VIB

KATHOLIEKE UNIVERSITEIT LEUVEN

HEART for the FUTURE

Scientific symposium in honor of the achievements of Désiré Collen
Leuven, October 6, 2008

The t-PA Story

HEART for the FUTURE

The t-PA story told by:

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Björn Wiman *(Stockholm, Sweden)*
Alfons Billiau *(K.U.Leuven)*
Dingeman Rijken *(Rotterdam, the Netherlands)*
Irène Juhan-Vague *(Marseille, France)*
Osamu Matsuo *(Osaka, Japan)*
Frans Van de Werf *(K.U.Leuven)*
Diane Pennica *(San Francisco, USA)*
Tsunehiro Yasuda *(Boston, USA)*
Marc Verstraete *(K.U.Leuven)*
David Stump *(Rockville, USA)*

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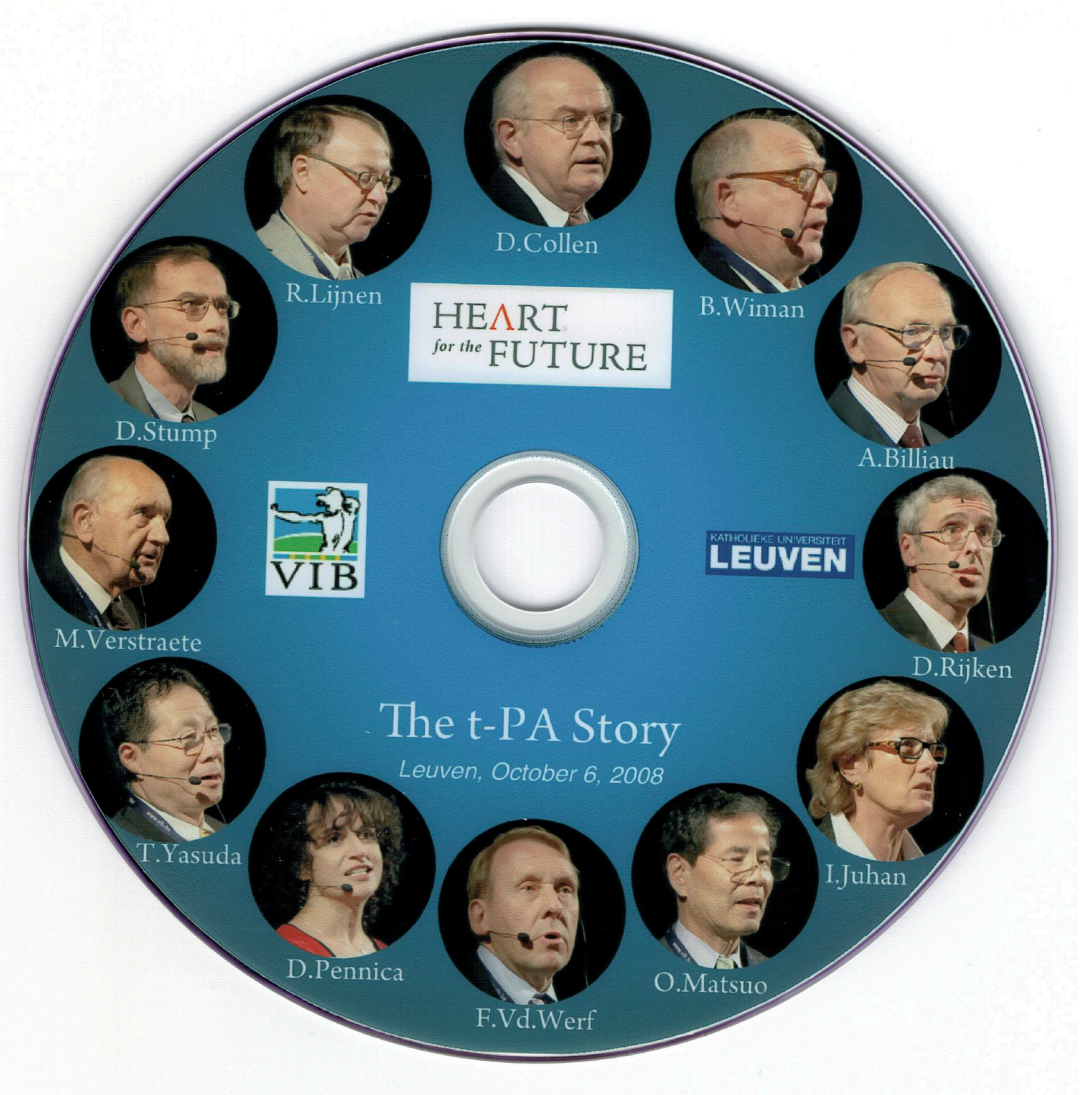


Acknowledgments:

This document is generated from the audiovisual DVD record of the symposium held on October 6, 2008 at KU Leuven in Belgium at the occasion of the official retirement of Désiré Collen. The lectures were transcribed and subsequently edited into a fluent English narrative, while preserving the speakers' original content and intent. Artificial intelligence-assisted editing was used during preparation of the manuscript, after which the text was carefully reviewed and finalized by Roger Lijnen, the moderator of the symposium. The figures included in the present document are a selection of the most relevant slides shown at the symposium. The original DVD transcript is archived on the website of the Désiré Collen Foundation (https://www.desirecollenfoundation.be/wp-content/uploads/2020/11/t-PA-story-slides_tekst2.pdf)

The t-PA Story: a Historical Account

Thrombolysis consists of the pharmacological dissolution of blood clots by administration of plasminogen activators that activate the fibrinolytic system. At the beginning of the 1970s knowledge on the fibrinolytic system was limited to identification of plasminogen that could be activated into the active enzyme plasmin by streptokinase or urokinase, and of the inhibitors α1-antitrypsin and α2-macroglobulin. Although it was known for many years that animal tissues contain a plasminogen activator that induces fibrin clot lysis, it was only in1981 that the first satisfactory purification of this plasminogen activator (t-PA) from human (uterine) tissue was achieved. In subsequent years t-PA could be purified from the culture fluid of Bowes melanoma cells in sufficient amounts to study its biochemical properties. These studies have resulted not only in a better understanding of the fibrinolytic system, but also in demonstration of the fibrin specificity of t-PA. Preclinical studies in various animal models and initial pilot studies in patients with acute myocardial infarction (AMI) have confirmed the potential of t-PA for efficient and fibrin-specific thrombolysis. Cloning of the t-PA gene in 1982-1983 and availability of recombinant t-PA (rt-PA) allowed large clinical studies in AMI patients, eventually demonstrating the superiority of t-PA over other available agents such as streptokinase.

The Food and Drug Administration (FDA) approved t-PA (alteplase) for the treatment of AMI patients in 1987, and the European Medicines Agency (EMA) followed 9 years later. Furthermore, in 1996 the FDA approved rt-PA for the treatment of acute ischemic stroke (AIS), and in 2019 the WHO declared t-PA to be an “essential medicine” for AIS treatment. It was also approved for treatment of acute pulmonary embolism by the FDA in 1990 and by the EMA in 2002. Since 2018 the annual global sales of medicines based on t-PA, mainly alteplase and its derivative Tenecteplase, exceeded the billion-dollar threshold and it thus became a “blockbuster”. Furthermore, increased usage is expected in patients with AIS.

The remarkable story of the development of t-PA from a laboratory concept into a life-saving drug is a perfect example of what collaboration between science, medicine and industry can achieve, and as such deserves to be told.

Roger Lijnen
Leuven, June 16, 2026

The t-PA Story: From Molecular Insight to Life-Saving Therapy

Over the past 3 decades, tissue-type plasminogen activator (t-PA), the physiological activator of the fibrinolytic system, has been developed from a laboratory concept into a life-saving drug for the treatment of thromboembolic diseases. This achievement was made possible because of the efforts of many scientists in academia and industry but would not have been realized without the leadership of Désiré Collen, “the father of t-PA”.

Today (October 6, 2008) recombinant t-PA, developed by Genentech Inc., has been used worldwide in over 2 million patients with acute myocardial infarction and in over 150,000 stroke victims.

“The t-PA Story” is a story of serendipity, but also of scientific intellect, resolve and determination. It still stands out as one of the fastest drug development projects in history.

At the occasion of the official retirement of Désiré Collen, a symposium with over 600 participants was organized by his main employers, VIB and KU Leuven, on October 6, 2008, in Leuven, Belgium. There, “the t-PA Story” was told by most of the main players who made it happen. This DVD transcript summarizes their contributions.

Leuven, November 7, 2008

Roger Lijnen, moderator.

P.S. Although prepared shortly after the symposium in 2008, the transcript has remained unpublished. Given the continuing importance of translational medicine and the historical significance of the development of alteplase, we believe that making these first-hand accounts publicly available will be valuable to future generations of scientists and clinicians

We hope that this volume will serve both as a historical record and as an educational resource illustrating how fundamental scientific discoveries can be translated into life-saving biopharmaceuticals through sustained collaboration between academia, clinicians, industry, and regulatory authorities.

Introduction

Roger Lijnen

It is my great pleasure to welcome you all to "*The t-PA Story*".

Between 1968 and 2008, Désiré Collen's scientific output comprised approximately 650 research papers published in peer-reviewed international journals, 170 review articles, and 28 issued U.S. patents. He ranked among the 100 most-cited scientific authors of the 1980s and is listed among the highly cited authors of both the 1980s and the 1990s.

Among his many scientific achievements, the most significant has undoubtedly been the development of tissue-type plasminogen activator, or t-PA, from a laboratory concept into a life-saving drug that is now used worldwide for the treatment of thromboembolic disease. This was translational research *avant la lettre*.

We are therefore delighted to have with us today many of the principal contributors to this remarkable story and to hear their firsthand accounts. As a colleague of Désiré for more than thirty years, I am particularly pleased to moderate this session. We are also deeply grateful to the Flemish Institute for

Biotechnology (VIB) and the Katholieke Universiteit Leuven for organizing this event.

At the origin of the development of t-PA was a molecular model for the regulation of fibrinolysis, developed jointly by Désiré Collen and **Björn Wiman**, our second speaker today. This model was presented in 1979 in a plenary lecture at the VIIth International Congress on Thrombosis and Haemostasis in London.

Initially, natural t-PA was obtained from the culture medium of the Bowes melanoma cell line. **Alfons Billiau** succeeded in producing this material in sufficient quantities to allow its purification by **Dick Rijken**, who subsequently characterized its biochemical properties and helped develop quantitative assays in collaboration with **Irène Juhan-Vague**.

The thrombolytic potential of t-PA was first evaluated in animal models in collaboration with **Osamu Matsuo**, who is also with us today. In 1981, together with Willem Weimar, the first patient suffering from renal transplant vein thrombosis was successfully treated with melanoma-derived t-PA. This was followed, in 1983, by the treatment of patients with acute myocardial infarction, primarily through the efforts of **Frans Van de Werf** and Burt Sobel.

Following the cloning and expression of the t-PA gene by **Diane Pennica** at Genentech, recombinant t-PA was evaluated in animal models in collaboration with the late Herman Gold and **Tsunehiro Yasuda**. With approval from the U.S. Food and Drug Administration, recombinant t-PA was administered to a patient for the first time in 1984.

The subsequent NIH Thrombolysis in Acute Myocardial Infarction trials in the United States, together with the European Cooperative Study Group led by **Marc Verstraete**, provided the foundation for numerous clinical investigations. These efforts ultimately culminated in the landmark GUSTO trial, which was monitored on behalf of Genentech by **David Stump**, our final speaker today. This study conclusively established the value of t-PA in the treatment of acute myocardial infarction.

During the next two hours, we will learn why Désiré Collen is widely regarded as “the father of t-PA.” As you will hear, this is a story of serendipity—of the right people being in the right place at the right time—but it is also a story of scientific insight, perseverance, determination, and exceptional leadership.

I hope you will enjoy this unique account of one of the most successful translational research projects in modern biomedical science.

Presentations

It is my pleasure to invite Désiré to tell us how it all started, and then I will subsequently briefly introduce the other speakers.

Désiré Collen

I would like to express my sincere gratitude for your presence here today and for your support of this endeavour. I would also like to take this opportunity to thank the many collaborators who, over the years, have made the t-PA story possible.

My involvement began in 1964, when I was a third-year medical student in the Laboratory of Blood Coagulation of Professor Marc Verstraete. At the time, I assisted Dr. Guido Tytgat, who was working on his PhD thesis on coagulation disturbances in liver cirrhosis. His research used accelerated turnover of radioiodine-labelled fibrinogen as a measure of in vivo coagulation.

When Guido moved on in 1968, I continued in the laboratory and embarked on my own doctoral research, using radiolabelled plasminogen to quantify

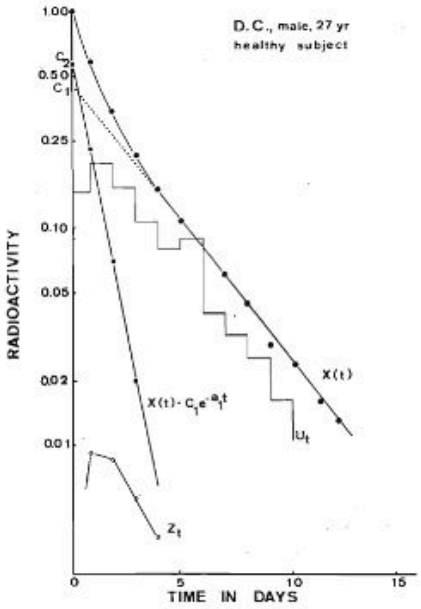
fibrinolytic activation. The plasminogen was purified in collaboration with Per Wallén and Björn Wiman.

This work led to our first publication, in which I served as the first of twelve healthy volunteers whose plasminogen turnover parameters were determined. As you can see in the figure, I was 27 years old at the time, and I am pleased to report that I had a perfectly normal plasminogen turnover. Following equilibration of the radioisotope, the plasma half-life was approximately 2.1 days.

Metabolism of plasminogen in healthy subjects: effect of tranexamic acid

D. Collen, G. Tytgat, H. Claeys, M. Verstraete and P. Wallén

The Journal of Clinical Investigation Volume 51, pp. 1310-1318 1972



Plasminogen metabolism in a control subject.
x(t) = plasma radioactivity:u_t = fractional daily urinary excretion of label; z_t = non-TCA-precipitable radioactivity in plasma

Subsequently, in studies of patients treated with streptokinase by Professor Jos Vermylen, we made a surprising observation illustrated in the figure. Although plasminogen activity declined very rapidly following streptokinase administration, the radiolabel disappeared from the circulation only with a half-life of about half a day (left panel). Clearly, the labelled plasminogen remained

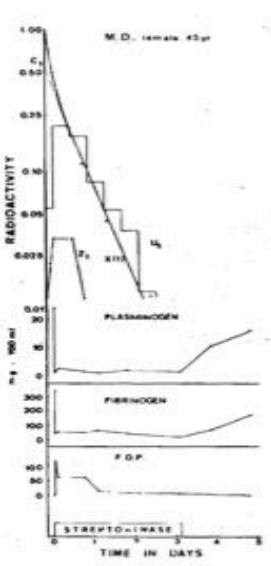
in the bloodstream even though its activity had been lost. The most plausible explanation was the formation of an inactive complex with an inhibitor.

Gel-filtration (size-exclusion) chromatography (right panel) shows the elution profile of plasminogen (upper part) and the appearance of a complex with an inhibitor (lower parts). In this patient, after only a few hours, the inhibitor had already neutralized most of the activated plasminogen.

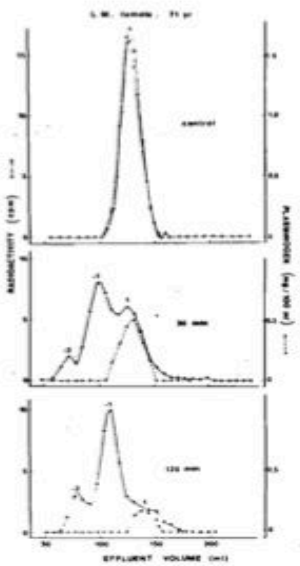
Metabolism of Iodine-labeled plasminogen during streptokinase and reptilase therapy in man

D. Collen and J. Vermylen

Thrombosis Research Volume 2, pp. 239-250 1973



Plasma levels of radiolabel and plasminogen during streptokinase infusion



gel filtration of serial plasma samples

At that point, we realized that if one could develop an assay for this plasmin–inhibitor complex, it might serve as a biomarker for in vivo activation of the fibrinolytic system. Since we had isolated and labelled the complex, and since it was inactive, this possibility appeared particularly attractive.

This was in 1971-1972 before monoclonal antibodies became available. The only approach that occurred to me was to purify the complex, raise a polyclonal

antiserum against it, absorb out the two individual components, and hopefully be left with antibodies that reacted specifically with “neoantigens” in the complex itself rather than with its precursors.

Together with Ed Plow, we studied this problem in great detail, and remarkably, the approach worked. The challenge then became whether this observation could be translated into a practical assay. Although Ed Plow and I worked very hard on the problem, we were ultimately unable to develop it into a commercially viable test.

When we subsequently analysed this polyclonal antiserum directed against the plasmin–inhibitor complex, it became clear that the findings were quite unexpected. At the time, most investigators assumed that the main inhibitor of plasmin was α1-antitrypsin.

Therefore we performed an old-fashioned crossed immunoelectrophoresis experiment using two antisera: one directed against α1-antitrypsin and the other against the plasmin–inhibitor complex after adsorption with plasminogen. The results clearly demonstrated the presence of a substantial amount of antitrypsin, but also of another component that was distinct from antitrypsin.

The outcome of these studies was the immunological identification of α2-antiplasmin. The next question, of course, was how this inhibitor functioned and what its kinetic properties were.

Initially, our efforts focused on developing an assay for the inhibitor complex. Subsequently, however, it became essential to isolate and characterize the inhibitor itself, work that was carried out in close collaboration with Björn Wiman.

At approximately the same time, in the mid-1970s, evidence was accumulating—particularly from the laboratory of Ed Reich—that malignant cells grown in culture produced fibrinolytic activity. Moreover, the aggressiveness of the malignant phenotype appeared to correlate with the amount of proteolytic and fibrinolytic activity secreted by the cells.

In collaboration with Fons Billiau, we discovered that the plasma protein capable of inhibiting this activity was, in fact, α2-antiplasmin.

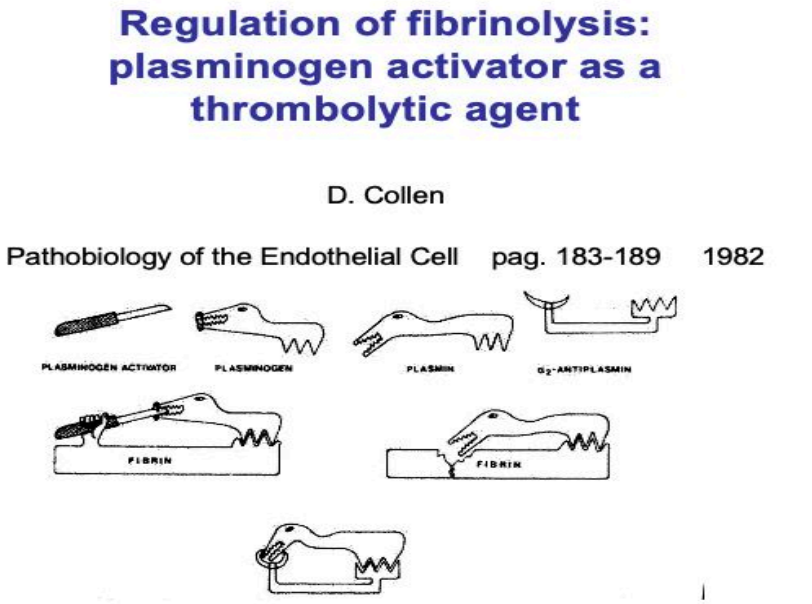
Our ambition was therefore to identify an inhibitor of cancer growth, an objective that, in retrospect, may have been somewhat naïve. Nevertheless, Fons' experimental system involved extremely laborious cell-culture titrations, and he eventually suggested that I attempt to purify one of these malignant proteases so that we could at least study its biochemical and kinetic properties.

At this point, serendipity once again played an important role.

The very first cell culture medium I received from Grant Barlow at Abbott originated from a melanoma metastasis grown in culture from a patient named Bowes. Although the patient had died three or four years earlier, her melanoma cells continued to produce extraordinarily high levels of what were then termed malignant proteases.This protease activity ultimately proved to be tissue-type plasminogen activator, or t-PA.

The reason why several groups had worked on this material for years without recognizing its potential as a fibrin-specific fibrinolytic agent is summarized in the cartoon. The concept emerged from our discussions with Björn Wiman concerning the kinetics of α2-antiplasmin inhibition of plasmin and the remarkable affinity of t-PA for fibrin.

This simple model, which has been shown many times over the years, provided the conceptual framework that linked one discovery to the next.



Schematic visualization of the molecular interactions regulating fibrinolysis. Plasminogen is converted to the proteolytic enzyme plasmin by plasminogen activator, but this conversion only occurs efficiently on the fibrin surface where activator and plasminogen are "assembled". Free plasmin in the blood is very rapidly inactivated by α_2-antiplasmin, but plasmin generated at the fibrin surface is partially protected from inactivation. The lysine-binding sites in plasminogen (represented as the "legs" of the animal) are important for the interaction between plasmin(ogen) and fibrin and between plasmin and α_2-antiplasmin.

The remainder of the story will be told by those who participated directly in the successive stages of this journey. Thank you very much.

Our second speaker is Bjorn Wiman. Bjorn was formerly at Umea University in Sweden and later at Karolinksa in Stockholm.

Björn Wiman

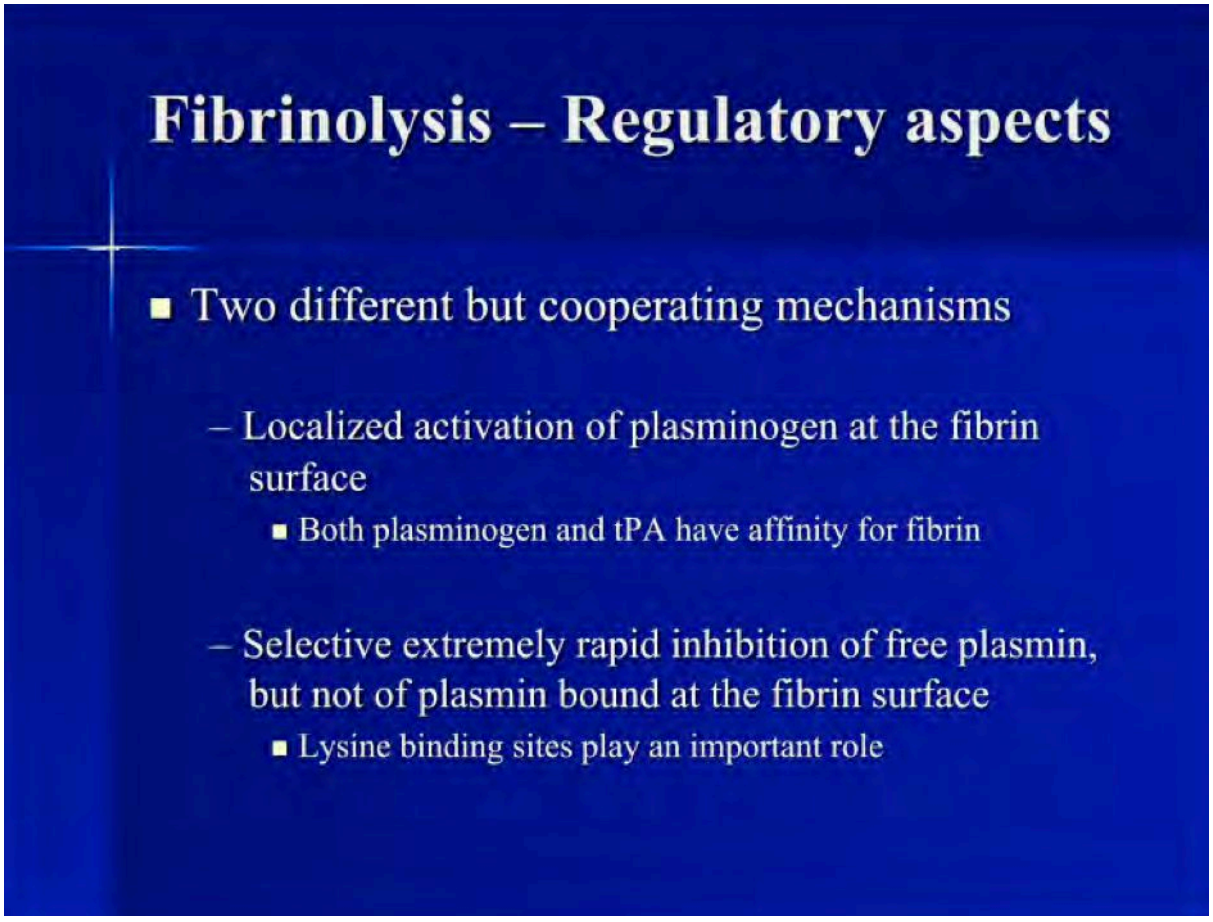
I am honoured and delighted to be here today, having been invited to participate in this distinguished symposium celebrating the retirement of Désiré Collen.

Although, on reflection, "retirement" may not be the appropriate word. I suspect that Désiré will simply shift gears slightly and then continue much as before.

My task today is to discuss some regulatory aspects of fibrinolysis and to place them in their historical context.

The regulation of fibrinolysis involves two distinct but closely cooperating mechanisms. The first is the localized activation of plasminogen at the fibrin surface. The second is the highly selective and extremely rapid inhibition of free plasmin, while leaving fibrin-bound plasmin relatively unaffected.

We knew that localized activation occurs because both plasminogen and tissue-type plasminogen activator (t-PA) possess affinity for fibrin. This leads to the formation of a ternary complex on the fibrin surface, where plasminogen activation proceeds extremely rapidly.



As to the second mechanism, we knew that the lysine-binding sites within the plasmin molecule play a crucial role.

Let me begin by discussing the affinity of t-PA for fibrin and its importance in localized plasmin generation.

The possibility that t-PA might have a specific affinity for fibrin had already been suggested many years earlier by Astrup and his colleagues. However, they were unable to provide convincing experimental evidence because the relevant proteins had not yet been purified. Consequently, the necessary tools to test this hypothesis were not available at the time.

Much later, during the summer of 1975, Per Wallén—my supervisor at the time in Umeå, Sweden—began work on the purification and characterization of t-PA from porcine heart tissue. One of his students was assigned the task of developing a new assay for measuring t-PA activity. Per wished to use a caseinolytic assay, a method with which we had considerable experience from studies of urokinase-mediated plasminogen activation. The problem was that we could detect virtually no activity using this approach, despite the fact that the same preparations displayed potent clot-lysing activity.

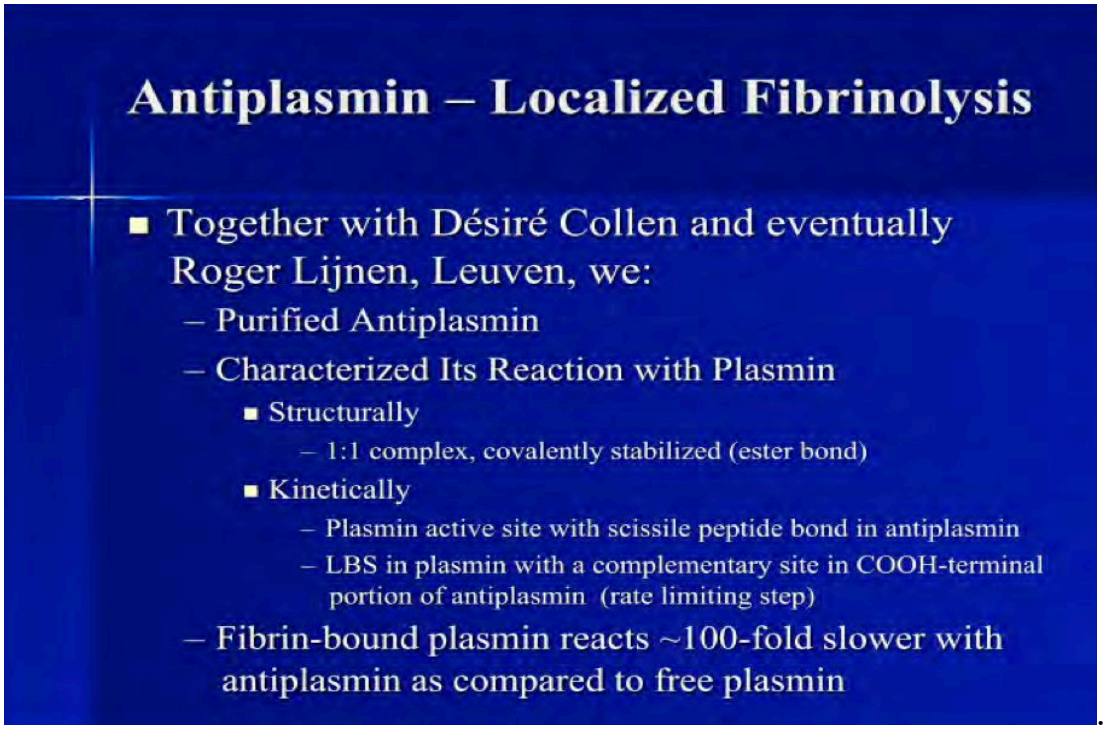
I began to wonder why this discrepancy existed. Eventually, I added a small amount of washed fibrin to the caseinolytic assay mixture and immediately observed a strong increase in caseinolytic activity. At that moment it became clear that plasminogen activation must occur preferentially at the fibrin surface.

Based on our growing understanding of the fibrin specificity of t-PA, I eventually succeeded in convincing Désiré that t-PA was indeed a fibrin-specific activator.

I am quite certain that, if not immediately, then within a matter of weeks, Désiré had already recognized the therapeutic implications and envisioned the development of a new thrombolytic agent. I certainly did not have that perspective. But Désiré did. He had worked for many years with Marc Verstraete, who was a pioneer in the treatment of thrombotic disease using thrombolytic agents such as streptokinase

A few years later, I was invited to Leuven to study antiplasmin together with Désiré. In reality, our collaboration had begun even earlier. While I was still in Umeå, we had already been attempting to purify native antiplasmin—a challenging undertaking at the time.

Together with Désiré Collen and, somewhat later, Roger Lijnen, we succeeded in purifying antiplasmin and characterizing its interaction with plasmin, both structurally and kinetically.



From a structural perspective, it became clear that plasmin and antiplasmin formed a covalently stabilized one-to-one complex. Eventually, we demonstrated that this stabilization involved the formation of an ester bond between the two proteins.

From the kinetic perspective, we found that two distinct interactions were responsible for the extraordinarily rapid reaction between plasmin and antiplasmin.

The first involved interaction of the active site of plasmin with a cleavable peptide bond in antiplasmin. Equally important, however, was the interaction between the lysine-binding sites of plasmin and complementary binding sites located in the carboxy-terminal region of antiplasmin. This interaction proved to be the rate-determining step.

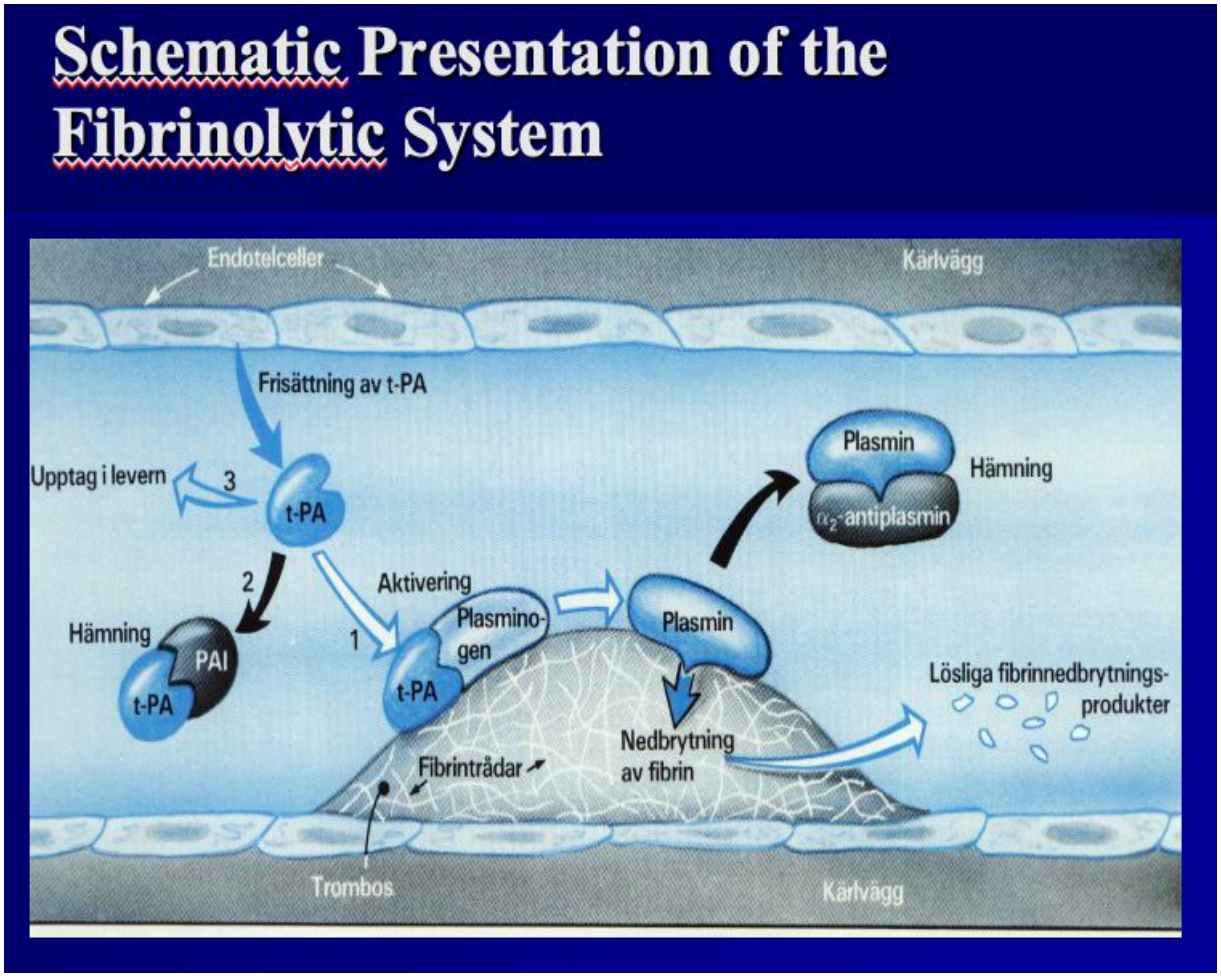
The reaction between plasmin and antiplasmin was astonishingly rapid, with a second-order rate constant exceeding 10^7 M^{-1} s^{-1}. Indeed, the reaction was so fast that it was difficult to understand how plasmin could function at all in vivo.

The answer lay in the lysine-binding sites. Because these sites are occupied when plasmin is bound to fibrin, fibrin-bound plasmin reacts approximately one hundred times more slowly with antiplasmin than does free plasmin.

This mechanism provides the second safeguard that keeps fibrinolysis localized to the site where it is needed.

The schematic shown here illustrates the regulation of fibrinolysis. Tissue-type plasminogen activator released from endothelial cells may follow one of three pathways. It can be cleared rapidly by the liver, inhibited by plasminogen activator inhibitor-1 (PAI-1), or, in the presence of fibrin, participate in formation of a ternary complex on the fibrin surface, leading to rapid generation of plasmin.

The fibrin-bound plasmin remains relatively protected. However, once released into the circulation, it is rapidly neutralized by α_2-antiplasmin.



Our current understanding indicates that PAI-1 is the principal determinant of t-PA activity under physiological conditions. High levels of PAI-1 result in very low concentrations of free circulating t-PA, whereas low PAI-1 levels permit greater t-PA activity and increased formation of plasmin–antiplasmin complexes. Such individuals exhibit a small but measurable increase in bleeding tendency.

Conversely, elevated PAI-1 levels are associated with an increased risk of thrombotic disease, particularly myocardial infarction. Prospective studies in healthy individuals have demonstrated that elevated PAI-1 is a major short-term risk factor for myocardial infarction, in some analyses proving more predictive than traditional lipid parameters.

Let me conclude with a few personal remarks.

I spent a memorable, enjoyable, and truly wonderful year in Leuven during 1977 and 1978.

I had the privilege of collaborating with a large number of talented, dedicated, and dynamic scientists. Together we published many papers, primarily concerning antiplasmin and its biological function.

We worked under a leader whom I came to respect more with every passing day and every passing year. That person was not Désiré Collen, but rather our common mentor at the time: Marc Verstraete.

Coming from the far North, I would like to compare the t-PA story to a snowball.

When I arrived in Désiré's laboratory and succeeded in convincing him of the remarkable fibrin specificity of t-PA, I created only a very small snowball.

Désiré then set that snowball in motion. He guided its course as it rolled downhill, growing larger and larger. Eventually it generated enormous momentum and substantial resources.

What I admire most about Désiré's achievements is the way he used those resources. He consistently reinvested them in creating better science, stronger research programmes, and new opportunities for scientific discovery.

That, in my view, is perhaps his greatest accomplishment.

Thank you very much.

Our next speaker is Alfons Billiau from the Rega Institute in Leuven..
He initially took care of the production of the melanoma cell culture medium, because we didn't have cell culture facilities at that tim. Equally important he made the initial contact with Willem Weimar at the Erasmus Medical University in Rotterdam, who treated in 1981 the first renal allograft patient with melanoma t-PA.
Dr. Weimar could not be here today, but Dr. Billiau kindly agreed to cover that part of the story himself.

Alfons Billiau

Dear Désiré, colleagues, and friends,

Like Désiré, I had the privilege of spending my entire academic career on the staff of this university, a career that formally ended in 2002.

When I first became acquainted with Désiré in the early 1970s, my research at the Rega Institute focused on the production of interferon from cultured mouse and human cells. Both research lines would later contribute to a fruitful collaboration between us.

As part of my work on retroviruses, I had developed several tumour cell lines by infecting mouse fibroblasts in vitro with murine sarcoma virus. Some of these cell lines were subsequently used at Ghent University to validate a malignancy assay based on the invasive behaviour of tumour cells in chick embryo blastoderms.

Therefore, I became interested—albeit somewhat indirectly—in the mechanisms underlying tumour-cell invasiveness. At that time, it was already believed that tumour invasion was due, at least in part, to an imbalance between proteases and protease inhibitors.

During discussions about our respective research interests, Désiré and I conceived the idea of testing my mouse tumour cell lines for protease production. We hoped that such studies might eventually provide an in vitro model for evaluating protease inhibitors with potential anti-tumour activity.

At that time, a British postdoctoral fellow, Vic Eddy, had joined my laboratory to work on interferon production. He was accompanied by his wife Judy, a biologist who was looking for employment. Désiré hired her to investigate the idea we had discussed.

The work proved successful. In 1977, Judy published a paper demonstrating that the mouse tumour cell lines from the Rega Institute produced significant proteolytic activity and that this activity resulted from the secretion of a plasminogen activator.

Almost as an aside, the paper also reported that a sample of culture fluid from a human melanoma cell line displayed similar fibrinolytic activity and that this activity could be inhibited by normal plasma. The origin of that culture fluid was not specified in the publication, but as Désiré has already told us, it was a sample he had brought back from New York—a culture that would later become famous as the Bowes melanoma cell line.

Several years later, during the winter of 1980–1981 (*correction added during editing: winter of 1978-1979, after the Bowes melanoma cell line was obtained in November 1978 from Dan Rifkin of Rockefeller University, New York)*, Désiré unexpectedly appeared in my office early one morning carrying a culture flask. He asked whether I could take care of the culture because, although his laboratory was exceptionally well equipped, it did not yet possess tissue culture facilities.

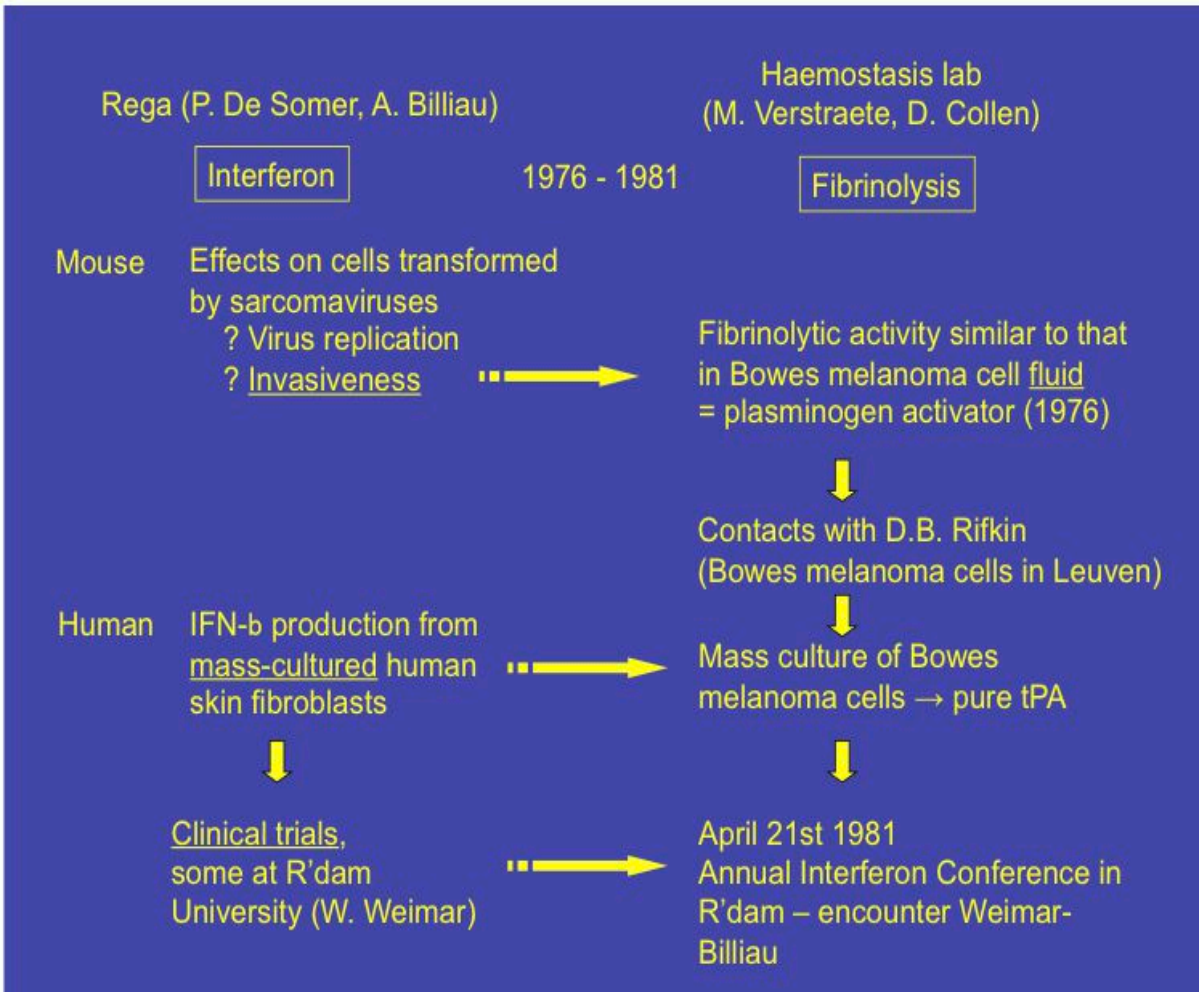
He explained that he had reason to believe that the cell line, known as Bowes, produced a protease with properties identical or very similar to those of normal

tissue-type plasminogen activator. He then gave me a brief introduction on t-PA—the same introduction that all of us have effectively received again today.

At the time, Désiré lacked the facilities to cultivate the cells, whereas at the Rega Institute we were already operating large-scale culture systems for the production of human fibroblast interferon. Growing the cells and collecting the culture supernatant therefore posed no difficulties.

Within a very short period, we had established a production system. Désiré hired a technician to expand the operation further at the Rega Institute, and before long sufficient quantities of purified protein became available to test the concept in animal models.

Meanwhile, we at the Rega Institute had begun clinical trials with interferon, attempting to demonstrate antiviral and antitumour activity. Some of these studies were being conducted at the Dijkzigt Hospital in Rotterdam under the supervision of Willem Weimar, who was then at an early stage of his career as a nephrologist.



Unfortunately, the overall results of the interferon studies were rather disappointing. Moreover, producing even small amounts of interferon required enormous effort and yielded little reward. In striking contrast, the production of t-PA was relatively straightforward and highly productive.

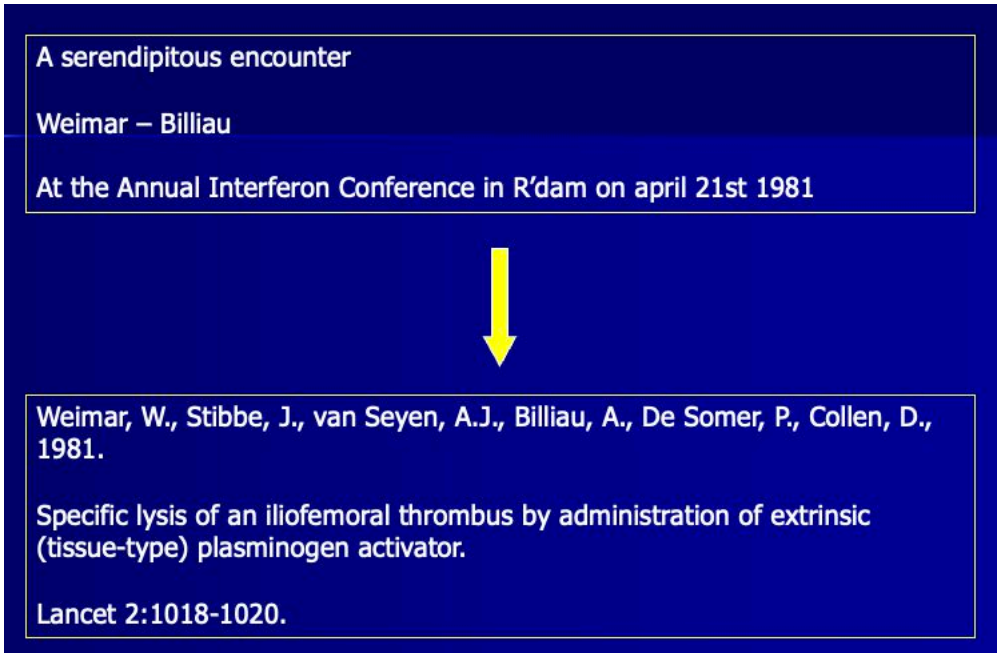
Against this background, an important and rather remarkable sequence of events unfolded.

In April 1981, the annual Interferon Meeting was held in Rotterdam. On April 21, or perhaps the day before, I drove to Rotterdam. Upon arrival at the conference venue, I searched for Willem Weimar but could not find him.

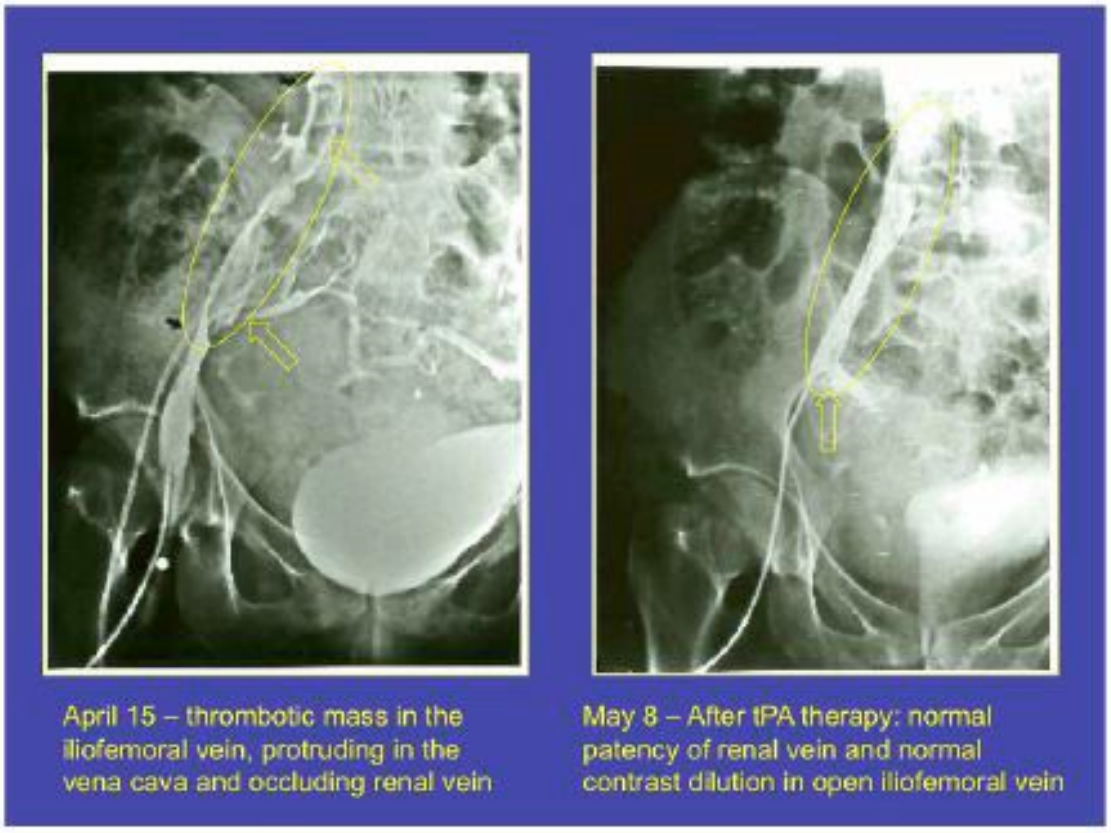
When he finally arrived, he explained that he had been delayed because he was caring for a renal-transplant patient who had developed a massive thrombosis in the vein draining the transplanted kidney. At that moment he had no effective treatment available. As he later recalled, he told me that he expected the patient either to lose the graft or possibly even to die within the next few days.

I then asked him a simple question: "Have you ever heard of t-PA?" The answer was no. I explained the story and told him that in Leuven we already had batches of purified t-PA available for administration. Willem immediately expressed a strong desire to obtain the material for treatment of his patient.

That same evening, I drove back to Leuven. The following morning, I telephoned Désiré, who immediately agreed. I collected the t-PA preparation and drove back to Rotterdam. Fortunately, I had my car.



Before the scientific meeting had even concluded, the patient's thrombus had dissolved.



The original angiographic images are shown here. On the left, obtained on April 15, the thrombus is clearly visible. On the right, obtained several weeks later on May 8, the obstruction has disappeared

Since Willem Weimar is unfortunately unable to be with us today, I would like to share some reflections that he sent to me a few weeks ago. He wrote:

"In those days, clinical research was based largely on trust between basic scientists, clinicians, and patients. Certainly, serendipity also played an important role. My renal-transplant unit happened to collaborate with the Rega Institute in Leuven. One of my patients developed an ascending thrombosis precisely during an Interferon Meeting in Rotterdam attended by Piet De Somer and Alfons Billiau. Désiré Collen had just produced the first batch of t-PA. And Fons Billiau had a car."

I would like to conclude by thanking Désiré for everything he has meant to me over the years—as a source of inspiration, as a scientific collaborator, and as a friend.

Of course, before these first patients could be treated with melanoma t-PA, the protein first had to be purified from the cell culture medium, which turned out not to be so simple, because in Leuven we did not succeed in purifying it to full homogeneity.
This is where Dick Rijken comes into the story. Dick Rijken was as a postdoc in Leuven from 1979 to 1982.
He was at the Gaubius Institute in Leiden and is now at the Erasmus University Medical Center in Rotterdam. He learned us a few tricks on how to purify t-PA.

Dingeman ("Dick") Rijken

Mr. Chairman, ladies and gentlemen, Désiré, dear friends,

I have been asked to speak about two subjects: the purification of melanoma plasminogen activator and the kinetics of plasminogen activation by tissue-type plasminogen activator, or t-PA.

My own involvement with t-PA began during my PhD studies at the Gaubius Institute in Leiden in the 1970s and continued during my postdoctoral years here in Leuven from 1979 to 1982.

Before discussing the t-PA story itself, I would like to make a few remarks about the history of t-PA.

The history of t-PA actually dates back to the first half of the twentieth century, when researchers began developing tissue culture techniques. During the cultivation of tissues on matrices containing clotted plasma, investigators frequently observed spontaneous liquefaction of the clot, suggesting that tissues contained substances capable of inducing fibrinolysis.

It is often difficult to define precisely when a protein has been "discovered," but for t-PA, the year 1952 was certainly a landmark.

In that year Astrup—already mentioned by Björn Wiman—published a seminal paper in *Nature* entitled "*Isolation of a Soluble Fibrinolytic Activator from Animal Tissue"*.

Using a powerful chaotropic agent, thiocyanate, Astrup succeeded in extracting t-PA from tissue. This was a remarkable achievement because t-PA is very strongly bound to tissues. To this day, I am not entirely certain why that binding is so strong, but Astrup's observation made purification possible for the first time.

From that moment onward, many investigators attempted to purify t-PA. Throughout the 1950s and 1960s, numerous PhD students devoted years of effort to this task.

In the 1970s, it became my turn. My supervisor, Professor Pieter Brakman, assigned me the challenge of purifying t-PA from human tissue. The first requirement was to identify an appropriate source. At that time, published data indicated that uterine tissue contained the highest concentrations of t-PA activity—approximately 720 Astrup-Albrechtsen units per gram of tissue. Other tissues contained substantially less activity. The liver, for example, contained virtually none, whereas the brain showed only modest activity. Interestingly, some of the most important contemporary t-PA research concerns the brain, where t-PA is now known to perform important non-haemostatic functions.

The second requirement was an assay. The assay we employed had also been developed by Astrup in 1952 and closely resembled his original tissue culture system. A thin layer of fibrin containing plasminogen was cast in a culture plate, and droplets containing t-PA were applied to the surface. After overnight incubation, the area of fibrin lysis could be measured as an indicator of activity.

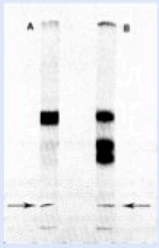
The third requirement was access to suitable purification methods. Here I was fortunate. During the 1970s, affinity chromatography became available, and this

technological advance probably explains why my efforts were more successful than those of many investigators who had worked on the problem in earlier decades.

After four years of work, I was finally able to publish a paper describing the purification and characterization of plasminogen activator from human uterine tissue. The yield, however, was rather modest. From five kilograms of human tissue, we succeeded in obtaining approximately one milligram of purified t-PA.

Purification and partial characterization of plasminogen activator from human uterine tissue

- D.C. Rijken, G. Wijngaards, M.Zaal-de Jong and J. Welbergen
  Biochim Biophys Acta 1979;580:140
- 5 kg tissue yielded 1 mg t-PA
  (5000-fold purified)



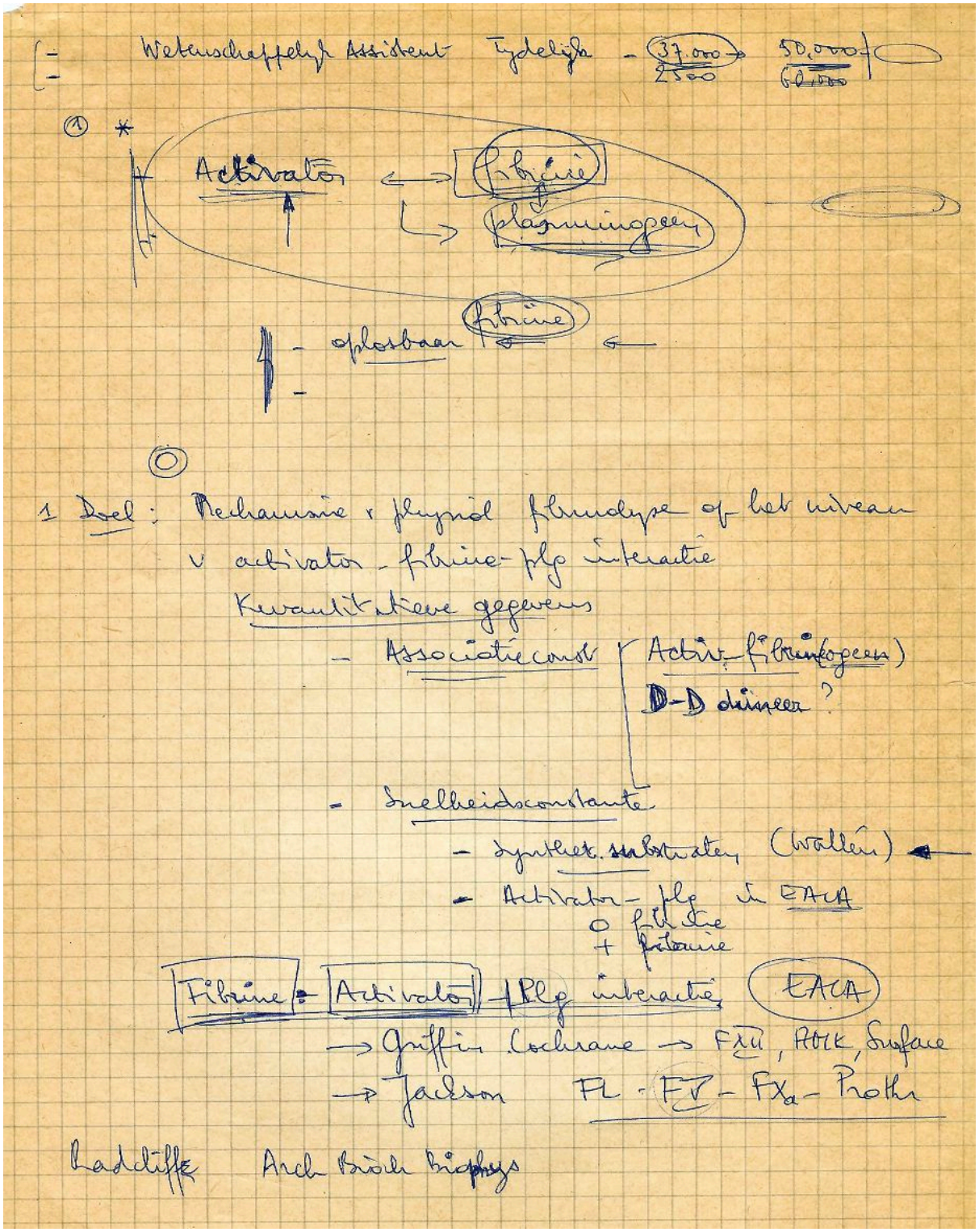
* Purified t-PA was stabilized by Tween 80

Detergent	Type	Residual activity (%)	
		After 0.5 h	After 24 h
Buffer alone	–	51	0
0.01% (w/v) sodium dodecyl sulphate	anionic	44	0
0.01% (w/v) *n*-hexadecylpyridiniumchloride	cationic	60	10
0.10% (v/v) Tween-80	non-ionic	99	114

Once a protein has been purified, the next step is to characterize it. Naturally, I began a series of experiments with my precious preparation. Unfortunately, every experiment failed. For more than six months, I repeatedly lost all measurable activity. Eventually, Pieter Brakman suggested adding the neutral detergent Tween 80 to the buffers. This simple modification solved the problem immediately by preventing adsorption of t-PA to glass and plastic surfaces. Only then could I complete the characterization studies and finish my doctoral thesis.

Afterward, I moved to Leuven as a postdoctoral fellow. One Saturday morning in 1978, I visited Désiré, and we discussed possible research projects. We eventually agreed that I would study the enzyme kinetics of plasminogen

activation by t-PA, with particular emphasis on the role of fibrin *(attached is Désiré's scribble of the meeting kept by Dick as a souvenir).*



The next time I met Désiré was during the 1979 International Society on Thrombosis and Haemostasis Congress in London, three months before my official arrival in Leuven.

There, Désiré told me about the melanoma cell culture system. He explained that it offered a much richer source of t-PA than human tissue, potentially containing as much as 500 units of activity per millilitre. As a result, one of my first tasks in Leuven became the purification of melanoma-derived plasminogen activator.

The conditioned medium contained approximately 20 units per millilitre rather than 500. Nevertheless, this represented an enormous improvement. It became possible to purify one milligram of t-PA from only ten litres of culture medium instead of five kilograms of tissue. As we have already heard, scaling up production of cell culture medium was relatively straightforward.

Our studies led us to conclude that the plasminogen activator secreted by cultured human melanoma cells was either identical or very closely related to the activator present in normal human tissues and clearly distinct from urokinase.

Purification and characterization of the plasminogen activator secreted by human melanoma cells in culture

- D.C. Rijken and D. Collen. J Biol Chem 1981;256:7035

TABLE I

Purification of human melanoma plasminogen activator

The figures represent the mean value with the standard error of the mean of (the first) three preparations.

	Volume	Total protein	10^{-3} × total activity	10^{-4} × specific activity	Yield	Purification factor
	ml	*mg*	*IU*	*IU/mg*	%	
Conditioned medium	10,000	610 ± 40	20 ± 2	0.032 ± 0.002	100	1
Zinc chelate-agarose	151	46 ± 2	17 ± 4	0.36 ± 0.08	83	11
Concanavalin A-agarose	84	4.5 ± 0.2	11 ± 2	2.5 ± 0.7	56	77
Sephadex G-150	37	1.0 ± 0.2	9 ± 3	9 ± 2	46	263

" All findings indicate that the plasminogen activator secreted by human melanoma cells in culture is very similar to, or identical with, the plasminogen activator found in normal tissue, but different from urokinase"

Because substantial quantities of purified material were now available, I could continue studying the fibrinolytic properties of both one-chain and two-chain forms of human tissue-type plasminogen activator.

In the meantime, Désiré suggested that Marc Hoylaerts begin work on the kinetic studies because purification was taking longer than expected. This proved to be an excellent decision.

Marc developed an elegant kinetic model for plasminogen activation by t-PA, based on the formation of the now famous ternary complex consisting of fibrin, plasminogen, and t-PA, within which activation proceeds at a greatly accelerated rate.

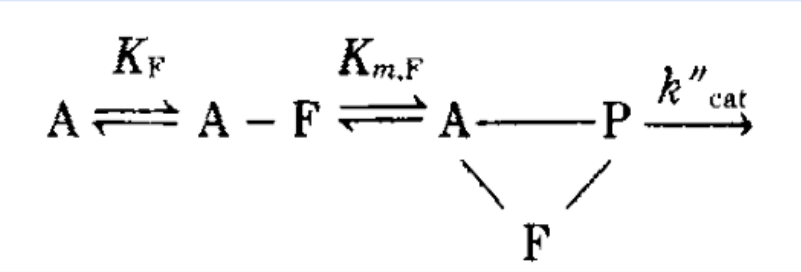
The resulting publication became extraordinarily influential and has been cited more than one thousand times. I suspect, however, that relatively few of those who cited it actually read the paper in detail, because enzyme kinetics is notoriously difficult to understand—even for me as the second author.

Fibrinolytic properties of one-chain and two-chain human extrinsic (tissue-type) plasminogen activator

D.C. Rijken, M. Hoylaerts, D. Collen. J Biol Chem 1982;257:2920

Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin

M. Hoylaerts, D.C. Rijken , H.R. Lijnen, D. Collen. J Biol Chem 1982;257:2912



A cyclic ternary complex is formed via a sequential ordered mechanism (>1000 times cited)

All of these findings, together with the treatment of the first patient, were presented at the 1981 ISTH Congress in Toronto.

Désiré, I would like to conclude by thanking you sincerely for the exciting and unforgettable years spent in your laboratory. Thank you very much.

Our next speaker is Irène Juhan-Vague from the Centre Hospitalier Universitaire Timone in Marseille. She has had a long-term collaboration with Désiré, spanning the period 1982-1989 and later up till today on many other subjects. Her efforts were in the determination of t-PA levels in pathological conditions, contributing to the discovery of PAI-1, as you have already heard, the main physiological inhibitor of t-PA.

Irène Juhan-Vague

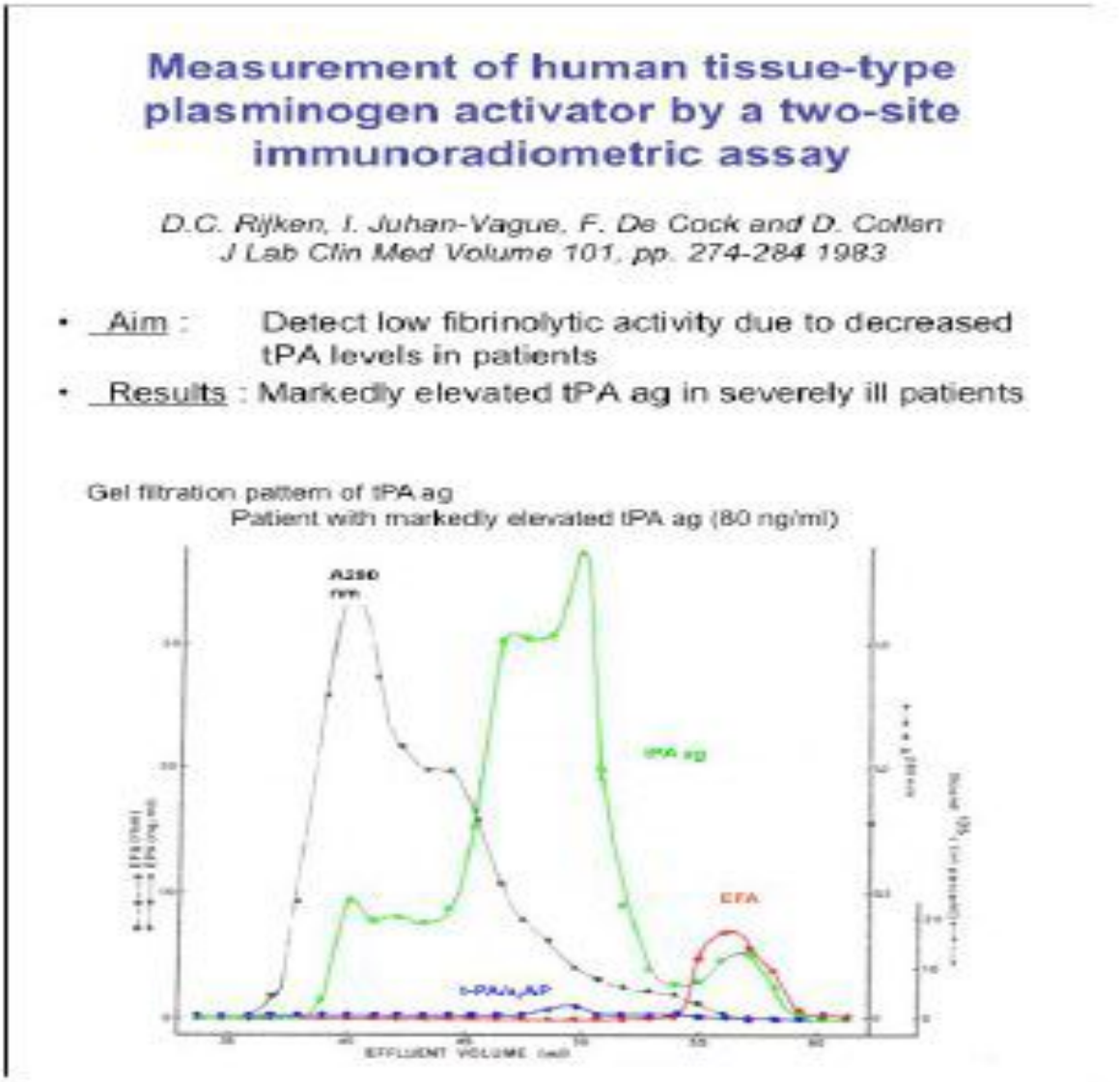
Désiré, in 1982 you agreed to become the supervisor of my PhD thesis, and I began studying variations in plasma tissue-type plasminogen activator (t-PA) antigen levels in different clinical conditions.

This work ultimately led to the identification of a specific inhibitor of t-PA, which later became known as plasminogen activator inhibitor-1, or PAI-1.

Initially, I used the radioimmunometric assay developed by Dick Rijken. Our objective was to identify patients with impaired fibrinolytic activity resulting from reduced t-PA levels, particularly among individuals suffering from thrombotic disorders.

However, we were quickly surprised to find markedly elevated levels of t-PA antigen in severely ill patients. When plasma obtained from a patient with very high t-PA antigen levels. was subjected to gel-filtration chromatography, the

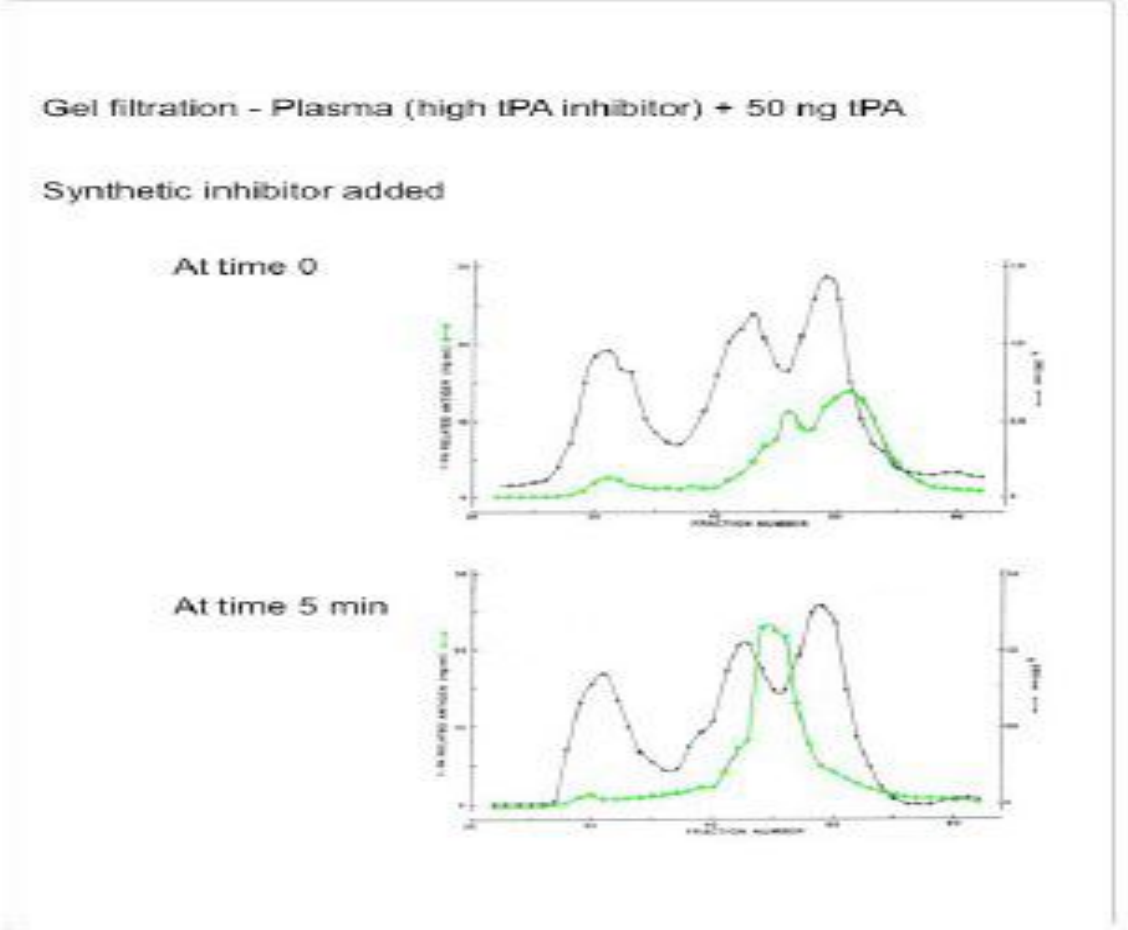
immunoreactive material was found to elute as a high-molecular-weight form that exhibited no fibrinolytic activity. Furthermore, it could not be identified as a complex with any of the known inhibitors.



We therefore proposed that the material represented a complex between t-PA and a previously unknown specific inhibitor. This observation prompted us to investigate the phenomenon further. We subsequently developed a functional assay for this inhibitor and found elevated inhibitor levels in many hospitalized patients.

The figure shown here illustrates plasma containing high levels of t-PA inhibitor. Active t-PA was added either immediately or after a five-minute

incubation period. As can be seen, the immunoreactive material shifted from a molecular weight of approximately 70,000 to 120,000 Daltons, indicating formation of a complex between t-PA and the inhibitor. This inhibitor would later be identified as PAI-1.



We then sought to determine the pathological conditions in which elevated PAI-1 levels occurred and to identify the factors responsible for its induction.

One of our first findings was that PAI-1 behaves as an acute-phase reactant. Following surgery, its concentration increased very rapidly, often within less than one hour.

We also observed that patients with deep vein thrombosis frequently displayed a normal release of t-PA following venous occlusion testing. However, this release was not accompanied by a corresponding increase in fibrinolytic activity because of the presence of high concentrations of the inhibitor.

Our primary interest subsequently focused on the dysregulation of PAI-1 in obesity and diabetes.

We demonstrated that elevated PAI-1 levels were not associated with all forms of obesity, but specifically with what is known as android obesity, characterized by preferential fat accumulation in the upper part of the body. This pattern of fat distribution is strongly associated with insulin resistance, metabolic syndrome, diabetes mellitus, and an increased risk of coronary artery disease.

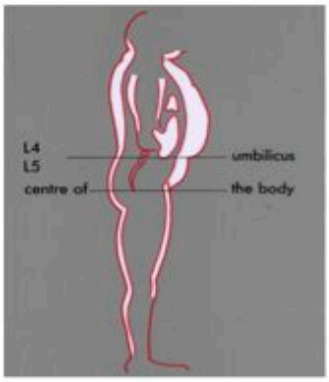
Correlation between blood fibrinolytic activity, PAI-1 level, plasma insulin level and relative body weight in normal and obese subjects

P. Vague, I. Juhan-Vague, M.F. Aillaud, C. Badier, R. Viard, M.C. Alessi and D. Collen
Metabolism Volume 35, pp. 250-253 1986

Plasma PAI-1 in angina pectoris Influence of plasma insulin and acute phase response

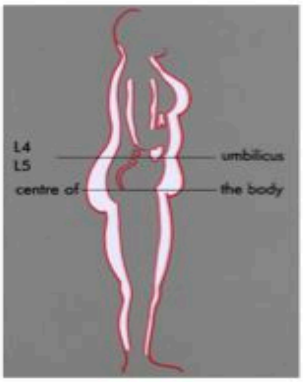
I. Juhan-Vague, M.C.Alessi, P. Joly, X. Thirion, P. Vague, P.J. Declerck, A. Serradimigni and D. Collen
Arteriosclerosis Volume 9, pp. 362-368 1989

PAI-1 is related to obesity and insulin resistance



Android obesity
Insulin Resistance
Metabolic syndrome
CHD risk

Plasma PAI-1 **31** ng/ml



Gynoid obesity

13 ng/ml

In contrast, individuals with gynoid obesity, characterized by fat accumulation predominantly in the lower part of the body, exhibited normal PAI-1 levels and generally did not display the same metabolic and cardiovascular complications.

These observations led us to conclude that elevated PAI-1 concentrations occur in a variety of pathological conditions, particularly obesity and diabetes, and that PAI-1 may play an important role in the development of thrombotic disease.

It is worth noting that even the highest PAI-1 concentrations observed in these patients remained below one microgram per millilitre. Such levels were not expected to interfere significantly with thrombolytic therapy using pharmacological doses of t-PA.

I am deeply grateful to Désiré. From the very beginning, he accepted and encouraged my decision to focus my doctoral research on clinical investigations. Without his support, it would have been extremely difficult to establish a research group in Marseille and to develop an independent scientific programme.

The gratitude of the Faculty of Medicine in Marseille for his contributions eventually led to Désiré being awarded the title of Doctor Honoris Causa of the University of Marseille.

Désiré, thank you for your mentorship, your confidence, and your friendship.

Thank you.

Our next speaker is Osamu Matsuo, who is at Kinki University School of Medicine in Osaka, Japan. He worked as a post-doc in Leuven in 1979-1980 and was actually the first one to do an experimental animal study with the melanoma t-PA, that was then just purified. This was done in rabbits with experimental pulmonary embolism.

Osamu Matsuo

It is a great pleasure to be here today and to participate in this impressive celebration in honour of Désiré Collen.

When I first came to Leuven in 1979, I had already been involved in research on thrombolytic therapy in Japan. Before joining Désiré's laboratory, I had actually spent considerable time arguing with our regulatory authorities. At that time, the Japanese government had approved only a very low dose of urokinase—10,000 CTA units—for the treatment of stroke.

I was convinced that this dose was ineffective and set out to generate experimental evidence to demonstrate this.

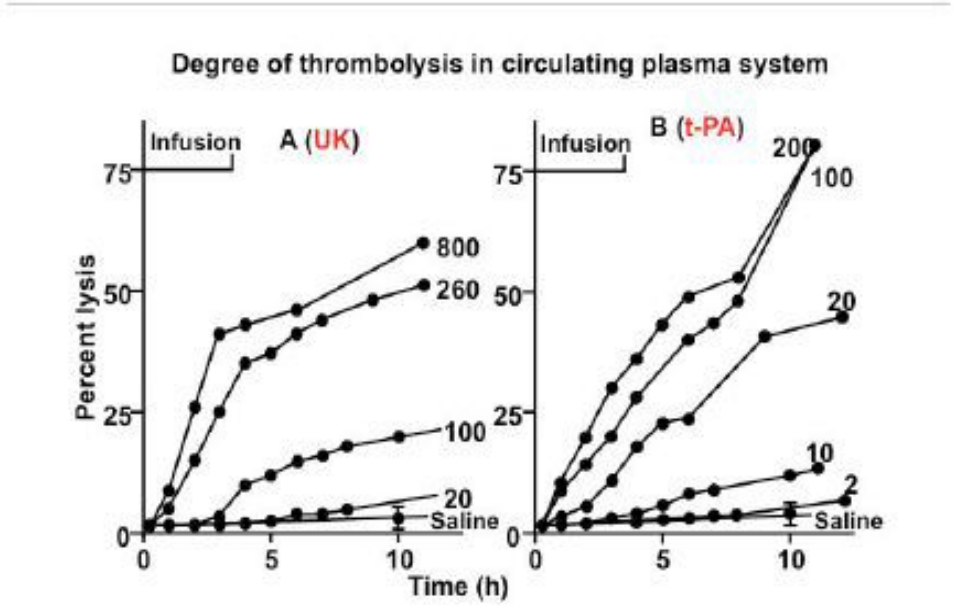
To do so, I developed a circulating plasma model. In this system, plasma circulated through an artificial loop resembling the cardiovascular system: a

reservoir connected through tubing representing arteries and veins. Urokinase could be introduced into the circulation, and its effects on fibrinolysis could then be measured. Using this model, I showed that low concentrations of urokinase produced virtually no thrombolytic effect.

When I joined Désiré's laboratory in 1979, I gained access to tissue-type plasminogen activator, or t-PA, and began comparing its activity with that of urokinase. The results were striking.

In the absence of α_2-antiplasmin, urokinase rapidly degraded fibrinogen. Tissue-type plasminogen activator also caused fibrinogen degradation, but much more slowly. However, in the presence of α_2-antiplasmin, t-PA no longer produced significant fibrinogen degradation, whereas urokinase continued to do so. This clearly demonstrated the fundamentally different behaviour of the two thrombolytic agents.

Using the circulating plasma model, I then examined the effects of both agents on thrombus dissolution. As shown in the left panel, urokinase produced thrombolysis in a dose-dependent and time-dependent manner. However, t-PA was substantially more effective (right panel). Even at relatively low doses, t-PA produced pronounced thrombolysis.



The degree of clot lysis was proportional to the mount of PA added, t-PA being about 10 times efficient than UK.

For example, 100 units of urokinase produced less than 20% thrombolysis, whereas comparable doses of t-PA produced more than 60% thrombolysis.

Thus, t-PA appeared to be approximately ten times more efficient than urokinase in this experimental system.

At the same time, we monitored activation of the fibrinolytic system in the circulating plasma. When 100 units of t-PA were administered, we observed substantial thrombolysis. In contrast, the same dose of urokinase produced only limited thrombolysis, despite causing similar degrees of fibrinogen degradation, plasminogen consumption, and α_2-antiplasmin depletion.

When much higher doses of urokinase were used, significant thrombolysis could indeed be achieved. However, this came at the cost of nearly complete depletion of fibrinogen, plasminogen, and α_2-antiplasmin.

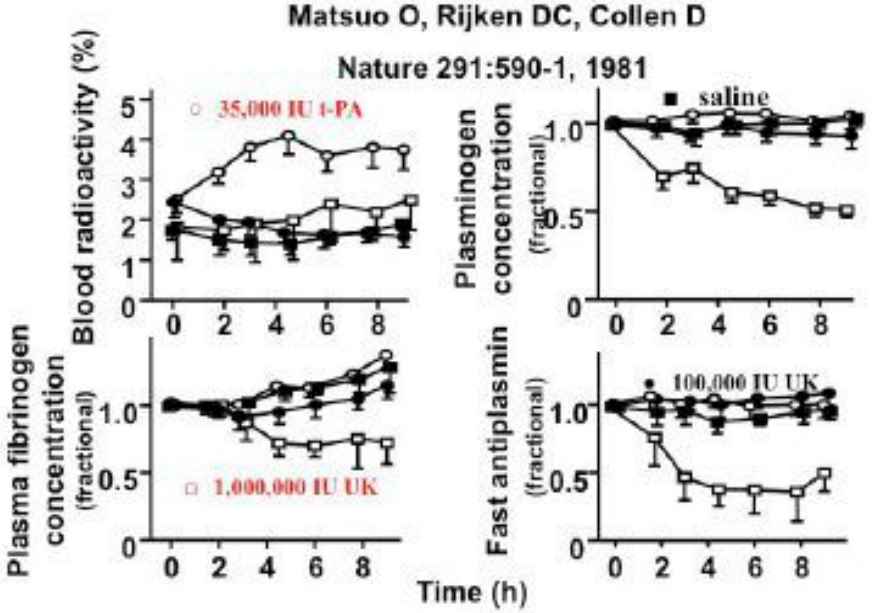
These experiments demonstrated that t-PA and urokinase behaved very differently in the circulation and provided strong evidence for the fibrin specificity of t-PA.

We then moved to in vivo studies using an experimental pulmonary embolism model. Radiolabelled fibrinogen was incorporated into thrombi, which were introduced into the pulmonary circulation. Thrombolysis was quantified by measuring the release of radioactivity into the bloodstream.

Thrombolysis by human tissue plasminogen activator and urokinase in rabbits with experimental pulmonary embolus

Matsuo O, Rijken DC, Collen D

Nature 291:590-1, 1981



t-PA induces thrombolysis at lower doses than UK, without extensive plasminogen activation in the circulating blood.

Thrombolysis by human tissue plasminogen activator and urokinase in rabbits with experimental pulmonary embolus

Matsuo O, Rijken DC, Collen D

Nature 291:590-1, 1981

Extent of thrombolysis and isotope recovery

Group	*n*	% Thrombolysis	% Recovery
Saline 9 h	4	3.2 ± 1.5	102.0 ± 2.4
Saline 24 h	6	5.8 ± 3.2	100.3 ± 4.4
UK 100,000	5	4.0 ± 1.2	103.3 ± 1.1
UK 1,000,000	3	11.5 ± 5.4	97.7 ± 2.9
TA 35,000	6	16.5 ± 2.7	100.4 ± 3.3
TA 70,000	3	23.1 ± 7.4	97.0 ± 0.8

Administration of 35,000 units of t-PA produced a clear increase in circulating radioactivity, indicating effective thrombus dissolution. By contrast, administration of one million units of urokinase caused marked depletion of fibrinogen, plasminogen, and α_2-antiplasmin, yet produced less efficient thrombolysis. Lower doses of urokinase behaved little differently from saline controls.

These findings were reproduced in subsequent experiments using higher doses. Infusion of 70,000 units of t-PA produced even greater thrombolysis, with approximately 23% clot dissolution. In comparison, 100,000 units of urokinase produced results similar to saline infusion, while even one million units of urokinase achieved less thrombolysis than t-PA despite causing extensive systemic fibrinolysis.

These experiments provided convincing evidence that t-PA could achieve effective thrombolysis while largely preserving circulating fibrinogen and other components of the haemostatic system.

Those were memorable days.

Our next speaker is Frans Van de Werf from the department Cardiology, University Hospital in Leuven.
As we have just heard the first patients ever treated with melanoma t-PA were renal allograft patients. Frans Van de Werf treated the first patients with Acute Myocardial Infarction with t-PA which eventually turned out to be the major indication for its clinical use.

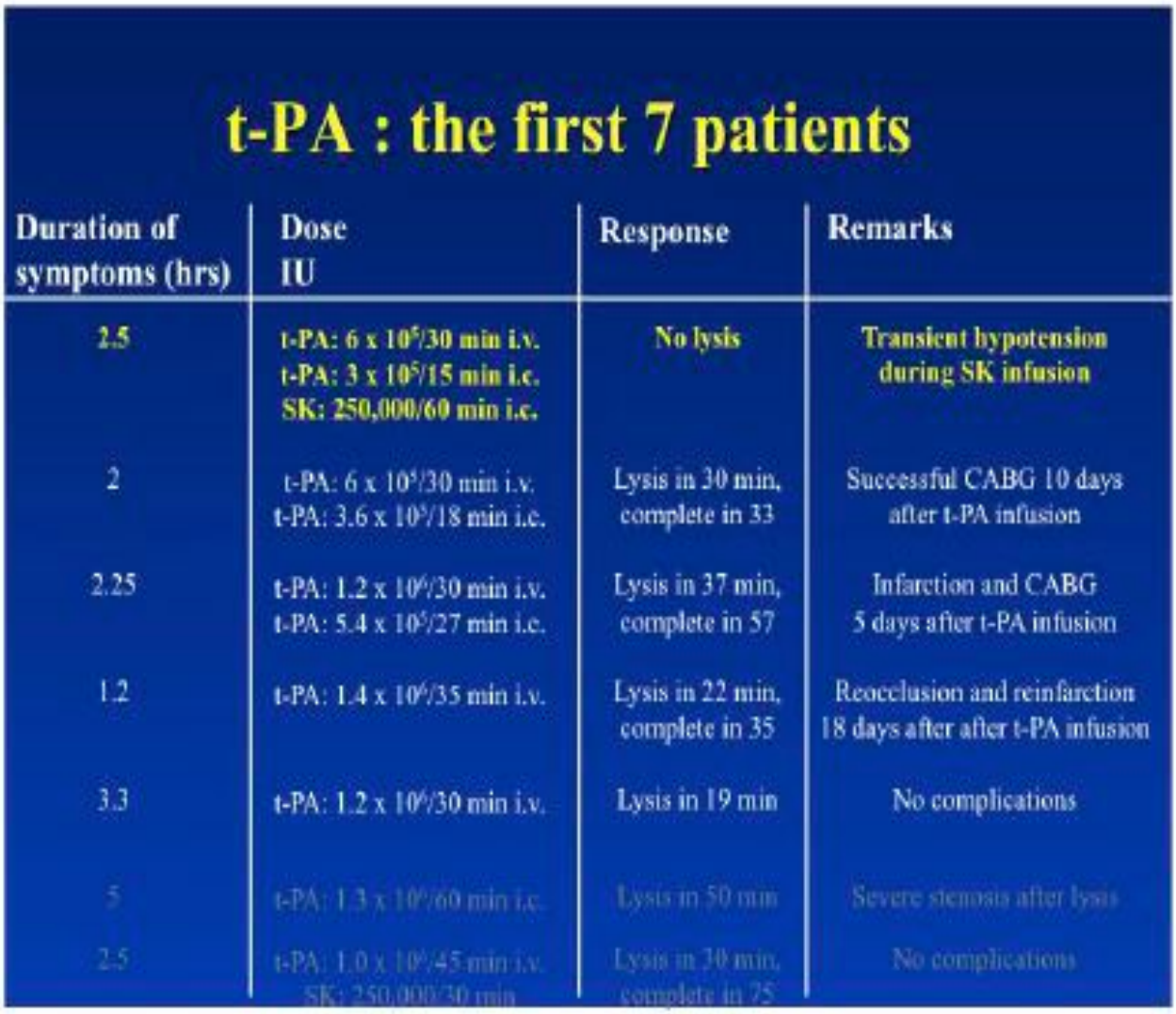
Frans Van de Werf

Ladies and gentlemen, Désiré,

I had the great privilege of treating the first five patients in the world with acute myocardial infarction using tissue-type plasminogen activator. Two additional patients were treated by Burt Sobel and his colleagues in St. Louis.

All of these patients received t-PA derived from the Bowes melanoma cell line and purified in Désiré's laboratory.

At that time, we had no idea what the optimal therapeutic dose of t-PA might be. Looking back, the doses we administered corresponded to only a few milligrams. As you can see, t-PA was given both intravenously and intracoronarily. These amounts were remarkably small compared with the doses used today, which are typically around 100 milligrams.



t-PA : the first 7 patients

Duration of symptoms (hrs)	Dose IU	Response	Remarks
2.5	t-PA: 6 x 10^5/30 min i.v. t-PA: 3 x 10^5/15 min i.c. SK: 250,000/60 min i.c.	No lysis	Transient hypotension during SK infusion
2	t-PA: 6 x 10^5/30 min i.v. t-PA: 3.6 x 10^5/18 min i.c.	Lysis in 30 min, complete in 33	Successful CABG 10 days after t-PA infusion
2.25	t-PA: 1.2 x 10^6/30 min i.v. t-PA: 5.4 x 10^5/27 min i.c.	Lysis in 37 min, complete in 57	Infarction and CABG 5 days after t-PA infusion
1.2	t-PA: 1.4 x 10^6/35 min i.v.	Lysis in 22 min, complete in 35	Reocclusion and reinfarction 18 days after after t-PA infusion
3.3	t-PA: 1.2 x 10^6/30 min i.v.	Lysis in 19 min	No complications
5	t-PA: 1.3 x 10^6/60 min i.c.	Lysis in 50 min	Severe stenosis after lysis
2.5	t-PA: 1.0 x 10^6/45 min i.v. SK: 250,000/30 min	Lysis in 30 min, complete in 75	No complications

The first patient was not successfully treated.

I still vividly remember the day of that procedure. It was January 1983. The intervention took place in the cardiac catheterization laboratory. First, we documented the coronary occlusion and then attempted to achieve recanalization.

Naturally, Désiré was present, but so were another twenty or thirty people. This was, after all, a completely new therapeutic approach.

Although the procedure was unsuccessful. ECG showed prominent T waves in the anterior leads, characteristic of an acute anterior myocardial infarction. The procedure began on January 20 at approximately three o'clock in the afternoon.

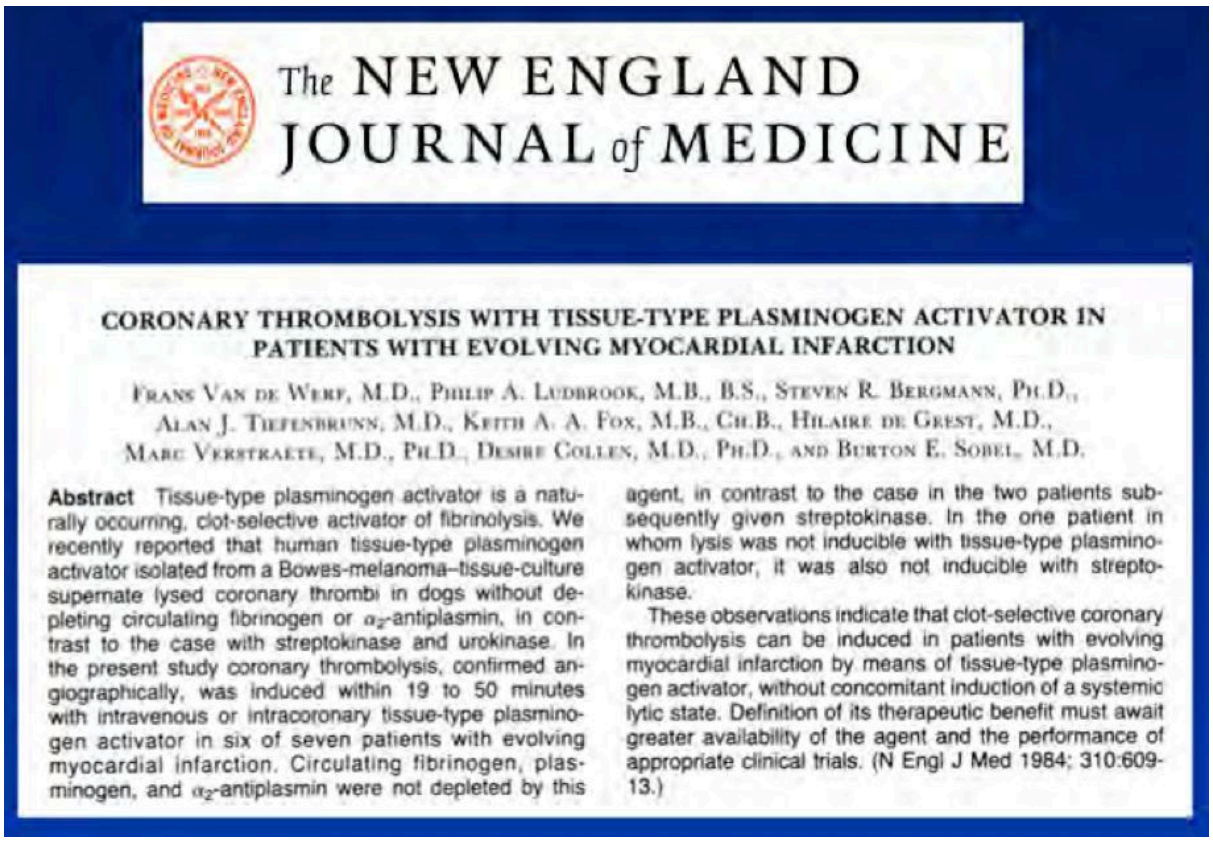
Despite our efforts, reperfusion could not be achieved. An electrocardiogram obtained later that evening demonstrated a large anteroseptal transmural

infarction.When the patient was subsequently evaluated prior to discharge, the ECG suggested the development of a ventricular aneurysm. We were therefore quite concerned about his long-term prognosis.

Yet medicine frequently surprises us. I continued to see this patient for many years. A few years ago, he required implantation of a pacemaker because of conduction disturbances.

Remarkably, he was still alive, 25 years after the unsuccessful thrombolytic treatment. He eventually died in July of this year at the age of eighty-three.His case reminds us that even patients who experience large anterior myocardial infarctions and unsuccessful reperfusion may survive for many decades.

The remaining six patients were successfully treated, and these experiences led to a publication in the *New England Journal of Medicine* in 1984. The paper described only seven patients.



The NEW ENGLAND JOURNAL of MEDICINE

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

FRANS VAN DE WERF, M.D., PHILIP A. LUDBROOK, M.B., B.S., STEVEN R. BERGMANN, PH.D., ALAN J. TIEFENBRUNN, M.D., KEITH A. A. FOX, M.B., CH.B., HILAIRE DE GREST, M.D., MARC VERSTRAETE, M.D., PH.D., DESIRE COLLEN, M.D., PH.D., AND BURTON E. SOBEL, M.D.

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.)

To my knowledge, this remains one of the smallest patient series ever published in the *New England Journal of Medicine*. If one calculates the number of citations per patient, it must surely represent a world record.

At the same time, I was also performing experimental studies in animals using a copper-coil model of coronary thrombosis. A small copper coil could be advanced over a guidewire and positioned within a coronary artery. Within minutes, the coil induced complete thrombotic occlusion of the vessel.

This model allowed us to study both coronary occlusion and subsequent recanalization using repeated angiographic examinations. Using this system, we conducted studies in 1983 that were subsequently published in *Circulation* in 1984.

Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Coronary thrombolysis with intravenously administered human tissue-type plasminogen activator produced by recombinant DNA technology*

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ABSTRACT Coronary thrombolysis was induced by intravenous infusion of human tissue-type plasminogen activator (recombinant human t-PA or rt-PA) obtained by expression of the cloned gene in a mammalian cell system. Thrombolysis was detected by the appearance of reperfusion arrhythmia and confirmed by repeat angiography in anesthetized dogs with 1-hr-old thrombi of the left anterior descending coronary artery that were induced with a copper coil. Infusion of 1000 IU (10 μg)/kg/min intravenous rt-PA (n = 9) elicited reperfusion within 13.7 ± 1.9 min (mean ± SE) without producing systemic fibrinolysis or distal coronary embolization. Infusion of urokinase at the same rate elicited thrombolysis in seven of 10 dogs within an average of 19.3 ± 2.2 min. However, distal coronary embolization occurred in two dogs and systemic fibrinolysis was observed in all. In three dogs treated with urokinase thrombolysis was obtained only with subsequent intracoronary infusion. Restoration of myocardial perfusion and metabolism assessed with positron-emission tomography was consistently noted in dogs treated with rt-PA. Thus, rt-PA, a clot-selective thrombolytic agent that does not activate the fibrinolytic system systemically and that is potentially available in large quantities, in view of its synthesis by recombinant DNA technology, offers a promising practical approach for coronary thrombolysis in patients with acute myocardial infarction.

Circulation 69, No. 3, 605–610, 1984.

This time we used recombinant t-PA produced by Genentech. Nine dogs received recombinant t-PA, while ten received urokinase.

The results demonstrated a dramatic difference between the two agents, not only in thrombolytic efficacy but also in fibrin specificity.

The study provided some of the first convincing evidence that recombinant DNA-derived t-PA retained the unique properties of the naturally occurring molecule.

Thereafter, I became involved in numerous international clinical trials, many of which were coordinated by our group. These studies consistently confirmed both the superiority of t-PA over streptokinase and the crucial importance of achieving rapid restoration of coronary blood flow.

In many ways, these investigations paved the road toward the development of mechanical reperfusion techniques, which are now routinely employed in hospitals with cardiac catheterization facilities.

In closing, I would like to thank Desire for all the support you have given me throughout my twenty-five years of academic life.

I would also like to thank you for everything you have done for our department, and above all for the friendship that you and Louisa have shown us over so many years.

Thank you very much.

Our next speaker is Diane Pennica, she is at Genentech in South San-Francisco.
As you have just heard the Bowes melanoma cell line has been instrumental in development of t-PA and Dick has explained how it has been purified, but it took an experienced technician about 2 weeks to purify 1 mg of melanoma t-PA. And you have just heard from Frans Van de Werf that the therapeutic dose is 100 mg of t-PA. So this could have been the premature end of the t-PA story, if not for a serendipitous meeting back in 1980 in Malmö, Sweden that resulted in the cloning of t-PA and large-scale production by recombinant DNA technology. It is Diane Pennica who cloned the t-PA gene.

Diane Pennica

Désiré, it is both a pleasure and an honour to be here today to help celebrate your many accomplishments.

Rather than presenting a large amount of scientific data, I would like to tell the story of how Désiré and I first met twenty-eight years ago, and how that chance encounter led to an extraordinary collaboration.

The story begins in May 1980, when I started my first job at a small and relatively unknown biotechnology company called Genentech. I was employee number sixty.

Today, Genentech employs more than eleven thousand people and has become one of the most successful biotechnology companies in the world. The success of t-PA played an important role in that transformation.

In 1980, Genentech's primary focus was cardiovascular disease. Heart disease was, and remains, the leading cause of death among men and women in both the United States and Europe. Every twenty seconds, someone suffers a heart attack, and more than one and a half million people experience myocardial infarction each year. There was therefore a tremendous need for a new therapeutic approach.

My very first assignment at Genentech was to clone the gene encoding a protein capable of rapidly dissolving blood clots in patients suffering from heart attacks. At the time, there were only rumours about a promising clot-dissolving substance called tissue-type plasminogen activator, or t-PA. Genentech asked me to attend a scientific meeting in Sweden to learn more about it.

I had been at the company for only a month. I barely understood what gene cloning was. But I was young, enthusiastic, and I had a passport. So I told my supervisor, "Sure, I'll go."

On June 12, 1980, in Malmö, Sweden, I found myself in exactly the right place at exactly the right time. Quite by accident, I met Désiré Collen. That accidental encounter would ultimately lead to our collaboration.

I arrived in Sweden a day before the meeting because I wanted to make sure I knew exactly where it was being held. My father had always taught me, "Never be late." When I checked into the hotel, I asked the receptionist where the conference would take place. She replied, "Oh, the doctors' meeting? That started today."

I was horrified. I thought I had somehow missed the opening day of the conference and imagined that Genentech had sent me halfway around the world only for me to arrive late. I dropped my suitcases in my room and ran to the meeting.

When I peeked into the conference room, I saw about thirty scientists seated around a large table. Convinced that I had simply missed the morning session, I quietly slipped into the room and took a seat.

Just as I sat down, Désiré walked to the podium and began speaking about t-PA. I was immediately captivated. He described a cell line producing t-PA, an antibody against it, and purified protein preparations. These were precisely the things I had come to learn about.

During the coffee break, people kept glancing in my direction. Eventually, someone approached me and asked politely, "Can I help you?" I introduced myself and apologized for arriving late. They looked puzzled. Then they explained that this was not the actual conference. It was a private pre-conference session attended by many of the world's leading researchers in thrombosis and fibrinolysis. I was not supposed to be there.

Had I not arrived a day early, had I not been so obsessed with punctuality, I might never have met Désiré. Later, several participants admitted that they had not asked me to leave because they assumed I was one of the scientists' daughters waiting for her father. So I was very fortunate.

That evening, the group gathered for dinner in a beautiful castle. There I had my first real conversation with Désiré. We discussed the possibility of cloning the t-PA gene. At the time, there was simply no practical way to obtain sufficient quantities of natural t-PA for widespread clinical use.

Désiré wondered whether cloning such a large gene was even possible. It is important to remember that this was 1980. No gene of that size had yet been successfully cloned.

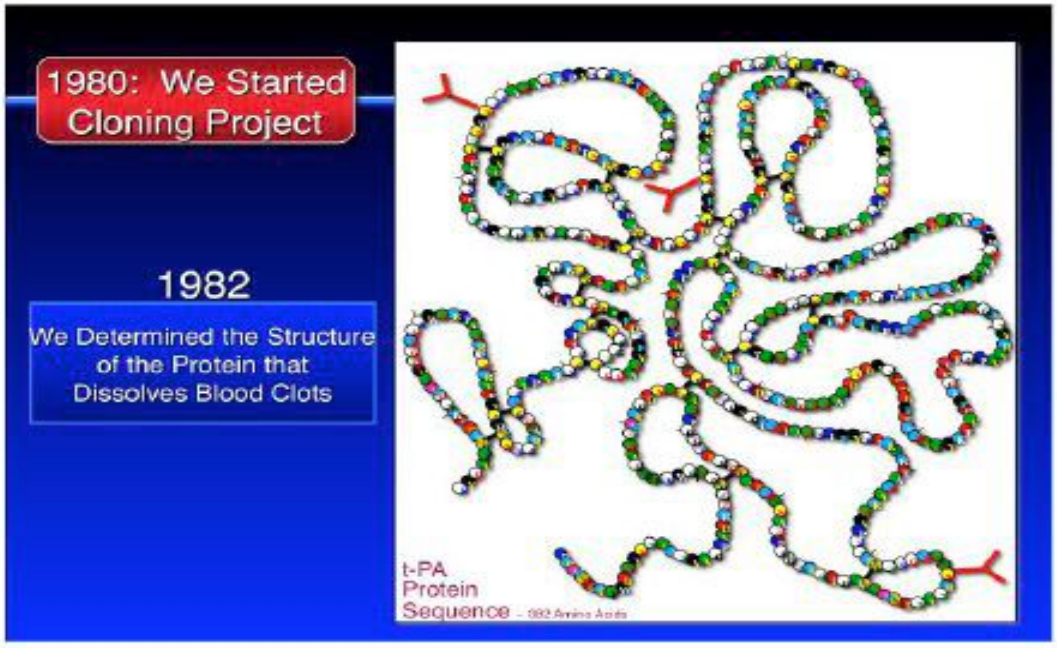
With all the confidence of youth—and very little practical experience—I immediately replied: "Of course we can do it." I suspect that my enthusiasm may have helped persuade Désiré to collaborate with us. What I did not tell him was that I had never cloned anything before.

I had absolutely no idea how we were going to do it. But I certainly was not going to admit that.

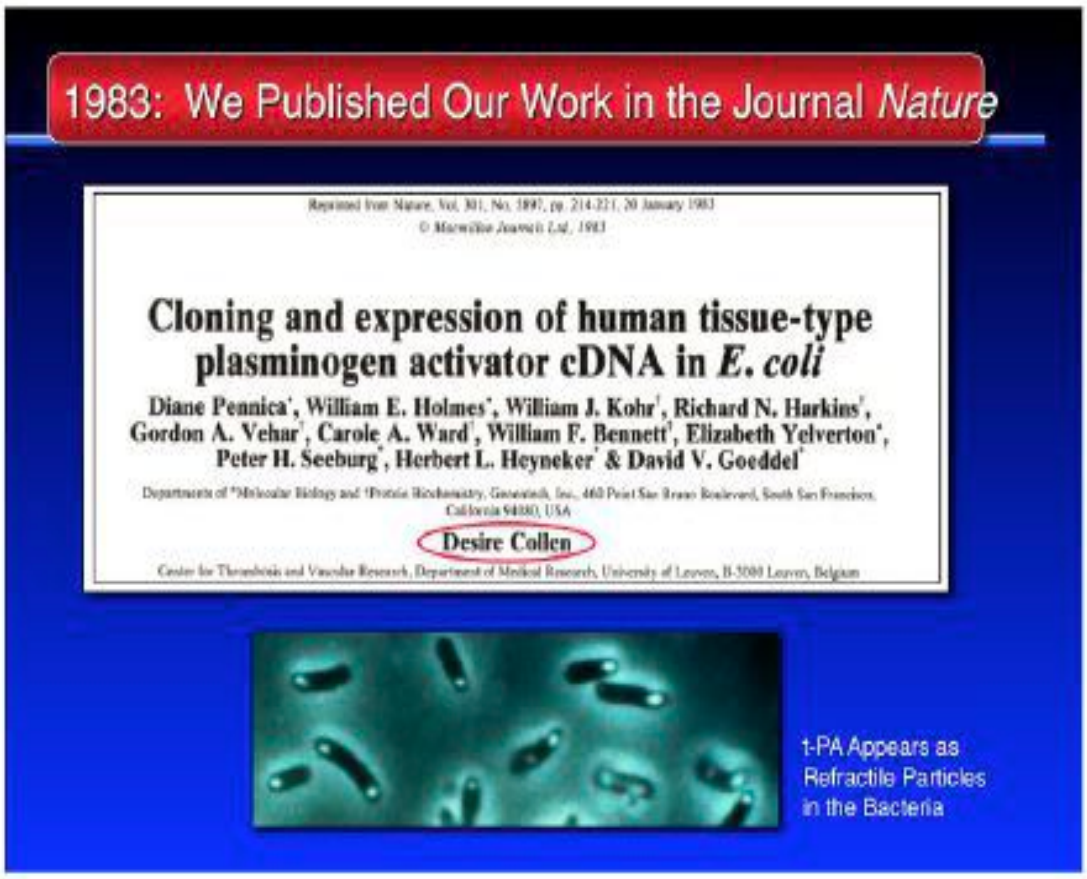
Looking back, the project proved to be enormously challenging. There were setbacks, disappointments, and intense competition from laboratories around the world. It quickly became a race.

For nearly a year, we worked almost continuously. I scarcely took a day off. Fortunately, the effort paid off. We eventually succeeded.

In 1980, we began the cloning project. In 1982, we determined the structure of the protein responsible for dissolving blood clots. It was a beautiful and remarkably complex molecule. That period remains one of the most exciting times of my scientific career.



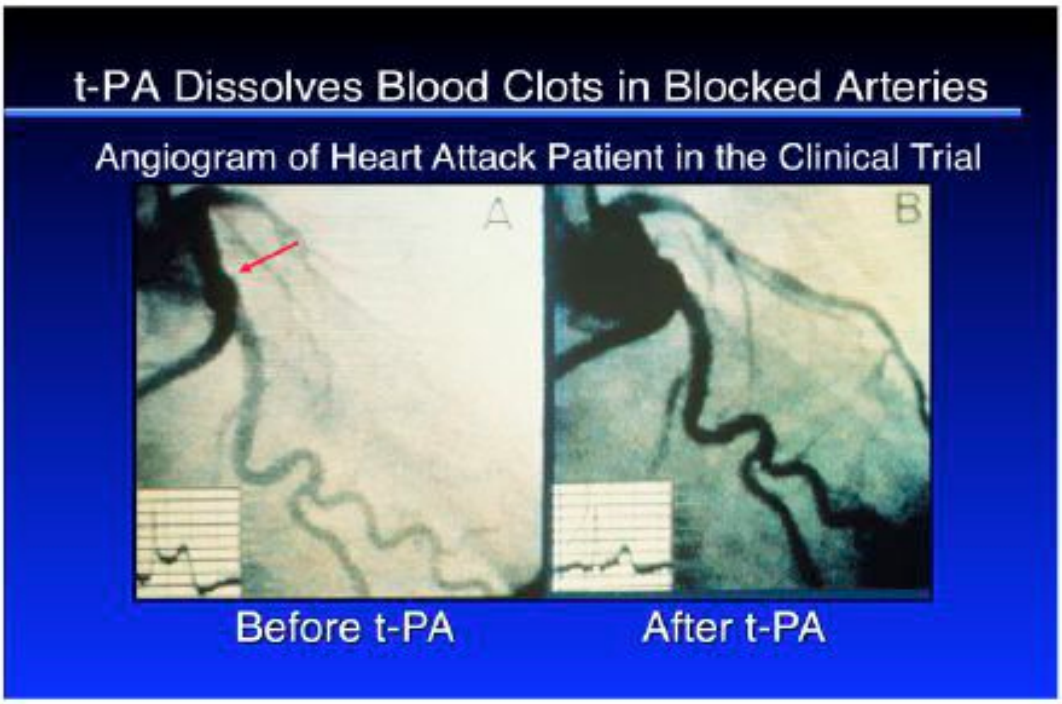
In 1983, we published the landmark paper describing the cloning and expression of human t-PA cDNA in *E. coli*.



To this day, Désiré, I still do not know how you managed to have your name appear on a line by itself in a *Nature* paper. I believe that may be a unique achievement.

By 1984, recombinant t-PA could be produced in large quantities. We named the drug **Activase** after a company-wide naming competition.

Clinical studies quickly demonstrated its effectiveness.



This angiogram from an acute myocardial infarction patient illustrates the result. Before treatment, blood flow is completely obstructed by a thrombus. After administration of recombinant t-PA, the artery is open and blood flow has been restored.

Another unforgettable milestone occurred on November 13, 1987. Seven years after that chance meeting in Sweden, recombinant t-PA received approval from the U.S. Food and Drug Administration for the treatment of acute myocardial infarction.

Newspapers across the country carried headlines describing the new clot-dissolving drug as a major advance in coronary therapy. Even today, the development of t-PA remains one of the fastest drug-development programs in modern pharmaceutical history.

One of the most rewarding moments of my career occurred shortly thereafter.

One day, while walking through the halls of Genentech, I was introduced to a visitor named Steve Birnbaum.

I was told: "Steve was the first heart-attack patient treated with recombinant t-PA." Then Steve was told: "This is Diane Pennica. She cloned t-PA." Without hesitation, he embraced me and said: "Thank you. Your drug saved my life."That moment remains with me to this day.

The High Point of My Career

Meeting Steve Birnbaum,
In the Halls of Genentech
the 1st Heart Attack Patient
Treated with t-PA

Since 1987:
~1.8 Million
Heart Attack Victims
Treated with t-PA

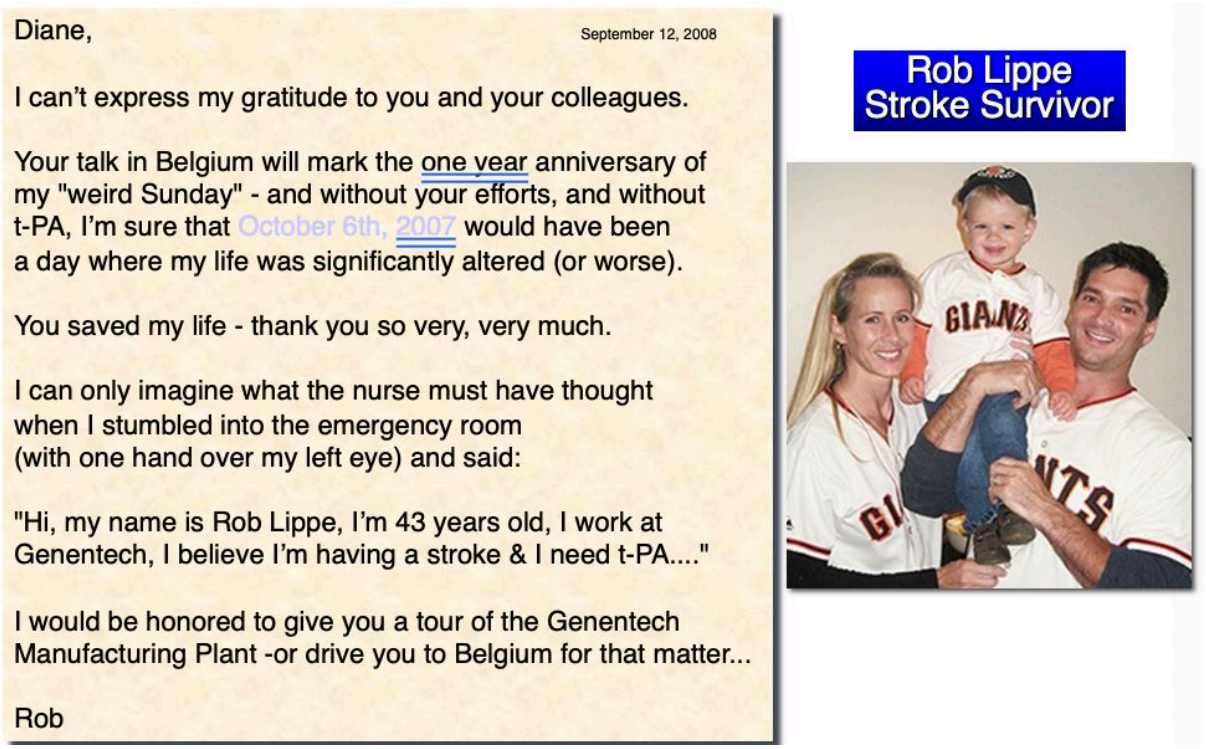
Since its approval, nearly two million patients suffering from acute myocardial infarction have been treated with t-PA.

I keep a Christmas card in my office from one of those patients. It reads:

Dear Dr. Pennica, Happy Holidays and thank you for helping to save my life. Of course, that gratitude belongs not to me alone, but to all of the people who contributed to this extraordinary achievement.

In 1996, t-PA received approval for the treatment of acute ischemic stroke. Every forty-five seconds, someone suffers a stroke, and more than 700,000 people each year in the United States and Europe are affected. Since then, more than 150,000 stroke patients have been treated with recombinant t-PA.

I would like to conclude by reading an email I received just three weeks ago. Like the Christmas card, its message truly belongs to everyone who contributed to the development of t-PA.



Diane, I can't express my gratitude to you and your colleagues. Your talk in Belgium will mark the one year anniversary of my "weird Sunday'- and without your efforts, and without t-PA, I'm sure that October 6, 2007 would have been a day where my life was significantly altered (or worse).

You saved my life – thank you so very, very much.

I can only imagine what the nurse must have thought when I stumbled into the emergency room (with one hand over my left eye) and said:

"Hi, my name is Rob Lippe. I am 43 years old. I work at Genentech, I tbelieve I'm having a stroke, and I need t-PA…"

I would be honoured to give you a tour of the Genentech Manufacturing Plant—or drive you to Belgium, for that matter…

Rob

Désiré, my first project at Genentech became the most exciting experience of my professional life. You helped transform a scientific dream into a therapy that has saved countless lives around the world. I wish you happiness, new adventures, and many more discoveries in the years ahead.

Thank you.

Our next speaker is Tsunehiro Yasuda; he is a former collaborator of the late Dr. Gold at Massachusetts General Hospital in Boston.
Now that this recombinant t-PA became available, they did the first experimental animal work with recombinant t-PA and subsequently also treated the first patients with acute myocardial infarction with the recombinant version of t-PA.

Tsunehiro Yasuda

Thank you very much for this invitation. It is a great pleasure to be here today and to share part of the t-PA story.

I should begin by emphasizing that this work could not have been accomplished without Dr. Herman Gold, who sadly passed away a few months ago. He was the principal investigator, while I was a junior member of the team working alongside him and Désiré. Together, we had a wonderful and exciting collaboration.

Recombinant tissue-type plasminogen activator (rt-PA) was produced by Genentech, thanks to the pioneering work described earlier by Diane Pennica. We tested this material first in animal models and later in human patients.

Herman K. Gold, M.D.

Massachusetts General Hospital
Harvard Medical School

Associate Professor of Medicine in Cardiac Unit

Our initial studies were performed in dogs. We developed a model of coronary thrombosis using an exposed canine heart. The left anterior descending coronary artery (LAD) was partially narrowed, after which blood and thrombin were injected to create an occlusive thrombus. Coronary blood flow was continuously monitored with an electromagnetic flow probe, and angiography was used to confirm the findings.

The angiogram revealed that initially the LAD is completely occluded. After administration of rt-PA, faint evidence of reperfusion begins to appear. Gradually the artery becomes more visible, blood flow increases, and eventually complete reperfusion is achieved.

When we first observed these results, we were tremendously excited. For the first time, we could clearly see that the therapy was working.

We then performed dose-response studies.

Acute Infarction in Dog

circulation, 70: 700, 1984

- rt-PA 4.3ug/kg/min
  - RP>40 min
- rt-PA 10ug/kg/min
  - RP=31±2 min
- rt-PA 15ug/kg/min
  - RP=26±7
- rt-PA 25ug/kg/min
  - 13±3

- 18 dog randomized in rt-PA and Saline
- Rt-PA
  - RPT: 28±3 min
  - LV Infarct size 2.5±0.5%
- Control saline
  - Not RP at all
  - Infarct size 16±3% ($p<0.0001$)

At infusion rates of 4.3, 10, 15, and 25 micrograms per kilogram per minute, we observed progressively shorter reperfusion times. Désiré immediately recognized the significance of these findings and pointed out that increasing doses resulted in increasingly rapid reopening of the occluded artery.

These encouraging observations led us to undertake randomized animal studies. Dogs were randomly assigned to receive either saline or rt-PA. The experiments were performed in a blinded fashion.

The results were remarkable. Virtually none of the saline-treated animals achieved reperfusion, whereas the majority of rt-PA-treated animals demonstrated reopening of the occluded coronary artery within approximately thirty minutes. Although we had hoped for positive results, the magnitude of the effect exceeded our expectations.

At that point we knew that animal studies alone were not sufficient. We needed to move into clinical investigations. The transition to human studies produced similarly encouraging results.

In patients with acute myocardial infarction, coronary arteries frequently reopened following treatment with rt-PA. In one randomized study, nine of thirteen patients achieved successful reperfusion, whereas none of the patients initially assigned to placebo demonstrated spontaneous reopening until they crossed over to active treatment.

These results were published in *Circulation* in 1984 and provided strong evidence that rt-PA could successfully restore coronary blood flow in patients suffering acute myocardial infarction.

Human Acute Infarction Trial
Circulation 70, 1012-1017, 1984

- 25 /33 Patients
  - with 0.5-0.75mg/kg of t-PA opened < 90min
- Only One patient /14-Control-saline patients
  - Opened, 13 remained closed
- The thirteen crossed over to t-PA,
  - 9/13 opened <45 min
- Circulating fibrinogen decreased by only 8% of the baseline
- Confirms the dose, safety and efficacy of rt-PA for acute infarction use.

I would like to share another aspect of those early studies. Désiré always emphasized the importance of moving quickly. I have never known him to do anything slowly. Dr. Gold adopted the same philosophy.

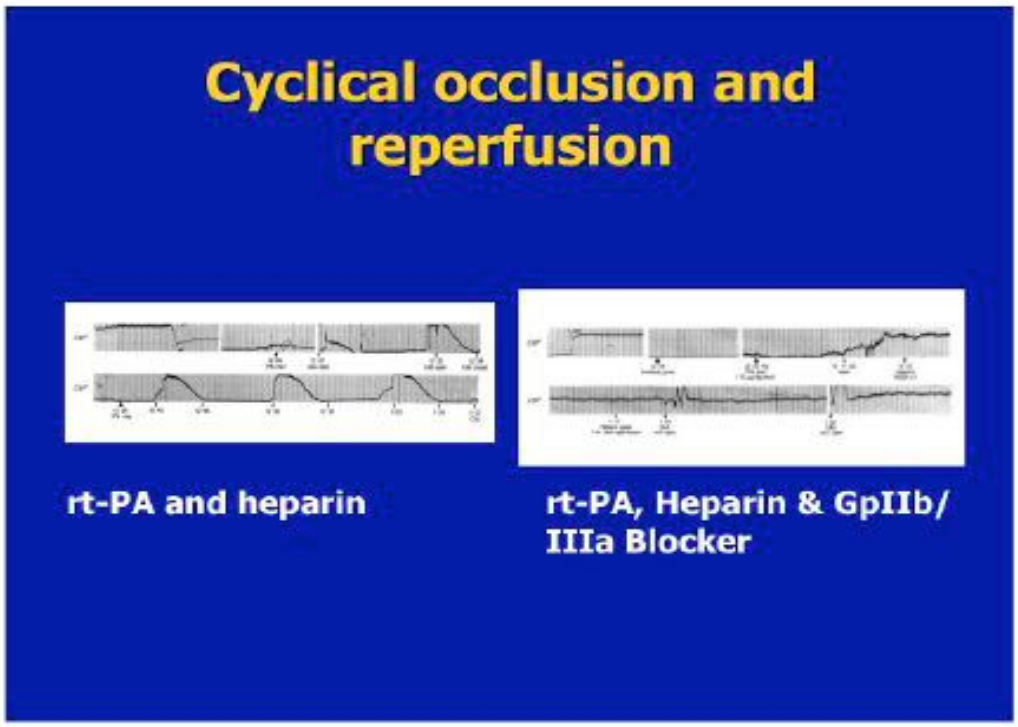
One challenge, however, was patient recruitment. Patients do not schedule heart attacks in advance. They arrive unexpectedly, usually through the emergency department, and by the time investigators become aware of them, the opportunity for treatment may already have passed.

We struggled with this problem until a former chief resident at our institution—Mark Fishman, who later became head of research and development at Novartis—offered an unusual suggestion.

His advice was simple:"Give them (added: the cardiac emergency technical staff) pizza." At first I thought he was joking, but surprisingly the system worked extremely well. It allowed us to identify eligible patients rapidly and substantially accelerated recruitment into the study.

Another important observation emerged from our animal experiments. Although t-PA could successfully reopen an occluded coronary artery, the artery often reoccluded minutes or hours later. Simply achieving reperfusion was not enough—we needed to maintain patency.

This tracing illustrates the problem. Following administration of t-PA, blood flow returns, only to disappear again as the artery reoccludes. The vessel repeatedly opens and closes in a cyclical fashion. Such unstable reperfusion is clearly not clinically satisfactory.



We therefore investigated the use of adjunctive antithrombotic therapies.

To study this problem, we developed a specialized canine circumflex-coronary model. A segment of artery was surgically inverted so that the adventitial surface became exposed to the arterial lumen, creating a highly thrombogenic environment. A distal stenosis was added, and coronary blood flow was continuously monitored.

One remarkable feature of this model was the speed of thrombus formation. Within only a few minutes after restoration of blood flow, complete coronary occlusion frequently developed.

Histological examination demonstrated that these thrombi were platelet-rich "white clots." Such thrombi responded relatively poorly to t-PA alone.

When platelet aggregation was inhibited using glycoprotein IIb/IIIa receptor antagonists, however, a very different result was observed.The coronary artery remained patent for prolonged periods, and sustained reperfusion could be achieved.

These studies demonstrated that rapid and durable coronary recanalization requires not only dissolution of fibrin but also inhibition of platelet-mediated rethrombosis. The combination of t-PA and potent antiplatelet therapy produced the fastest reperfusion times and the most stable maintenance of blood flow.

These findings eventually influenced clinical practice and contributed to the widespread use of combined thrombolytic and antithrombotic strategies in patients with acute coronary syndromes. Today, many patients arriving at hospitals with acute coronary thrombosis or ischemic stroke continue to benefit from principles that emerged from these early investigations.

In closing, I would like to thank Désiré for his inspiration, his leadership, and his friendship.

Désiré, I wish you many healthy, productive, and enjoyable years in what I would call not retirement, but rather your second scientific career.

Thank you very much.

Our next speaker is Marc Verstraete, who as Désiré said earlier, was the director of our lab when Désiré started his scientific career and has been that throughout the whole development period of t-PA. He coordinated the European clinical studies with recombinant t-PA and that is what he will tell us about.

Marc Verstraete

Désiré, ladies and gentlemen,

Thank you very much for inviting me to participate in this symposium.

When recombinant tissue-type plasminogen activator became available in Europe, I contacted a number of colleagues and friends who had previously collaborated with me in clinical trials involving streptokinase. Together, we formed what became the European Cooperative Study Group and embarked on a new series of investigations, initially focused on thrombolytic therapy in acute myocardial infarction.

Before discussing t-PA, it is worth recalling some of our earlier work with streptokinase.

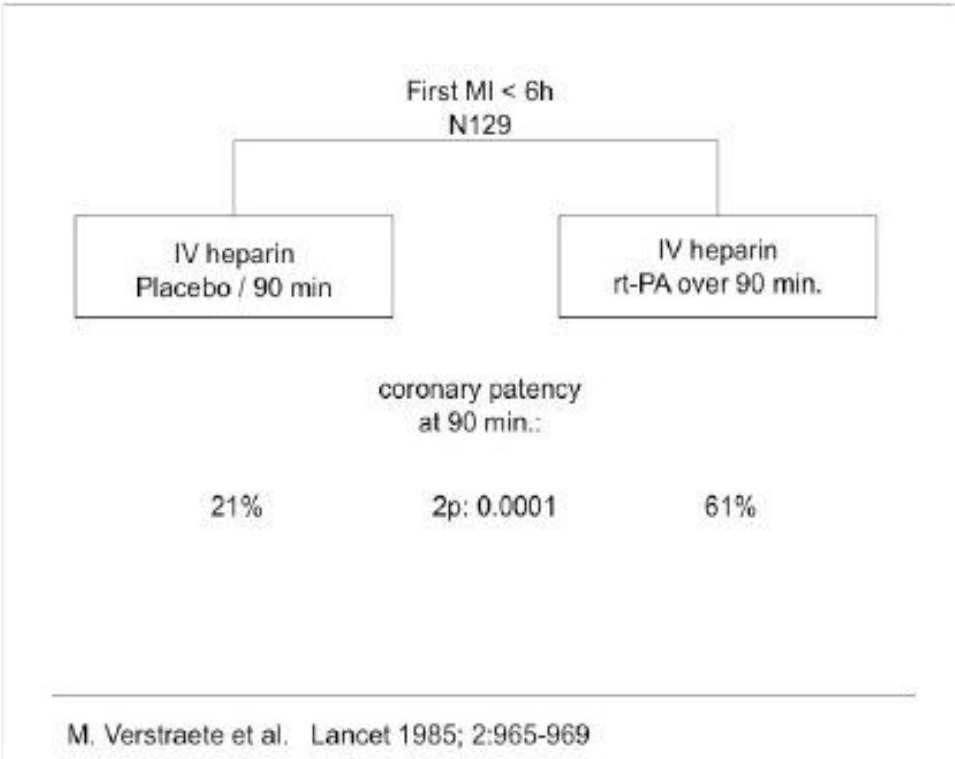
In one of our first studies, we enrolled patients who were treated up to 72 hours after the onset of myocardial infarction. Patients received either prolonged streptokinase infusion or heparin. Unfortunately, no significant difference emerged between the two groups.

We then refined our approach and selected patients presenting within 24 hours of symptom onset. In this trial, streptokinase treatment produced a reduction in 24-day mortality of approximately 30%.

The next study yielded even more striking results. By restricting enrolment to patients presenting within 12 hours of infarction, we observed a reduction in six-month mortality approaching 50%.

This was an extraordinary finding. The results were published in *The New England Journal of Medicine*, yet, somewhat surprisingly, they initially attracted relatively little attention from the cardiology community.

When recombinant t-PA became available in Europe, we continued our collaborative programme and initiated the first European trial of this new thrombolytic agent.



In this study, 129 patients were randomized to receive either t-PA administered over 90 minutes or placebo. Coronary angiography was performed 90 minutes after initiation of treatment.

The results were remarkable. Coronary artery patency was observed in only 21% of patients receiving placebo, compared with 61% of those treated with t-PA. The difference was highly significant and provided compelling evidence of the thrombolytic efficacy of t-PA.

This encouraging experience led directly to a second European trial. In this study, patients received either t-PA plus heparin or streptokinase combined with heparin and aspirin. At that time, we were still somewhat cautious and did not yet feel comfortable administering aspirin routinely together with t-PA.

Once again, angiography was performed at 90 minutes. The t-PA-treated patients demonstrated superior coronary patency compared with those receiving streptokinase. Equally important, we observed substantial preservation of circulating fibrinogen. In the streptokinase group, fibrinogen levels fell to approximately 12% of baseline values, whereas in the t-PA group roughly 61% of the original fibrinogen concentration was preserved. These findings confirmed what experimental studies had already suggested: t-PA was not only more effective but also more fibrin-specific and therefore potentially safer than streptokinase.

Our next question concerned dosing strategy. Had we identified the optimal dose of t-PA, or might prolonged administration further improve outcomes? To address this issue, we studied 123 patients and performed coronary angiography at the end of the initial t-PA infusion.

Approximately 27% of patients still had an occluded coronary artery, whereas 72% had achieved successful reperfusion. Patients with patent arteries were then randomized to receive either an additional infusion of t-PA or no further thrombolytic treatment.

The results showed that reocclusion rates between six and twenty-four hours were essentially identical in both groups. We therefore concluded that prolonging t-PA administration did not provide additional protection against reocclusion. Of course, reopening a coronary artery is important, but the ultimate objective is reduction of mortality.

In a subsequent study, patients received either t-PA together with heparin and aspirin or placebo combined with heparin and aspirin.

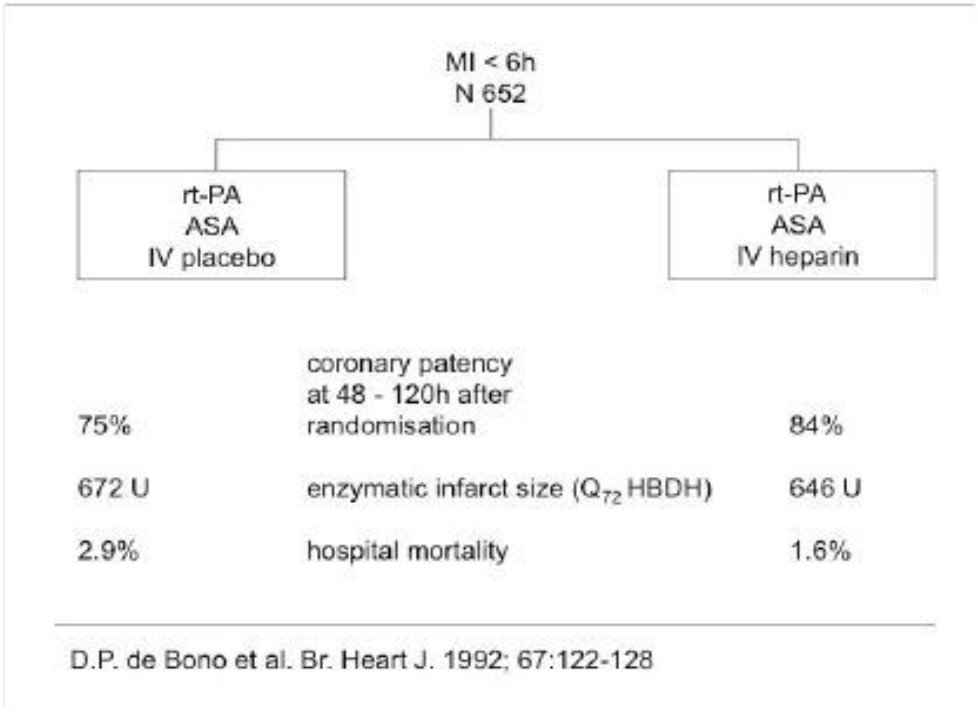
The results were highly encouraging. Fourteen-day mortality was reduced by approximately 50% in the t-PA group compared with controls. This survival benefit persisted at three months and was accompanied by a significant reduction in infarct size.

We then explored whether immediate mechanical intervention could further improve outcomes. All patients received aspirin, heparin, and 100 mg of t-PA administered over three hours. They were then randomized either to immediate percutaneous transluminal coronary angioplasty (PTCA) or to conventional management.

To our surprise, angiographic patency rates did not differ between the two groups. Moreover, recurrent ischemia during the first twenty-four hours was actually more frequent among patients undergoing immediate PTCA.

Mortality was also approximately 50% higher in the immediate-PTCA group. The conclusion was clear: in patients already receiving t-PA, heparin, and aspirin, routine immediate angioplasty offered no additional benefit and might even be detrimental.

Another important question concerned the role of heparin. To address this issue, we compared patients receiving t-PA and aspirin together with intravenous heparin to patients receiving t-PA and aspirin with placebo instead of heparin.



Coronary patency between 48 and 120 hours after randomization was slightly higher in the heparin group. More importantly, infarct size was reduced and hospital mortality was lower among patients receiving heparin. These findings strongly supported the routine use of adjunctive heparin therapy together with t-PA.

Naturally, these European studies involved relatively modest numbers of patients and could not match the scale of the major American trials such as TIMI and TAMI, which enrolled far larger patient populations.

Nevertheless, the European and American programmes complemented one another remarkably well.

Despite being conducted independently and on different scales, both sets of investigations reached essentially the same conclusions. Together they established, in a remarkably short period of time, the clinical value of tissue-type plasminogen activator and transformed the treatment of thromboembolic disease.

For all of us who participated in these efforts, it was a privilege to witness the translation of a scientific concept into a therapy that has benefited millions of patients worldwide.

Thank you very much.

Our next speaker is David Stump.
Dave was a post-doc in Leuven from 1984 to 1986, he subsequently went to Genentech and is now at Human Genome Sciences in Rockville, Maryland.
Dave will give us an overview of the United States' studies with recombinant t-PA in myocardial infarction and at the end of this symposium reflect on translational research between academia and industry.

David Stump

What a pleasure it is to be here on this special occasion.

I would like to continue the story where Professor Verstraete left off by describing the parallel developments that were taking place in North America and then returning to what I believe was the defining moment in the clinical development of t-PA: the international effort that ultimately established its therapeutic value beyond doubt.

When I returned to the United States in 1986 after two wonderful years in Leuven, I joined the faculty of the University of Vermont. My collaboration with Désiré continued uninterrupted.

At that time we became closely involved with two highly influential American cardiovascular research networks. The first was the TIMI Study Group, led by Eugene Braunwald and supported by the National Institutes of Health. The

second was the TAMI Investigators, led by the then-young Eric Topol and Rob Califf, an academically driven consortium independent of government sponsorship.

Our group in Vermont also participated in studies being conducted with Herman Gold and his colleagues. Désiré took the lead in maintaining many of the international collaborations, while I worked closely with Rob Califf and Eric Topol. Together we sought ways to coordinate efforts between the various research groups. Looking back, those collaborations proved enormously important.

The TIMI studies yielded two particularly significant observations.

First, in a relatively small, randomized trial comparing t-PA with streptokinase, reperfusion of occluded coronary arteries was superior with t-PA. Importantly, this advantage appeared relatively independent of the age of the thrombus.

Second, a larger study, TIMI II, was undertaken following a dose-finding phase that had become necessary after modifications in the manufacturing process for recombinant t-PA. During those investigations it appeared that a dose of 150 mg might produce more effective thrombolysis. Consequently, the initial phase of TIMI II employed this higher dose.

However, after approximately 900 patients had been treated, it became evident that the risk of intracranial haemorrhage was unacceptably high. The dose was therefore reduced to 100 mg. At this lower dose, the incidence of intracranial haemorrhage fell to approximately 0.5%, while overall mortality remained highly favourable at approximately 4.5%.

As a result, enthusiasm for t-PA among TIMI investigators and practising cardiologists throughout the United States grew rapidly following FDA approval in 1987. The ability of t-PA to reopen occluded coronary arteries appeared obvious and compelling.

A series of studies then focused on understanding and optimizing coronary artery patency. Several important findings emerged.

First, both TIMI and TAMI investigators consistently demonstrated that maintaining an open coronary artery was strongly associated with improved survival. Patients who achieved sustained reperfusion experienced substantially lower mortality than those with failed reperfusion or reocclusion.

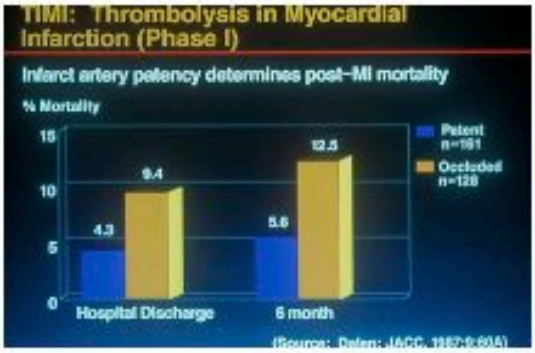
Second, three independent studies—including one performed in Europe—demonstrated that reocclusion after successful thrombolysis occurred

significantly more often when intravenous heparin was not administered concomitantly with t-PA.

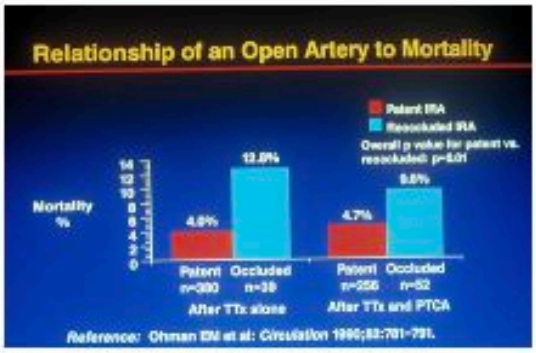
Third, and perhaps most importantly, three separate studies demonstrated that administration of 100 mg of t-PA over ninety minutes rather than three hours resulted in higher early coronary patency rates, particularly during the critical first hour of treatment.

The Nature of the Open Artery with rt-PA

TIMI

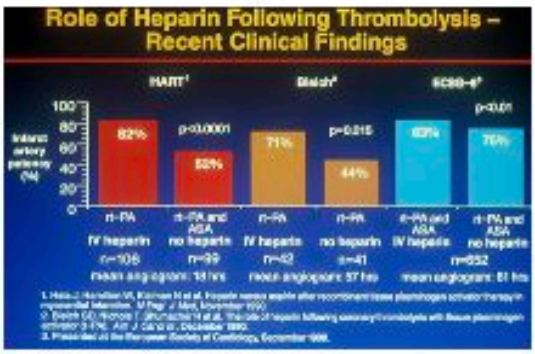


TAMI



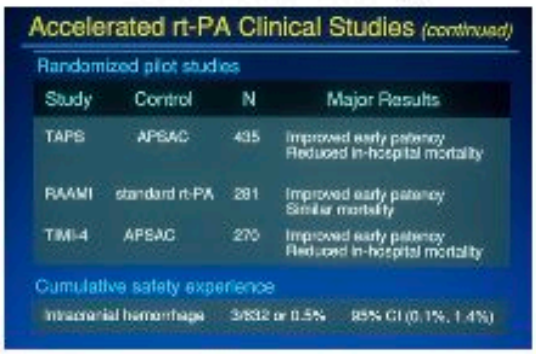
Long term mortality improved with achieving and sustaining coronary patency.

Heparin



Sustained coronary patency is superior following rt-PA when followed with IV heparin.

Accelerated dosing



Accelerated administration of rt-PA enhances coronary patency. Risk of intracranial hemorrhage appears to be acceptable but uncertainty remains.

Although these findings were encouraging, concerns remained regarding the potential for increased intracranial haemorrhage with accelerated administration. The available studies were too small to provide definitive reassurance.

At that point, further progress slowed while the cardiology community awaited the results of large multinational mortality trials comparing t-PA with the considerably less expensive streptokinase.

The first major results emerged in 1990 from the GISSI-2 and ISIS-3 studies. Importantly, both trials employed minimal concomitant heparin therapy and used the standard, non-accelerated t-PA regimen.

When both studies failed to demonstrate a mortality advantage for recombinant t-PA, the consequences were profound. Those of us involved at the time recognized immediately that this represented a genuine crisis for the future of t-PA. Indeed, it threatened the future of Genentech itself.

At that moment, the scientific concept in which many of us had invested years of effort appeared to be in jeopardy. The situation was particularly personal for me because I had joined Genentech in 1989 to lead the further clinical development of t-PA.

As a haematologist interested in thrombolytic therapy, I suddenly found myself participating in intense discussions involving many of the world's leading cardiologists—including our colleague Frans Van de Werf—as well as senior executives and scientific leaders at Genentech.

Everyone was asking the same question: What should we do next?

A broad consensus quickly emerged. Without a definitive clinical trial, t-PA would inevitably become an expensive therapeutic curiosity rather than the standard of care. The answer was the GUSTO trial.

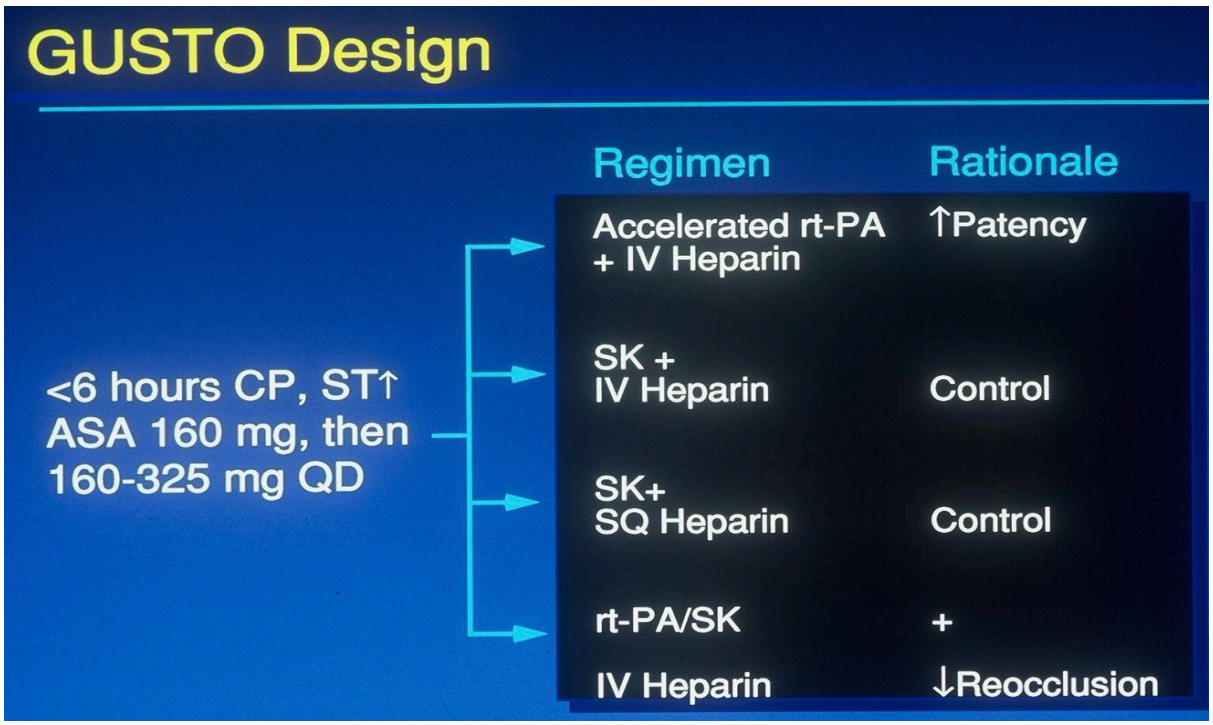
The design of GUSTO represented one of the most ambitious clinical undertakings of its era. Perhaps the most difficult decision I personally faced was whether to support use of the accelerated ninety-minute t-PA regimen.

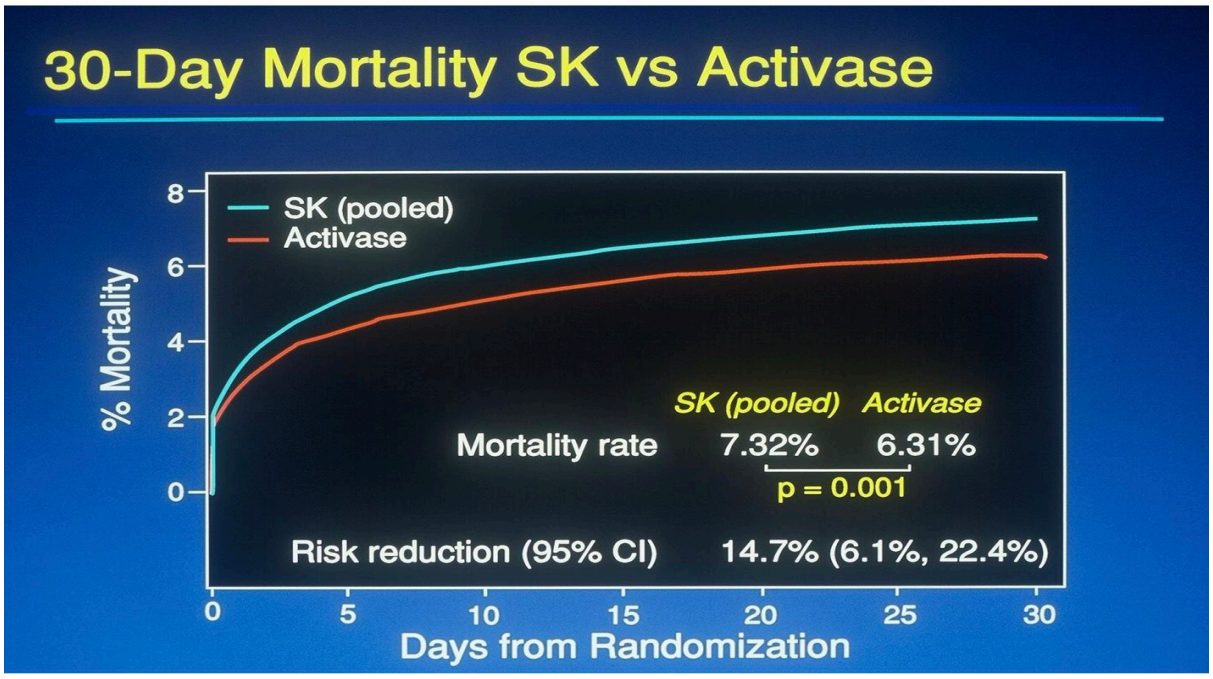
My own modelling of the relationship between coronary patency and mortality suggested that both accelerated t-PA administration and concomitant heparin would be required if we were to achieve our target: an absolute reduction in mortality of one percent. That may sound modest, but in acute myocardial infarction a one-percent reduction represented an enormous clinical benefit.

At the same time, uncertainty regarding intracranial bleeding risk remained. The answer would not become clear until thousands of patients had been enrolled.

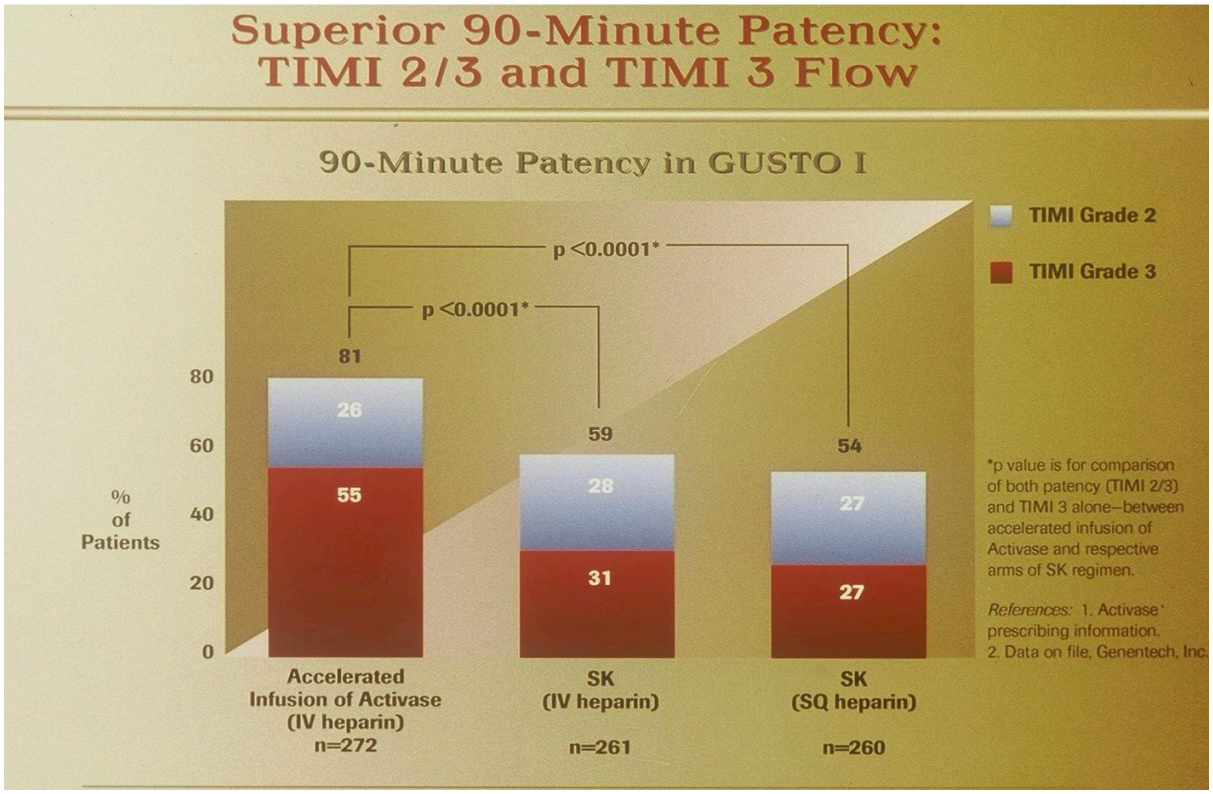
Over the next three years I watched the trial unfold with enormous anticipation. When the results became available in 1993, they were decisive.

The GUSTO trial enrolled 41,021 patients and demonstrated a statistically significant reduction in mortality for patients treated with accelerated t-PA compared with those receiving streptokinase.





An angiographic substudy confirmed higher coronary artery patency rates with accelerated t-PA and demonstrated corresponding improvements in left ventricular function and survival.



The trial did reveal a small but statistically significant increase in intracranial haemorrhage. However, when overall clinical benefit was assessed using stroke-free survival, accelerated t-PA still demonstrated a significant net advantage.

The implications of GUSTO were far-reaching.

Recombinant t-PA became established as the leading thrombolytic therapy for acute myocardial infarction. The mechanism of benefit was clearly linked to rapid restoration of coronary blood flow.

An innovative but expensive biotechnology-derived therapy was shown to be clinically valuable and economically justifiable. More broadly, GUSTO demonstrated that cutting-edge science and technology could successfully be translated into meaningful patient benefit.

Finally—and not insignificantly for some of us—Genentech survived and prospered.

Having reviewed the history, I have been asked to reflect briefly on the broader lessons of translational research.

The t-PA story has frequently been cited as an example of successful collaboration between academia and industry. Because I had the privilege of participating from both perspectives—first in academia and later in industry—I have often been asked whether this model truly offers advantages beyond what either sector could achieve independently.

I usually answer this question by proposing a simple thought experiment.

Suppose we remove one of the essential contributors from the story. What would have happened?

Let us first imagine a world without the science. By science, I mean the fundamental biochemical and biological discoveries made by many of the people gathered in this room.

Without those discoveries, streptokinase and urokinase would likely remain the principal thrombolytic agents. Mortality from acute myocardial infarction would be higher. Effective thrombolytic treatment for acute ischemic stroke would not exist.

Now imagine a world without medicine. By medicine, I mean the clinical and interventional disciplines that identified arterial thrombosis as the central mechanism underlying myocardial infarction and ischemic stroke.

Without those contributions, t-PA might have remained an interesting biological molecule with only limited clinical applications. Reperfusion therapy would never have become standard care for heart attack and stroke patients.

Finally, imagine a world without industry. Without biotechnology, there would have been no practical way to clone, express, and manufacture sufficient quantities of t-PA.

The molecule would have remained a scientific curiosity. Patients would have benefited little, if at all. And Genentech's subsequent innovations—including therapies such as Rituxan, Herceptin, and Avastin—might never have been possible.

My conclusion is straightforward. The success of t-PA required all three components: science, medicine, and industry. Together they achieved something that none could have accomplished alone.

Yet because we are gathered here today to celebrate Désiré Collen, let me propose one final thought experiment.

What if there had been no Désiré Collen? I am convinced that the t-PA story would not have unfolded with anything approaching the urgency, efficiency, and effectiveness that characterized its development. Indeed, it might never have happened at all.

I am equally convinced that my own life—and I suspect the lives of many people in this room—would have been considerably less interesting, rewarding, and fulfilling. Most importantly, however, fewer patients with acute myocardial infarction and acute ischemic stroke would be alive and well today.

Fortunately, this final scenario exists only as a theoretical exercise. And that is why we are here.

Désiré, on behalf of your colleagues, collaborators, friends, and most importantly the countless patients whose lives have been touched by your work, we offer our congratulations and our gratitude.

It is a remarkable story. I am proud to have been a part of it. Thank you.

I would now like to invite professor Verstraete to share with us some brief concluding remarks.

Closing Remarks by Marc Verstraete

Ladies and gentlemen,

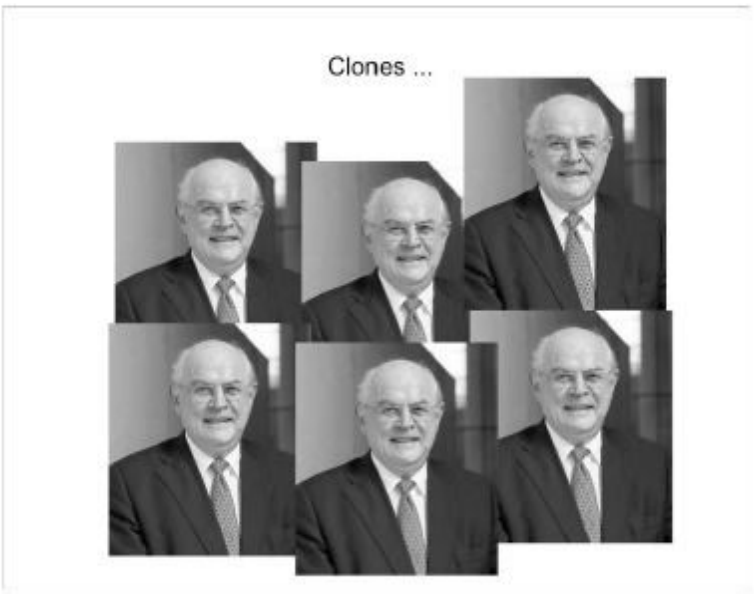
When one is asked to draw conclusions after such a remarkable day, a certain degree of hesitation is inevitable.

The word *conclusion* suggests the end of an event, the completion of an undertaking, or the closing of a chapter. Yet that is precisely what today should **not** represent. Although this day marks an important milestone in the life and career of Désiré Collen, it is certainly not the end of his scientific journey. Far from it.

We continue to need his inspiration, his advice, his encouragement, his support, and his critical insight—not only in the laboratory that was once mine, but throughout the Faculty of Medicine, the University of Leuven, and the wider scientific community.

The question, therefore, is how we can ensure that we continue to benefit from the presence and influence of Désiré Collen in the years ahead.

One possible solution comes to mind. We should create a number of clones of Désiré Collen, thereby ensuring that he remains with us indefinitely. And once we have successfully produced these clones, I strongly recommend that we immediately apply for a patent.



On a more serious note, Désiré, thank you for inspiring so many people.

Thank you for serving as an example to generations of students, scientists, physicians, and colleagues. Thank you for demonstrating that scientific excellence can be combined with generosity, friendship, integrity, and humanity. You have worked extraordinarily hard throughout your career, yet you have always done so with enthusiasm, warmth, and respect for others.

For me personally, you have been much more than a colleague. You have been a friend, a trusted adviser, and a loyal companion throughout many years of scientific endeavour.

And therefore, ladies and gentlemen, I would like to ask you to join me in honouring this gentle giant of biomedical science, Désiré Collen, with a standing ovation.

Thank you.