only a 10.2% cure rate in the patients who had had a placebo injection. In 3.7% of cases, there were also side effects here. In respect of the FTMH (full thickness macular hole) variant, the results showing a recovery rate of 40% were even better.

At the request of the investment bank Jefferies, market research was also conducted among 50 specialists. The results do not appear in the annual report but were evidently positive

enough. It was revealed that up to 300,000 patients were waiting for ocriplasmin in the United States, and as many as 840,000 worldwide. As ThromboGenics points out in its annual report, there was little competition in sight let alone approved products. The market for diseases and disorders of the eye represented USD 14 billion in 2009, mainly driven by the success of Lucentis as a remedy for combating AMD (age-related macular degeneration).

Another important factor was that “retina specialists are traditionally inclined to use innovative medicines.” Now that they had gained plenty of experience with injections in the aqueous humor, thanks to the other medicines, ocriplasmin could catch on quickly. “Ocri is likely to experience a rapid uptake,” the board of directors said. Ocriplasmin is an exceptional therapy for a problem for which there is no medicine, and prescribing practitioners are receptive to innovation. “This means that ocriplasmin could turn into a commercial success,” the board added. Once again, the company indicated that it also wanted to attract other ophthalmological products.

ThromboGenics also pressed ahead with its other development paths. TB-402 to combat peripheral thrombosis could be better than the existing Lovenox produced by Sanofi-Aventis and underwent Phase II testing on patients. TB-403 was tested for brain cancer and liver cancer. Roche had already paid EUR 65 million for the license.

The deal of the year

Up until 2012 everything evolved as desired. Collen seemed to be on the way to his second medicine, something that few in Belgium had done before him, except of course for Dr Paul Janssen of Janssen Pharmaceutica, and those were easier times. Microplasmin convincingly survived Phase III for the syndrome that would henceforth be known as ‘sVMA’ (*symptomatic vitreomacular adhesion*), after the American regulator included its precise description in the syndromes register. ‘Macular hole’ was added for microplasmin as a clinical picture, but not diabetic retinopathy and still less AMD, age-related macular degeneration. With Fujifilm Corporation it was already agreed in 2011 that they would produce and deliver large amounts. Microplasmin had in the meantime been renamed ocriplasmin, but a genuine brand name was needed with a view to its approval by the American FDA and Europe’s EMA. This ended up being Jetrea®, after the more evident ‘Vitreoclear’ had to be scrapped because a company in Spain owned that name.

In March 2012, Patrik De Haes concluded a major contract with Alcon, the ophthalmological subsidiary of the Swiss pharmaceutical giant Novartis, for the commercialization of microplasmin worldwide, except for the USA. There, a couple of dozens of ThromboGenics’ own specialized sales agents would go on tour with the aim of convincing ocular surgeons. Alcon must really have believed in the product, since it immediately paid EUR 75 million. A further EUR 90 million would be added upon first sales in the US and in Europe, with the possibility of 210 million more, together making 375 million. On top of that, ThromboGenics would receive a royalty on net sales. The trade press talked of 30% and put annual sales at an estimated USD 500 million in the USA alone, and an equivalent amount in Europe. (19) Anyone familiar with these kinds of contracts knows that 30% is a very high percentage, certainly because it comes in addition to the other generous payments. It could be that in so doing, Alcon/Novartis was already taking account of a subsequent acquisition of

ThromboGenics or at least of Jetrea®. For the purchaser, the agreed fees would then be deducted from the amount offered. In such cases, the buyer is buying its own turnover. “But an offer was never made,” Buyse and Collen have confirmed.

Half a billion in turnover twice over, means you have a blockbuster on your hands! And if you have a blockbuster, a takeover bid is a probability, despite the relatively high selling price of USD 3,950 for 125 micrograms of Jetrea® and despite the relatively short term of the patent on microplasmin (which expires in 2023) and also without taking account of the field of application being extended to cover two other syndromes. For its deal with Alcon/Novartis, ThromboGenics landed the *SCRIP Licensing Deal of the Year Award*. Presentation of the SCRIP awards is an annual event staged by the communication agency Informa, that always elicits great expectation in the sector. Désiré Collen collected the award in London. In August 2012, the test results for Jetrea® were published in the *New England Journal of Medicine*, a medical journal devoted exclusively to breakthroughs in the field of medicine. In a nutshell, ThromboGenics performs phenomenal work. For this path in an academic environment, one would receive a *summa cum laude*.

In October 2012, Patrik De Haes is quoted as saying in *De Tijd*: “Analysts are predicting that at its peak the medicine could bring in hundreds of millions of EUR a year, but actually that’s still guesswork. Give us a year and then we’ll be able to make a realistic forecast. And don’t forget that we’re still busy testing whether the remedy could be useful for eye disorders in diabetes patients. If that proves successful, we would be looking at several hundred million more.” No investor wants to miss out on a goldmine like that! (20) In 2013 De Haes was chosen by the readers of the magazine *Trends* as one of the 12 candidates for the title of Manager of the Year.

10 out of 10 with the FDA

The FDA agreed to a priority review of the file. Wednesday, 17 October 2012, was D-day. The FDA would return its verdict at around 11 p.m. Belgian time. First the US regulator asked for more information. Would this entail the decision being deferred by six months after all, which was the ‘*plan B’* for ThromboGenics? ThromboGenics’ managers sat down to eat at the Leuven fish restaurant Beluga, BlackBerry phones in hand. “What made it even more stressful was the fact that we were being swamped with text messages and e-mails. Did we know anything yet? Was there white smoke? We also received e-mails from smaller investors who, much to our surprise, were really caught up in the excitement of it all. It seemed as though the whole of Flanders was on watch for that one piece of news,” recounted Patrik De Haes in *De Tijd*. The situation was saved when at 2.30 a.m. the answer in the affirmative was received.

“Yes, we were asleep when the news came,” admits Chris Buyse. But much more important than the decision of 16-17 October was the Drugs Advisory Committee hearing on 17 July 2012 in Washington. Just as had occurred at the time with t-PA, a new drug is always first assessed by a panel. This takes place in a public auditorium where some 120 people are in attendance. The panel listens to an introductory exposé of no more than 35 minutes and then to the questions that are posed by the FDA’s regulators, among others. In this process a representative of the company goes up to the podium on each occasion to answer the

question. “We had delegated those answers to well-prepared people on our team,” says Buyse. “We ourselves didn’t say anything, and were not expected to, either. This kind of panel consists of scientists, but the chairman of the association for the blind was also on it, as was an ethics expert, and so on – a total of 10 people. We had hundreds of slides ready with answers to all possible questions, and one of our staff members oversaw the structure of that huge file. So that person knew: now we need slide no. 121. Underpinning that were the 70,000 pages of the file. Witnesses were also called, including a patient of ours. A hearing like this is a well-oiled machine that lasts two hours. Then there is a vote in real time on whether the benefits outweigh the disadvantages and risks. The vote in our case was 10 against 0. Everyone was in favor!” In theory, that advice is then followed by the FDA but the FDA has three months in which to arrive at a verdict, which led to extra tension, according to Buyse: “17 October was therefore cutting it very fine.” In the meantime, *De Tijd* rightly cheered ThromboGenics as “the first Belgian company to have wholly independently developed and financed a biotech drug and brought it onto the market worldwide.” Admittedly this did not prompt the Belgian social security system ever to reimburse the costs of the medicine. But the share price did rocket to EUR 27 at the end of July 2012.

At the end of October 2012, more than half of ThromboGenics’ shares were held by American investors, despite the company not being listed on an American stock exchange. What was the reason for this huge interest among investors in the US, *De Tijd* asked Patrik De Haes. “The doors always stayed open for us in the United States. Why? We promised and we delivered. That’s quite simple. But the moment you let people down once, the door shuts in your face. And you’re back to square one,” he replied. ThromboGenics did, however, still have to deliver the goods at that point.

Not everything ThromboGenics touched in 2012 turned to gold. At the beginning of the year Roche pulled out. After EUR 42 million in advance payments to ThromboGenics and 20 million to BioInvent, Roche dumped TB-403. That decision terminated potential milestones of USD 450 million, 60% of which would have been for ThromboGenics! The rights and even the results were returned to the company. ThromboGenics and its partner BioInvent announced that they would look into whether TB-403 might be relevant for the problems at the back of the eye.

“Roche had Avastin and that worked,” says Collen. “But the issue was whether there was any synergy with our product. That would then have led to a combination drug. At the beginning, that hypothesis was reasonably hopeful, but in the end, they found that the costs were no longer justified. There wasn’t enough of a synergy. Roche worked on that with a good 50 people for three years. When they pulled the plug on it, ThromboGenics got back many grams of material and all the discoveries and rights.”

The story came to an end for TB-402 as well. In Collen’s view, ThromboGenics had not tested it correctly in hip operations, with administration too soon after the intervention – resulting in hemorrhages. That was a blow, and the board came to the conclusion that continuing to devote time and money to this was not worth the while. Collen was not happy with the study or with this decision, but amidst the euphoria surrounding Jetrea® there was no grieving over this loss. ThromboGenics put a stop to one of the seven development paths it had started out with – antifactor VIII.

ThromboGenics' share price skyrocketed in 2012 to EUR 45. The company was in celebratory mood. Buyse was named CFO of the Year, and De Haes and Buyse were presented by Véronique Goossens in the weekly *Knack* (21) as 'the odd management', because they were exuberant during interviews and would even fall about laughing. Buyse: "We called ourselves *les rigolos de Thrombo*" [the Thrombo wags]. And De Haes: "We're very thrifty. We have a corporate jet, but we don't use it much." *Knack* talks of "a Flemish story that cannot be told often enough. It provides inspiration for the scientific world and the business world and the combination of the two." Expectations were running high, and the financial analysts were outdoing each other in optimism. One had his sights on a share price of EUR 75! A gold rush was under way on the stock market. Quite a few inexperienced investors suddenly discovered the Leuven miracle, and nobody tempered the expectations; nobody spoiled the party.

ThromboGenics' total market value exceeded EUR 1.5 billion. This meant the share met the conditions for listing on the Bel20 index, a quotation which indeed occurred in March 2013. ThromboGenics was the first medical company of stature in the Bel20 since the likewise listed UCB, the Belgian pharmaceutical group that came into being after various mergers in 1928. Collen was a fêted man. He was presented with the *Lifetime Achievement Award* by Scrip and by BelCham, the Belgian-American Chamber of Commerce. At the beginning of 2014, director Staf Van Reet wrote in the 2013 annual report that Collen's contribution to ThromboGenics and to medical science "cannot be overestimated". Collen had no regrets about having reinvested EUR 71 million in t-PA royalties into research. He was celebrating the second major success of his life! Or the third, since staphylokinase was also a terrific remedy! It's just that it never became a medicine.

Chapter 2: Collen overboard

To: Dr. Staf Van Reet
Chairman Nomination and Remuneration Committee
ThromboGenics NV

London, November 1, 2013

Mr. Chairman

Herewith I wish to terminate my (via Patcobel NV) relationship with ThromboGenics NV. This termination could formally take one of two forms:

1. A resignation because of lack of confidence expressed by a consortium of shareholders with respect to our 2013 Warrant Plan and a lack of support by the Board for the response proposed by its Chairman.
2. A retirement due to a change in status from Belgian resident ("verblijfhouder") to UK domiciled resident with a permanent home in London.

I propose that we put this termination issue formally on the agenda of the next Board meeting in Iselin, NJ on December 15, 2013. This will give the Board ample time to elaborate a succession and presumably prepare a press release. At that time my resignation/retirement as Chairman of ThromboGenics Inc could be simultaneously be handled.

I indeed believe that it is better that I leave now walking out through the front door before my situation deteriorates further.

Best regards

Désiré Collen

Genentech farewell party of the Heyneker team to Collen.
In front, left to right: Jane Henner, Désiré Collen, Greg Gray and Holly Lipetzky.
At the back, left to right: Bill Holmes, Herb Heyneker, Mike Rye and Mark Matteucci.

Summary. *Pride goes before a fall. Collen the academic, clashed with De Haes, the manager. Chairman and Founder Désiré Collen no longer recognized his company and stepped down. Chris Buyse also had enough of ThromboGenics. And then that one promising bird proved to have Icarus wings: Jetrea® flopped. Fortunately, in the wake of the euphoria surrounding the discovery, ThromboGenics had raised a lot of money, with which the company could persevere in its new ophthalmological strategy... stubbornly, in Collen's view. But perhaps the research paths could yet meet expectations, and the Icarus would turn into a Phoenix. We'll see, said the blind man.*

Tu quoque Brute!

The Tarpeian Rock lies in the immediate vicinity of the Roman Capitol! The rock from which alleged Roman traitors were hurled was conveniently close to the place where the greatest glory was to be reaped. Against a background of an industrial jubilee, a genuine tragedy was playing out in the senior ranks of ThromboGenics at the end of 2012 and in 2013. Jean-Luc Dehaene and Landon Clay felt the time was ripe to hand over the torch. Dehaene resigned on 5 March 2012, but the general meeting re-elected him to the post of director in May, albeit for two years. Prior to this, in July 2011, Landon Clay had been succeeded as director by his son Thomas. At 26, Thomas Clay became the youngest ever director of a listed Belgian company, but his father's shoes proved large for him to fill.

To avoid inheritance problems stemming from too large a gift, Collen, who was now 69, moved from Herent near Leuven to London. Belgian law does not allow putting one's heirs at a disadvantage by giving away a disproportionately large amount of one's estate – something that Collen had done by virtue of his donations in the Collen Charitable Trust, Biggar and Thromb-X. British legislation is more flexible in this respect. The move officially took place in April 2012.

Collen had already handed over most operational responsibility to Patrik De Haes in 2008 but did continue to keep a grip on the company. He wanted to fulfil his role by means of an '*Office of the Chairman'*, which would ordinarily take the form of a fortnightly meeting of the Chairman with the CEO and the CFO. This 'committee' was also formally included in the company's governance code in 2010. The meeting had to have a very specific task and minutes had to be taken. All major issues in the field of human capital, intellectual rights, financial operations and collaborative partnerships had to be discussed at these sessions. Decisions falling under the board of directors' remit were prepared here.

CEO De Haes never contributed actively to this committee and from the middle of 2012 onwards he decided to report exclusively to the board of directors. A breach of trust arose with the Chairman. The Office of the Chairman was removed from the company's governance code. The Chairman-Founder of the company felt he was being maneuvered aside and the board failed to blow the whistle on De Haes.

At first sight, the Office of the Chairman was an intelligent structure for a company like ThromboGenics, which was after all the brainchild of its Chairman-Founder. Collen's wide-ranging experience, network and nose for opportunities and risks were institutionalized, as it were. After all, hadn't the company been started up with Collen's money and with substances

that he had discovered together with colleagues of his? In those fortnightly meetings, the CEO and CFO would have in the Chairman a sounding board for the many decisions that would have to be taken by the company's senior management. As it happens, in addition to the audit committee and the nomination and remuneration committee, many companies appear to have room for a committee that prepares the board's decisions. The fact that the Belgian statutory Corporate Governance Code did not make provision for this is a formalistic point. In this case there seemed to be numerous arguments for departing from the code, something which for that matter is permissible under the law.

"It was our sincere intention to give the CEO our backing and endorsement," says Buyse. "But Patrik De Haes took it as a threat on his position and a sign of distrust. As CEO I would even have asked for it. Financial ambitions may also have played a part," thinks Buyse. "Whatever the case, the board of directors didn't regard an administrative oddity such as this as being acceptable. What's more, the problem of De Haes and Collen had already been festering for at least a year. In November 2012 it came to the surface, but not a single director, not even Jean-Luc Dehaene, called for a stop to the animosity between the two until November 2013 when Désiré tendered his resignation."

"Jean-Luc Dehaene really helped us," acknowledges Collen. "He knew his stuff, but in early 2013 it was clear that I was going to lose and in situations like that Dehaene was a political realist. He didn't step into the breach for me and went along with the majority."

"Dehaene had Dexia, a capsized Belgo-French bank, landed on his plate in October 2008," explains Buyse, in an attempt to put a good face on it. "He then became chairman of what he himself called 'a gigantic hedge fund', which needed no less than EUR 90 billion in state guarantees. That was an enormous responsibility for someone approaching the age of 70. And he was saying farewell at ThromboGenics. In 2010 his directorship had been extended by just two years, and then in 2012, after much insistence, by a further two years. Dehaene was the only person on the board of directors who could have defused the conflict between Collen and De Haes, but he didn't. When Collen tendered his resignation, Dehaene, as oldest board member, even became Chairman of ThromboGenics for a few minutes until Staf Van Reet filled the post, although as Van Reet said: "I'm not jumping to do so."

A management crisis of this kind subsequently proved instructive. "You discern the quality of a management team when things are going badly or when major problems arise," concludes Buyse. "At ThromboGenics everything went swimmingly between 2006 and 2012; it was a party. Not much was needed in the way of leadership during those years. Maybe we should have made use of the good state of affairs and of the departure of Jean-Luc Dehaene and Clay senior, to substantially strengthen the board, but we didn't. We thought our bed was made."

So Collen the academic clashed with De Haes the manager – a collision with all the sudden violence of a total loss. And time did not heal all the wounds.

The donkeys of ThromboGenics

Other problems were also smoldering below the surface of ThromboGenics' spectacular success in 2012. Chris Buyse was also reproached by some members of the board for being Collen's mate. Buyse was and is indeed director of Collen's non-profit association LSRP,

which also held some of the rights to ocriplasmin (and therefore Jetrea®) and to PIGF and anti-PIGF. And LSRP also invested in companies left and right, including in Leuven's Bio-Incubator of which ThromboGenics was one of the lessees. Buyse was also criticized for having needlessly increased ThromboGenics' capital by EUR 77 million in 2012. That obviously led to a dilution of the sizeable capital gain accruing to the existing shareholders. From early 2012 there were vociferous calls for Jetrea® to be sold so that the shareholders could cash in.

"I myself left ThromboGenics by mutual arrangement," says Buyse. "But I also had trouble with a number of situations. People did indeed criticize me for working with Désiré in LSRP and for sitting on the board of the Bio-Incubator of which we were a lessee. We had invested EUR 2 million in that with LSRP, but at the same time had always said that the rents should be reduced when the bank loan was paid off. We weren't in it to make money; we wanted to house and help start-ups. No, I was a director with LSRP, that's true, but there had never been a situation entailing incompatibility with ThromboGenics. And it's correct that LSRP held rights to ocriplasmin, and still does to this day. Désiré didn't originally plan to claim those rights but in view of the tensions, that's what we did. At the beginning of 2012, we converted the advance payment from Alcon/Novartis into turnover and applied a percentage to it. That was fair and Dehaene and the board approved it. In 2012 LSRP received EUR 3.1 million, and in 2013 around EUR 3.4 million. I heard after the event that I was criticized for having drawn the Collen card and not the ThromboGenics card. That's nonsense. What I was also reproached for by Rudi Mariën, who himself never held all that many shares but did wield influence over the directors, was that I kept on raising money for ThromboGenics. Mariën felt we ought to sell it. Now, we never ruled out a sale but it's fairly obvious that such a sale is best negotiated with a hefty war chest, in other words based on a situation of not *having to* sell. You know, governance is absolutely vital but it can also be misused for accounts to be settled. Anyone who looks for a stick..."

> Rudi Mariën (°1945) is an active player in the Flemish biotech sector. This pharmacist by training first developed the BARC chain of clinical labs. In 1985 he set up Innogenetics, of which he became Chairman. The company was the first biotech company to be floated, with a development path aimed at a vaccine for hepatitis C. That failed in Phase II, but the company went ahead with a test for blood poisoning, the rights to which were acquired by Roche. In 2008 it was taken over by Solvay Pharma, whereupon that company ended up with Abbott in 2009. The regulator forced Abbott to dispose of Innogenetics and in 2010 it was acquired by the Japanese company Fujirebio. Most of the investors suffered great losses with Innogenetics. Rudi Mariën himself invested the EUR 37 million he ended up receiving for his stake in Innogenetics in Devgen, among others, which was taken over by the Swiss company Syngenta in 2012. Mariën invested and continues to invest in other biotech companies such as Biocartis, MdxHealth, Ablynx, Galapagos and also for a couple of years in ThromboGenics. Mariën is known to be an investor who does not hesitate to sell his participating interests and who has little patience for companies that 'waver' and 'do not know their limits'. In the case of ThromboGenics it seems that in 2013 he was operating mainly as a looking back prophet. (1)

"Chris and I used LSRP funds, i.e. non-ThromboGenics money, to invest in Celyad, Bone Therapeutics and iTeos Therapeutics, among others," concedes Collen. "We later brought

the best participating interests into our Fund⁺ fund. We did that with my money. Patrik De Haes had qualms about that and it was a stumbling block for Clay junior as well. Clay was adamant about wanting Chris to report exclusively to Patrik, and that also had to be evidenced by the two men's pay; De Haese's salary had to be higher than Buyse's. Up until that point they had earned more or less the same. But Chris was the man of four capital increases! In that period we also lost our senior Americans Laermer and Pakola, who didn't want to align with the new hierarchy."

Chief Business Officer Stuart Laermer left the company at the end of 2011, and went on to set up a consultancy firm. Chief Medical Officer Steve Pakola tendered his resignation in May 2012. He was taken on by the new Belgian ophthalmological company Amakem where he was appointed CMO. In September 2011 this start-up company established in Diepenbeek near Hasselt, had raised EUR 18 million for what had been presented as Phase II research into a medicine for glaucoma, a disorder in which an increase in the pressure on the eyeball reduces the field of vision and eventually leads to blindness. This problem would be tackled with a kinase inhibitor. Kinases are very important enzymes, proteins that set in motion biochemical processes, and could possibly also curb undesired developments when rendered inoperative by an inhibitor. It was surmised that kinase management might also prove useful in diseases other than glaucoma.

Amakem was a path developed by the Ghent based company Devgen, which opted to specialize in rice seeds and therefore hived off its medical activities. After contacts with ThromboGenics, which indicated that it was not interested in cooperation, the company was established in 2010 with Chris Buyse as chairman and LSRP as investor, along with three Devgen executives. Upon the capital increase in 2012 Buyse left the board, but LSRP stayed on as shareholder, alongside Vesalius Biocapital, the Limburg investment holding company LRM, the Flanders Venture Capital Company (*Participatiemaatschappij Vlaanderen - PMV*), the Dutch-German investment group Forbion and the French Crédit Agricole. In September 2013 Jean-Marie Stassen also left ThromboGenics to join Amakem, where he became head of R&D. However, the research ran aground in Phase II and LSRP wrote off its investment of EUR 2.1 million in 2014.

According to Buyse, this excursion undertaken by Collen and himself into ophthalmology did not cause any raised eyebrows at ThromboGenics. The ThromboGenics board saw no point in taking this risk and if Collen wanted to embark on it with his money, that was his business. Glaucoma is a very different syndrome to retinopathy. And if Pakola and Stassen no longer saw it working out at ThromboGenics, they could set to work with Amakem in the wider circle around Collen. When this LSRP investment stranded three years later, ThromboGenics came off well. Whatever the case, with the departure of key players such as Laermer, Pakola and Stassen, ThromboGenics was a pigeon loft even at the height of its capacity.

The problem in the upper echelons at ThromboGenics also ran deeper than the far-reaching understanding between Collen and Buyse and the lack of hierarchical clarity that De Haes and some directors wanted to do away with. Collen and his board of directors were also growing away from each other as regards business strategy. When it became clear that with Jetrea® ThromboGenics might have a real gem on its hands, dollar signs appeared in the eyes of the directors and, in the background, of the impatient investors. De Haes liked the idea. ThromboGenics would become a very profitable and exclusive ophthalmological

company and the thrombosis and cancer research paths would be downsized. These were regarded, so Collen says, as ‘Collen’s little toys’, yes, ‘folklore’ that merely continued to cost more and more money. Bottomless pits! ThromboGenics had to turn into ‘a profitable ophthalmic company’, sporting the splendid baseline ‘*advancing science, enhancing vision’*. “There’s a limited pot of money, and biotech players want to develop too many products,” De Haes said to Véronique Goossens in Z-Talk on the tv business station Kanaal Z, on 7 September 2012. “They should work on far fewer molecules. But you need courage to put a stop to research.” Quoted in *De Morgen* on 5 January 2013, Collen clearly didn’t agree with this. Ten days before the actual launch of Jetrea® in the USA, he prophetically said: “To make ThromboGenics a lasting success, we will have to take extra steps. I don’t doubt our product’s success, but with only one medicine a company is vulnerable. Something negative can always happen in the coming years. With a single product you’re a one-trick pony.”

But the board opted for De Haes. The decision intrinsically meant that there would no longer be any translational research between academia and Big Pharma. ThromboGenics would bring Jetrea® onto the market now and its field of application would be extended as soon as possible to cover diabetic retinopathy. Collen then in vain referred to the prospectus that had accompanied the flotation, in which it had been expressly promised that ThromboGenics would take on more molecules from the KUL and the Flemish Institute for Biotechnology, in addition to the seven with which the company started out in 2006. The board appeared to have forgotten that promise contained in the prospectus. “One prize pony is better than a stable full of donkeys,” opined Patrik De Haes in January 2013 in newspaper *De Standaard*. (2) Later Collen would reply that “a stable full of donkeys is better than one lame pony”, but in early 2013 nobody yet knew that the pony was lame.

Sell that thing now!

The conflict between the Chairman and the CEO escalated. Success has many fathers, and the paternity of Jetrea® was now also disputed. In the picture painted by Patrik De Haes in all kinds of presentations, the emphasis was placed on the far-reaching changes that had taken place under his leadership since 2008. Collen by no means disputed De Haes’ merits, but for Collen truth has its rights: as far back as 2001 Steve Pakola wrote out the whole scenario that led to a medicine six years after the flotation. In those six years he had only been mistaken in thinking that it could come about more quickly. He was the true father of Jetrea®, together with Collen and with Peter Stalmans as the main contributor to its clinical development.

“What I was able to press ahead with was to tell the true story about the history of Jetrea® on the website and point out that the management could only lay claim to the sales part of that merit, says Collen with a degree of satisfaction. “In the meantime Jetrea® had been approved by the FDA and directors and shareholders were smelling money. I could not turn that around. For them Jetrea® and ophthalmology were the future.”

But if it were all only about dollars and euros and ThromboGenics had no ambition other than Jetrea®, why was so much unnecessary and diluting capital raised? And why wasn’t the company simply sold? The market capitalization amounted to nearly EUR 1.6 billion at the end of 2012. The shareholders would have been quite happy with EUR 40 per share.

According to Collen, Rudi Mariën was one of those who wanted to sell ThromboGenics. “Mariën had never held many ThromboGenics shares, but he created a profile of himself as a kind of activist. He came to me at my house. He said: ‘Your CEO has to go and live in the USA and you have to dump your CFO.’ ‘And what am I supposed to do with those 100 people in Leuven?’ I asked him. ‘That’s not your problem, they’ll have to go and look for another job,’ he said. Now he may well be saying: they should have listened to me! If they had, they would have gotten a billion for the company. That’s not actually true, since no-one ever really made an offer. The ‘shorters’ (who sell hired shares in the hope of later being able to buy them back more cheaply and thus speculate for a stock price fall) also at once saw that the market value was untenable. The shorters earned a lot of money, but the shareholders did not, unless they had bought cheaply and sold in good time.”

But with hindsight wasn’t Mariën indeed right? In mid-February 2014 he said in the economic magazine *Trends* (1): “I know it’s a tough thing to say, but ThromboGenics should also have been sold last year. As a matter of fact, I had already said that a couple of years earlier. They had brought their product onto the market, and well done to them for that. Selling the rights for Europe to Alcon was the right way to go. How were they to do it otherwise, in those countries with all those rules? But then they decided to go and sell the product in America themselves and that was a blunder. ThromboGenics thought it was going to go and sell the product there with 15 people. They don’t know how vast the US is! What’s more, if your business is in the United States, your senior managers have to be there too, and not in Leuven.” Mariën did not make it, albeit due to the fact that, according to Collen and Buyse, there was never a buyer or an offer. And as for Mariën’s assertion that the company should have been sold “a couple of years earlier”, i.e. years before the beginning of 2014, Collen and Buyse laugh at the notion. In the euphoric atmosphere of 2012 there were no dissident voices to be heard, except that of Collen himself, who kept insisting that all the money should not be put on just one horse. The “tough thing to say” that Mariën alludes to sounds to them more like hindsight bias. For that matter, anyone not believing in a share can always sell it on the stock exchange. Then you have no need to make retroactive statements after the event. At Collen’s headquarters, people do not exactly think highly of Mariën.

The turn taken by ThromboGenics in 2012 was unacceptable for Chairman Collen. The board of directors was faced with a lacerating choice in mid-2013: the CEO or the Chairman? From February 2013 it became clear that there was no longer room for both of them. Collen saw straight away that he did not stand a chance and prepared to leave. But if ThromboGenics was no longer interested in paths other than microplasmin for ophthalmological purposes, he would take those other molecules with him. This led to a somewhat indecorous argument about the licenses that had been granted by the KU Leuven and LSRP for substances that had been ‘thrown in the rubbish bin’. Collen: “I tried to see to it that the programs that had been discontinued were given back to the university. There was then an argument about the ‘best effort to exploit’ obligation that had been stipulated in the contracts and whether this undertaking had been complied with. But if the university wanted to retrieve those programs, ThromboGenics would have to be paid for them.”

The battle-weary Collen relinquished of his intellectual property rights. “They thought I’d give in, also because I had pumped so much money into it. But I wasn’t about to be a merely decorative Chairman, that wasn’t something for me. I kept the honor to myself and quit.”

The doubt adversely affected the ThromboGenics share price, which peaked at over EUR 40 at the end of 2012 but fell away after that. ThromboGenics lost its Bel20 listing and swiftly fell to the status of a small cap, with a market value of 100 million. “This had however no consequences for the CEO’s compensation packet of over EUR 0.5 million, quite on the contrary,” says Collen. The expectation value, i.e. what investors are prepared to pay over and above the company’s cash assets, melted like snow in the sun. The speculators, or ‘shorters’, earned a lot of money from the slump in the share price. One of the shorters who at the end of December 2013 made no secret of the fact that he regarded ThromboGenics’ share price as madness, was the American Joseph Edelman of Perspective Advisors. (3) The credulous investors who were still jumping on the bandwagon at EUR 40 lost a lot of money. The stock market can be merciless.

Year		2012	2013	2014	2015	2016	2017
	EUR million						
Turnover		75.11	112.78	13.78	11.20	7.10	9.05
	Real		21.72	13.78	11.20	7.10	9.05
	Paid in advance	75.04	90.03				
Profit/Loss		30.42	26.40	-51.12	-37.93	-60.35	22,61
Capital and Reserves		227.97	258.77	208.01	170.02	109.86	133.36
	Increase (excl. warrants)	*77.18*					
Intangible Assets and Goodwill		74.92	71.60	64.97	58.29	25.90	23.60
Cash (incl. “investments”)		148.23	172.36	127.08	101.39	80.07	105.70
Number of shares		36,008,391	36,094,349	36,094,349	36,094,349	36,094,349	36,094,349
Capital/shares (book value):		6.33	7.17	5.76	4.71	3.04	3.69
Cash/shares		4.12	4.78	3.52	2.81	2.22	2.93
Market value last week of December		46.42	19.50	6.34	3.56	2.53	3.94
Expectation value		40.09	12.33	0.58	-1.15	-0.51	0.25

In 2016 EUR 26.6 million was written off for Jetrea®, which partly explains the sizeable loss.

”It was mismanaged”

On 1 November 2013 Collen tendered his resignation as director and Chairman. He was succeeded by Staf Van Reet. The American ophthalmologist David Guyer was co-opted to the post of director. CFO Chris Buyse also left the company eight months later, on 26 June 2014. The Canadian Paul Howes took his place. Collen delegated his voting rights to director Luc Philips and set about looking for someone to buy his last shares. He found a buyer in the form of Baron Philippe Vlerick’s Luxembourg company Bareldam, which already held 1.8

million shares and bought Collen's last 500,000 shares at a unit price of EUR 2.77. Vlerick also became a director of ThromboGenics.

After staphylokinase, the thrombosis remedy for poor people, Collen received a second blow with ThromboGenics, a wound that left scars that are still apparent today. Collen: "A good many shareholders, including friends of mine, had bought ThromboGenics shares because they believed in Collen, so I couldn't leave them in the lurch. But on 1 November 2013 I threw the towel in nonetheless. ThromboGenics then urged me to sign a document in which I renounced all intellectual property rights. I consulted a lawyer who said: claim your rights, you can still renounce them later. But in November I said: you can have it. Also because KU LRD had warned me, saying: Collen, you won't get far in the courts with 'best efforts to exploit', but more importantly because I did not want to turn against shareholders who had given me their confidence."

But not all was lost. Collen is carrying on with two projects. "We are continuing to develop the PIGF molecule in CoBioRes, separately from ThromboGenics," says Collen. "I'm working on that in CoBioRes with by now eleven people, with my own funds, but I did have to conclude a licensing agreement with ThromboGenics. And a second path is an idea of UCL Professor André Trouet that I had suggested be brought into ThromboGenics but had been rejected. You know, a lot of opportunities were missed. For example, at one time Euroscreen could have been bought for about EUR 10 million. And now, as Ogeda, it's worth 800 million! We've since reaped handsome rewards from that with Fund+! The failure of Jetrea® wouldn't have been a disaster for ThromboGenics if the company had had more irons in the fire."

He regards it as much more awful for ThromboGenics than for himself. "Of course, 25 years of dedication and work have been lost, but what hurts are the losses that the investors sustained from 2012 onwards. I recovered most of the EUR 71 million that I had invested in ThromboGenics, in Ireland and in Belgium. I sold my last 500,000 shares. I don't look at it anymore. With Fund+ Chris Buyse and I are now writing a new much more successful story."

ThromboGenics was a missed opportunity – an opportunity to build a new, large and diversified Belgian pharmaceutical company. In the name of focus and goal-orientedness, and of profitability in the short term, all the stakes were placed in just one medicine and on ophthalmology, a difficult and heavily grazed pasture. In 2013 ThromboGenics did have almost EUR 180 million in cash, a solid reputation and the trust of financial backers with deep pockets. Add to that strategic vision, the good fortune that seasoned medical entrepreneurs and businessmen always have, and you have a world player. *Quod non*, and that continues to rankle in Collen's mind. "It was mismanaged. It was somewhat akin to what happened with Steve Jobs at Apple," he says. "John Sculley showed him the door and they had to beg him to come back. I won't be going back. In my view ThromboGenics is lost unless they reinvent themselves, as Celyad or Tigenix did. In 2016 they sold for USD 6 million Jetrea®: that's no longer break-even. They're now going to try to roll out that medicine for diabetic retinopathy. As meanwhile confirmed, there was little chance of that succeeding."

Steve Pakola agrees. He is currently involved in the development of an easily administered medicine to combat non-proliferative diabetic retinopathy. Relaunching Jetrea® with a broader use is not self-evident, he says. "They'll need to have very solid data. The medicine will have to really slow down the proliferation of diabetic retinopathy, and do so over a long enough period."

The fall of Icarus

As if the Gods wanted to punish the protagonists of this tragedy, which for some amounted to a patricide, the sale of Jetrea® indeed got under way with far more difficulties than had been anticipated by salesman De Haes. The medicine's undisputed qualities were far outweighed by the loss of income it caused among eye surgeons. Both in the USA and in Europe, where Alcon/Novartis marketed Jetrea®, sales figures were well below expectations. The ThromboGenics management did what it could, but the ophthalmologists and eye surgeons continued to swear by their surgical interventions. Jetrea® turned into a flop. Annual sales peaked at a paltry USD 22 million in the first year, 20 million of which in the USA, and then dwindled to USD 7.1 million in 2016. Novartis wrote off the investment and in April 2016 announced its intention to completely wind down its ophthalmological subsidiary Alcon. (4)

De Haes decision to organize the sales in the USA himself was most probably reckless, but Alcon had even less success in generating turnover in the rest of the world. And although that decision was perhaps inspired by De Haes, it was accepted by the other directors, including Collen and Buyse. The board took this decision in 2008, that is to say a couple of years before there was a medicine. And when the decision was already irreversible, many investors also applauded ThromboGenics' choices, as evidenced by the capital increase in 2012. So everyone was living in a glass house.

When in October 2012 the economic newspaper *De Tijd* raised the question as to whether it wasn't a major risk for the company to handle the sale of Jetrea® itself when it did not have any experience, De Haes retorted: "Hang on a minute. In the 1990s I set up a company with more than 100 employees in the USA. I know how things work there. And we took on people in the US from Genentech and Novartis. We were looking for 28 salespeople and we received 3,000 applications. It's no exaggeration to say that our medicine is a genuine innovation, and that attracts people."

"Patrik does have a medical diploma," acknowledges Buyse. "But he's not a scientist. He's a manager, specialized in sales and marketing and in making deals, and for a long time he was good at that. We followed him. The negotiations with Roche first, and then with Alcon, were great work. The way in which future turnover was converted into advance payments compelled admiration. And then, it's true, we all shared the illusion that we had on our hands a medicine that would sell itself. We really were mistaken in that appraisal. There was never any kind of fraud, never any attempt to present the situation as being better than it really was; no, strategic mistakes were made. '*Putting the right product in the right place, at the right price, at the right time'*, the four Ps of marketing. That's where we neglected the rules. We did lower the price that had initially been put forward at over USD 5,000, although definitely not by nearly enough, and we were wrong about the target group, the surgeons, etc. The tragedy is that the product works, but that now it's no longer certain that it will even continue to be available."

Buyse and Collen describe De Haes as a traditional manager, with everything that is assumed to entail, i.e. focus on value accrual and value realization, with a sense of systematics, rigor and an aversion to risky undertakings and figures in the red: someone a board of directors could place their trust in, but a miscast for a company like ThromboGenics?

“That’s an accurate picture,” reckons Buyse. “He came from Big Pharma and that kind of profile can clash with the entrepreneurial spirit and academic freedom to take the occasional detour without knowing in advance where it will take you. Innovative companies are also often something of an organized chaos. Companies like ThromboGenics call for a different form of management than a large pharmaceutical group. You always need discipline. If you’re burning cash you obviously need to be more careful about keeping an eye on your pennies, but you have to find the right balance. We also see that in the companies in which we have a participating interest now with Fund^{+}. It’s not easy to find good managers. A previous failure can be an asset.”

“And that also applies to the independent directors,” adds Collen. “To Staf Van Reet and Luc Philips: they, too, were mainly focused on risk limitation, as had been expected of them in the large companies they came from. For our directors it was all about moving out of the red as quickly as possible, and that’s an understandable attitude, but you don’t do pioneering work that way.”

Collen has his idea about how things should have evolved, and who could have been held up as a model. “I emulated Paul Janssen. That man got things right, right across the board, and what’s more was shrewd enough to sell Janssen Pharmaceutica to Johnson&Johnson in good time. What a career!”

“And we had another example in our extended environment,” relates Buyse. “I myself never met the people who set up Genentech, but what venture capitalist Bob Swanson and scientist Herbert Boyer did there in our sector is worthy of great admiration. They also began with a few dozen people.”

“Yes, that’s a role model,” endorses Collen. “They had a staff of 69 when I started working with them at the end of 1980. The scientific staff they took on were all academics – strong willed and unconventional. But their scientific output was comparable to that of the famous Whitehead Institute of Biomedical Research in Cambridge, Massachusetts, US. At Genentech they compiled a long list of first-rate publications, but also worked day and night. They were hardly paid, but they all held options and many have become millionaires. Actually, they invented biotechnology. When I started working with Genentech the company was still housed in a warehouse in South San Francisco, with two little windows, one in the reception and one in Swanson’s office. Diane Pennica, who cloned t-PA, sometimes didn’t know whether it was day or night, and often slept there. Now 12,000 people work there on a beautiful campus, it’s unrecognizable. When you come in you are immediately informed of the significance of the site: a sign in huge letters reads: *Here biotechnology was invented.* And that is true.”

A motley crew

“Maybe we needed that kind of manager at ThromboGenics,” concedes Buyse. “Pioneers, creative people who are bold enough to think *out of the box*.” Genentech, which is today a division of Roche, is a legendary corporate success and now forms part and parcel of the collective memory of the State of California. In the oral history of Genentech such as the *Online Archive* of California (5), which has recorded the oral testimonies of surviving witnesses, Herb Heyneker recalls the pioneering years of Genentech and the cooperation with Désiré Collen with obvious pleasure. Without being prompted, he refers several times to

Collen and to the motley crew that did pioneering work on molecular biology and cloning at Genentech.

Herb Heyneker: “Collen spent an entire summer in my laboratory in order to learn about molecular biology and cloning. He spent a lot of time with Bill Holmes, myself and Gregory Gray and we had a lot of fun. It was absolutely fantastic how this established Professor from the university in Leuven came here to learn the ABC of cloning with a motley crew. Collen settled in quickly. He was a good mate. Despite being a professor he wanted to gain practical, hands-on experience, and to that end he took a four-month sabbatical and it was a wonderful time.”

Herb Heyneker again: “When Désiré Collen visited my laboratory, we had already begun with the t-PA project. Collen eagerly wanted to understand what was involved in cloning a gene such as t-PA and what the difficulties were at that time. I was really impressed by Désiré. He was not only a very enthusiastic scientist but also a good organizer. He spent a lot of time with Bill Holmes, even though Bill was a very eccentric man. Collen looked on in amazement at the way we molecular biologists set up our experiments. In Belgium he was a respected Professor but here he had to get into the trenches, as it were, and humbly listen to what Bill and I told him. It must have been a strange business for him but he gladly went along with it. In no time he became one of the team.” The aforementioned Bill Holmes gave up going to a barber or hairdresser on his fourteenth birthday and sported a ponytail that extended down to the small of his back. Later he came to Leuven with Collen and took his doctorate there.

And as in the comic albums of Asterix and Nero, with Collen it also ended with a party. Herb Heyneker: “I’d like to say something else about Désiré Collen’s visit, since he made quite an impression. We became good friends and before he left, we threw a little party in San Francisco. After a nice meal in North Beach, we found a tent at Fisherman’s Wharf where the guests could get changed and we all donned mediaeval outfits. The photo that bears witness to that occasion is one of the funniest ever taken in my scientific career.”

A nice story, and one in which the funniest photo also has its place. But how far do you slacken the reins in a company? You battle complexity with creativity rather than rigid discipline – that much goes without saying. But how much creative craziness is justified in a disciplined organization? Most probably not as much as was made allowance for in the early years at Genentech. Sometimes the referee does have to blow his whistle and point to the penalty spot. The world of the thoroughbred researcher and enthusiastic entrepreneur ends where the world of the manager begins. Yet even in an efficient organization there is likely to be more freedom possible than was still allowed at ThromboGenics in Leuven after they thought they had struck gold with Jetrea®; gold that was to slip through their fingers.

The tension between *’drifting off in hobbyland’* and ‘*systematic abandonment’* is as old as translational research. Some discoveries have stemmed from manifest disobedience, and companies such as Google and 3M even encourage this to some extent, but of course it can only be permitted in an environment where there is no shortage of money. (6)

Marc de Smet, the Amsterdam ophthalmologist who at the time alerted Pakola and Collen to the potential of microplasmin for eye problems: “People should listen more to the scientists who are behind the product: they should be involved more in the management and the board of directors. Désiré knew very well what research was. He may not have known so much

about the eye but for a long time he had Steve Pakola as his advisor in that area. But this perfect team was outflanked by a team of sales staff."

Further to Pfizer's decision at the beginning of 2018 to discontinue all research into Alzheimer's and Parkinson's, Jan Rosier, Professor in pharmaceutical development in Dublin, denounced in the newspaper *De Standaard* the domination and short-sightedness of the managers running pharmaceutical companies. "The pharmaceutical industry no longer wants to conduct basic research. That's because it is no longer run by prominent scientists, but mainly by investors, MBAs, economists and managers. That also explains why the industry developed shares buy-back programs to the tune of USD 261 billion between 2006 and 2015, representing about 80 medicines that were not developed." Rosier also refers to the cultural differences between managers and scientists. "Managers in large companies demand order, structure, authority and in particular obedience. That leads to a culture intolerant of both the creativity and the sharp, critical academic attitudes needed for such research." (7)

And of course, it gets very awkward indeed when those managers then bungle things up in the way they run things. Koen Hoffman (formerly of KBC Securities): "The market for Jetrea® was completely misjudged. It might have been known that the eye surgeons would be only too happy to trip up Jetrea®. Collen is a charismatic man and an exceptionally enterprising Professor, but he's not a salesman. And it was in commercial flair that the entire management was found to be wanting."

For sale but no buyers

Did ThromboGenics shake off a burden with Collen's departure? That self-assurance is not apparent from the first decision taken by the board without Collen. After the last board meeting with Collen on 5 December 2013 in New York, the company had to be run without a figurehead. A cornerstone was missing. Collen's departure was sold to the outside world as a kind of retirement, but what now? A medicine that was having trouble catching on, a new strategy based on new uses for that same disappointing medicine: *quo vadis* ThromboGenics? And what if a former Chairman were to turn against the company? Collen was not planning to do that. He knew his departure was already a major disappointment for the shareholders and there was no need for any further conflict on top of that. He signed a 'termination and settlement agreement' which neatly wound up all relations between Collen and ThromboGenics. But in the board there was a lack of self-assurance, that much is clear. In such circumstances an external expert has to provide an umbrella, i.e. to supply an analysis and a strategy under which the directors can shelter.

And that is indeed what happened. The Board entrusted a third party with the task of "examining the different strategic possibilities liable to enable the company to tap the commercial potential of Jetrea® more effectively and develop its sufficiently proven capacity for product development." In February 2014, two months after Collen's departure, the US investment banker Morgan Stanley was thus instructed to sell the company. This is what it boiled down to, although it was couched in veiled terms: to find a "strategic reference shareholder for everything related to Jetrea® in the USA". Elsewhere it was literally stated that: "The board is of the view that a transaction is in the company's interest."

And the board was in earnest. To encourage the CEO and CFO to make a 'transaction' possible, a bonus in the event of success was promised to the tune of EUR 2.6 million for Patrik De Haes and EUR 1.56 million for Chris Buyse. That is not unusual, and during the examination by Morgan Stanley and possible acquirers, they were also paid a retention fee, i.e. remuneration for not stepping down, which amounted to 50% of their pay for 10 months. According to insiders, that *is* unusual. The cat was evidently away. The CEO's severance pay was also doubled to 12 months. The Americans David Guyer and Thomas Clay stipulated that ThromboGenics had to safeguard them as comprehensively as was legally possible against any lawsuits and third-party claims. That is something you would ask for if you fear there will be lawsuits and claims. Obviously the directors concerned abstained during the vote on these proposals.

Then on 15 May 2014 the then 73-year-old ThromboGenics director Jean-Luc Dehaene died unexpectedly. In his capacity as director of the biscuit company Lotus Bakeries he was on a visit to a subsidiary of the company in Brittany when he suddenly felt unwell and fell into a coma. The former Belgian prime minister had previously been diagnosed as suffering from cancer of the pancreas. We will never know how Dehaene saw ThromboGenics' future panning out after Désiré Collen had left. For that matter, as director he too had delegated the answer to that question to Morgan Stanley.

In August the study conducted by the American consulting firm revealed that the company should continue on its own. Morgan Stanley had thus failed to find a buyer. Was it too late? Or more to the point, what was a buyer other than Novartis supposed to do with the agreements, entered into by Novartis on the sale of Jetrea® throughout the world except in the USA? ThromboGenics had made itself unsaleable, other than to Novartis. So Morgan Stanley's assignment was therefore over quite quickly.

Chris Buyse then called it a day as CFO. In the media the two events were seen as linked. Buyse: "Yes, my departure and the end of Morgan Stanley's mandate were announced in the same press statement. Morgan Stanley came to the conclusion that ThromboGenics should carry on as a stand-alone company. It boiled down to the fact that they hadn't found a buyer. In those circumstances I opted for a position with LSRP and with Collen. That was the right decision." That day the share price fell from EUR 14 to EUR 10.

Failure is an orphan

Success has many fathers, but failure is an orphan. Jetrea® flopped and ThromboGenics' market value withered. In 2012 the board clearly committed a strategic blunder. Double or quits came up quits. There was evidently not enough critical sense on hand. Account should have been taken of one of the risks cited in the prospectus: sometimes doctors balk at using a medicine. It is not always clear what their reasons are, and nobody will ever admit that when taking decisions they are more mindful of their wallet than of the patient's welfare. And on top of that, was Jetrea® ever really a threat to established surgery? Or was that, too, a Leuven pipe dream?

"Patrik De Haes negotiated efficiently with Alcon/Novartis," Collen acknowledges. "They paid a lot of money and that is largely Patrik's merit. But the decision for the company to take care of the commercialization in the USA itself was reckless. And what's more we went about it

the wrong way. Instead of going to the 2,000 surgeons, we should have approached the ophthalmologists, but there are 20,000 of them there."

But anyone familiar with American medicine knows that apart from the insurance companies and large pharmaceutical companies, the associations of specialists are very important. According to experts, they are often the policy-making lobby. They can make or break a medicine. (8) And they do that through their affiliation, but not always in the patient's interest. Didn't ThromboGenics know that? And couldn't they have brought in an expert to carry out a critical assessment of the market? Not someone you pay to tell you what you want to hear! "With hindsight of course we now know that we should have licensed the product," concedes Collen. "But De Haes convinced us that we could do it independently. We misjudged the degree to which we represented a threat to surgeons, who were of course fond of their scalpels, their bistouries. Try breaking through that."

"The market and market potential were indeed studied," says Collen. "There are 600,000 cases worldwide, and if you have a penetration of 10%, you're doing big business. But what will one of these ophthalmologists say to the patient when he doesn't know better: you have vitreomacular traction; we'll monitor it and when it gets serious enough, we'll send you to see the surgeon. That's called the '*watchful waiting approach'*. The surgeon then says: wait a bit, since this is a serious operation. You have a 10% chance of spontaneous improvement. Eventually the patient is operated, requiring to lying face down for as much as 10 days in order to keep the retina permanently in its place. It now will continue much like that for the foreseeable future."

Steve Pakola wants to be very cautious in his assessment: "First and foremost it is by no means exceptional for approved medicines to fail. In fact, it happens rather often. People forget that. But I think that two things may have gone wrong. Firstly, the price. It was simply too high. You have to view this kind of retinopathy treatment as a whole. The price of a new medicine thus becomes a part of an available amount. What goes towards a new medicine is no longer available for other parts of the treatment. That was completely misjudged. Even USD 2,000 was too much, let alone USD 3,950. Secondly, a new medicine has to really catch on, particularly if it calls for a change of paradigm. Here you're going from surgery to medication. That only succeeds if the medicine delivers on all the promises. However, Jetrea® only works in carefully selected patients – something that was not always clearly stated at the outset. And a very expensive medicine that doesn't always work quickly earns itself a bad name – and that's irreversible."

Marc de Smet: "There were experienced, motivated people at Thromb-X and ThromboGenics: people who were driven by the scientific ambition to understand the process and to develop a product that could have a positive effect in people. The economic interest was secondary. Jetrea® came out of that research, with a different application to what I had expected. I saw it as an aid to surgery, but it was developed to make surgery unnecessary. Of course, that is a more attractive market. Jetrea® works in 30% of cases, and in 50% in the event of a very careful selection, for a price of around EUR 3,000, which is more than the cost of a surgical vitrectomy in many countries. And surgery works; it is the daily source of income for many ophthalmologists. Eyes are vulnerable and for every patient at risk of losing their sight or in whom loss of sight has occurred, the eyes are organs to which they are greatly attached. They don't want to run risks and expect us to give them a

permanent solution. It is also awkward to explain to an insurance company that an even higher amount has to be paid out on a surgical vitrectomy because Jetrea® hasn't worked! So that price is far too high. At the time I told the financial department at ThromboGenics this. The answer was: for hepatitis C a treatment costs EUR 90,000 (EUR 1,000 per pill), so in comparison Jetrea® is cheap! Cheap it most certainly is not. Had the price been EUR 500, it would be used, with new indications. Adaptations to microplasmin could have made it more effective. Version 2 could then no doubt have cost more and would have been much more successful." The reference to Gilead Sciences' hepatitis C medicine is painful. After 2015 Gilead had everyone talking about it on account of the very high price it charged for its revolutionary Sovaldi and Harvoni medicines. (9)

It takes two to tango

Eppur si muove! Still the earth revolves around the sun! ThromboGenics persevered. And the latest capital increase meant there was money for a few years more: some EUR 125 million at the end of 2014. Incidentally, Jetrea® was still an excellent remedy, as Professor Peter Stalmans testified in the 2013 annual report. In a careful selection of patients Jetrea® worked in 70% of cases. But a surgical intervention works in 100% of cases, the American surgeons reiterated. To win over the sceptics nonetheless, ThromboGenics organized three new studies, including Orbit among 150 patients in 120 retina centers in the USA. But it takes two to tango, and if the ophthalmologist can't see it happening...

The 2015 annual report opens with a statement that looks like an open door. The first sentence reads as follows: "The management and the board of directors back ThromboGenics' strategy fully." Further on the text reads: "The management never acts without consulting the board. The directors are unanimously behind the management team's strategy." With a new director and shareholder in the shape of none other than Philippe Vlerick, nobody had any doubts about that. The prominent Flemish businessman already held shares and bought Collen's last remaining shares. With a stake of 6.44% he clearly believed in the venture and with him the company once again had a heavyweight on board.

From that point on, the report said, Jetrea® was to be subject to cost-neutral planning, i.e. costs could not exceed sales. There was no talk of profit, but the bleeding was stopped. The TB-403 research into a drug to combat brain cancer in children was accommodated in a separate company, Oncurious, in which VIB also acquired a participating interest. The medicine in question was an orphan medicinal product with limited economic potential, but which could possibly meet a dire need. It was to ThromboGenics' credit that this research was continued.

The available cash was then fully devoted to the possible use of Jetrea® to combat diabetic retinopathy. This research could start in Phase II, as the annual reports kept claiming year after year. With the Circle trial it was hoped at the end of 2017 to be able to confirm in Phase II that Jetrea® helped in non-proliferative diabetic retinopathy, a preliminary stage of proliferative diabetic retinopathy. The latter leads to blindness. A meagre consolation was that Jetrea®'s results, this time in the OASIS study, remained good. Twenty thousand patients had already been treated worldwide. Jetrea® was now offered in diluted form. Half of the patients treated with sVMA were cured after one injection, at least if a careful pre-selection had been carried out and patients with a particular anomaly, epiretinal membranes

(ERM), were excluded. But a sales volume of EUR 11 million was not much to show for all this.

After the calvary, the resurrection?

ThromboGenics was thus rowing unavailingly against the current at the end of 2016. The share vegetated on the stock exchange, whilst analysts digested their mistakes. Memory became blurred. The cash supply gradually shrank. Time ticked by. In the spring of 2017 directors Patricia Ceysens and Luc Philips left the company on their socks. Ceysens' mandate had been extended by a further four years in 2016 until December 2019. The shareholders were not informed of the departure of Luc Philips and Patricia Ceysens, both of whom had been members of the board's audit committee. The fact that they had left could be inferred from the composition of the board as this appeared on the ThromboGenics website. In mid-2017 Staf Van Reet passed on the chairman's gavel, also prematurely, to the young Thomas Clay – like director Philippe Vlerick, a major shareholder. Were the financial backers thereby seizing power? Was playing time over? Evidently. ThromboGenics scrambled to its feet.

CEO Patrik De Haes wiped a large part of the slate clean when he put Alcon/Novartis out of its misery with a shrewd deal – his third at ThromboGenics. Under this agreement ThromboGenics took over the commercialization of Jetrea® worldwide, providing for a compensation of no less than EUR 53.7 million and an investment by Novartis in new ThromboGenics shares to the tune of EUR 10 million. The new shares thus acquired are non-marketable ('lock-up') until 15 September 2020 and Novartis renounces any attendance at general meetings for a period of ten years. This means that the acquired shares de facto have no voting right.

De Haes clearly forced Novartis onto its knees. Maybe he was able to demonstrate that Alcon/Novartis had failed to observe its agreements. Worldwide turnover (excluding the USA) had fallen in 2016 to the chickenfeed figure of EUR 2.5 million, vis-à-vis a similarly paltry sum of EUR 4.4 million posted by ThromboGenics itself in the USA. Novartis did some spring-cleaning in 2017 in the whole of its ophthalmological division, resulting in the possibility of the specialized subsidiary Alcon even ceasing to exist. Separately from the deal the head of ophthalmology at Novartis, the Belgian doctor Vinciane Van Geersdaele, moved over to ThromboGenics to head up the new Global Jetrea® Unit. She had to see to it that by 2019 Jetrea® became cost-neutral worldwide, as was already the case in the USA.

Taking account of the advance payments of 165 million it had made, Novartis was stripped by Jetrea® to the tune of EUR 228.70 million, plus the costs of the sales team that had tried to sell it for four years and the lawyers' costs that in this case no doubt also ran to millions. We know that in the pharmaceutical industry a lot of money goes up the chimney. But ThromboGenics itself was able to keep going and by the end of September 2017 could reckon on a cash supply of EUR 113.4 million, not counting the capital increase of EUR 10 million. 2016 was closed with EUR 80 million in liquid assets. With more than EUR 120 million in the bank, the company should be able to continue at the present cost level for three years. As far as cash was concerned, ThromboGenics was almost as well off as when Collen left the company in 2013.

To counteract the dilution of that capital increase, the share price had to be as high as possible in the 30 days leading up to 20 November 2017. Novartis had to pay as high a price as possible per share. It thus boiled down to convincing the shareholders that a new and promising ThromboGenics was rising up. And ThromboGenics' credibility among investors proved to be better than expected. Patrik De Haes deal had a persuasive effect. Communication manager Wouter Piepers also addressed Belgian private investors, the small speculators who are marginal as shareholders but can be important when it comes to the price formation. Belgium's investment journals rediscovered ThromboGenics. A stock market valuation corresponding to or lower than the available cash, with zero valuation for the product pipeline, i.e. with no expectation value, was also unreasonable. ThromboGenics was not a house of mourning! The specialist media also felt that ThromboGenics was more than Jetrea®.

Inside Beleggen (Inside Investment) promptly included ThromboGenics in its model portfolio and later recommended it as a promising tip for 2018. *De Belegger* (The Investor) said that it expected there to be price triggers in the news feed in 2018; in other words, news that could prompt a rise in the share price. Pierre Huylenbroeck of *Mister Market* also took a sympathetic view of the second life of a failure. Only *De Tijd* refused to let itself be won over straight away. (10) But the share price started moving, and rose from EUR 3.50 briefly to EUR 5.49 and at the end of 2017 to EUR 3.40 again. In January 2018 ThromboGenics rode the swell of the bull trend caused by two takeover bids for Belgian biotech companies. Whatever the case, there was expectation value again. ThromboGenics was again deemed to be worth more than the cash per share. At the end of 2017 the cash supply after the capital increase by Novartis amounted to some 120 million with 38.3 million shares. This therefore meant EUR 3.13 per share – less than the introduction price. We thereby base ourselves on Novartis having paid EUR 4.59 per new share for a stake of 5.69%.

Was the ThromboGenics story so convincing as to justify higher expectations again? At first sight ThromboGenics appeared to be old wine in new bottles. Of the original research paths, the eternal promises of ocriplasmin and anti-PIGF remained up to the end of 2017. Research was still ongoing on both substances to ascertain whether they could be useful against forms of diabetic retinopathy: the old story that had been recounted since 2006, in other words. There appeared to be no getting past Phase II in these cases. And yet there is more to it than a case of 'throw more money at it', one of Eroom's laws. The molecules used and their combinations have been enriched with a great deal of research over the last four years, and knowledge of the back of the eye has come a long way in the meantime. Clinical picture selection is much more advanced than it was a few years ago, when the back of the eye still held many secrets. In diabetic retinopathy a distinction is now made between four clinical pictures: proliferative and non-proliferative diabetic retinopathy, which in both cases can be accompanied by DME, an edema, an accumulation of fluid in the macula, the little yellow spot on the retina.

ThromboGenics was thus continuing to place its money on two old horses. With anti-PlGF ThromboGenics was looking for a combination with the three existing anti-VEGF medicines to combat certain forms of diabetic retinopathy. THR-317 is currently being administered to 50 DME patients with sight-threatening proliferative retinopathy. Given the medical urgency, the recruitment of patients is reported to have gone smoothly. For DME the initial results with

anti-PlGF in early April 2018 looked promising. Should a combination of anti-PIGF with one of the anti-VEGF remedies succeed, anti-PIGF may yet turn into the success that had been deemed possible in the 2006 prospectus. The specialized *Grand View Research* asserts that diabetic retinopathy could represent a market of USD 10 billion in 2025. ThromboGenics will certainly try to bring an anti-VEGF partner on board.

And as for the second path that was dragging along, what could still possibly be done to combat diabetic retinopathy with ocriplasmin, the core of Jetrea®? The answer was: nothing! At the beginning of December 2017, after Novartis had acquired a stake in the capital, ThromboGenics unexpectedly discontinued this research. No patients could be found who would be prepared to have three injections in the eye to avert a problem that had not yet arisen. Exit ocriplasmin for diabetics! The stock market took fright and sent the share price tumbling again.

And then there are two more embryonic paths in ophthalmology. ThromboGenics hopes to be able to get two newly acquired substances, THR 687 and THR 149, past the preclinical phase in 2018. THR 687 comes from the Belgo-Dutch listed company Galapagos *(integrin antagonist*) and THR149 from Bicycle Therapeutics *(plasma kallikrein inhibitor)*. In 2013 that American oncological biotech company sold ThromboGenics the rights to deploy '*bicycles*' (bicyclic peptides) primarily to combat DME, an oedema in the macula. Bicyclic peptides are stretches of amino acids, comparable to very small antibodies, i.e. proteins that are mobilized by the immune system to fight viruses and bacteria. One of Bicycle Therapeutics' major partners is the pharmaceutical company Astra-Zeneca. Progress again depends on the speed with which patients can be recruited. The criteria are stringent and there is therefore a high drop-out rate, but experts believe that the approach sounds worthwhile. We will hear more about the wider ophthalmological potential of THR 687 from the Galapagos stable in the first half of 2018.

Susan Schneider, ThromboGenics' new CMO (Chief Medical Officer), is the person now overseeing all this. As an American ophthalmologist she had already been involved in the launch of three ophthalmological products, including Genentech's Lucentis, the most successful medicine for combating a number of problems at the back of the eye. Schneider was also involved in the study of Lucentis' active substance ranibizumab. She is a director of the American Glaucoma Service Foundation to Prevent Blindness. A seasoned expert of this calibre does not join the team if there is nothing going on in the company. Has she brought ocriplasmin's Via Dolorosa to an end?

Since 2016 a second use of anti-PIGF is to be found in a new 50/50 collaborative partnership with BioInvent and is spun out into a new company, Oncurious, in which the Flemish Institute for Biotechnology, VIB, has a 15% holding. Here researchers are hoping to make an orphan medicinal product against medulloblastoma, an uncommon but extremely malignant pediatric brain tumor. The research is in Phase I/IIa, with results expected in 2018, and is being conducted in ThromboGenics' American site in Iselin, New Jersey. This could become major medical news, but most probably not major financial news, since the remedy then would be prohibitively expensive. However, should results prove to be better than expected, it could lead to further research into the usefulness of anti-PIGF in the fight against other forms of cancer.

Oncurious has also been given a licence by the VIB for five potential immuno-oncology remedies. These are substances in the preclinical phase, and it is hoped that one of them can be brought to the clinical phase by 2019. The substances come from the VIB-KU Leuven labs under the management of Massimiliano Mazzone and Gabriële Bergers and from a VIB-VUB lab headed up by Jo Van Ginderachter. Hundreds of millions are invested worldwide in attempts to mobilize the human immune system against cancer, often in combination with radiotherapy. Should one of the five new remedies make it through to the clinical phase, VIB would acquire 33% of Oncurious. In the meantime, ThromboGenics has invested an extra EUR 2.1 million in Oncurious.

With a product pipeline such as this, it would virtually be impossible to go public as a newcomer today. But ThromboGenics is a listed company and also boasts a recently reinforced management, seasoned executives and researchers who are yearning for a new pharmaceutical success – and a commercial success, for new warrant issues have since taken place. Perhaps the luck with which ThromboGenics was blessed in its first six years will come back. There would be much rejoicing if that were the case, on the part of Désiré Collen too. He knows full well that facts are to be respected. But we haven't gotten that far yet. We'll see, said the blind man.

Postscript June 2020

The final editing of the original book in Dutch 'Désiré Collen, Biotechpionier' was completed end of June 2018. In July 2018 the board of ThromboGenics proposed during its General Assembly to change the name of the listed Belgian company ThromboGenics to Oxurion (OXUR).

In August 2019, the company announced the results from an exploratory 70-patient phase 2a study evaluating the efficacy and safety of intravitreal THR-317, an anti-PIGF antibody, administered in combination with ranibizumab (Lucentis® Novartis), a VEGF inhibitor, for the treatment of DME, Diabetic Macular Edema. The study showed that the combination did not produce an increase in BCVA, Best Corrected Visual Acuity, in the overall population at Month 3. Consequently, the research into the use of anti-PlGF in existing medicines based on anti-VEGF was terminated.

At the end of 2019 Oxurion announced that the company would transfer the distribution of its Jetrea® medicine to the American-German company Inceptua Group. The Jetrea®-turnover decreased in 2019 to a disappointing EUR 4 million after EUR 5.3 million in 2018. Since 2012 some 35,000 patients have been treated with micro- or ocriplasmin.

Oxurion, the renamed ThromboGenics, continues two research programs: THR-149, a plasma kallikrein inhibitor and THR-687, a pan-RGD integrin protagonist (see above), both promising medical paths against DME, Diabetic Macular Edema. Decisive results of both THR-149 and THR-687 are expected in 2022/2023. With EUR 50 million available cash after the IPO, EUR 196 million private placements by Chris Buyse, EUR 42 million payments by Roche and EUR 228.7 million milestone and severance payments and a capital contribution by Novartis, Oxurion has 'burned' approximately EUR 464 million. End of 2019 EUR 52.7 million cash was still available and according to the 2019 annual report, the company is 'actively pursuing new funding'.

PART V: THE PROFESSOR EMERITUS KEEPS MOVING

Mark Waer

Chris Buyse

Arnoud de Pret

Pierre Drion

François Fontaine

Hilde Laga

Debasish Roychowdhurry

Urbain Vandeurzen

Désiré Collen

Fund+ Board of Directors, end 2018 (www.fundplus.be)

Désiré Collen

Chris Buyse

Nele Kindt

CoBioRes Board of Directors, end 2018 (www.cobiores.be)

Chapter 1: Fund+ makes up for the minus signs

Fund+ team in 2015
Left to right: Paul Magrez, Jan Van den Bossche,
Philippe Monteyne, Diane De Wyngaert, Chris Buyse, Désiré Collen

Fund+ team end of 2018:

Mark Waer

Chris Buyse

Philippe Monteyne

Jan Van den Bossche

Alexandra Tolia

Diane De Wyngaert

Sarah Van De Sype

Louis Declerck

Désiré Collen

Summary: *After his departure from ThromboGenics, Désiré Collen organized his public legacy. He strengthened his existing non-profit association LSRP and the Collen Charitable Trust with a private foundation, the Désiré Collen Foundation, and with an evergreen investment fund, Fund⁺. In the process he received support from the government and from several wealthy Belgian families. With the foundation, Désiré and Louisa Collen are seeing to it that the investment fund Fund⁺ is firmly established and will last indefinitely. In 2017 a single investment, Ogeda, promptly provided for a sizeable increase in the young fund's capital. Over time Chris Buyse and Désiré Collen can henceforth contribute to the continued advancement of medical science. With nearly EUR 200 million on the books at the end of 2018, the fund represents an additional source of prosperity for Belgium and Flanders*

Philanthropy in Belgium and abroad

At the end of 2013 Collen was professor emeritus and former chairman of ThromboGenics. With awards, recognitions, and an impressive curriculum vitae to his name, he had turned seventy. What next? He and Louisa had become established Londoners, and he had become member of the Reform Club, a private members club, the first one to admit women as from 1981, and he had become a fellow of the Royal Society of Medicine.

After leaving ThromboGenics, he still had quite a lot on his plate. New allocations had to be found for the Genentech royalties he had recovered from ThromboGenics, an amount which ran to several tens of millions. Collen channelled this money into new initiatives, with the non-profit association LSRP and the Collen Charitable Trust as the bases of operations. LSRP was the successor of his non-profit association Désiré Collen Research Foundation, which had to be renamed when in 2007 a new law dictated specific conditions to the statute of foundations.

For 19 years prior to 2007, LSRP and its predecessor had already been a generous funding office for the university, where justified calls for sponsorship were seldom dismissed. The Faculty of Theology had received sponsoring for an air conditioning system in its library with a unique collection, and the Faculty for Agricultural Sciences for a new experimental garden in Rillaar. That sponsorship continued after the change of name to LSRP.

Collen also supported projects abroad. Professor Sarah Harper's Oxford Institute of Population Ageing received financial support between 2012 and 2017 for research into the population explosion in sub-Saharan Africa, within the framework of the Collen Programme on Fertility, Education, and the Environment. (1) The programme studies the link between education of girls and birth control. "For five years Sarah was granted EUR one million a year to develop programmes aimed at heading off a demographic disaster in the region. At present there are 800 million people in sub-Saharan Africa but by the end of the century that figure will have risen to between 2 and 4 billion. The African continent can't handle that!" says a concerned Collen. "An economic migration of at least 60 million people from that region to Europe is anticipated. The only way to address this is to strengthen education overall and that of young girls in particular, so they can stand up for their rights. We also need to alert politicians and religious leaders to the problem." However, in May 2019 Collen stopped sponsoring Sarah Harper's programme, because he felt too small a part of his

sponsoring had been spent on field work. He earmarked the remaining EUR 650,000 that had not yet been transferred to the Oxford project, to be used for the initial intention.

The *Fundación Tejedores de Sueños* in Costa Rica was set up in 2010 by Collen's former HR Manager, Linda de Donder, who had moved to this Central-American country in 2001.(2) The *Fundación'*s main aim is to invest in the education of teenagers from poor families. LSRP had pledged USD one million to that project. In 2011, the first study grants were awarded, and the *Fundación* provides for transport, school materials and school uniforms for its pupils on a grant and expects them to regularly forward their school reports. To continue benefiting from the grant system, the students must pass every year. Together with his friend and wine connoisseur, Urbain Boutelegier M.D., Collen is now a member of the organization's Belgian supporting team.

Boutelegier (°1947) had been involved for many years in '*Casa Hogar'*, a project for street children in Toluca, Mexico. It was set up in 1986 by Michel D'Hooghe, M.D., then delegation head of the Belgian national football team, with the help of several 'Red Devils' (Belgium's national soccer team). Boutelegier was deputy chairman of the project until 2011 and he was one of the main fundraisers by organising regular wine events in the casino of Middelkerke, a Belgian sea resort. Désiré Collen sponsored the Casa Hogar for several years.

Until September 2014, Boutelegier was a GP in Assebroek, near Bruges. His practice was so popular that patients would sometimes sit on the stairs because the waiting room was full, according to an article in the newspaper *Het Nieuwsblad* on 11 October 2014. Up to the early 1990s, he had also been sports doctor for the Cercle Brugge soccer team. But the combination of off-site games and having to be in his practice early the next day constituted a pace that was difficult to keep up. There also were his wine activities and the Casa Hogar in Mexico, so he stopped his job at Cercle Brugge for other pursuits. "I had a very busy practice, but the work eventually numbs you, unless you have hobbies," Boutelegier explains. Through his knowledge of wine and his friendship with former fellow students, such as the ex-rector of the Leuven University, Mark Waer and cardiologist and professor emeritus Frans Van de Werf, he extended his network. At a dinner with Van de Werf he met Désiré Collen, which led to a close and lasting friendship.

Boutelegier's knowledge of wine was not something he had picked up from home. His father was a schoolteacher and there were six children. "We knew nothing about wine. But I did know a lot about farming and wine growing." His liking for wine came much later. During his time as a practising GP he had a patient with diabetes. Each time things were not going his way, the man would get angry and lock himself up in his wine cellar and drink himself comatose. "At his wife's suggestion, a friend and I bought his entire wine cellar," says Boutelegier. "The first time we opened one of his bottles at home, my wife and I agreed: this was not bad at all! And my interest grew from there." His appreciation grew even more when on a trip in France he was on his own at the breakfast table and got talking to the hotel guest on a neighbouring table, who introduced himself as Michel Bettane. It turned out that Bettane was a renowned wine critic and the author of numerous wine guides. Their chance meeting grew into a new friendship, which subsequently opened many doors, particularly those of wine chateaux.

In 2015, Boutelegier followed his friend Désiré Collen to the *Collen Programme of the Oxford Institute of Population Ageing*, where he became visiting fellow, until Collen's sponsorship ended. When Collen decided to grant more support to the project in Costa Rica, Boutelegier immediately put his shoulder to that wheel. Boutelegier now coordinates the *Sana Juventud* programme of the *Fundación Tejedores de Sueños*.(2) "I share Désiré's view about reproduction and education. The best way of setting about family planning and avoiding a population explosion is through education. Pregnancies at an early age are a major problem in Costa Rica, and through scholarships, sports and culture we try to keep girls longer in school and help them finish their secondary education." The Foundation has already organized a collaborative partnership with *Seprojoven*, an NGO for disadvantaged and indigenous girls using football as a way to cope with problems such as violence and poverty. The NGO links its activities to the education and empowerment of these girls. This project was supported by FIFA. Since then, Boutelegier has been traveling back and forth between Belgium and Costa Rica to support the Foundation in winning people over and in contacting other projects, such as the soccer school *Escuela de futbol mixta del Caribe* in Limon, or *Casa de los Niňos*, which started with a canteen for underprivileged children but also supports school attendance. Other alliances include the scout movement *Guías y Scouts Tropa 180* or *Merienda y Zapatos*, an organization giving tutoring to children from favelas and to immigrant children, as Costa Rica is coping with a growing number of immigrants from poorer neighbouring countries. *Tejedores de Sueňos* wants to play a coordinating role in these alliances with other NGOs that focus on education, and the organization works together with the Ministry of Public Education to achieve a larger impact. "There has to be a collaboration in some way or other," Boutelegier insists. "For instance, we give increased visibility to *Merienda y Zapatos*, and in turn they help us contact university graduates to mentor secondary students with all kinds of difficulties." Through this system of 'Aliados' or allies, the project becomes more widely known, and is now influential in 5 of the 7 provinces of the country. We have received a handsome budget from LSRP, and we will be spending that money effectively and transparently," Boutelegier assures. He is convinced that with the alliances they have set up, the project will continue even beyond the present sponsorship.

More recently Collen's lifelong colleague Roger Lijnen introduced him to his friend Fons Maex. Lijnen and Maex had studied chemistry together in the early seventies. In 1987 Maex had founded his own chocolate factory in Aarschot, north east of Leuven. His company, Kim's Chocolates, soon grew out to be one of Belgium's biggest chocolate producers and a major exporter, selling chocolate under its own brand names 'KC Chocolatier' and 'Cachet' and producing private label chocolate products for third parties. In 2007 Maex moved production to a new and larger plant of 11,556 m^2 or 120,000 sq.ft in Tienen, south east of Leuven. Maex who has always been committed to sustainability, had the new factory built with 100 percent recyclable material. The plant has solar panels on the roof providing up to 20 percent of the energy needs, and a state-of-the-art wastewater treatment installation based on bio fermentation. "We probably have the greenest chocolate factory in the world," Maex boasts.

When in 2010 he was looking for biologically produced cocoa beans in Tanzania, he was deeply affected by the living conditions of the local farmers. In conversations with the elders in the villages he heard their two main concerns were 'madrasses and magi', or schools and drinkable water. On his travels through the region Maex had seen many derelict schools and

school buildings that were unfinished because halfway construction the money had run out. As a hands-on planner he therefore decided to concentrate his efforts on schools. He made an inventory of the needs in 141 villages in the Kyela Busokelo and Rungwe districts of the Mbeya region in Tanzania and he set precise targets: renovating 700 schools, completing another 700 that were halfway finished, and building 700 new ones, that is, if he could find enough teachers for these new schools. The funds, about EUR 1 million per year, come mainly from premiums his company Kim's Chocolates, pays on every batch of cocoa beans. Hence the name of the project: 'Cocoa for Schools'. (3) A smaller part of the funds comes from private sponsors.

During his first meeting with Collen end 2018 Maex presented his project and Collen without further ado donated EUR 10,000. A second meeting followed in May 2019. Maex, who in 2017 had already stepped down as CEO of Kim's Chocolates was by then spending all his time on his 'Cocoa for Schools'-project. He had decided to build a boarding school for girls in Kafundo, where they could safely finish their schooling with no risk of sexual assault, unwanted pregnancies or forced marriages. The schooling of girls in underprivileged regions as a means to curb overpopulation has always been one of Collen's concerns. Hence Collen committed another EUR 75,000 towards the building of this boarding school. And if it turns out more is needed, Maex can count on additional funds from LSRP.

With more than half of the intended number of classrooms realised - close to 1,000 in the first quarter of 2020 -, the project has impressive results: in only 3 years the passing rate in the last year of the primary schools increased from about 50-60% to 85-98%. This in turn had an impact on the secondary schools: in 2019 an extra 4,300 pupils enrolled, the following year another 2,000 more and these figures are expected to continue to increase the coming years.

LSRP also supports cultural initiatives. Musicologist and Beethoven specialist Jan Caeyers could count on a contribution for his *Le Concert Olympique*, formerly the Beethoven Academy and prior to that the New Belgian Chamber Orchestra. Collen, who is not a connoisseur of music but rather a music lover, has carried on his sponsoring until the Beethoven Year 2020.

He also sponsored the Francqui Foundation with a gift of EUR 15 million. The Francqui Foundation was set up in 1932 by former US president Herbert Hoover and the Belgian diplomat Emile Francqui who were both life and soul to the Commission for Relief of Belgium and to the reconstruction of Belgium after WW I. The Foundation was set up to promote higher education and scientific research in Belgium and it finances scholarships, endowed chairs, and the highly esteemed Francqui-prize, which the Belgian king personally hands out every year to a scientist. Désiré Collen won the award in 1984.

As a laureate of the Louis Jeantet Prize for Medicine in Geneva in 1986 which awards scientists who have distinguished themselves in biomedical research, Collen wishes to set up a another Jeantet prize for translational medicine: 'the Jeantet-Collen Award', towards which he will set apart 20 million Swiss francs to generate an annual grant of CHF 600,000.

More recently LSRP helped finance the acquisition of the Ton Koopman library by the Ghent Orpheus Institute, the European center for artistic research into music. Ton Koopman (°1944

Zwolle) is a Dutch conductor, founder of the Amsterdam Baroque Orchestra, and a virtuoso on the organ and the harpsichord. He is specialized in Bach and baroque music and is known for his historically accurate interpretations of these compositions. During his yearlong career as a musician and a researcher Koopman has collected many prints and original manuscripts of baroque music and added his own annotations and comments about the interpretation and execution of many pieces. His collection, consisting of thousands of volumes from 1600 till 1800, supplemented by many 19th and 20th century works, is now part of the Ghent Orpheus Institute, where it will serve as a library for artistic research, and as such will also be digitally accessible to music researchers in Belgium and beyond.

In May 2017, Collen became a 'Permanent Resident' of the UK and in March 2019 he obtained 'Settled Status' under the EU Settlement Scheme of the UK. He set up Patcobel UK Ltd, a UK company limited by shares according to the Companies Act of 2006, registered by the Companies House of England and Wales on 19 December 2017 (Company Number 11118392). This company will manage his future limited remunerated activities as a UK domiciled resident. Collen also founded a UK based charity that was registered by the Charity Commission of England and Wales on 28 February 2020 as the 'Foundation for Education to improve Family Planning' (Registered Charity Number 1188260). This charity will administer the residual funds earmarked by LSRP for the field activities of the Oxford Programme, the Tejedores de Suenos project and the Cocoa for Schools Building project, as well as any future philanthropic activities and donations of Collen as a UK domiciled resident.
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LSRP accelerates

Collen's non-profit association got into gear in the wake of Chris Buyse's transfer from ThromboGenics to LSRP in 2014. The objectives were still to award study grants, organize lectures, set up biotech infrastructure in Flanders and Leuven, support scientific cooperation with the KU Leuven and other academic institutions, and last but not least to help young companies with advice and venture capital. LSRP quickly turned into a success story. Four years after Buyse had left ThromboGenics, LSRP was the medical research institute with the greatest financial means in Leuven.

The institute is located in Leuven, but not in KU Leuven's sphere. After Collen's removal from LRD in 2001, the KU Leuven has less control over this non-profit association. LSRP is not one of KU LRD's 76 divisions. The university is represented on the board of directors, but 'statutory director' Collen has the last word, together with fellow director Chris Buyse. Collen can appoint a successor and, should the non-profit association ever be dissolved, the capital would go to "the KU Leuven and/or the Flemish Institute for Biotechnology", according to the articles of association. The term 'and/or' is used, which could yet make things interesting. "Collen likes to decide himself what happens and what ought to happen. And he only invests in what he understands," says Jo Bury (VIB). "And he understands the Flemish Institute for Biotechnology, whereas he does not always understand the KU Leuven."

LSRP has been investing in biotech since as far back as 2006, when ThromboGenics came back to Belgium. With Chris Buyse as an occasional right-hand man, Collen acquired participating interests in a few young companies, two of which would subsequently go public.

Those flotations occurred at much higher valuations than the amounts LSRP had invested in the companies. LSRP's holding in the Liège company Cardio3Biosciences, which is now called Celyad Oncology, increased sevenfold, whilst the capital contribution it had made to Bone Therapeutics quadrupled. The increases in value are not always cashed and therefore remain latent and invisible. These successes more than made up for the one setback, with Amakem, meaning that LSRP gradually built up a substantial amount in savings. LSRP is also co-owner to the tune of one third of the Bio-Incubator building in which ThromboGenics is housed. In addition, LSRP still holds licensing rights to the substance that had given rise to ThromboGenics' drug. Were Jetrea® to catch on, something that was still a possibility at the end of 2013, LSRP would have to find a use for that money too. But in the end that led to disappointment; income from microplasmin rights remained dismal.

"With our royalties, since everything is always to be traced back to that, we made around ten investments with our non-profit association LSRP," says Collen. "That was done off the cuff, without us properly keeping check of it all. But some participating interests did well. Cardio3Biosciences, which is now Celyad Oncology, went public, as did Bone Therapeutics. And when Chris also left ThromboGenics, I suggested he turn it into something professional."

Chris Buyse left ThromboGenics in August 2014, and Collen included him in his ambitious new plans. In February 2015, the Désiré Collen Foundation (DCF) (*Désiré Collen Stichting* – DCS) was established, and in May 2015 a new investment fund, Fund$^+$ was set up. Collen soon had four operational Belgian legal entities, DCF, Fund$^+$, LSRP and a new biotech company CoBioRes, in addition to Belgian and offshore structures housing his more limited family assets. Collen thus embarked on a new adventure that was soon to be many times more successful than ThromboGenics.

The Désiré Collen Foundation

A foundation is an instrument that defies time, outliving its founders. It is a structure that can be used to leave funds for a particular purpose, for perpetuity if so desired. The foundation continues to carry out its task long after its founder has died. Belgium has around 1,200 foundations, half of which are institutions of public utility and the other half private charitable establishments. By far the largest foundation of public utility is the Koning Boudewijn Stichting, which is itself responsible for managing some 600 foundations, all together representing slightly less than half the total asset value of all Belgian foundations. That value can be put at EUR 1.5 billion *(estimated in 2018)*. Private foundations can serve to protect vulnerable people when family protection ceases, but also to convert shares into depositary receipts and lock-in a family fortune. However, most private foundations have a public utility, i.e. culture, health, social affairs and, like the Désiré Collen Foundation, science. A major advantage is the tax treatment of the capital under management, on which an annual tax of 0.17% is due. And at the time of establishment and when capital contributions are made, there are favorable donation taxes (5.5% in Flanders) and duties on legacies. Besides his own foundation, Collen also made gifts to existing foundations, such as EUR 15 million to the Francqui Foundation, which had awarded him the Francqui prize back in 1984. (3)

In actual fact, Désiré Collen already had a 'foundation'. The legal predecessor of the non-profit association LSRP was called the Désiré Collen Research Foundation, but when the legislator reorganized Belgian foundations along different lines in 2002, ordinary non-profit

associations were no longer allowed to call themselves foundations. The Foundation thus became a non-profit association, bearing the name LSRP (Life Sciences Research Partners). The Collen Charitable Trust and the offshore company Biggar into which Collen had for years channelled a large part of the Genentech royalties, were some sort of foundation as well. Collen thereby relinquished his proprietary right to a trust that was also of 'public utility', namely the support of scientific research. But with the Genentech royalties coming to an end and with the new Belgian Désiré Collen Foundation, these offshore structures gradually shifted into sleep mode.

The object of the new Désiré Collen Foundation was "the promotion, advancement and fulfilment of economically and socially innovative developments and acquisition of knowledge in the field, chiefly but not exclusively, of biosciences, medical science and science in general, chiefly but not exclusively in Belgium". The foundation's name and object may only be changed during the founder's lifetime, and not thereafter, and that is the 'perpetuity term'. The board consists of three (and a maximum of five) people whose mandate is unpaid. The first directors are Désiré Collen, his wife Louisa Reniers, and Chris Buyse. The initial capital was EUR 25,000, but that figure increased by three zeros in the following two years. The Désiré Collen Foundation became in no time one of the larger Belgian private foundations. (4)

A first stop

Three months later, in May 2015, the full purpose of the Désiré Collen Foundation grew apparent. The foundation became a 'perennial' anchor for a new investment fund, Fund$^+$. Collen told the newspaper *De Standaard* in July 2015: "The capital contributions I make, remain in the fund. I have set up a special foundation for this purpose. The money can no longer leave it. And I will also reinvest any capital gains I receive on the participating interests."

Alongside the Collen foundation, the non-profit association LSRP was also a co-founder of Fund$^+$. The foundation brought in EUR 2.5 million and LSRP EUR 6.5 million, of which 5 million in kind and 1.5 million in cash. All together that totaled EUR 9 million. LSRP exchanged its participating interests in a number of companies for Fund$^+$ shares. The capital contribution consisted of 111,474 shares in Euroscreen from Gosselies, a company that would later cause a stir under the name of Ogeda. LSRP also brought in its stake in iTeos Therapeutics, 10% or so of the total number of shares in this company. iTeos is a spin-off of the French-language university UC Louvain and is active in immunological cancer research. A quarter of the Ghent-based company Q-Biologicals constituted the third contribution, which was sold a little later to a French group. There were also 250 convertible loans from Masthercell and finally 11,912 of the existing 289,922 shares in Promethera Biosciences. Promethera started out at the UC Louvain as well and was aiming to use stem cell therapy to tackle liver diseases other than those caused by alcoholism. The presence of Japanese and Korean partners was noteworthy.

These participating interests were recorded in LSRP's books to the value of EUR 5.4 million, but LSRP brought them into Fund$^+$ with a 7.5% discount, i.e. for 5 million. LSRP's listed holdings, i.e. Celyad and Bone Therapeutics, were not brought in. Fund$^+$ soon acquired part

of Novadip, another UC Louvain offspring that wanted to help repair bone fractures using stem cells.

With Fund⁺, Flanders and in fact the whole of Belgium, bearing in mind the significant French-speaking portfolio, has an investment company that will be specialising in biotechnology. The whole set-up is well prepared. Fund⁺ starts out with soundly formulated articles of association as an 'investment company'. Investments will be made in life sciences, but also science in general. The company clearly has no desire to be a passive investor but wants "to be directly or indirectly involved in the establishment, management, running and monitoring of its participating interests". It may also do this on behalf of third parties, i.e. on the instruction of investors who do not have specialists at their disposal.

This makes Fund⁺ an initial stop for young specialized companies in their search for financing. The aim is to have a lot of dossiers sent in – a critical mass from which potentially the best ones can be picked. To be able to do that Fund⁺ has to have the know-how in house to make judicious selections, and so the availability of this specialist skill is included as an objective in the Fund⁺ articles of association. In addition to a general meeting and a board of directors, provision is made for a strong executive committee, with wide-ranging authority for Chairman Collen and his three to six fund managers. Collen and managing partner Chris Buyse formed part of this executive committee until the end of 2017. Since then Mark Waer (°1951) has taken office as the new Chairman in Collen's place, a replacement officially confirmed at the general meeting in June 2018. Collen will remain on the board initially as a director and thereafter as an 'observer', and Buyse is to continue as a managing partner.

In addition to Diane De Wyngaert, who had already been Buyse's and Collen's organizational number two for some time, Fund⁺ took on three experienced fund managers as 'partners'. Paul Magrez, an MD with a PhD in medical computer sciences from the ULB, acquired 30 years' experience with the pharmaceutical companies UCB and GSK, among others. He started up his own consulting firm and later used this to support Fund⁺ with the selection and monitoring of the shareholdings. He was director of Aelin Therapeutics, in which Fund⁺ became shareholder in 2017. He remained with Fund⁺ until the middle of 2018. Philippe Monteyne is a neurologist from the UC Louvain with a PhD in viral immunology. He worked for GSK and Sanofi and is a director with Novadip and eTherRNA (until end 2018) and with the French gene therapy specialist Horama in which Fund⁺ acquired a stake in 2017. The third partner is Jan Van den Bossche, a KU Leuven economist, who had been a biotech analyst with the broker Petercam for 12 years and went on to become investor relations manager at the Dutch chemical group DSM. He is director of Octimet Oncology and the German company Immunic in which Fund⁺ became a shareholder in 2017. Immunic works on drugs to combat colitis ulcerosa (an inflammation of the large intestine), Crohn's disease, and the skin disease psoriasis. He took over the directorship at Aelin Therapeutics after Paul left Fund⁺. In December 2018 Alexandra Tolia joined Fund⁺ as Partner. Previously, Alexandra led venture capital investments in private and public companies at PMV and Hunza Ventures. She holds a PhD in Medical Sciences from the KU Leuven, followed by a postdoctoral fellowship at UCSF (San Francisco, USA), and an executive degree in General Management from the Solvay Brussels School of Economics and Management. Alexandra has served as board member in several companies active in life sciences and has taken over the Fund⁺ board position at eTherRNA.

The clear intention with Fund+ is to develop a center of knowledge that not only selects investments carefully but also subsequently monitors them with specialists. In many private investment vehicles this knowledge is not on hand, in which case investment risks are sometimes taken on a hit-or-miss basis and are limited by a broad spread of those investments. But that only makes monitoring more difficult. The main restriction laid down by Fund+'s articles of association also relates to that spread, since an investment may never exceed 10% of the equity capital plus the committed amounts.

The fund is also open-ended, which means the intention is not to ever have it liquidated. "Most high-risk funds are discontinued after seven to eight years," explains Chris Buyse. "The invested money, preferably with substantial capital gains, then reverts to the investors. That is the case, for example, with the funds of Capricorn, another Leuven investor. But in the case of Fund+, the time limitation does not exist, and that's really for the better in the biotech sector. The studies carried out in these areas are quite simply very time consuming. For some investors that constitutes an obstacle, but for the type of investor we were looking for, it was a positive factor." So, anyone in Belgium wishing to invest sustainably in the biotech industry and its related disciplines can now look to Leuven's Beguinage (Begijnhof) where Fund+ and DCF are established – a carefully selected spot in a private residence that Désiré Collen once built for professors temporarily staying in Leuven.

The Fund+ board made provision for a special status for the Désiré Collen Foundation (DCF) until the end of 2017. Until then Collen was in all respects the Chairman, whereupon DCF can be one of the three main shareholders that together appoint the managing director. At the beginning that was of course Chris Buyse. Via his company Pienter-Jan, Buyse and Collen are the first directors, with an unpaid mandate until 2020. A maximum of nine directors may be elected for a term of up to six years, and of these, four are independent directors. In principle there should always be two candidates for a mandate. The authorized capital amounts to EUR 200 million, but Fund+ already started straight away with an available capital amounting to EUR 125 million. This is callable (i.e. available on demand from the shareholders) when larger investment projects present themselves – a proviso to which the shareholders are committed.

Add a plus sign

Why is there a plus sign in the name after the word Fund? For Collen, the plus sign refers to societal commitment, something he did not find on the stock exchange. He wants the fund to be more than a purely financial fund, as he said in De Standaard two months after Fund+ was set up. (6) "With Fund+ I want to invest, together with others, in the continued growth of bio- and pharmaceutical companies. We want to help young promising companies to get established here. We want to keep the intellectual property and the jobs here." When De Standaard points out that there are already numerous funds, Collen replies: "The moment a traditional venture capitalist grasps the front door handle, he already has his eye on the exit through which he can get out as quickly as possible. Our aim is to keep promising companies that are important for the economy and the prosperity in our country for as long as possible. Obviously, we are not averse to posting a return on that, but not in the context of early exits."

ThromboGenics has left Collen with an abhorrence of certain stock market practices, in particular the role played by 'shorters', i.e. speculators who sell shares they do not own but in the best case have borrowed from somewhere. In these sales, they rake in a certain amount and then, sometimes with the help of naive media, cause the share price to fall. In every company one can find bad news that can be dramatized. If the difference between the guaranteed price the speculators get when they sell and the new lower price is big enough, they buy back the shares they sold and pocket the difference between the higher selling price and the lower purchase price. This happened on a large scale at Dexia Bank between 2008 and 2011. The fall in the ThromboGenics share price also provided the shorters with a jackpot. Since Collen's departure as Chairman of ThromboGenics at the end of 2013, the share price has tumbled from EUR 40 to less than EUR 5.

In the middle of 2015, Collen was indignant about that stock market slump, as evidenced by his words in *De Standaard*: "It's not normal that people who only speculate should earn so much money. This practice should be taxed. On the other hand, people who invest in company growth should be given financial incentives. When it comes to the shorters, who borrow shares on a purely speculative basis in order to sell them at a profit, why not have them pay 50% tax on those profits? There's too much highway robbery on the stock market and too few people who invest in growth." (5)

Collen hates the cynicism that comes with this speculation on failure and decline. "As long as I live, don't expect to find Fund$^+$ on the stock market," he says. Collen frowns doubtfully when one suggests that a solution might be the Expert Market of Euronext – where pricing occurs only once a week – because then the small investors could also be fellow companions.

Fund$^+$ makes a break with the remuneration system that paves the way to quick gains on the stock markets. Fund$^+$ executives are paid correctly and in line with the market, but bonuses are at all times subject to the board of directors' discretionary decision and are therefore never an enforceable rule. In biotech funds, up to 20% of the capital gain goes to the managers in the event of an exit. At Fund$^+$, that is not the case. The board's remuneration committee decides on the fixed amounts and the variable remuneration, partly on the basis of a particular formula and partly in a wholly discretionary manner. Heated disputes about fund managers' pay are unlikely at Fund$^+$. But, of course, an excellent fund manager has his/her market value.

Collen wins over families

When Collen voiced his criticism of the shorters at ThromboGenics in the middle of 2015, his Fund$^+$ had already notched up substantial growth. Despite ThromboGenics, the aura around Collen still had an impact. His personal commitment, both financially and in the day-to-day management of Fund$^+$, his undisputed expertise and his business talent still opened purses. The fact that Collen himself had covered Fund$^+$'s operating costs in the first three years also had a persuasive effect.

A week after its establishment by the Désiré Collen Foundation and the non-profit association LSRP, the pick of the Belgian business community had joined up to become shareholders. Two big names particularly stand out. Arnoud de Pret is a widely respected nobleman hailing from one of the families behind the international brewing group AB Inbev.

He had considerable industrial experience, including at operational level, among other things as financial manager of Umicore from 1991 to 2000. He was also director at the Emsens family's large Sibelco sand group and at the Janssen family's UCB chemical group. The then 71-year-old Arnoud de Pret entered Fund⁺'s capital with his family holding company Multifin, of which his three daughters are also directors. He bought 1,500 new Fund⁺ shares for EUR 1.5 million. His pledge was four times larger. The pledged capital can be mobilized by the board of directors at any time within the context of the statutory authoried capital.

Public holding companies also believed in the Collen-Buyse duo. Two holding companies pledged EUR 10 million each, paying up EUR 2.5 million of this amount immediately. These were: Wallonia's SRIW (the *Société Régionale d'Investissement en Wallonie*), a holding company with a balance sheet total of close to one billion and accounting for some EUR 100 million in investments every year; and the federal Belgian public holding company FPIM (Federal Holding and Investment Company), which also invested EUR 2.5 million, with four times this amount as a commitment. The lack of Flemish public investors has to do with the fact that the Flanders Venture Capital Company (*Participatie Maatschappij Vlaanderen* – PMV) has a biotech portfolio of its own and has a preference for funds with a termination date.

A participating interest of the same size was also acquired by the Leuven industrialist Urbain Vandeurzen, who developed and then sold his company LMS, Leuven Measurement and Systems. With his family, Vandeurzen manages the holding company VF Capital with a balance sheet total of EUR 360 million, of which in excess of EUR 300 million took the form of investments and liquid assets at the end of 2016. Finally, with half a million EUR and a pledged EUR 1.5 million, the Brussels financier Pierre Drion, a friend of Collen's, also came on board. Drion, Vandeurzen and de Pret became board members, with de Pret as an independent director. This was possible because he was not one of the reference shareholders mentioned in the articles of association, i.e. the three largest shareholders. These are still LSRP and, on the same footing, SRIW, FPIM, Vandeurzen, and additionally DCF, the Désiré Collen Foundation – with a special status until the end of 2017. DCF will also be represented in Fund⁺'s board in perpetuity, after that 'initial period' running to the end of 2017, albeit as an 'observer' should DCF and Désiré Collen no longer be elected to the post of director. That gives Fund⁺ the timelessness of the Désiré Collen Foundation. Fund⁺ thus became a wholly evergreen fund, in contrast to most other funds that only wish to invest if the planning includes an exit.

Capital kept pouring in. Two months later, Vic Swerts paid his respects. A self-made man, Swerts is the sole owner of Soudal, a flourishing silicone company in the Campine region, set up in 1996 and with its head office in Turnhout. The Soudal holding company with which Swerts invested in Fund+ had an equity capital of EUR 77 million, largely consisting of carried over profit. The operating company Soudal posts a worldwide turnover of EUR 670 million with a net profit of around EUR 25 million after a sizeable tax burden *(figures 2017).* The company is specialized in joint filling compounds, adhesives, foams, lubricants and cleansing agents.

Swerts pledged EUR 5 million and immediately invested EUR 1.25 million in Fund⁺. Another new shareholder was the KU Leuven, via KU LRD chief executive Koen Debackere and his

team, with EUR 1 million and a further commitment of EUR 3 million. DCF, Collen's foundation, then slipped a further EUR 1.25 million into Fund+ and de Pret's Multifin EUR 325,000. Another new shareholder was Tolefi NV, a company owned by the French-speaking Goblet family. With Tolefi, Goblet had built up a capital base of EUR 100 million, primarily in property. This led to the paid-up capital contribution in Fund+ rising from EUR 18.5 million to EUR 22,825 million.

Five months later, in December 2015, Fund+ refuelled again. DCF put another EUR 2.5 million into Fund+, taking the foundation's total investment in the fund to EUR 6.25 million. Multifin contributed another EUR 250,000. Sambrinvest reinforced the public capital contribution to Fund+ with EUR 0.5 million. This entity is a Walloon holding company with a balance sheet total of EUR 31 million, of which EUR 10 million is equity capital. The board of directors comprised no fewer than 16 members. With a contribution of EUR 0.5 million, another newcomer was the limited partnership Omnivale from Schilde, near Antwerp. Wouter Vandersypen is the business manager. We are talking here of heirs of the founders of the J. Van Breda & Co bank. Now Fund+ had EUR 25,325,000 in fully paid up capital at its disposal. And the board was given some legal reinforcement with the arrival of the renowned lawyer Hilde Laga, at the request of Collen and four reference shareholders.

In the meantime, the Fund+ team had obtained new investments. In March 2016 Fund+ took part in a capital increase of eTheRNA, a promising Flemish-Brussels company (VUB) incorporated in 2013. Professors Kris Thielemans and Bart Neyns set about attacking skin and breast cancer using immunotherapy, based on an mRNA technology (messenger RNA) they called TriMix. Still in 2016, Fund+ strengthened its board of directors and its scientific committee with Debasish Roychowdhury, an experienced American Indian cancer specialist who had worked in major pharmaceutical companies in the past. In Belgium, Roychowdhury is also director at Celyad, in which LSRP had a holding until 2017.

Federal holding company gives a boost

The development of Fund+ as a center of knowledge in the field of biotechnology became known in broader circles. There was a need for such a knowledgeable fund. Financiers are regularly approached by whizz kids in biology and medicine who claim to have discovered the Holy Grail. Most financiers have little clue as to what this is about. The world of biotech is complicated, and the specializations so numerous that in the end everyone is largely illiterate. The major Flemish financiers include a few experienced old hands and perhaps also former pharmacist Marc Coucke – people who do understand the sector. With his capital of EUR 1.3 billion, Coucke holds direct participating interests in a few pharmaceutical and biotech companies that he monitors personally. But other financiers who lose their bearings when they listen to boastful biotech stories can find their way to Fund+. If need be, Fund+ can also send a competent director to adventurous companies in which a lot of money disappears every year.

Fund+ gradually became a compass and a guide for investors. That was the case on 27 September 2016 for the Federal Holding and Investment Company (FPIM), which was already a shareholder in Fund+. On that date, the FPIM brought its participating interests in three companies into Fund+. This concerned a contribution in kind which was paid for with

new Fund+ shares. The total value of the contribution was EUR 13,038,000, which was paid for with 12,300 shares of EUR 1,000, so EUR 12.3 million. The rest of the amount was posted as issue premium, a component of equity capital. This meant that a new share no longer cost EUR 1,000, but EUR 1,060, EUR 60 of which being issue premium. FPIM's contribution resulted in Fund+'s capital rising from EUR 25,325,000 to EUR 37,625,000 plus EUR 738,000 in issue premium. And the commitments on top of that mean that Fund+'s clout was three times greater. After this contribution, François Fontaine joined the Board of Directors as a representative of FPIM/SFPI. Fontaine had previously been a senior advisor to the socialist (PS, Parti Socialiste) politician Laurette Onkelinx for eight years.

Of the three participating interests brought in by FPIM, one would later prove to be of great significance. This concerns 991,430 Euroscreen shares. The value of this contribution was fixed at EUR 5 million. Fund+ already held 111,474 shares in Euroscreen, so Fund+'s total participating interest in Euroscreen rose to 17.27%. In addition to that FPIM also contributed 28,852 B-shares in Promethera Biosciences. Here, too Fund+ already held shares in this company, to the tune of 11,921 in this case, so Fund+'s stake in Promethera rose to around 13%. Finally, 22,274 Novadip Biosciences shares moved from FPIM to Fund+.

It did not stop there. At the same time FPIM also brought in EUR 752,600 in cash, for which it received 710 new shares. This turned FPIM into a reference shareholder of Fund+, and some shareholders therefore in turn increased their stakes in Fund+. The shareholders Drion, LSRP, KU Leuven, Sambrinvest, Tolefi, SRIW and Omnivale renounced their preferential rights and were thus prepared to have their stake in the share capital diluted. The other shareholders subscribed to new Fund+ shares. Once again it was Collen's foundation that made the largest effort. DCF subscribed to 2,600 new shares at a price of EUR 2,756,000, which brought the private foundation's fully paid-up contribution to EUR 8 million. The de Pret family's Multifin followed with EUR 1,150,000 and 1,085 new shares. The Vandeurzen family's CF Capital paid in EUR 1,250,000 and received an extra 1,180 shares. Vic Swerts of Soudal kept it to EUR 503,500 and received 475 new shares. And four new shareholders also subscribed to shares. Meusinvest is a Walloon public holding company with a balance sheet total of close to EUR 400 million, with an investment of EUR 503,500 for which it received 475 shares. The Brussels noble financier Marc Nolet de Brauwere van Steenland brought in the same amount, as did the Luxembourg company EVDC Invest of Luc Van de Casseye. And with a contribution of EUR 254,400 Spinventure NV, a small Liège investment company with a balance sheet total of 17 million consisting almost entirely of liabilities, was accepted as the smallest shareholder. In total, Fund+ thereby acquired an additional EUR 8,177,900, of which EUR 462,900 in issue premium, in exchange for 7,715 new shares.

The shareholder breakdown in the wake of all these new contributions and operations was as follows:

Shareholders	27.09.2016 (before FPIM contribution)		27.09.2016 (after FPIM contribution)	TOTAL 45,340	Percentage
DCF	5,000		2,600	7,600	16.76
LSRP	6,500		0	6,500	14.34
Drion	500		0	500	1.10
Multifin	2,075		1,085	3,160	6.97
FPIM	2,500		710+12,300	15,510	34.21
SRIW	2,500		0	2,500	5.51
VF Capital	2,500		1,180	3,680	8.12
KU Leuven	1,000		0	1,000	2.21
Soudal Holding	1,250		475	1,725	3.80
Tolefi	500		0	500	1.10
Omnivale	500		0	500	1.10
Sambrinvest	500		0	500	1.10
Spinventure			240	240	0.53
Meusinvest			475	475	1.05
Marc Nolet			475	475	1.05
EVDC			475	475	1.05
				45,340	100

The largest shareholder was now FPIM, i.e. the federal government, with 34.2% of the shares. Collen's foundation held 16.7% (and together with LSRP, 31%). With 8.1%, Vandeurzen is the third-largest shareholder, followed by de Pret with almost 7%. SRIW had also made a sizeable commitment. Fund⁺ closed 2016 with a balance sheet total of EUR 47.2 million, of which EUR 23 million was still in cash. Total callable capital amounted to EUR 125 million.

The miracle of Cana

Fund⁺ therefore moved into 2017 with solid financing and sound management. Four new participating interests were added that year. Octimet was carrying out preclinical studies to ascertain whether research into certain cancer-suppressing substances from the Janssen Pharma stable in Beerse would be worthwhile. Fund⁺ was also invited abroad. That led to interests in the German company Immunic and the French company Horama. Immunic was also still at the pre-clinical phase in its studies into the relevance of a number of substances to combat intestinal problems, Crohn's disease and psoriasis. And with Horama, Fund ⁺ was again venturing into eye diseases, since Horama was looking for a genetic intervention against hereditary defects of the retina.

The year 2017 was to be an extraordinary one for Fund⁺. On 3 April, the Japanese pharmaceutical group Astellas Pharma made a fantastic bid for Ogeda, which had gone by the name of Euroscreen until 2016. With a view to a possible flotation in which EUR 50

million to EUR 70 million would be raised, Euroscreen had changed its name to Ogeda, but the flotation did not happen. Astellas bid EUR 500 million immediately and a further EUR 300 million if certain milestones were attained. That meant EUR 800 million for a company with a nominal capital of less than 40 million – nominal capital that had been almost completely wiped out by carried over losses.

Ogeda-Euroscreen from Gosselies, a spin-off of the *Université Libre de Bruxelles*, had been incorporated in 1994 and had a capital of EUR 34 million further to a capital increase in 2015. During this time, it conducted research in various directions, based on GPCR technology (*G protein-coupled receptors*). These are a large family of proteins that receive signals through the membrane of somatic cells and then set certain processes in motion via the G protein. Smell, for example, can lead to hormonal surges via these receptors. The discovery of these receptors led to Robert J. Lefkowitz and Brian K. Kobilka being awarded the Nobel Prize for Chemistry in 2012, after Alfred Gilman had received the Nobel Prize for Physiology and Medicine in 1994 for the discovery of the G proteins that pass on the signals from the receptors into the cell. Since then the effect of G protein-coupled receptors has been revealed down to the smallest detail. This research was and is of great pharmaceutical significance. Around half of all current medicines, including beta blockers, antihistamines and various psychiatric medicines, achieve their effect by acting on these receptors.

But Euroscreen had been evaluating the possibilities of GPCRs for years to no avail, until the Frenchman Jean Combalbert took over at the helm in 2007. With Euroscreen's substantial know-how, he set about looking for a drug to combat hot flushes experienced by women during the menopause, a condition that affects 80% of women, of whom one in four seeks medical help. There are medicines available for the complaint, but these act on the oestrogen hormone and that causes side effects, even including cancer and vascular diseases. The wide-ranging search for HRTs, hormone replacement therapies, yielded little in the way of results, until Combalbert and his team successfully brought their fezolinetant through Phase IIa. Fezolinetant acts on the neurons that regulate body temperature. The results were announced in January 2017. After a four-week treatment, complaints decreased in 89% of the cases, compared with 38% for a placebo, and after 12 weeks the ratio was 93% vis-à-vis 54%. This concerns the frequency of the flushes. The intensity of the flushes decreased in 60% of the cases after four weeks, and in 70% after 12 weeks. Side effects are limited and not serious. (7)

Astellas' bid for Ogeda was a most significant opportunity for the shareholders. Ogeda's largest shareholder is Vesalius Biocapital II – Vesalius Biocapital's second investment fund with EUR 78 million at its disposal. It was set up in 2011, after the success of a previous fund from 2007 that raised EUR 76 million. This Belgian investment fund is run from Luxemburg and has similarities with Fund^{+}, although it seeks exits much more explicitly than Fund^{+}, on the stock exchange or by means of a takeover. Since 2007 the partners and financiers have carried out some 21 investments, and for six successes there has been just one write-down. Management of Vesalius is in the hands of four specialists. The man who managed Ogeda from Vesalius is Alain Parthoens, a biochemist, computer scientist and financier and a well-known figure in Belgian and European associations for venture capital. After the success of Ogeda, Parthoens announced that he wanted to set up a new fund, Newton Biocapital, with a capital of EUR 200 million by the end of the following year. Ajit Shetty, former chief executive

of Janssen Pharma (Johnson&Johnson) would be chairman, and three investors had come forward: FPIM, Belfius Insurance and Sambrinvest. At the end of 2017, after three months of fundraising, the counter stood at EUR 30 million.

With 18.01% of the capital, Fund$^+$ was the second largest shareholder in Ogeda, and thus immediately pocketed EUR 90 million of the 500 million on 17 May 2017. EUR 84 million of this is capital gain, the difference between the amount actually invested and the share in the takeover price. Most Fund$^+$ shareholders could thus more than double their capital stake since Fund$^+$ had been set up in May 2015. Collen's foundation, DCF, pocketed a capital gain of EUR 14.1 million for a total investment of EUR 7.9 million. LSRP received EUR 12 million against an investment of EUR 6.5 million. Multifin was entitled to EUR 5.8 million against a contribution of EUR 3.1 million and the Vandeurzen family's VF Capital was looking at EUR 6.8 million for a contribution of EUR 3.6 million. Barely two years after Fund$^+$ had been established, on 29 September 2017, EUR 45 million flowed back to the shareholders – a sum equal to the total capital that had been called up until then.

A EUR 200 million fund

After that EUR 500 million, there was a further 300 million to come. For that two more steps had to be taken with fezolinetant in Phase III, and that could still take years. Regulators could also be responsible for some delay. But given the results in Phase II there was little reason to doubt success. Otherwise Astellas would never have coughed up 500 million. When that extra EUR 300 million is paid, there is a further capital gain of 54 million coming Fund$^+$'s way. For DCF that means a total capital gain of 23.2 million. That amount has to be added to the 7.9 million DCF has already allocated to Fund$^+$. With EUR 31 million, as a private foundation of public utility DCF then becomes one of Belgium's major private foundations. And the non-profit association LSRP becomes the KU Leuven's largest non-profit-making association. The total capital gain for LSRP amounts to 19 million, to be added to a balance sheet total of 24 million.

The story is also positive for FPIM but on account of Ogeda shares brought into Fund$^+$ at the end of September 2016, the capital gain on that block of Ogeda shares had to be shared with the other Fund$^+$ shareholders. The federal investment company secured a capital gain of EUR 28.9 million from Ogeda's 500 million via Fund$^+$ and has the prospect of a further EUR 20 million or so from the remaining 300 million.

Chris Buyse: "We started out with a potential capital of EUR 125 million, a quarter of which we called up in cash. That became approximately 45 million further to LSRP's and FPIM's contribution in kind. After all manner of costs and taxes, an extra EUR 70 million has now been added to that from Ogeda, which could later become 120 million. We carried out a capital reduction and dividend issues, so that a part of our resources flowed back to the Fund$^+$ shareholders. But DCF has undertaken immediately to re-contribute this payment as capital. FPIM did that in respect of half of the capital gain. When we base ourselves on our callable capital of 125 million, we add Ogeda's 70 to that and take 30 million off for the capital reductions and dividend issues, that leaves us with EUR 140 million in actual, available funds. We will temporarily have a lot of cash available."

The shareholders' commitment is also strengthened. Where they used to commit themselves to a capital increase when extra funds were needed, now the existing shares are only fully paid up for a quarter. That makes contributions easier. The unit share price is now EUR 1,400. After these operations, the Désiré Collen Foundation holds 26.29% of the shares, against a contribution of EUR 21.16 million and a further EUR 17.4 million in pledged funds. LSRP made a contribution in kind of 4.0 million and holds 3.47%. The second largest shareholder is the Federal Holding and Investment Company (FPIM), with a stake of 20.18% and an additionally committed 5.6 million. The Vandeurzen family is the third largest shareholder with 12.51% after a contribution of 8 million and 8.4 million pledged. The de Pret family holds 10.13% after an investment of EUR 6 million and a commitment of 7.3 million.

When Ogeda later pays its second instalment, Fund^{+} will have funds totalling close to 200 million. "Nobody in Belgium can match that and we are becoming a respectable fund in Europe," says a proud Buyse. "Now we are receiving invitations for all manner of financing syndicates in Europe. We'll be working together with a Swiss fund, Omega, with LSP in the Netherlands, which is co-run by Rudy Dekeyser, former chief executive of the Flemish VIB, and with the French company Kurma. Those are all funds of between EUR 120 and EUR 150 million, which means they're comparable to Fund^{+}. But we're doing that without overlooking our original mission: we're still a Belgian fund that invests first and foremost in Belgian companies. We might earmark an amount for investment in a slightly earlier stage, but always clearly after the seed money." By seed money Buyse means money with which start-ups get going, i.e. what they raise from among their 'FFF' circle: family, friends and fools.

In the meantime, Fund^{+} continues to invest. A promising newcomer is Aelin Therapeutics, one of the spin-offs of the Flemish Institute for Biotechnology. Never before has a VIB start-up raised such a large amount; from the start it has EUR 27 million at its disposal. Ten years ago, two scientists, Joost Schymkowitz and Fréderic Rousseau, developed a process to get proteins to aggregate and thereby to neutralise them. At the time the discovery found its way into the pages of the specialist journal *Science*. The question now is whether proteins involved in the division of bacteria or cancer cells can also be neutralised. In viruses, fungi and even in plants, the process appears to work. If Aelin can further develop this technology, it can lead to a completely new type of antibiotic. The fact is that bacteria are becoming increasingly resistant to existing antibiotics and there is therefore a pressing need for an alternative. (8) A new type of antibiotics really would be world news.

Fund^{+} is also attracting competitors. Biotech is 'hot' and everyone wants to get a piece of the pie. There was already biotech know-how on hand at the (also Leuven-based) Capricorn Venture Partners, at the Colruyt family's investment fund Korys, and at the Flanders Venture Capital Company (PMV). Some ten Belgian families recently asked former VIB chief executive Rudy Dekeyser to set up a new biotech fund. EUR 280 million was immediately made available for the 'Health Economics Fund', the aim of which is to invest worldwide in initiatives that are advanced far enough. Fund^{+}'s local focus is therefore not to be found at the Health Economics Fund, but Dekeyser will undoubtedly seize Belgian investment opportunities too. After all, biotech is played out on a world stage which, like ailments and complaints, knows no borders. The 'Flemish' company Galapagos began in Mechelen, was listed in Brussels, Amsterdam and New York, and recently opened offices in Basle and Boston, because of the specialist knowledge available there.

There is also private money in Flanders for basic medical research, even where no medicaments are immediately in sight. With 35 other financial backers, Désiré Collen, via LSRP, is supporting the 'Opening the Future' initiative of Urbain Vandeurzen to make funds available *inter alia* to the Leuven Alzheimer specialists Bart De Strooper and Wim Vandenberghe for the next five years. They have meanwhile reached their goal of EUR 10 million.

Investors are queuing

There is money available in the world and in Belgium. A lot of money is put aside passively in savings and current accounts. The question is how to turn this potential capital into economic growth and employment – and just as importantly, how to provide those savers with the prospect of real income and capital growth. That transformation will no doubt run for a significant part through funds such as Fund$^+$. Many ambitious entrepreneurs with new ideas need to be supported by what is gradually becoming an oversupply of capital. That is a luxurious environment in which there should also be a place for motives other than a quick financial return.

"Fund$^+$ dovetails with what is sometimes referred to as impact investing," says Chris Buyse. "That's sustainable investment with a broader field of vision than just return." In the banking sector, Triodos was a precursor, but now most banks are trying to factor in the societal added value of the services they provide. "As an investor, our DNA is indeed unique. We're an open fund, and therefore we're not focused on short-term profit. We have a marked local focus and support a Flemish-Belgian ecosystem of scientific research. Thanks to the VIB and other initiatives, a culture has grown in Belgium that's genuinely respected internationally. All biotech companies in France, the Netherlands, Portugal and Belgium are in the Euronext Biotech index, and Belgium, small though it is, accounts for more than 50% of that index! Of course, Galapagos represents a weighty share, but that company has also developed from this ecosystem. With a market capitalisation of 1.5 billion, ThromboGenics was once very important, too. There's coming and going. And that's just the top of the iceberg; those are the companies that are visible because they're listed. I think there currently are 350 biotech companies in Belgium, about a hundred of which are really promising. There are already twenty or so in our Bio-incubator in Leuven. Désiré Collen has made a sizeable contribution to this ecosystem, and with Fund$^+$ we want to take that contribution forward. Obviously, we also keep an eye on the financial return; we're not a charity. But there's more to it than that. Our plus!" *(This interview was conducted in April 2018)*

The issue as to what will happen post-Collen has been solved. With his foundation and his Fund$^+$, Collen has developed effective medicaments to combat his own transience. He represents a socially committed monument that will stand the test of time.

Postscript June 2020

Two years after this book was first published in Dutch, Professor Collen's legacy is flourishing. His new investment projects might become his second great achievement after the discovery of t-PA. The Désiré Collen Foundation, supports several cultural and scientific programmes and remains the cornerstone of the evergreen investment fund Fund$^+$ www.fundplus.be

In five years, Fund+ has acquired a prominent place among European biotech investment funds. This success is based on several factors: the long-term nature of DCF and its stake in Fund+; the seasoned medical and financial know-how of the investment team; a patient but also alert investment attitude; and some astonishing early successes. Today, Fund+ has 13 companies in its portfolio, and several more are standing in line.

CEO Chris Buyse: "We can say that we examine nearly all Belgian investment opportunities and many European ones as well. We know what goes on in the biotech world and we can be very selective. Sixty percent of our EUR 200 million investment capacity is now put to work and if ever we need more money, the existing and many new shareholders will readily provide the necessary amounts. We remain part of the Belgian ecosystem, but science has no borders and our playground is now Europe, with some excursions in the USA and on the Nasdaq stock exchange."

Three board members retired from the board mid-2020, but Pierre Drion and Arnoud de Pret remain supporting shareholders. They were replaced on the board by two distinguished personalities. Luc Debruyne was President of Global Vaccines and member of the Corporate Executive team of the pharmaceutical giant GSK. He is now strategy advisor to the CEO of the Coalition for Epidemic Preparedness Innovations, CEPI, one of the international institutions coping with the COVID-19 pandemic. The second new appointee to the Fund+ board is Gérard Lamarche. He was co-CEO of the Belgian holding company GBL and is still a director of that EUR 15 billion group. In 2019, he joined Multifin S.A. as Chairman of the Board; this company is the patrimonial office of the de Pret family, an anchor investor in the global brewing group AB Inbev and a shareholder of Fund+.

News about the companies in which Fund+ has invested, is constantly being released. Fund+ is a busy biotope. Since the financial contribution of the Japanese Itochu Group in 2019, the specialist company in liver diseases *Promethera* is ready for a stock-exchange listing. After a reversed merger, *Immunic* is already listed on Nasdaq, and recently capital and liquidity has been added to that listing. With its broad antiviral IMU 838, Immunic might provide a cure for Covid-19. After the contribution of USD 125 million by, among others, the American investors RA Capital and Boxer Capital*, iTeos Therapeutics* is also ready to be listed on Nasdaq. iTeos is a specialist in immuno-oncology. *EyeD Pharma* will announce an important investment in the Liège region. Its subsidiary Uni ID Pharma Manufacturing will start producing high tech medical devices. *Minoryx* started in Barcelona but will continue in Belgium its research into genetic diseases of the central nervous system. For example, the Friedreich Ataxia disease is often fatal for 1 in 17,000 new-borns. The French biotech company *Horama* focuses on gene therapy for the treatment of rare genetic diseases in ophthalmology. In March 2020, the company announced an exclusive licensing agreement with the Leiden University Medical Center (LUMC) for global rights to a gene therapy program to treat Inherited Retinal Dystrophy, a rare but devastating ophthalmic condition leading to blindness. In June 2020, *eTheRNA* added capital from Fund+ and others, especially the new partner China Grand Pharma. The company is working on a vaccine against Covid-19. *Novadip, Confo Therapeutics, Aelin Therapeutics* with possibly alternative antibiotics*,* are the other promising jewels in the Fund+ crown. And in *Epics*, Jean Combalbert, the key scientist behind one of Ogeda, one of Fund+'s great successes, continues his RNA- epigenetic research. Ogeda was one of two successful exits of Fund+, the other being *Q-Biologicals* in 2016.

Five years after its creation, Fund^{+} is an enthralling never-ending story.

Chapter 2: CoBioRes, a favor to a friend

CoBioRes Team, end 2017
Left to right: Peter Pokreisz, Andrea Casazza, Marzia De Petrini, Nele Kindt, Diane De Wyngaert, Kylie Clarke, Leentje Cosemans, Tom Janssens

CoBioRes Team, beginning 2019:

Nele Kindt

Geert Reyns

Peter Pokreisz

Andrea Casazza

Kristien Van Belle

Lawrence Van Helleputte

Leentje Cosemans

Marzia De Petrini

Tom Janssens

Marijke Pellens

Diane De Wyngaert

Summary: *Collen did not entirely leave the world of entrepreneurship. In 2013 he set up CoBioRes (Collaborative Biotech Research) with a starting capital of EUR 1 million, which was LSRP money. It began as favor to his colleague, Professor André Trouet. In this new company, Collen once again brought together promising ideas that perhaps with partners could be developed into medicines. With CoBioRes there might be a new ThromboGenics in a few years' time, but in this case, it would be the translational company Collen had dreamed of, straddling university and industry.*

André Trouet's strategy

The origins of CoBioRes go back to 2009, some years before Collen had left ThromboGenics in 2013. In 2009 Collen was contacted by his French-speaking colleague André Trouet (1936-2014), professor emeritus of the UCL and trained by Nobel Prize winner Christian de Duve. Trouet worked in Leuven with a Franco-Belgian biotech company, Diatos, which had wanted to go public in 2006, three months after ThromboGenics' stock exchange introduction. But by then the stock market climate had further deteriorated. ThromboGenics had succeeded by lowering its introduction price, but the heydays for IPO's were over. Diatos, with a 17.35% stake by the Flemish investment company Gimv, wanted to raise a total of EUR 25 million on its initial public offering on Euronext Paris and Euronext Brussels. The first listing was scheduled for 11 July, but on that day their press release announced that the prevailing market conditions had prompted Diatos to cancel its IPO. Three years later Diatos went into liquidation, and a disappointed Trouet approached Collen asking for help. Professor Trouet had devised a strategy which made existing chemotherapy with doxorubicin less toxic for the patient. He had been working on this project for 25 years, and all that research would now be lost. Would Désiré Collen be able to help out?

Doxorubicin is a frequently used and highly efficient drug in cancer treatment and has been in use since the 1970s. It causes cancer cells to die off, but its effect is not very selective: it also causes considerable damage to healthy cells. It therefore can only be administered a limited number of times. Above a certain threshold, it becomes too toxic for the heart, and white blood cells.

Professor Trouet's strategy, which he wanted to develop in Diatos, involved a so-called prodrug, a medicine administered in a non-active format, which only after administration turns into a biologically active substance. The affixing of a tetrapeptide structure (a little group of four amino acids, for example ALAL) to that doxorubicin molecule, renders doxorubicin inactive. But enzymes from the micro-environment of cancer cells selectively cut the peptide structure loose in two steps, thus releasing active doxorubicin, and this occurs only in the vicinity of cancer cells. That way the drug is selectively activated, and danger to healthy cells is reduced. Doxorubicin can then be used in a much more targeted manner and in higher concentrations to combat the tumour.

Collen agreed, and decided to set up a small company for Trouet's project, BioTra NV (Biotechnical Translational Research), of which Collen himself became CEO. Professor Trouet had his lab in the buildings of Leuven's Sint-Rafael hospital and worked there with two Msc (Masters of Science) and two Bsc (Bachelors of Science). "I decided to buy his equipment from the liquidated Diatos through the receiver," Collen recalls. "The staff had been referred to the plant closures fund, from which they received very little. I rehired them,

told them they could take their annual holidays first, and then continue their work in our new structure. But I also told André that we could not develop this ourselves, he would have to license it out." After two years of research into new prodrugs, Trouet submitted a patent application and tried to license it out, but the approached pharmaceutical companies felt the project was still at too early a stage.

After two and a half years, the money (EUR 1.6 million) had been used up. Collen informed Biotra's four employees that the company was to be discontinued, and that he would pay them three months' severance pay, but that in the meantime they would have to look for another job. One of the staff members, unsatisfied with this arrangement, lodged a complaint with the labor court, which deemed the employment contract with Diatos and subsequently with BioTra to be one continuous contract. Collen therefore would have to give a much higher severance pay. "That would have cost me a further EUR 170,000. I then decided to formally liquidate BioTra and to drop the patent application."

Trouet himself did not give up. He had new ideas with tetrapeptide prodrugs that were sensitive to other cancer-related enzymes. He applied for a patent for this invention and licensed it to the company CoBioRes that Collen set up in 2013. This newly established company was housed in Gasthuisberg, the KU Leuven university hospital, occupying Collen's former office on the ninth floor of the Central Services building. The research work was carried out in labs rented from KU Leuven via the non-profit association LSRP. Professor Trouet died the following year, 2014, but the American patent for his new ideas had been filed. A contract with Trouet's widow guaranteed her rights as André Trouet's heir.

Nele Kindt (°1981), who obtained her Biomedical PhD in 2008, joined the CoBioRes team in February 2016 and became CEO eight months later. She had done her doctorate in Collen's lab, and she still recalls the way students looked at him in awe, when he came bursting in halfway through a doctoral student's presentation and would then often be the only one to have a question at the end. "And that query was straight to the point, even though he had missed half the presentation," says Kindt.

Kindt began her career as a researcher with ThromboGenics and, in 2012, moved to the Limburg-based Amakem, which focused among other things on the development of innovative eye drops for glaucoma patients. "I also worked in the ophthalmology programme at ThromboGenics, but unlike ThromboGenics, Amakem was a small company with a staff of 15, so you were much more closely involved in everything that went on." Unfortunately, Amakem closed its doors in 2016. Nele Kindt was recruited by Chris Buyse, Désiré Collen's right-hand man. "His phone call came just at the right time," she says. CoBioRes was still in its infancy with three members of staff and two projects in its portfolio, and Kindt was expected to infuse extra life into the small company.

The two projects that were immediately placed in her charge were already looking promising. First there was the oncology programme inherited from Professor Trouet, which was being further developed. Kindt: "We are in the first instance focusing on breast cancer because that model is relatively easy to induce in laboratory animals. But we didn't rule out the possibility of applying the same strategy for other cancer types. With a significant grant from the VLAIO (the Flemish Agency for Innovation and Entrepreneurship), we studied the effectiveness of

our prodrug in triple negative breast cancer, a sub-type of breast cancer that currently has a very bad prognosis."

In triple negative breast cancer, the tumour does not have any receptors for oestrogen or progesteron and also lacks the HER2 protein. These are three negatives: hence the name triple negative breast cancer. Treatment with hormone therapy or trastuzumab (Herceptin) makes use precisely of these receptors and proteins and is therefore not applicable in the case of triple negative breast cancer. This form of the disease often grows faster and is more aggressive than hormone-sensitive breast cancer.

Safety and toxicity are being tested on animals, and the first results are promising. "Our version of Trouet's prodrug allows us to double the dose of Diatos' DTS-201, the first version of prodrugs, for which a Phase 2 study had already been started, just before the company failed. We could even increase 15 times the dose compared to doxorubicin, indicating that the treatment might be more effective. We have meanwhile included sarcoma in our tests, a cancer of connective tissue, such as bone, cartilage, fat, vascular and hematopoietic tissues, and our data are very good. We even have positive results in models where doxorubicin was no longer effective," says Kindt, referring to tumours that have become resistant against doxorubicin. They might react positively again when the prodrug is administered.

The tests are done in PDX models (patient derived xenografts), cancer models where tissue from a human tumour is implanted and expanded into mice. This allows the natural growth of the cancer and makes the results more relevant than when cell lines are used. By 2021 this project should enter its clinical stage with a first patient.

The second venture, the cardiovascular project with PlGF (Placental Growth Factor), was meant to be ready for tests on patients in 2019. This study was performed in close collaboration with Professor Stefan Janssens of the cardiology department at Gasthuisberg, The purpose of their research was to check if after a heart attack PlGF makes enough new small blood vessels so that part of the heart muscle, which had been in need of oxygen, is irrigated again. With less muscular tissue dying off, the heart is able to function more effectively. The PlGF was tested on pig models, because a pig's heart has many similarities to the human heart.

The PlGF research was originally a ThromboGenics project but was discontinued there. Collen bought back the licence from ThromboGenics, but with a re-licence stipulating that ThromboGenics would receive 25% of the income during the first ten years, so that if the project was successful and a contract with a pharmaceutical company was secured, royalties would be coming in every year.

But the project had to be abandoned by the end of 2019. Although PlGF might have led to the creation of new small blood vessels, there was no significant improvement of the heart function, which was the primary endpoint of the study, and clinically the most relevant one. Further analyses are being made, but merely for academic purposes.

At the beginning of 2018, a third project was taken on by CoBioRes. This concerned a new medicine, an improved drug against transplant rejection, and was the result of research conducted by immunologist and former rector of the KU Leuven, Mark Waer (°1951) and his Rega Institute colleague, Piet Herdewijn.

Waer often had the opportunity to talk informally about his research with Désiré Collen, whom he regarded as one of his best friends. They had the scientific background in common, but they also shared a love of wine and a friendship with Urbain Boutelegier. Mark Waer had first met Désiré Collen when he had to do his rotations as a young assistant in Professor Verstraete's vascular diseases department, where Désiré was the big man at the time. "We assistants were often kindly asked to donate blood for Désiré's tests," Waer remembers. Later there were various wine-tasting trips organized by Boutelegier. And during the rectorship elections in 1995, Waer was on Désiré's advisory council. Collen failed to win the election. "But I did pick up a lot from that experience," laughs Mark Waer, who himself became rector of the KU Leuven from 2009 to 2013.

Piet Herdewijn with many years' experience in synthesising new drugs and Mark Waer with basic training in kidney diseases and kidney transplants, were focused on immunology. "A lot of progress has been made in this area in recent years," Waer confirms. "Whereas 25 to 30 years ago around half of those kidney transplants were lost after just six months, those results have improved enormously with new medicines. Today most kidney transplant centers manage to ensure survival of the patient and the transplant in around 90% of cases after one year." But beyond the first year, more than half are still lost, as immunosuppression to combat rejection symptoms is not yet ideal. In addition, there are side effects. The immune system serves to protect people against infections and cancers, so if people have to be treated with immune suppressives for many years, the risks are real.

The search was therefore on for drugs that could enable this immunosuppressive treatment to be discontinued after 6 or 12 months. The drug already worked in mice, but these were young mice bred in a laboratory and living in ideal conditions. In older mice that had already had infections or that moved around in the natural environment like people do, and thus have a stronger immune system, it proved to be much more difficult. At CoBioRes they tried to confirm the initial results of Waer's and Herdewijn's research, but the outcome was not sufficiently promising, and the project was discontinued by mid-2019.

"Well that is how science works," says Kindt resignedly, although she realizes a lot of research money has been lost. After the EUR 1 million start-up amount, two capital increases were carried through, resulting in CoBioRes having EUR 3 million at its disposal by the end of 2016. In October 2017, an extra 5 million was paid in, and a grant from the VLAIO yielded an extra EUR 1.7 million.

Looking for investors

Although two of the three projects failed to give the expected results and had to be discontinued, Kindt thinks the fact that CoBioRes is now mainly focusing on cancer drugs might make it easier to find new investors. "It is extremely difficult to find investors who are prepared to put money in research that covers different fields, from vascular to immunology or cancer."

Finding investors might not be easy, because although their only remaining project, the prodrug, is no longer early stage, they don't yet have proof of concept in patients either. As soon as they can start the clinical stage, CoBioRes will have more chances of finding an investor or a partner with whom it can further develop this medicine, or to whom it can grant a licence. "What we have now is a technique, which can serve as a platform, easily

adaptable and widely applicable. The first patent had Trouet as inventor and CoBioRes as owner, but we want to generate new IP (intellectual property) and file new patents, giving us an extra protection of 20 years, because by the time our medicine is on the market, there might be only less than a decade left of the patent's term."

CoBioRes continues to keep a close eye on additional projects. "We are looking around in academic labs where research is being conducted that we think dovetails with the CoBioRes strategy, and which we think deserves further development. And for which we could eventually look for a major partner who would be able to oversee the clinical studies. Or sometimes we hear about projects that are put to Fund^{+} but fall by the wayside there because they are still at too early a stage. At the moment it is certainly not our intention to develop projects beyond Phase II. Currently that is simply not feasible both financially and from the point of view of manpower. We are a small team, even though we have grown from three to the current eleven members of staff plus two consultants, and more staff might be taken on as and when we secure new projects in the portfolio. Right now, if new personnel were to be hired, we would be very cramped in our three rooms at Gasthuisberg," says Kindt, who is not averse of the idea of a move.

Up to what phase does Collen wish to carry on developing projects in CoBioRes? "Well, as far as we can and hopefully to the inflection point, when clinical development arrives, since the investments will then be very sizeable," he says.

And how long does he see himself actively engaged in it? "I was born in 1943, so the best is behind me, but I will keep going for as long as I can," he assures us. Scientific research has not just been Collen's work, but also his hobby, his vocation, and the meaning of his life.

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PART I: FROM NATURAL T-PA TO RECOMBINANT T-PA

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Personal reflections from Roger Lijnen

(July 2020)

Reading this book has made me realize how lucky and privileged I have been to witness these past 4 decades of the life and work of Désiré Collen. I have very much appreciated the professionalism of the authors, Paul Huybrechts and Frieda Van Wijck, in telling this remarkable story.

In February 1978, a few days after obtaining my PhD in Biochemistry at KULeuven, I learned about a vacancy in the lab for 'Blood Coagulation' of Professor Marc Verstraete. I made an appointment, and after about 10 minutes I was accepted as an 'assistant'. Then Professor Verstraete said to me 'Go to Professor Collen next-door, he can use a biochemist.' Désiré's main question was 'When can you start?' and my reply was 'Now'. I actually started weeks before I got a contract and stayed for 40 years. When I joined the lab of Désiré I really still had to learn what biochemical research was all about. He taught me how to focus on a problem, come up with a hypothesis and test it experimentally. A major asset to the lab has always been his clear vision on what would become important in the near future, and his focus on the relevant issues ('Where is the meat?' used to be his standard question when somebody proposed a new experiment or topic).

The focus was always on fundamental research, but with an open mind for applications. Thus, Désiré was the first to realize the potential of t-PA as a commercial drug. Part of the royalty income earned on t-PA was invested in the lab, which allowed us to continue our research under the best possible conditions. As a consequence, the CMVB has grown from about 15 collaborators when I joined to over 60 in 2018, the year of Désiré's retirement. In addition, the recognition as a department of the Flemish Institute for Biotechnology (VIB) allowed the foundation of the Center for Transgene Technology and Gene Therapy (later the Vesalius Research Center, and now the Center for Cancer Biology). It is clear to all of us that Désiré has been the driving force behind these achievements, thanks to his scientific capacities and his management skills.

As a consequence we were usually among the first in the field to introduce new technology, starting with hybridoma technology in the 1980's, recombinant DNA technology and genetic engineering in the 1990's, transgene technology and gene transfer in the 2000's, and later on stem cell technology.

Monoclonal antibodies were first introduced by Paul Holvoet and later by Paul Declerck. Antibodies against most proteins of the fibrinolytic system were raised, and have been extremely valuable tools for structure/function studies, but also for the construction of immunological assays to be used in clinical studies. The generous gift of many of these antibodies to colleagues around the world has greatly facilitated their work. Moreover, some of these are -still today- sold to reagent companies and form a (minor) source of income for the CMVB.

To introduce recombinant DNA technology, Luc Nelles, a young PhD form UIA, was attracted. Furthermore, when Désiré was at Genentech, he convinced one of their 'stars', Bill

Holmes, to come to Leuven to set up recombinant DNA technology. The introduction of genetic engineering in mice by Peter Carmeliet and Mieke Dewerchin is extensively described in this book. A project with the aim to generate transgenic rabbits, led by Luc Schoonjans, was less successful due to persistent stem cell problems. To carry out this project, Désiré had a mobile lab installed by a giant crane on top of the CDG building. It probably is still there, and now home to a large pigeon population. Gene transfer studies included the work of Bart De Geest on LDL gene transfer and of Thierry Vandendriessche/ Marinee Chuah on haemophilia. For the staphylokinase mutagenesis program Désiré employed a team of 4 modelling experts from Plant Genetic Systems (Marc De Mayer, Laurent Jespers, Yves Laroche and Ignace Lasters). They designed numerous mutants and variants but, as described elsewhere in the book, this turned out to be a dead end.

The work of the team headed by Jean-Marie Saint-Remy and Marc Jacquemin, eventually led to the characterization of the antifactor VIII antibody TB-402. The team of Saint-Remy later founded the spin-off company ImCyse.

The scientific output of Désiré between 1968 and 2008, the year of his official retirement from the university, consists of approximately 650 research papers in peer-reviewed international journals, 170 survey articles and 28 issued US patents. He ranked among the most highly cited authors of the 1980's and 1990's. (1) *PubMed* lists 234 joint publications for Collen/Lijnen! This does not include about 70 chapters in textbooks and congress proceedings.

While development of t-PA for thrombolytic therapy has undoubtedly been the main scientific achievement of Désiré, it is by far not the only one. Since the foundation of the Center for Transgene Technology and Gene Therapy, together with Peter Carmeliet and colleagues, landmark contributions were made to the fields of vascular biology, tumour biology and neurobiology. Many of these achievements were only possible thanks to the vision of Désiré to invest early on in new technology and to attract top-level collaborators. This also applies to the many devoted and skilled technicians we have worked with; they constituted the strong backbone of the lab, with sometimes unique expertise. When some of them were at risk to lose their job because of reorganizations in the university lab, Désiré offered them a job at ThromboGenics, illustrating his concern for the people working with him.

The story of t-PA as it is told in this book is a story of serendipity at several levels: the finding that Bowes melanoma cells produce large amounts of t-PA, the contact through Billiau with Weimar leading to treatment of the first patient, the unexpected way Désiré met Diane Pennica. Indeed, serendipity amounts to having the right people at the right place at the right time. But above all, the development of t-PA from a laboratory concept to a life-saving drug used world-wide for treatment of thromboembolic diseases, is a story of scientific intellect, resolve and determination. This is an example of translational research *avant la lettre*. It still stands out as one of the fastest drug development projects in history, with only 7 years between the first meeting with Diane Pennica and the approval of rt-PA by the FDA.(2,3)

To illustrate that Désiré is considered to be 'the true father of t-PA', our collaborators presented him with a drawing of the t-PA structure in which the single letter amino acid symbols were replaced by pictures of his many collaborators. As there are 527 amino acids in t-PA, even he did not have so many different co-workers. Therefore, if you look closely,

you will also see pictures of Mickey Mouse, Minny Mouse, Popeye, and so on, showing some artistic freedom.

Less known probably is that Désiré was one of the founders of the *International Society for Fibrinolysis and Thrombolysis* (ISFT) in 1992, later renamed as *International Society for Fibrinolysis and Proteolysis* (ISFP). (4) ISFP is a not-for-profit organization that aims at the "furtherance of scientific research relating to fundamental and medical aspects of fibrinolysis, thrombolysis and proteolysis". During the heydays of thrombolysis, this society had over 500 members, and it still exists today, with financial support from LSRP. One evening in 1992, Désiré called me at home and said 'I have good news and bad news. The good news is that, together with M. Samama, F. Bachmann, P. Brakman and J. Davidson, we have founded the ISFT; the bad news is that we ask you to be the Executive Director.' I accepted and kept this position until 2018, when I retired myself. The XIIth international congress of the ISFP was organized in Leuven in 1994, with Désiré as President and myself as Program Chairman. There were over 500 participants, which outnumbered the available hotel rooms in Leuven at that time. One of the highlights of the meeting was the presentation by Peter Carmeliet on the phenotype of his first knock-out mice. Because of the unexpectedly high number of registrants, we made a significant profit, which went entirely to the ISFP, to support its goals, and to provide travel grants to allow young investigators to participate in future congresses. In addition, at the bi-annual congresses of the ISFP, five '*D. Collen Young Investigator Awards*' are given to colleagues under 35 years, who submitted the highest rated abstracts.

Désiré was also Vice-President of the XIth *International Congress on Thrombosis and Haemostasis* (Brussels 1987), with Professor Verstraete as President; it brought more than 3000 participants to Belgium, until then the highest number ever for an ISTH congress, testifying for the international appeal the CMVB had achieved.

When Désiré retired from the university in 2008, I became his successor as Director of the *Center for Molecular and Vascular Biology* and Chairman of the *Molecular and Vascular Medicine Department*. I became a full professor at the faculty of medicine in 1995, which I would probably not have achieved without the continuous support of Désiré Collen and Marc Verstraete. (5)

I have always highly appreciated the opportunities they have provided me to attend numerous international congresses and to present communications, frequently in response to invitations they had received. Furthermore, I strongly enjoyed the academic freedom in the lab, where Désiré would always allow you to explore a new idea, that means if you could convince him of the potential. If no funds were available for such project, he would even generously supply these.

At the scientific level, I would like to reiterate that I did not financially benefit from our projects on t-PA, staphylokinase or microplasmin. I was a co-inventor on the staphylokinase patent but, as reported extensively in this book, that patent never had any commercial value. I am actually one of the unlucky ones that lost money on ThromboGenics shares, buying at EUR 20 and eventually selling at EUR 9.

During all the years of our collaboration, we did not only have contacts at the scientific level, but also many at social events not in the least thanks to the generous hospitality from Désiré and Louisa. I vividly remember all the evenings at their home, with excellent wines, and for a

given time with grilled salmon that Louisa prepared; until she found out that I actually don't like fish at all, but didn't dare to tell in the beginning. Also, at international congresses, Désiré always tried to re-unite all former '*Leuven guys*' for a nice dinner. For the Concert Olympique performances in Leuven, there were always at least 10 VIP tickets available for lab members.

When Désiré retired in 2008, we have prepared an *Anthology of Scientific Collaborations* to recognize his research contributions on fibrinolysis, thrombosis and haemostasis. Many colleagues, as well from Belgium as from abroad, have collaborated with Désiré at a given moment in their scientific career. A list of the 86 foreign graduates who worked with Désiré in Leuven over the last 40 years is shown in Addendum 1. The academic Belgian co-workers are listed in Addendum 2. As it was not possible to ask all of them for an account on their stay in Leuven, we invited in total 40 past and present collaborators who published papers jointly with Désiré that received more than 100 literature citations. Of those, 38 have kindly provided a narrative and personal appreciation of their collaboration with Désiré. (6) The picture that emerges speaks for itself: a great scientist, a visionary entrepreneur, a superb teacher, a delightful friend and above all a gentleman. All have experienced that Désiré has only one word, and always keeps to a given word.

All of these colleagues testify that their stay in the Leuven lab has determined to an important extent their future career. Many of those indeed have established their own research groups in countries all over the world, but Leuven has stayed their 'second home city'.

A prominent example is Professor Osamu Matsuo, first-minute collaborator on the t-PA and staphylokinase projects. The first time I met Professor Matsuo was in April 1979, when he visited our lab in preparation of a one-year sabbatical he would start later that year. Désiré was delayed and had asked me to welcome him and to show him our laboratory. Because I thought he would be thirsty after such a long trip, I kept asking him 'Do you want coffee? … Do you want a coke? … Do you want some water? …' Every time he said yes and drunk it all. I didn't realize that I was being rude and that for him it would have been difficult to say 'No'. This was my first experience with the Japanese way; since I have learned much more of it and came to appreciate the Japanese style and courtesy. A second mistake I made when he asked me where he could buy a car. I tried to direct him to a second-hand Toyota car dealer, not realizing how strong the Japanese economy was at that time. A few weeks later he was driving a brand-new white BMW, series 5. Unfortunately, it took a while before he realized that not the Belgians were driving at the wrong site of the road! And then off course there was the dinner at Désiré's home with his experimental rabbit in the bathroom.

During our long-term collaboration, Professor Matsuo has sent other co-workers to be trained in our lab in Leuven. First Dr. Shigeru Ueshima in the early 90's and subsequently Dr. Kiyotaka Okada in the late 90's. In addition, Dr. Nobuo Nagai, who spent about 10 years in our lab, joined the lab of Matsuo in Osaka in 2007, but has later moved on to a new career opportunity.

Both Désiré and I travelled to Japan in 2011 to attend the official retirement ceremony of Professor Matsuo from Kinki University in Osaka. At the occasion of the XXIIIth ISTH congress in Kyoto in 2011, Osamu organized a '*Leuven evening'*, inviting all 25 congress participants from Leuven for an exquisite dinner.

My first project in the lab was on α2-antiplasmin, supervised by Björn Wiman. He gave me a very hard time, but I learned a lot from him, particularly on enzyme kinetics. When Björn returned to Umea, I went to work with him for 3 months at the end of 1978, and we became good friends. I remember Umea as a stimulating place to work but freezing cold and depressively dark already in the afternoon.

At the early 1980's we had a real '*Spanish-Italian colony*' in Leuven, with Nuria Sala (Barcelona), José Paramo (Pamplona), Mario Colucci (Bari) and his wife Angela Todesco, who had a project in the CMVB lab of Professor Vermylen, as did Paolo Gresele (Perugia) and Carla Zoja (Bergamo). They actually followed in the footsteps of Jordi Felez (Barcelona), Nicola Semeraro (Bari), Maria-Benedetta Donati and Giovanni De Gaetano (Pozzilli) who had worked with Professors Verstraete, Vermylen and Collen in the previous decade. The similarity between the Italian and Spanish language was clearly sufficient to understand each other. They all worked very hard in the lab, but also liked to party very hard after work. Me and my wife Ingrid have joined them many times, and all stayed long-life friends visiting each other whenever possible. In November 1983, when we moved to our new house in Herent, the whole colony came to help us, and Angela prepared spaghetti for the whole crowd.

The most colorful colleague at all, without any doubt, was Bill Holmes who came from Genentech to teach us recombinant DNA technology. With his long hair and beard, he really looked like Jesus Christ. One day, the Pope was visiting Leuven, and Bill took a taxi; the driver looks at him and says *'I knew that the Pope was coming, but I didn't know that he was also bringing his Boss.*' During his stay he took his wife Roxanne to Switzerland, to get married somewhere on a mountain top. I learned a lot from him, and in exchange I could help him with the biochemical aspects of this PhD thesis on α2-antiplasmin. When we were in San Francisco, years later, he insisted on having a BBQ with us and with Dave Stump's family.

Professor Philip Badenhorst (Bloemfontein, South Africa) spent a sabbatical in the team of Professor Vermylen in 1981-1982. In 1986, we were both invited to participate in a conference in Bloemfontein and enjoyed hospitality in his guesthouse. During the development of staphylokinase, Jean-Marie Stassen went to Bloemfontein for thrombolysis experiments in baboons. Before his retirement, Philip came to Leuven and invited the CMVB staff for dinner in The Botaniq; off course Désiré offered the wines.

Also, our colleagues from Marseille, Irène Juhan-Vague and Marie-Christine Alessi, became good friends during their stay in Leuven, lasting long after that with us visiting the Provence several times. Many more colleagues from these days in Leuven have remained good friends: at the risk of forgetting many, I recall Henri Bounameaux (Geneva), Ed Plow (Cleveland), Douglas Vaughan (Chicago), Ed Conway (Vancouver), Michel Hanss (Lyon), Christian Korninger (Vienna)… They have all made a great career in the field, and it has always been a pleasure to meet them again at an international congress or in Leuven for a visit.

When I retired from the university in 2018, Dick Rijken, Nuria Sala, Carla Zoja and Bill Holmes came to Leuven with their partners for the official ceremony and party. Dick was actually one of the speakers at the official ceremony.

Besides our scientific achievements, I have always felt that having such good friends all over the world has been a great enrichment of my life. These relations were strongly strengthened by the most generous way Désiré has always shared reagents, sometimes uniquely available from our laboratory. This generosity has greatly promoted research on fibrinolysis and proteolysis and has had a major impact on the career of many colleagues in the field.

As extensively reported in this book, at the occasion of Désiré's retirement in 2008, a world-class symposium '*Heart for the Future'* was organized in Leuven, at which occasion in the afternoon 'The t-PA story' was told by the main contributors to the development of t-PA. I had the pleasure to be the master of ceremony, and to introduce my old friends.

In 2018 Désiré proposed to celebrate the 10-year anniversary of this symposium by organizing a second edition. According to Désiré 'this gathering will be a purely social event to recall and chat about the good old days.' Diane De Wyngaert and I started by contacting the speakers of the first edition. To our great satisfaction B. Wiman, A. Billiau, D. Rijken, I. Juhan-Vague, O. Matsuo, F. Van de Werf, D. Pennica, and D. Stump enthusiastically accepted the invitation to come to Leuven for a 3-day event. Only T. Yasuda could not make it. It was a great pleasure for Désiré to have the main players in the t-PA story together in Leuven again.

The programme included 2 very nice dinners at the restaurant of Désiré's son Peter, The Botaniq, with excellent wines offered by Désiré; there was a guided tour in Leuven and in Mechelen, a nearby art city, followed by lunch in the Tivoli Castle; a concert by the Berliner Philharmonika Strinq Quintet in the Music Chapel Queen Elisabeth in Waterloo, followed by a gastronomic dinner organized by Fund^{+} at the occasion of the retirement of its founder and first chairman, Désiré Collen. The main event, however, to which all had been looking forward, was an academic session at the Begijnhof Congresshotel where I presented a selection of slides taken from the first symposium in 2008. We were very pleased that also Marc Verstraete (93 years old!) could attend this session and enjoy the vivid discussions and recollections by the participants. A second highlight was the wine tasting at Désiré's house which, in my opinion, was as good or even better than the one in 2008, with exceptional wines such as: Château Latour 1988, Lynch-Bages 2000, Château Pape Clément 1998, Carruades Château Lafite Rothschild 1988, Charmes-Chambertin 1988 (Domaine Joseph Roty), Bâtard-Montrachet 2005 (Domaine Leflaive)…

No doubt this gathering was very much enjoyed by all, and all agreed with Désiré's proposal to have a third edition in 2028, at least if some of us would still be alive by then.

I think that it is appropriate to end with a personal reflection of our mentor, the late Professor Marc Verstraete, at the conclusion of the symposium in 2008. (6) 'Under Désiré's determined leadership, the research center became world-wide one of the 10 highest-ranking laboratories active in the field of thrombosis, haemostasis and fibrinolysis.'

On behalf of all present and former colleagues, I wish Désiré good health, happiness, success and exciting new experiences in the years to come.

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Personal reflections from Peter Carmeliet

(June 2008)

After graduating at the University of Leuven as M.D. (1984) and Ph.D. (1989), I was fortunate to meet Désiré, almost accidentally, at a seminar, he had organized for a top-class speaker from Harvard. This fortuitous meeting turned out to be the start of a long-lasting productive, inspiring and collegial collaboration, which has changed the face of my scientific and academic career. Even though I only knew Désiré then from his reputation, I could not know at that moment what a pioneer in molecular medicine he was, years ahead of his time.

Initially, Désiré offered me the opportunity and means to perform a postdoctoral training at Harvard Medical School as 'D. Collen Research Foundation Fellow' (1989-1990). He thereafter had the vision to invest, at a very timely moment, in the gene-targeting technology, and offered me the unique opportunity to learn this technology during a second postdoctoral training at the Whitehead Institute (MIT; 1990-1991). To maximize and accelerate the learning process, Désiré also supported the salaries of two technicians, and, after my return to Belgium, that of Mieke Dewerchin, in addition to very substantial investments in equipment. This has allowed the entire team to acquire the gene-targeting technology at a very early time, shortly after its discovery. During this stay, t-PA, u-PA and PAI-1 knockout mice were generated. Anecdotally, Désiré carried the first t-PA knockout mouse home in his hand luggage on the airplane, and literally – and, justifiably, so – introduced the first knockout mouse into Belgium. I am extremely appreciative to Désiré for giving me the opportunity to perform this postdoctoral training, as this experience has been of unmeasurable value, allowed me to become an independent scientist and to acquire frontline technology, convinced me of asking important questions in science, taught me how to provide answers to difficult questions, and shaped my view on cutting-edge research in general.

After finishing this postdoc, Désiré offered me a position to work in his laboratory. This was a decision, that not many others in his position would have dared to take, when considering that substantial funding had been invested in a postdoc, who had generated three mutant mice, but not a single publication. It showed, however, that Désiré was not shy of investing in high-profile, high-risk projects – an important example to many of us.

Upon returning to Leuven, Désiré spared time, effort nor support to build a state-of-the-art SPF mouse facility, expand a phenotyping laboratory, and core group of excellent collaborators to establish, in a record time, a prime mouse knockout center in Europe – a tour de force, and evidence of his dynamic determination. During this period, several studies on the fibrinolytic and coagulation knockout mice were generated. Under his leading impulse, with his never-ending enthusiasm and vital energy, and with his motto 'better to write than to read about your data', lab meetings and other discussions were seeding grounds for papers and projects. His supreme writing skills and sharp critiques have undoubtedly contributed to the success of getting papers published in top journals. Désiré stressed the importance of focusing on important questions and publishing high-profile papers, and created a unique environment where young scientists had the chance of taking such risks, and persisting with determination until the goal was reached. He often provided important guidance in

publication strategies, yet was not afraid of appealing peers and inform them that it was not feasible ‘to conduct the entire study once more at the backside of the moon.’

Some anecdotes (besides the many other more important cases) may, perhaps, illustrate his dedication to science. For instance, I vividly remember that both of us were sitting on May first’s holiday in the secretariat, waiting many hours for a fax from *Nature* with a final decision about the tissue factor knockout paper, as promised by the editor. Or, the days I slided a prefinal draft of a paper under the door of his home at 1AM in the morning, upon which he would wake up at 5AM in the morning to revise the paper so that it could be submitted by noon the same day. Or, the times when we disturbed Désiré in holidays (even in the wake of Christmas) to ask advice or discuss strategies how to proceed with urgent scientific or publication matters – never did I get an answer that the time was not right. I therefore consider it a great honor to have received such an outstanding training and a true privilege to have been able to learn so much from his example of combining innovation, excellence, efficiency, entrepreneurship, perseverance, speed and leadership.

While still at the Whitehead Institute, the interest in angiogenesis and the plan to knockout out the VEGF gene grew. Though this endeavor differed from past projects in the laboratory, Désiré was generous and open-minded to support this new research avenue – as he has done ever so thereafter, for any of our new research directions. Again, Désiré supported us, in most challenging times, to generate the VEGF knockout mouse, and, in order to be competitive, he supported another (short-term) stay for myself and Lena Kieckens at the Lunenfeld Institute, Toronto. Thanks to enormous investments from Désiré, the VEGF knockout paper was finalized, and became a landmark paper in the field, from which the laboratory has greatly benefited. Around the same time, VIB was established, and Désiré offered me to become adjunct director of the Center for Transgene Technology and Gene Therapy at the VIB3 department (1996). Unique research and career opportunities became available, but, as Désiré warned, ‘there is no free lunch at VIB.’

With the growing interest in angiogenesis, we explored alternative animal model systems to accelerate angiogenesis research, including zebrafish. Once more, Désiré has been instrumental and key in establishing this state-of-the-art technology, at a record tempo, in VIB3, allowing the department to generate high-profile papers, and become recognized as a leading zebrafish angiogenesis laboratory. More recently, Désiré has invested once again in frontline technology, human genetics, thereby offering a junior staff member, Diether Lambrechts, promising career opportunities.

Besides science, I could not have imagined a better tutor to introduce me to the world of research valorization, technology transfer and intellectual property – an experience, very few scientists get a chance to become exposed to early during their career. His leading example to translate research into medicine has inspired all of us to combine fundamental research with a vision on translational medicine. Perhaps one of the best examples of such translational efforts is the work on PlGF. Because of an interest to study the role of other VEGF family members, we characterized the role of PlGF, an underappreciated gene in the angiogenesis field at that time. Genetic studies revealed that PlGF was a disease-specific angiogenic factor, leading us to suggest that this growth factor might be an attractive drug target. Through a productive collaboration with his newly founded company (ThromboGenics), we explored the therapeutic potential of anti-PlGF antibodies in preclinical

animal models, which ThromboGenics is now further exploring in clinical trials. The depth and quality of the study could not have risen to its current level without the input and collaboration of Désiré.

From all the above, it is evident that the success of my personal career as well as that of many others in VIB3 would not have reached the current level without Désiré's scientific excellence, pioneering vision, entrepeneurship, dynamic leadership, managerial skills, financial support, understanding of important questions and goals, interest *avant la lettre* in translational medicine, and intuition and appreciation for top science. In name of all collaborators of VIB3, I therefore express my sincere gratitude and great appreciation, and wish Désiré the best of luck with his future ambitions.

ADDENDUM 1

Foreign graduates who collaborated with Désiré in Leuven (compiled in 2008)

Alessi Marie-Christine (France)
Angelillo-Scherrer Anne (Switzerland)
Aparico Christina (Sweden)
Autiero Monica (Italy)
Bartha Katalin (Hungary)
Benotmane Abderrafi (Morocco)
Bini Alessandra (Italy)
Bounameaux Henri (Switzerland)
Breviario Ferruccio (Italy)
Bykowska Ksenia (Poland)
Cederholm-Williams Stewart (U.K.)
Challis Phil (U.K.)
Chan Joyce (U.S.A.)
Colucci Mario (Italy)
Conway Edward (Canada)
De Falco Sandro (Italy)
Ding Hao (China)
Drew Angela (Australia)
Edy Judith (U.K.)
Felez Jordi (Spain)
Ferreira Valerie (France)
Fischer Christian (Germany)
Fukao Hiro (Japan)
Garcia de Frutos Pablo (Sweden)
Gerard Robert (U.S.A.)
Hanss Michel (France)
Hashimoto Yutaka (Japan)
Holmes William (U.S.A.)
Ibanez Ines (Spain)
Idusogie Esohe (Nigeria)
Imura Yoshimi (Japan)
Jalbert Louise (U.S.A.)
Jang Ik-Kyung (U.S.A.)
Kiss Robert (Hungary)
Korninger Christian (Austria)
Kurokawa Tomofumi (Japan)
Laermer Stuart (U.S.A.)
Levi Marcel (The Netherlands)
Li Xian-Kui (China)
Lu Hua Rong (China)
Mann Kenneth (U.S.A.)
Matsuno Hiroyuki (Japan)
Matsuo Osamu (Japan)
Mattot Virginie (France)
Molla Ayesha (Bangladesh)
Nagai Nobuo (Japan)
Nong Zeng-Xuan (China)
Ny Annelii (Sweden)
Oei Liem Som (Indonesia)
Okada Kiyotaka (Japan)
Owen Whyte (U.S.A.)
Pakola Steve (U.S.A.)
Paramo José (Spain)
Perez Graciela (Argentina)

Ploplis Vicky (U.S.A.)
Plow Edward (U.S.A.)
Pokreisz Peter (Hungary)
Prandini Marie-Hélène (France)
Praus Michael (Germany)
Quarck Rozenn (France)
Rakoczi Istvan (Hungary)
Rapold Hansjörg (Switzerland)
Rijken Dingeman (The Netherlands)
Rosen Elliot (U.S.A.)
Rouvier Jorge (Argentina)
Sabovic Miso (Yugoslavia)
Sala Nuria (Spain)
Salwa Jan (Poland)
Semeraro Nicola (Italy)
Singh Indy (U.S.A.)
Sobel Burton (U.S.A.)
Spriggs Douglas (U.S.A.)
Stump David (U.S.A.)
Suzuki Yasuhiro (Japan)
Szelid Zsolt (Hungary)
Theilmeier Gregor (Germany)
Thompson Anne (Australia)
Ueshima Shigeru (Japan)
Ugwu Francisca (Nigeria)
Varenne Olivier (France)
Vaughan Douglas (U.S.A.)
Warmerdam Petra (The Netherlands)
Wiman Björn (Sweden)
Zamarron Concha (Spain)
Zhao Zhi-An (China)
Zoldhelyi Pierre (U.S.A.)

ADDENDUM 2

Belgian collaborators, who work(ed) with Désiré Collen in Leuven (compiled in 2008)

Aerts Frans
Arnout Jef
Ballegeer Veerle
Barbeaux Philippe
Belayew Alexandra
Benhida Abdellah
Bloemmen Frans
Bouché Ann
Bulens Frank
Buyse Chris
Cambier Patrick
Carmeliet Peter
Carlier Vincent
Casazza Andrea
Cauwels Karen
Ceustermans René
Chuah Marinee
Claeys Hendrik
Clarke Kylie
Compernolle Veerle
Cosemans Leentje
Crabbé Gert
Criel Arnold
Danloy Sophie
Darras Veerle
De Cock Frans
De Deene Andy
De Donder Linda
De Geest Bart
De Geest Natalie
De Haes Patrik
De Maeyer Marc
De Mol Maria
De Petrini Marzia
De Vreker René
De Vriese Astrid
De Wyngaert Diane
Deckmyn Hans
Declerck Paul
Delobelle Katrien
Demarsin Eddy
Descheemaeker Koen
Dewerchin Mieke
Dhoest An
Diricx Marjan
Eelen Marianne
Frederix Liesbeth
Freson Kathleen
Gijsen Lore
Gilles Jean-Guy
Gillijns Hilde
Gils Els
Hageman Gilles
Hermans Bart

Ngo Thu Hoa
Holvoet Paul
Hoylaerts Marc
Huybrechts Erwin
Jacquemin Marc
Janssens Stefan
Janssens Tom
Jespers Laurent
Jonckx Bart
Keppens Maria
Kiekens Lena
Kindt Nele
Lambrechts Diether
Laroche Yves
Lasters Ignace
Lavend'homme Renaud
Lemmens Gudule
Louwette Sophie
Lijnen Roger
Luttun Aernout
Mahau Tanja
Manderveld Ann
Moerenhout Maureen
Moons Lieve
Moreau Huberte
Morren Jan
Nelles Luc
Noppen Bernard
Nuyens Dieter
Oosthuyze Bert
Peerlinck Kathelijne
Pellens Marijke
Pieters Griet
Plaisance Stephane
Purushothaman Suresh
Raemdonck Laurence
Reyns Geert
Roelants Ivo
Saint-Remy Jean-Marie
Schetz Rita
Silence Karen
Sinnaeve Peter
Souza Castro Luciene
Stassen Jean-Marie
Stockmans Filip
Stoffels Katinka
Terras Franky,
Thys Chantal
Tollet Magda
Tricot Jean-Pierre
Tytgat Guido
Van Belle Kristien
Van Geet Christel
Van Geyte Katie
Van Hoef Berthe
Van Helleputte Lawrence
Van Horenbeek Madi
Van Nuffelen An
Vancoetsem Trees
Vandegaer Marie-Louise

VandenDriessche Thierry
Vanderelst Luc
Vanderschueren Steven
Vandervoort Petra
Vanhulst Kelly
Vanlinthout Ingrid
Vanrusselt Marleen
Vercruysse Claire
Verdrengh Evelien
Verhaegen Arlette
Verhaeghe Raymond
Verhamme Peter
Vermassen Elke
Vermylen Jozef
Verstraete Marc
Verstreken Maria
Vranckx Christine
Wittebols Hans
Wittevrongel Marie-Christine
Wyns Sabine

Register

Désiré Collen, Biotech Pioneer

A thrilling story of daring scientists and their medical breakthroughs.

How a trailblazing discovery led to many leaps forward and made KU Leuven one of the world's leading scientific institutions.

Désiré Collen, Biotech Pioneer relates the fascinating story of scientific discovery in a time when biotechnology was not yet a science. Although the cultivation and cross fertilization of plants were, strictly speaking, biotechnological techniques, modern biotechnology dates from the early 1970s, when pioneers such as biochemist Herbert Boyer from the university of California managed to transfer genetic material into a bacterium. Together with venture capitalist Robert Swanson, Boyer set up Genentech, one of the first genetic engineering companies.

Just a few years later, on the other side of the Atlantic, in Leuven, Désiré Collen discovered t-PA, the enzyme responsible for fibrinolysis, or the dissolving of blood clots. Clogged arteries were then still one of the major causes of death. The ensuing cooperation between Collen and Genentech was the beginning of a long-lasting success story, from which not only Collen but also scientific research and the University of Leuven benefitted greatly for many years. According to a Reuters ranking, KU Leuven has been, from 2016 onwards, the most innovative university in Europe. Flanders and Belgium served as the cradle of several highly successful biotech companies.

t-PA was a relatively expensive medicine, and Collen went on to develop a much cheaper clot-dissolving remedy to benefit patients in less affluent countries. He failed, however, to find the necessary finances for Phase 3 trials. Meanwhile, he had set up ThromboGenics, a company which later specialized in ophthalmology. Collen continued to stimulate and finance research in other fields, such as the cardiovascular research of Peter Carmeliet. In 2013 he left ThromboGenics, following a difference in views on the company's focus, and in 2015 he set up Fund^{+}, a biotech-oriented investment firm. Fund^{+} has meanwhile acquired a prominent place among European biotech investment funds and has scored some astonishing early successes. In June 2020, Fund^{+} had 13 companies in its portfolio, with several more waiting to come on board.

Frieda Van Wijck (1950) holds a master's degree in media and communications from the University of Leuven (KU Leuven) and started her career as a regional journalist for the newspaper *Het Laatste Nieuws*. She later worked as a journalist for radio and television station *VRT*, the Dutch-language public broadcaster in Belgium, and as a talk-show host for the same station. She has interviewed numerous Belgian and international personalities, such as former US Secretary of State Madeleine Albright, actress Jane Fonda, Nazi hunter Simon Wiesenthal, Spanish chef Ferran Adrià, author Isabel Allende, natural historian David Attenborough and athlete Michael Johnson. She has written columns and occasional articles for several magazines and is a keen gardener, a regular moviegoer and an avid reader. She is also involved as a volunteer with *Luisterpunt*, a library for the visually impaired.

Paul Huybrechts (1946) studied at the University of Leuven (KU Leuven), graduating with a master's in political sciences. During his student years, he wrote for Dutch-language magazines, and later became a full-time journalist for the newspaper *De Morgen*, where he specialized in economic and financial news. He moved to the financial newspaper *De Tijd*, first as a journalist and later, after a brief period at the private banker *Dewaay-Servais,* as managing director. He is the author of *SOS NMBS* and *Hugo's Heilige Vuur*, and the co-author of *Naar Grijsland* and *Een lang leven gezond.* In his spare time, he likes listening to classical music, going to concerts and visiting museums of modern art. He also spends time cycling and tending his vegetable garden.

The authors live in Leuven, and *Désiré Collen, Biotech Pioneer* is their first project together.