The t-PA Story

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 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 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 K.U.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 summarises their contributions. We hope that it will serve as an educational document, illustrating the importance of translational research in the development of novel biopharmaceuticals.

Leuven, November 7, 2008
Roger Lijnen, moderator.

ROGER LIJNEN

Moderator


HEART for the FUTURE
The t-PA story told by:
Désiré Collen (VIB - K.U.Leuven),
Björn Wiman (Stockholm, Sweden)
Alfons Billiau (K.U.Leuven)
Dingeman Rijken (Rotterdam, the Netherlands)
Irène Juhan-Vague (Marseille, France)
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David Stump (Rockville, USA)
The t-PA story
KATHOLIEKE UNIVERSITEIT LEUVEN
VIB

It's my pleasure to welcome you all to "the t-PA story".
The scientific output of Désiré between 1968 and 2008 consists of approximately 650 research papers in peer-reviewed international journals, 170 survey articles and 28 issued US patents.
He ranked among the 100 most cited scientific authors of the nineties and is listed with the highly cited authors of the 1980's and 1990's.
Among these many scientific achievements, the landmark has undoubtedly been the development of tissue-type plasminogen activator or t-PA from a laboratory concept to a life-saving drug, that is used worldwide for the treatment of thromboembolic disease
(This was indeed translational research avant-la-lettre).
Therefore, we are very happy to have here today the main players in this story and to hear their testimony first-hand.
As a colleague for over 30 years now, I am very happy to be able to monitor this session, and we are very grateful to the Flemish Institute for Biotechnology and the University of Leuven for organizing it.
At the basis of the development of t-PA was a molecular model for the regulation of fibrinolysis, that was developed together with Bjorn Wiman, our second speaker, which was presented in a Plenary Lecture at the VIIth International Congress on Thrombosis and Haemostasis in 1979.
Initially, natural t-PA was obtained from the culture medium of the Bowes melanoma cell line, which was produced by Alfons Billiau in sufficient amounts to allow its purification by Dick Rijken, and to study its biochemical properties and to develop quantitative assays (which was done in collaboration with Irène Juhan-Vague).
Its thrombolytic potential was first evaluated in animal models (together with Osamu Matsuo who is also here today).
In 1981 in collaboration with Willem Weimar, the first patient with renal transplant vein thrombosis was successfully treated with melanoma t-PA, followed in 1983 by patients with acute myocardial infarction (mainly by 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 studied in animal models (that was in collaboration with the late Herman Gold and with Hiro Yasuda).
With the approval of the Food and Drug Administration, recombinant t-PA was first administered to a patient in 1984.
The subsequent NIH Thrombolysis in Acute Myocardial Infarction trials in the USA, and the European Cooperative Study Group (led by Marc Verstraete) provided the foundation for numerous clinical trials, culminating in the GUSTO trial (that was monitored on behalf of Genentech by David Stump who is our last speaker for today).
This trial conclusively established the potential of t-PA for treatment of acute myocardial infarction.
We will learn in the next 2 hours why Désiré is considered to be "the father of t-PA".
As you will hear, it is a story of serendipity (or the right people being at the right place at the right moment), but it is also a story of scientific intellect, resolve and determination.


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

DESIRE COLLEN

The t-PA story

From labeled plasminogen turnover, over α_2 antiplasmin, to the plasminogen activator secreted by the Bowes Melanoma cell line

- a brief account

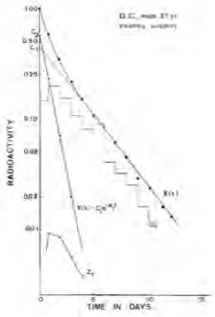
Désiré Collen

0

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



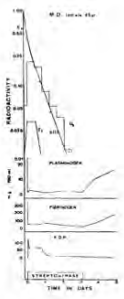
Plasminogen metabolism in a control subject.
x(t) = plasma radioactivity; u_1 = fractional daily urinary excretion of label; z_1 = non-TCA-precipitable radioactivity in plasma

1

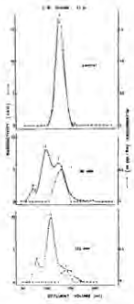
Metabolism of Iodine-labeled plasminogen during streptokinase and reptilase therapy in man

D. Collen and J. Vermylen

Thrombosis Research Volume 2, pp. 239-250 1973



Plasma levels of radiolabel and plasminogen during streptokinase infusion



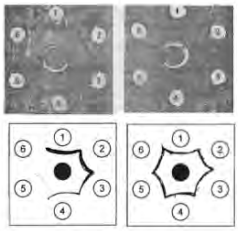
gel filtration of serial plasma samples

2

Emergence in plasma during activation of the coagulation or fibrinolytic systems of neoantigens, associated with the complexes of thrombin or plasmin with their inhibitors

D. Collen

Thrombosis Research Volume 5, pp. 777-779 1974



Immunodiffusion in 1 % agarose gel.
Center wells contain serum (left) and serum treated with 250 CTA units Urokinase per ml (right).
Well 1 contains rabbit antiserum raised against purified plasmin-antiplasmin complex.
Wells 2-6: same antiserum adsorbed with respectively 4, 8, 12, 16 and 20% (vol/vol) of human serum.
The precipitin lines against serum are adsorbed whereas a precipitin line against Urokinase activated serum persists.

3

I would like to express my sincere gratitude for your presence here and for your support of this endeavour.
I would also like to take this opportunity to thank my many collaborators over the years who have really made this t-PA story be possible.
I actually started as a third year medical student in the Laboratory of Blood Coagulation of prof. Marc Verstraete in 1964 (so I have been around a little while), to help Dr. Guido Tytgat, who was making his PhD thesis on the coagulation disturbances in liver cirrhosis, using accelerated radioiodine labeled fibrinogen turnover as a measure of in vivo coagulation.
After Guido moved on in 1968, I continued working on my own thesis using radiolabeled plasminogen to quantitate fibrinolytic activation.
Plasminogen that was actually purified in collaboration with Per Wallén and with Bjorn Wiman.
This led to a first paper which you see here in which I served as the first of twelve healthy volunteers used to determine the turnover parameters of plasminogen.
You can see that at that time I was 27 years and I can tell you that I have a perfectly normal plasminogen turnover.
The half-life after the equilibration of the radio isotope clearance was about 2.1 day.
Now following that study in patients which were treated by Jos Vermylen with streptokinase we saw very much to our surprise initially that although with streptokinase the plasminogen activity goes down very very quickly that the isotope from the blood disapeared only with a half-live of half a day.
So clearly the plasminogen label stayed in the blood but the activity was gone and obviously this could be explained by the formation of an inactive inhibitor complex.
And you see here this is the plasminogen on gel filtration size exclusion chromatography and this is the main inhibitor which here in this patient after a couple of hours had taken care of most, of the activated plasminogen.
Now at that time this gives us the suggestion that if you could make an assay for this plasmin inhibitor complex that you might have a biomarker for in vivo activation of the fibrinolytic system and so as we had the complex here labeled, it was inactive.
Now in these days before the monoclonal antibodies, we are talking here about 1971, I believe or 72 the only way known to me was to try to purify the complex, make a polyclonal antiserum to it, absorb the two components and hopefully be left with some residual antibodies that react with the complex only and not with the precursors.
We studied this in great detail with Ed Plow and it actually did work.
What you have here is the plasma in the middle and here the anti-serum which is progressively adsorbed with plasma and you see that you can adsorb everything away.
At least this is how it looked originally.
Whereas if you would take activated plasma and you adsorb it away there is still residual activity.
And so the problem was can we make this into an assay and although we tried very hard in collaboration with Ed Plow, we couldn't really make this into a commercial development.

Immunochemical distinction between antiplasmin and alpha $_1$-antitrypsin

D. Collen , F. De Cock and M. Verstraete

Thrombosis Research Volume 7, pp. 245-249 1975

"It is generally believed that α_2-macroglobulin and α_1-antitrypsin are the main plasmin inhibitors in human plasma.
We now present immunochemical evidence that the low molecular weight ("non α_2-macroglobulin") antiplasmin, contrary to the general belief, is different from α_1-antitrypsin and probably also from the other known protease inhibitors in plasma."

Immunoelectrophoretic evidence indicating that antiplasmin is different from α_1-antitrypsin.
A. Human plasma versus a mixture of antisera against antiplasmin and α_1-antitrypsin.
B. Plasma from a patient with α_1-antitrypsin deficiency versus antiserum against anti-plasmin.
C. Urokinase-activated plasma from the same patient with α_1-antitrypsin deficiency versus a mixture of antisera against antiplasmin and α_1-antitrypsin.

4

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

D. Collen

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

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

5

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

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

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

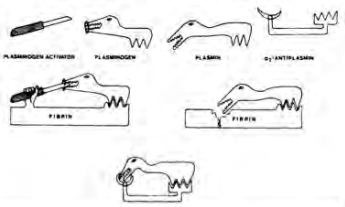
Mixed cultures of mouse fibroblasts and mouse fibroblasts transformed with Kirsten murine sarcoma virus were grown in petri dishes and overlayed with casein. The appearance of focal lysis zones required the presence of transformed cells in the culture and plasminogen in the overlay, indicating that caseinolysis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

6

Regulation of fibrinolysis: plasminogen activator as a thrombolytic agent

D. Collen

Pathobiology of the Endothelial Cell pag. 183-189 1982



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.

7

However when we looked at this polyclonal antiserum against plasmin inhibitor complex everybody thought at that time that it probably would be α_1-antitrypsin. This is an old-fashioned crossed immuno electroforesis with two sera, one against α_1-antitrypsin, one against this inhibitor plasmin complex, but adsorbed with plasminogen, and you clearly see that there is a lot of antitrypsin and something else which is not antitrypsin.
The results of all this was that α_2-antiplasmin was identified immunologically and then the problem was how does this work, what are the kinetics.
So initially we tried to go after an assay for this inhibitor complex but subsequently of course it became important to activate and to purify the system and this was actually done in collaboration with Björn Wiman.
Now around that same time, in the middle of the seventies, there was evidence accumulated mostly in the group of Ed Reich that malignant cells in culture produced fibrinolytic activity and that the malignant phenotype was much more severe or correlated with the amount of protease fibrinolytic activity secreted.
And in collaboration with Fons Billiau we found then that the protein in plasma that was able to revert this was actually the α_2-antiplasmin.
So we wanted to go after an inhibitor of cancer growth which in retrospect proved to be a little bit naive, but in any case Fons' system was so complicated with titration of cell cultures that eventually he suggested that I try to purify such a malignant protease so that at least we could do some biochemistry and some kinetics on it.
And there serendipity has helped me once again because the very first cell line medium that I got from Grant Barlow from Abbott was from a patient's melanoma, metastasis in culture, a patient called Bowes.
And that patient Bowes, produced her melanoma, a patient that already died three or four years earlier, but produced malignant proteases in very high amounts which eventually proved to be t-PA.
The reason that other people had been working with this for three or four years already and not recognized the potential as a fibrin specific fibrinolytic agent is actually summarized in this cartoon here, which was based on the interactions with Wiman, the kinetics of α_2-antiplasmin inhibition of plasmin and the affinity of t-PA for fibrin.
And it is based on this cartoon, which has been shown many many times that one thing went to the other, the rest of the story will be told to you by those people who have participated in those different steps.
Thank you very much.

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

BJORN WIMAN

Regulation of Fibrinolysis - Historical

Björn Wiman

Department of Clinical Chemistry
Karolinska Hospital, Karolinska Institutet
Stockholm, Sweden

Fibrinolysis – Regulatory aspects

- Two different but cooperating mechanisms
  - Localized activation of plasminogen at the fibrin surface
    - Both plasminogen and tPA have affinity for fibrin
  - Selective extremely rapid inhibition of free plasmin, but not of plasmin bound at the fibrin surface
    - Lysine binding sites play an important role

tPA – Affinity to Fibrin – Localized Plasminogen Activation

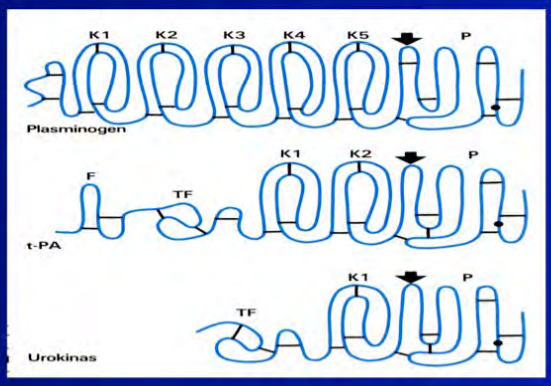
- An affinity between tPA and fibrin was first suggested by Astrup and coworkers in the early seventies. However, no solid experimental data was presented.
- In the summer of 1975, I worked with the development of a caseinolytic assay for tPA, in collaboration with Per Wallen. Practically no activation of plasminogen occurred, in spite of high concentration of active tPA. However, by addition of a tiny amount of washed fibrin, a rapid caseinolysis occurred. Activation of plasminogen took place at the fibrin surface.
- In the autumn of 1977, as visiting professor in Leuven, I convinced Désiré Collen that tPA indeed was fibrin specific.
- A few weeks later, Désiré already had the goal to develop tPA as a new thrombolytic drug.

I'm honoured and very pleased to be here today to be invited to this distinguished symposium in the celebration of the retirement of Désiré Collen.
I guess that the retirement is not a correct word because I guess that he will just switch gears a little bit and then continue as usual.
My task today is to talk about some regulatory aspects of fibrinolysis and the mostly historical remarks.
The regulatory aspects of fibrinolysis that I will talk about, involves two different but cooperating mechanisms.
The first one is localized activation of plasminogen at the fibrin surface and the second one is the selective extremely rapid inhibition of free plasmin but not of plasmin that is bound to the fibrin surface.
We knew that the localized activation is occurring just because both plasminogen and t-PA have affinity for fibrin forming the ternary complex and therefore at the fibrin surface activation goes extremely rapidly.
For the second part, we knew that the lysine binding sites in the plasmin molecule play a very important role in this mechanism.
Let me first start to talk a little bit about the affinity to fibrin of t-PA and its importance for the localized plasmin activation.
It has been suggested, that there was an affinity between t-PA and fibrin and that was actually suggested very long time ago by Astrup and coworkers.
However, they really didn't present any solid experimental data to proof this hypothesis mainly because all these proteins were not purified at that time so they didn't really have the possibilities to proof it.
Much later, in the summer of 1975, Per Wallen, my boss at that time working up in Umea, in another part of Sweden, he had started the work of purification and characterization of t-PA from pig heart muscles one of his students at that time, got the task to set up a new method to measure t-PA.
Per Wallen would like to see a caseinolytic assay for measuring t-PA, a method that we were very used to, at that time, looking at urokinase activation of plasmin.
The problem was that we couldn't find any activity at all using this method.
The t-PA was very active in clot lysing system.
So then I was thinking myself actually, what is the reason for this?
And it ended up with that I added a small amount of washed fibrin to the caseinolytic mixture and immediately we got a very strong caseinolysis. So from that point of view, it was quite clear that activation of plasminogen must occur at the surface of fibrin.
A few years later, I was actually invited to come to study antiplasmin, together with Désiré here in Leuven, already our cooperation started before that because already, when I was in Umea, we tried to purify the native antiplasmin which was indeed, a difficult task.
Nevertheless, having the knowledge about the fibrin specificity of t-PA, I eventually happened to convince Désiré that t-PA indeed was fibrin-specific.
So, and then I'm certain that if not immediately, just a few weeks later, Désiré already had a goal to develop a new thrombolytic agent I mean, I didn't have that perspective but Désiré had.
I mean he had been working with Marc Verstraete for a long time, who was the master in using treatment of thrombotic disease with thrombolytic agent using streptokinase at that time.

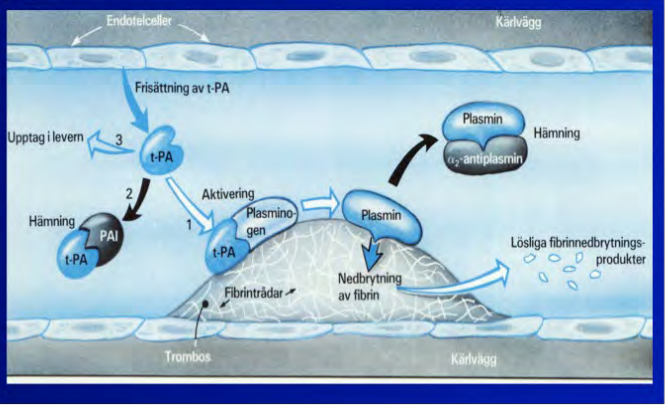
Antiplasmin – Localized Fibrinolysis

- Together with Désiré Collen and eventually Roger Lijnen, Leuven, we:
  - Purified Antiplasmin
  - Characterized Its Reaction with Plasmin
    - Structurally
      - 1:1 complex, covalently stabilized (ester bond)
    - Kinetically
      - Plasmin active site with scissile peptide bond in antiplasmin
      - LBS in plasmin with a complementary site in COOH-terminal portion of antiplasmin (rate limiting step)
  - Fibrin-bound plasmin reacts ~100-fold slower with antiplasmin as compared to free plasmin

Structure of some enzymes in the fibrinolytic system



Schematic Presentation of the Fibrinolytic System



Well, together with Désiré Collen and eventually Roger Lijnen who, a little bit later, joined the group, we purified antiplasmin.
We characterized its reaction with plasmin, both structurally and kinetically and from the structure point of view, it was clear that a one to one covalently stabilized complex was formed.
Eventually we were able to demonstrate that an ester bond had been formed between those two proteins.
Nevertheless, from the kinetic point of view, we were able to demonstrate that actually two different interactions were of importance for the extremely rapid reaction that occurred between plasmin and antiplasmin.
In the first one, it's a plasmin active site interacting with a scissible peptide bond in antiplasmin.
But, very important, the lysing binding site in plasmin, also interact with complementary site in the carboxyterminal portion of antiplasmin and that is actually the rate limiting step.
I mean, in this case, the reaction between plasmin and antiplasmin was so rapid with a rate constant of over 10^7 M^{-1} s^{-1} that we could not really understand how plasmin could be active at all in vivo.
But, I mean, since the lysing binding sites in plasmin were very important for this rapid reaction.
That actually meant that plasmin that were fibrin bound reacted at about 100-fold slower with antiplasmin as compared to the free plasmin.
So this is then the second mechanism keeping the fibrinolytic process localized.
This is a schematic presentation of how the fibrinolytic process is regulated, t-PA release from the vessel wall endothelial cells can take one of three pathways either is taken up extremely rapidly in the liver.
It could be inhibited by PAI-1 or if fibrin is present, it forms a ternary complex with plasmin at the fibrin surface and rapidly plasmin is formed which is sitting here relatively alone without problems until its released out into the bloodstream when it's rapidly captured by α2-antiplasmin it is clear from knowledge that we have now, that PAI-1 is really the important determinant of the action of t-PA under physiological fibrinolysis and that means, if you have a high levels of PAI-1, you have very little free t-PA in circulation.
If you have low levels of PAI-1, then actually you have a lot of free t-PA causing increased levels of for example plasmin, antiplasmin complex and those patients indeed had small, but significant risk of getting bleeding problem.
On the other hand, high levels of PAI-1, that really causes an increased risk of thrombotic disease mainly of myocardial infarction and it is now known from prospective studies in healthy individuals that in the short term follow up this is actual a major risk factor for myocardial infarction, it's much stronger than any of the lipid parameters.

Concluding Remarks

- I spent a memorable, funny and fantastic year in Leuven (1977/1978)
- I cooperated with a great number of devoted and dynamic scientists
- We published a lot of papers, mostly about antiplasmin
- We had a boss that I respected more for every day
- We were drinking a lot of fantastic french wines and had a large number of superb dinners cooked by Désirés wife Lovisa

Last, I have some concluding remarks.
I've spent a memorable, funny and fantastic year in Leuven during the years '77 and '78.
I cooperated with a great number of devoted and dynamic scientists.
We published a lot of papers, mostly about anti-plasmin and its function.
We had a boss that I respected more for every day and year to come and that was not Désiré Collen, but it was our common boss at that time, Marc Verstraete.
And we also were drinking a lot of fantastic French wines and we had a large number of superb diners cooked by Desiré's wife, Louisa.
Coming from the North, I rather would like to compare the t-PA story with a snowball and what I did was, when I came to Desiré's lab and were able to convince him about the fabulous specificity of t-PA, I made a very little snowball.
What Desiré did was that he put that snowball in rolling and he directed its rolling so that it get bigger and bigger and bigger and it actually created a lot of resources and what I admire mostly about the work of Désiré is that he has actually used those resources in a fantastic way.
I mean, he has really used the resources so that they created more and better science.
So that's my concluding remarks. Thank you very much.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Our next speaker is Alfons Billiau from Rega Institute in Leuven, who collaborated with Désiré in the period 1980-1982.
He took initially care of the production of the melanoma culture medium, because we didn't have cell culture facilities at that time, but maybe even more importantly he made the initial contact with Willem Weimar at the Erasmus Medical University in Rotterdam, who treated in 1981, I believe the first renal allograft patients 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

HEART for the FUTURE
Monday October 6, 2008 Leuven
www.heartforthefuture.be

Congratulations Désiré !

Alfons Billiau
Rega Institute
University of Leuven

Rega (P. De Somer, A. Billiau) — Haemostasis lab (M. Verstraete, D. Collen)

Interferon — 1976 - 1981 — Fibrinolysis

Mouse: Effects on cells transformed by sarcomaviruses
? Virus replication
? Invasiveness

Human: IFN-b production from mass-cultured human skin fibroblasts

Rega (P. De Somer, A. Billiau) — Haemostasis lab (M. Verstraete, D. Collen)

Interferon — 1976 - 1981 — Fibrinolysis

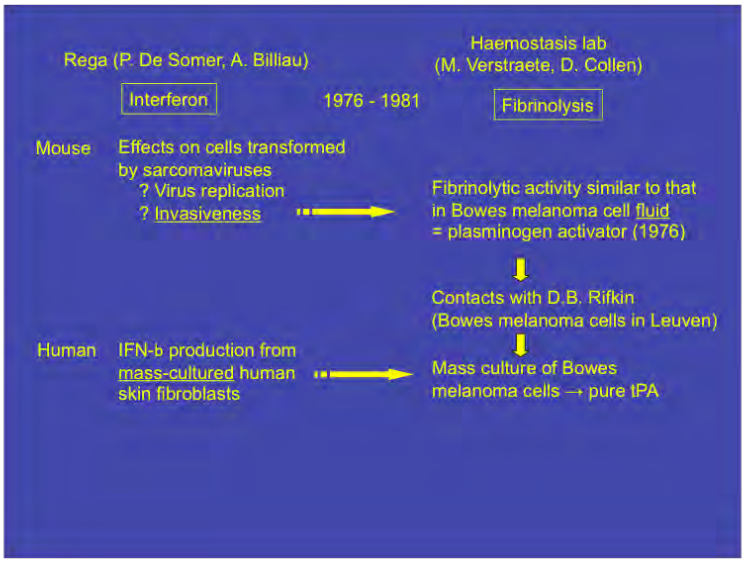
Mouse: Effects on cells transformed by sarcomaviruses
? Virus replication
? Invasiveness

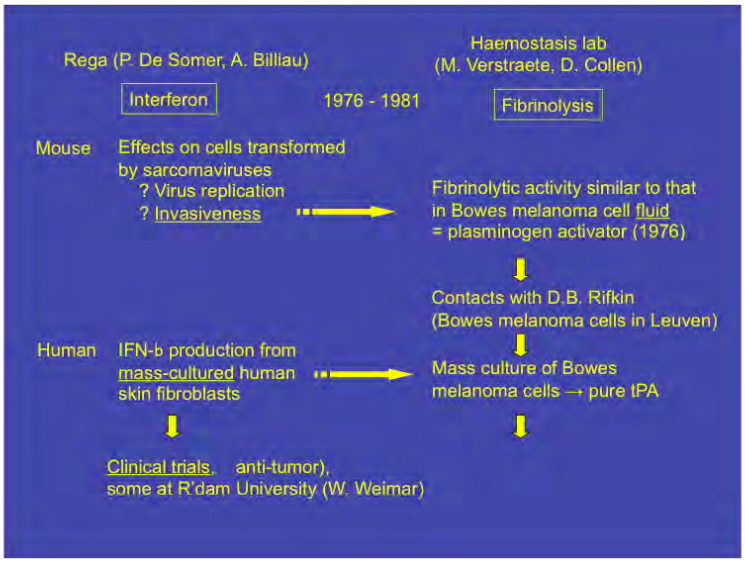
→ Fibrinolytic activity similar to that in Bowes melanoma cell fluid = plasminogen activator (1976)

Collen D, Billiau A, Edy J, De Somer P
Identification of the human plasma protein which inhibits fibrinolysis associated with malignant cells.
Biochim Biophys Acta 1977, 499,194-201

Human: IFN-b production from mass-cultured human skin fibroblasts

Dear Désiré, colleagues and friends.
I've had the pleasure, like him, to have belonged to the academic staff of this university for my entire career, which already ended in 2002.
In the early 1970's when I first made Désiré's acquaintance, my own research at the Rega Institute included the production of interferon from cultured cells both of mice and men.
Both these research lines, in mice and in human interferon would be important for the development of a fruitful collaboration between us.
For my retrovirus research line, I had developed several tumour cell lines, as Désiré already mentioned, by in vitro infection of mouse fibroblasts with mouse sarcoma virus and some of these cells, had been used at the University of Ghent, to validate a test for malignancy by looking at invasiveness in vivo in chick embryo blastoderms.
Thus, I had become interested (more or less sidewise) in the invasiveness of tumor cells and as Désiré already mentioned, already at that time invasiveness of tumor cells was believed to be due, at least, in part to the disbalanced secretion of proteases and protease inhibitors.
So, when Désiré and I happened to exchange views on our mutual interests, the idea rose to test my mouse tumor cell lines for production of proteases, and to see whether this could perhaps lead us to develop an in vitro model for studying protease inhibitors which might then prove to have anti-tumor potential.
Now at the time, a British postdoc, Vic Edy, had come to my laboratory at the Rega Institute to work on interferon production and he was accompanied by his wife Judy, a biologist and she was looking for a job and Désiré employed her to explore the idea that we had and she published her paper on the subject, in 1977, showing that, indeed, the mouse tumor cell lines, that we had at the Rega Institute, were producing a proteolytic activity, and that this activity resulted from secretion of a plasminogen activator.
Now in her paper, Judy also reported, rather parenthically, that she tested fibrinolytic activity in a sample of culture fluid from a human melanoma cell line and found that it resembled that of my mouse tumour cell lines in being inhibited by normal plasma.
Now the origin of the fluid is even not specified in the paper, but as Désiré told us, this was the sample that he had brought, I guess from New York, a sample of fluid from a culture that would later become the famous Bowes melanoma cell line.


Rega (P. De Somer, A. Billiau)
Haemostasis lab
(M. Verstraete, D. Collen)
Interferon
1976 - 1981
Fibrinolysis
Mouse
Effects on cells transformed by sarcomaviruses
? Virus replication
? Invasiveness
Fibrinolytic activity similar to that in Bowes melanoma cell fluid = plasminogen activator (1976)
Contacts with D.B. Rifkin (Bowes melanoma cells in Leuven)
Human
IFN-b production from mass-cultured human skin fibroblasts
Mass culture of Bowes melanoma cells → pure tPA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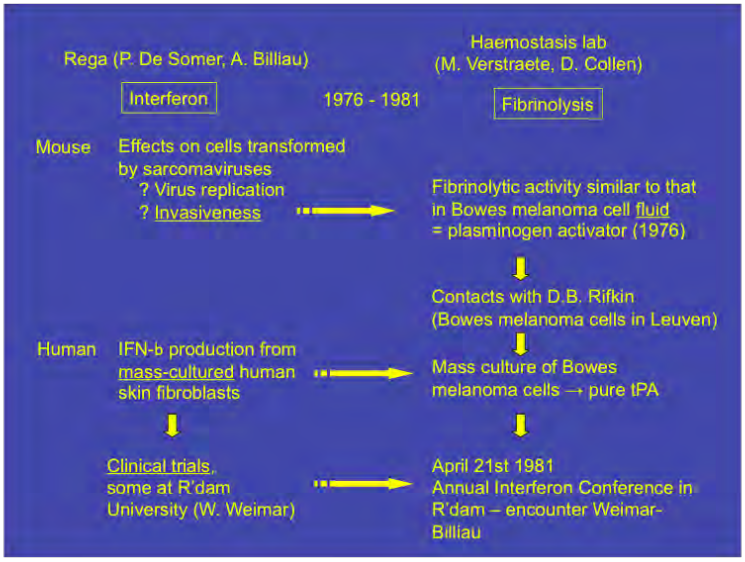
Mass culture of Bowes melanoma cells → pure tPA
Clinical trials, anti-tumor), some at R'dam University (W. Weimar)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Human
IFN-b production from mass-cultured human skin fibroblasts
Mass culture of Bowes melanoma cells → pure tPA
Clinical trials, some at R'dam University (W. Weimar)
April 21st 1981 Annual Interferon Conference in R'dam – encounter Weimar-Billiau

But then, it must have been wintertime 1980-1981, one day, rather out of the blue, Désiré stepped into my office on an early morning I guess, handing me a culture flask and asking me: 'Could I take care of this culture because in his own laboratory, although it was very well equipped, he did not have yet, at that time, tissue culture facility.
He explained having information that the line, called 'Bowes', produced a protease with properties similar or identical to those of the normal tissue-type plasminogen activator and he gave me of course the initiation course on t-PA that we have just received five minutes ago.
Now as I said, he didn't have the equipment to cultivate the cells, while we at the Rega Institute, we were fully operational in producing human fibroblast interferon from a mass culture system.
So producing the cells and producing the supernatant was very easy and in a very short time, we could set up the system.
Désiré hired a technician to even increase it in the Rega Institute and very soon he accumulated sufficient pure protein to deliver proof of principle in the animal model.
Meanwhile, at the Rega Institute, we had started to do clinical trials, trying to proof that the interferon, that we were producing, had some antiviral or some anti-tumor potential.
And some of these trials were being done in Dijkzigt Hospital in Rotterdam under the guidance of Willem Weimar, then in his early career as a nephrologist.
The overall results of our trial with interferon were in fact very discouraging and on top of that it was really very difficult and it involved a lot of pains and aches to produce very small amounts of interferon, while producing the t-PA was very easy.
So this contrast between these difficult and discouraging trials with interferon were really striking and then in April the annual interferon meeting of that year 1981 was to take place in Rotterdam.
So on the 21st of April, or the day before, I drove to Rotterdam, I took my car, which, as you will see was important, and arriving at the Town hall, I was looking for Willem Weimar, but I couldn't find him.

A serendipitous encounter

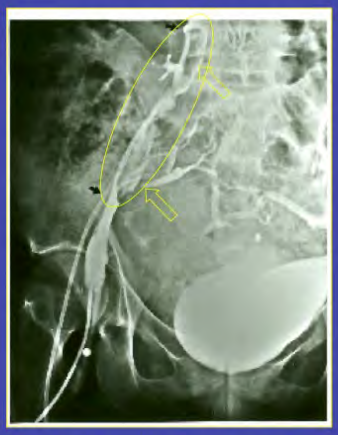
Weimar – Billiau

At the Annual Interferon Conference in R'dam on april 21st 1981

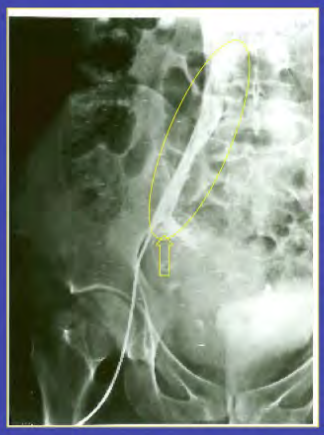
Weimar, W., Stibbe, J., van Seyen, A.J., Billiau, A., De Somer, P., Collen, D., 1981.

Specific lysis of an iliofemoral thrombus by administration of extrinsic (tissue-type) plasminogen activator.

Lancet 2:1018-1020.



April 15 – thrombotic mass in the iliofemoral vein, protruding in the vena cava and occluding renal vein



May 8 – After tPA therapy: normal patency of renal vein and normal contrast dilution in open iliofemoral vein

Thank you, Désiré, for everything you have signified to me in

the past in terms of inspiration, collaboration and friendship.

Thanks to the organizers of this meeting.

Thanks to the audience for attention.

He arrived late telling me that his being late was due to the fact that he was taking care, at that same moment, of a patient, a renal transplant patient who had developed a big thrombus in the vein, of the kidney and he had no solution for this.
He said "well, I'm really – perhaps I have to go back to see the patient, but the patient – in fact I expect the patient to die tomorrow or the day after tomorrow".
So I told him and these are the words that I read from his account that he just sent me a few weeks ago, I apparently told him: "did you ever hear of t-PA?"
He had never heard of t-PA.
I explained the story, I told him that, here in Leuven, we had badges of t-PA ready to be injected and he really insisted that he would receive the product to inject it.
I immediately, that same evening, came back with my car to Leuven, in the morning I called Désiré, Désiré immediately agreed, I brought the t-PA sample to Rotterdam – which was easy because I had my car – and before the meeting was over, the patient's thrombus was dissolved.
These are the original pictures taken.
On the left side you see the thrombus on April 15 where you can see it, here I suppose and it has gone on, a few days later with the picture here taken on May the 8th.
I will add to this few reflections of Willem Weimar since he could not be here today.
His reflection is: in those days clinical research was purely based on trust between basic scientists, clinicians and patients. But certainly, the serendipity factor played a role too.
My *renal transplant* unit happened to cooperate with the Rega Institute in Leuven.
One of my patients was developing an ascending thrombosis exactly during an Interferon meeting in Rotterdam, which was attended by De Somer and Billiau.
Désiré Collen had just produced the first t-PA batch and Fons Billiau had a car.
I wish to stop here by thanking Désiré for everything he has signified to me in the past, in terms of inspiration, collaboration and friendship and I wish to thank the audience for attention.

tPA – Affinity to Fibrin – Localized Plasminogen Activation

- An affinity between tPA and fibrin was first suggested by Astrup and coworkers in the early seventies. However, no solid experimental data was presented.
- In the summer of 1975, I worked with the development of a caseinolytic assay for tPA, in collaboration with Per Wallen. Practically no activation of plasminogen occurred, in spite of high concentration of active tPA. However, by addition of a tiny amount of washed fibrin, a rapid caseinolysis occurred. Activation of plasminogen took place at the fibrin surface.
- In the autumn of 1977, as visiting professor in Leuven, I convinced Désiré Collen that tPA indeed was fibrin specific.
- A few weeks later, Désiré already had the goal to develop tPA as a new thrombolytic drug.

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 ’83.
He was at that time at the Gaubius Institute in Leiden and is now at the Erasmus University Medical Center in Rotterdam and he learned us a few tricks on how to purify t-PA.

DINGEMAN RIJKEN

The t-PA story

- Purification of melanoma plasminogen activator
- Kinetics of plasminogen activator by t-PA

Dick Rijken
Erasmus University Medical Center
Rotterdam, the Netherlands

PhD-student in Leiden: 1974-1979
Post-doc in Leuven: 1979-1982

History of t-PA

- 1900-1950: During tissue culture on a matrix of clotted plasma, the clot may liquefy.
- 1952: Historical year !

Why ?

History of t-PA

- 1900-1950: During tissue culture on a matrix of clotted plasma, the clot may liquefy.
- 1952: Historical year for t-PA!

Why ?

Désiré was nine years old

Mister chairman, ladies and gentlemen, Désiré, dear friends, so I have been asked to speak about two things.
About the purification of melanoma plasminogen activator and about the kinetics of plasminogen activation by t-PA.
I worked with t-PA as a PhD student, at the Gaubius Institute in Leiden, in the Netherlands in the seventies, and then as a postdoc, here in Leuven from 79 to 82.
Before I will speak about the story of t-PA, I want to make a few remarks about the history of t-PA.
The history of t-PA goes back to the first half of the previous century, when people developed the technique of tissue culturing.
And during tissue culture on a, for instance a matrix of clotted plasma, people frequently observed that the clot may liquefy, suggesting that tissue contains something that induce fibrinolysis.
It's sometimes difficult to say when a protein, was really discovered, but for t-PA 1952 was a very historical year.
And the question is: why?
I was born in 1952, but that was not the reason.
What about Désiré?
Désiré was nine years old.
He was playing outside and did not yet think about t-PA.
I think, he was thinking about already flying in the air like in his airplane.

History of t-PA

- 1900-1950: During tissue culture on a matrix of clotted plasma, the clot may liquefy.
- 1952: Historical year !

Why ?

Tage Astrup (1908–2006)

- Astrup T and Stage A
  Isolation of a soluble fibrinolytic activator from animal tissue. Nature 1952; 170: 929

Purification of human t-PA

Source: Uterus

Assay: Fibrin plate method (Astrup & Mullertz, 1952)

Human Organ	Astrup & Albrechtsen U/ml
Uterus	720
Adrenals	410
Prostate	334
Lung	223
Kidney	119
Heart	82
Brain	35
Spleen	20
Liver	0

(Albrechtsen, 1957)

Separation techniques: Affinity chromatography

Purification and partial characterization of plasminogen activator from human uterine tissue

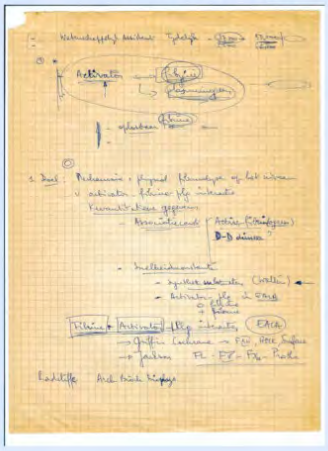
- D.C. Rijken, G. Wijngaards, M.Zaal-de Jong and J. Welbergen
  Biochim Biophys Acta 1979;580:140
- 5 kg tissue yielded 1 mg t-PA
  (5000-fold purified)

* 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-hexadecyltrimethylammonium chloride	cationic	60	10
0.10% (v/v) Tween-80	non-ionic	99	114

The real reason was that Astrup, who has been mentioned already by Bjorn Wiman.
Astrup published in 1952 in Nature a paper entitled “Isolation of a soluble fibrinolytic activator from animal tissue”.
So by using a very strong chaotropic agent, thiocyanate, two molar thiocyanate he was able to extract t-PA from the tissue, because t-PA was, or is, strongly bound to the tissue and I still don’t understand why that is.
But anyhow, it was possible by this observation to extract t-PA from a tissue and from that time on people started to try to purify t-PA and in the fifties and in the sixties, several PhD students spent their time to purify t-PA.
And in the seventies it was my turn and my director and supervisor, Professor Pieter Brakman he told me that I had to purify t-PA from human tissue.
And the first thing that you need then is a source and in that time, this table was available in the literature, showing that uterine tissue contains the highest amounts of t-PA.
Here is 720 Astrup & Albrechtsen units per gram of tissue.
Other tissues contained less t-PA activity, for instance the liver, no activity at all, brain a low activity.
It’s interesting that most relevant papers at this moment, nowadays, are about brain t-PA because t-PA seems to have an essential role there, a non-haemostatic function.
The second thing you need is an assay and the assay that we used was, again, developed by Astrup, in 1952 and it was an assay which was very similar to his tissue culture technique.
So he used a tissue culture plate and put a thin layer of fibrin, containing some plasminogen, into the plate and then a droplet containing t-PA was applied on the plate and the plate was incubated overnight and then the next morning you can see and measure how much fibrin has been lysed during the night.
And then, the third thing that you need for purification are separation techniques and I was lucky in the seventies, because affinity chromatography became available and that’s probably the reason that I was more successful than people in the fifties and in the sixties.
So after four years, I was able to publish my first paper about the purification and characterization of plasminogen activator from human uterine tissue, here you see the material.
So we were able to purify one milligram of t-PA from five kilogram of tissue.
And after you have purified a protein you want to characterize the protein, and of course I tried to do that but I spent more than half a year doing experiments with my purified t-PA and all experiments failed.
Because in each experiment, I lost my activity until Pieter Brakman suggested me to use a neutral detergent Tween 80 in my buffers and indeed that trick stabilized my t-PA activity just by preventing adsorption losses to plastic and glassware.
So, I could perform my characterization experiments and complete my thesis.

Post-doc in Leuven



Visit to Désiré on a Saturday morning in 1978

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^{-4} × 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"

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

$$A \overset{K_F}{\rightleftharpoons} A - F \overset{K_{m,F}}{\rightleftharpoons} A \longrightarrow P \xrightarrow{k''_{cat}}$$
$$\qquad\qquad\qquad\qquad\qquad\quad F$$

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

Then I went to Leuven as a postdoc.
So on a Saturday morning in '78, I visited Désiré and we discussed about the possibilities and then finally we agreed that I would study the kinetics, the enzyme kinetics of plasminogen activation by t-PA and in particular the role of fibrin.
The next time that I met Désiré, was in the summer of 79, three months before I would really come to Leuven, it was in London during the ISTH congress.
Désiré told me about the melanoma cell culture, so he told me that he has a much better potential role for t-PA, having at least 500 units t-PA per ml.
And so, I started here in Leuven by purifying melanoma plasminogen activator.
The melanoma conditioned medium contained 20, only 20 units per ml instead of 500, but still it was a huge amount because it was possible to purify one milligram of t-PA from 10 litres of conditioned medium.
10 litres medium instead of 5 kilogram tissue and as we heard from the previous speaker, this one is easily to scale-up.
We concluded in this paper that all findings indicate that 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.
So then, because of the availability of a lot of purified t-PA, I continued with studying the fibrinolytic properties of one chain and two chain human extrinsic tissue-type plasminogen activator, a long name for just t-PA.
Désiré suggested in the meantime that Marc Hoylaerts could already start with the kinetics because it took too long before I could start.
So, and that was a very good decision because Marc was able to develop a very nice model for the kinetics of plasminogen activator by t-PA.
Here you see the ternary complex already mentioned by Bjorn Wiman, plasminogen activator, plasminogen and fibrin in which the activation rapidly occurs.
This is a very interesting paper, more than one thousand times cited, although I believe that only a few people have really read the paper because enzyme kinetics is very difficult to understand, even for me as a second author.

ISTH Congress in Toronto, 1981

Low-budget accommodation in the student rooms of the university campus

Désiré ??

Désiré,

Many thanks for the exciting years in your lab!

So all these results, including the treatment of the first patient were presented at the ISTH Congress in Toronto, in 1981.
Professor Verstraete was sponsored by a business class ticket to go to Toronto and that was sufficient money for our chairman of this session and for Marc Hoylaerts and for me, to fly with cheap tickets to Toronto and it was also sufficient for the low budget accommodation in the student rooms of the university campus.
And Désiré would join us and he had also booked for a room in that student campus.
But when he arrived at the campus it appeared that he had got a room in the ladies department because they didn't know whether Désiré was a male or a female.
So, without hesitating one second Désiré took his luggage and went to the city for a more luxurious hotel.
I want to finish by saying, Désiré, many many thanks for the exiting years in your lab.
Thank you.


HEART
for the FUTURE
The t-PA story told by:
Désiré Collen (VIB - K.U.Leuven),
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)
The t-PA story
KATHOLIEKE UNIVERSITEIT
LEUVEN
VIB

Our next speaker is Irène Juhan-Vague from the Centre Hospitalier Universitaire Timone in Marseille.
Se 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.

IRENE JUHAN-VAGUE

1982…

Determination of circulating tPA levels in pathological conditions

In vivo complex formation with a specific inhibitor

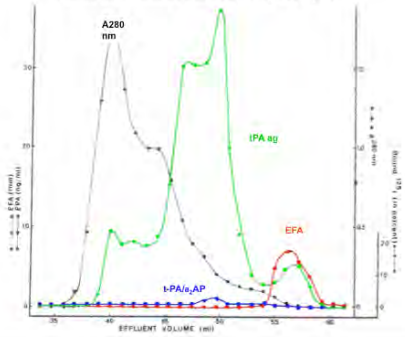
I Juhan-Vague

Measurement of human tissue-type plasminogen activator by a two-site immunoradiometric assay

D.C. Rijken, I. Juhan-Vague, F. De Cock and D. Collen
J Lab Clin Med Volume 101, pp. 274-284 1983

- Aim : Detect low fibrinolytic activity due to decreased tPA levels in patients
- Results : Markedly elevated tPA ag in severely ill patients

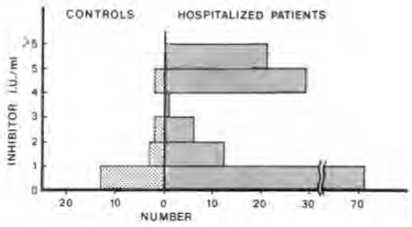
Gel filtration pattern of tPA ag
Patient with markedly elevated tPA ag (80 ng/ml)



Plasma levels of a specific inhibitor of tPA and uPA in normal and pathological conditions

I. Juhan-Vague, B. Moerman, F. De Cock, M.F. Aillaud and D. Collen
Thrombosis Research Volume 33, pp. 523-530 1984

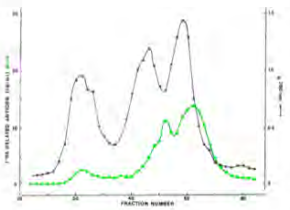
- Assay : 1 ml plasma + 5 u (50 ng) tPA, 5 min, 37°C
  Euglobulin fraction
  Fibrinolytic activity on bovine fibrin film
- Results : Markedly elevated tPA inhibitor activity in several clinical conditions



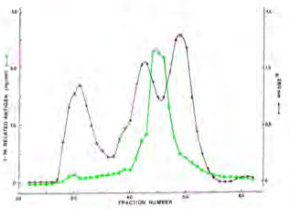
Gel filtration - Plasma (high tPA inhibitor) + 50 ng tPA

Synthetic inhibitor added

At time 0



At time 5 min



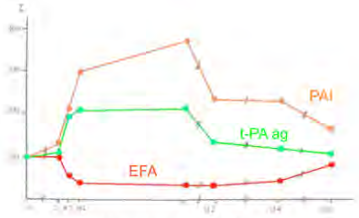
Désiré, in 1982 you accepted to be my mentor for my PhD thesis and I began to study plasma t-PA antigen variations in clinical situations.
This led to the identification of a specific inhibitor of t-PA, which became PAI-1.
I used first the radioimmunometric assay described by Dick Rijken with the aim to detect low fibrinolytic activity due to decreased t-PA levels in patients with thrombotic disorders.
But we were very rapidly surprised to find markedly increased t-PA levels in severely ill patients.
Here a plasma of a patient with very high level of t-PA antigen levels and this plasma was gel filtered and we see immunoreactive material eluted at high molecular weight form, without fibrinolytic activity, and it could not be identified as a complex with known inhibitors and thus we suggest that this was a complex with an unknown specific inhibitor.
This observation prompted us to further investigate this phenomenon and we developed a functional assay for this specific inhibitor and we found high level of inhibitors in many hospitalized patients.
Here we have a plasma with high t-PA inhibitor levels and active t-PA was added and a specific inhibitor was also added immediately or after 5 minutes incubation and we see that immunoactive material shifts from a molecular weight of 70 000 to 120 000 and this means that there was a complex formation of the t-PA added with the inhibitor which was called later on PAI-1.

High levels of PA inhibitor were found in several pathological conditions.

Increased t-PA inhibitor levels in the post-operative period

M.F. Aillaud, I. Juhan-Vague, M.C. Alessi, M. Marecal, M.F. Vinson, C. Arnaud, P. Vague and D. Collen
Thrombosis Haemostasis Volume 54, pp. 466-468 1985

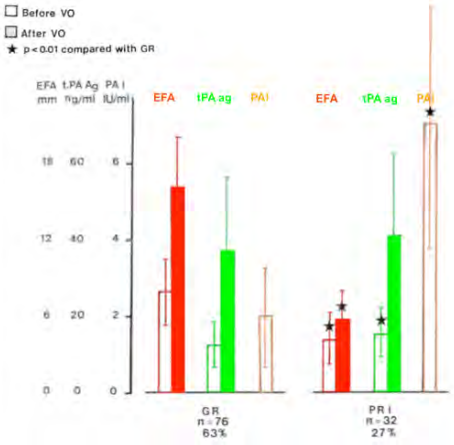
PAI-1 : acute phase reactant protein



post operative period

Deficient tPA release and elevated PAI-1 levels in patients with spontaneous recurrent DVT

I. Juhan-Vague, J. Valadier, M.C. Alessi, M.F Aillaud, J. Ansaldi, C. Philip-Joet, P. Holvoet, A. Serramigni, D. Collen
Thrombosis Haemostasis Volume 57, pp. 67-72 1987



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

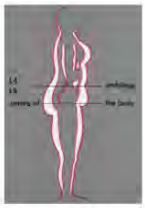
Plasma PAI-1 in angina pectoris Influence of plasma insulin and acute phase response

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

Then we tried to determine in which pathological conditions high levels of t-PA inhibitor were found and what were the inducers.
We first showed that PAI-1 is an acute phase reactant protein; it increased very quickly after surgery, in less than 1 hour.
In patients with deep vein thrombosis it was often found that after venous occlusion test, t-PA was normally released but it was not accompanied by an increased fibrinolytic activity and this was due to high levels of the inhibitors.
We were mainly interested by the disregulation of PAI-1 in obesity and diabetes.
And we showed that PAI-1 is increased, not in all form of obesity but in this kind of obesity which is called android obesity and which is due to a repartition of the fat in the upper part of the body and this kind of obesity is accompanied by complications such as insulin resistance, metabolic syndrome, diabetes and increased risk of coronary disease.
And in this form of obesity, called the gynoid obesity with fat in the lower part of the body, PAI-1 is normal and this form of obesity is not accompanied by all these complications.

• High levels of PA inhibitor were found in several pathological conditions.

They could play a role in the thrombotic process.

• The highest concentrations observed were still below 1 µg/ml.

Such levels were not expected to interfere significantly with thrombolytic therapy with tPA.

Therefore, high PAI-1 levels were found in several pathological conditions and especially in obesity and diabetes and we suggest that it could play a role in the thrombotic process.
The highest concentrations observed were still below one microgram per millilitre.
Such levels were not expected to interfere significantly with thrombolytic therapy with t-PA.
I am extremely grateful to Désiré.
He accepted at the very beginning, that I focus my thesis work on clinical research and without his help it would have been extremely difficult to initiate a research team in Marseille and gratefulness of the faculty of medicine in Marseille lead to the nomination of Désiré as doctor honoris causa of the university of Marseille.
Thanks for all Désiré.


HEART for the FUTURE
The t-PA story told by:
Désiré Collen (VIB - K.U.Leuven),
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)
The t-PA story
KATHOLIEKE UNIVERSITEIT LEUVEN
VIB

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.
He 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

First experimental animal studies with melanoma t-PA

-- from UK to t-PA --

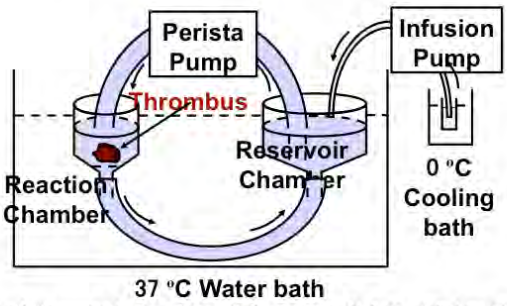
Osamu Matsuo

Present address:
Department of Physiology,
Kinki University School of Medicine, Osaka

Assessment of the thrombolytic effect of low urokinase concentrations

Matsuo O, Mihara H, Rokushima Y

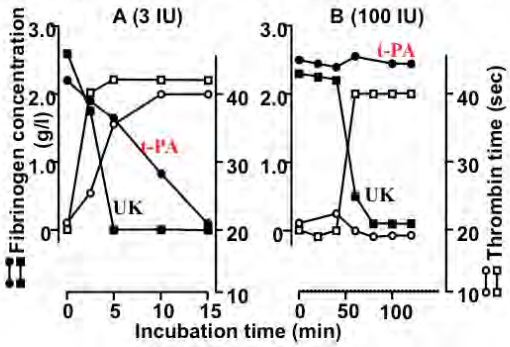
Acta Haematologica Japonica 39:298-305, 1976



The degree of thrombolysis at low UK was negligible. Higher UK induced thrombolysis accompanying extensive activation of fibrinolysis.

Comparison of the relative fibrinogenolytic, fibrinolytic and thrombolytic properties of tissue plasminogen activator and urokinase in vitro

Matsuo O, Rijken DC, Collen D Thromb Haemost 45:225-9, 1981



Purified system without (A) and with (B) antiplasmin (1 uM)

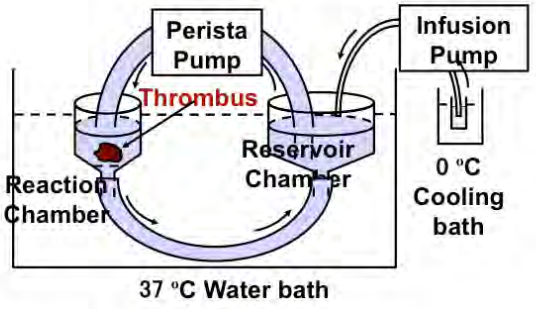
A: much rapid fibrinogenolysis by UK, B:no fibrinogenolysis by t-PA

Thank you.
It is my great pleasure that I can be here and these very impressive days for Désiré Collen.
When I came here it was 1979, before joining Désiré's lab, I was fighting against our government, because at that time, in our government approved very low concentration of UK, urokinase, only 10,000 CTA units was approved for the treatment of stroke at that time.
So I guess raising the evidence that the low concentration of UK doesn't work.
This is a circulating plasma model.
This kind of techniques is used for the t-PA experiments.
This is like a heart, the arteries connect to the circulation and come back to the vein in the reservoir where we injected the urokinase and I checked it and find out low concentration of UK doesn't work at all.
In 1979 I joined Désiré lab and I can use t-PA and I compare the activity of t-PA with urokinase.
You see in the absence of the anti-plasmin which is introduced already the UK digested fibrinogen very quickly, but the t-PA also digested, but the speed is very slow, but in the presence of the anti-plasmin the t-PA doesn't digest fibrinogen but urokinase digested fibrinogen.

Comparison of the relative fibrinogenolytic, fibrinolytic and thrombolytic properties of tissue plasminogen activator and urokinase in vitro

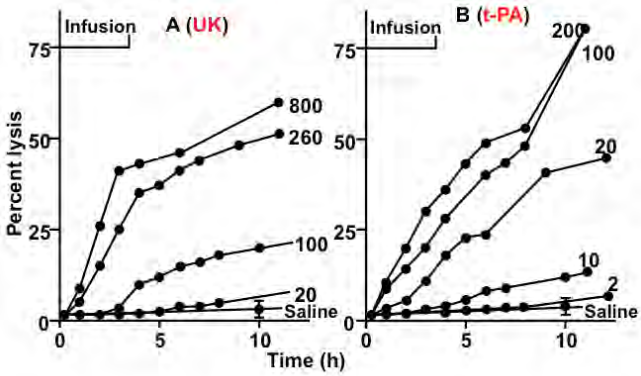
Matsuo O, Rijken DC, Collen D

Thromb Haemost 45:225-9, 1981

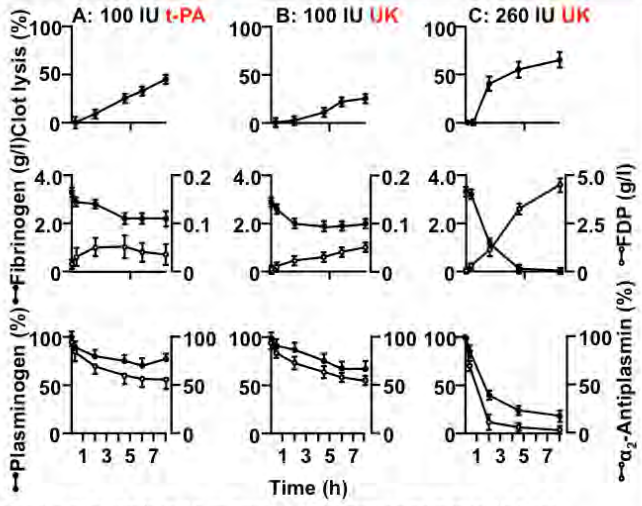


Circulation model to examine thrombolysis

Degree of thrombolysis in circulating plasma system



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



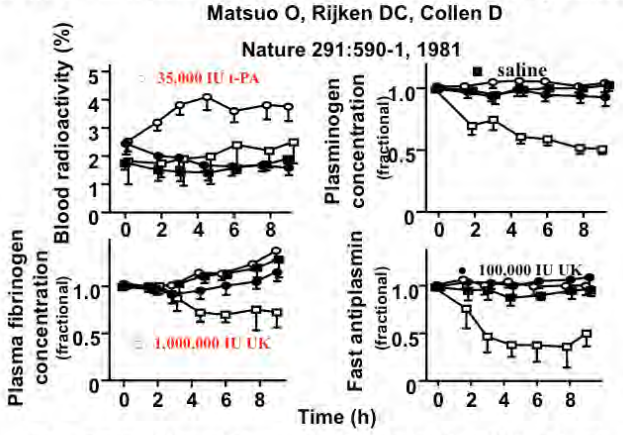
Moderate clot lysis with moderate fibrinogenolysis with t-PA.
Marked clot lysis and extensive fibrinogenolysis with high UK.

Then I use these kind of circulating plasma models and how is the t-PA acts on thrombus and compared it with urokinase.
You'll see the left side urokinase induced thrombolysis in time dependent and also dose dependent.
But here right side the t-PA dose dependent by induced the thrombolysis, but if you take a look very carefully even the 100 units of urokinase induce only less than 20 % thrombolysis, but here over 60% thrombolysis is observed in the case of t-PA.
So thus almost t-PA is 10 times efficient than UK.
During such experiments we checked the activation of fibrinolytic system in the circulating plasma.
If we take a look, in that case of a t-PA 100 units it's got very good thrombolysis.
But UK 100 units induced very low thrombolysis, but almost same degree of the fibrinogenolysis and plasminogen depletion and antiplasmin depletion.
But when we use very high amount urokinase we get very clear thrombolysis, but instead of such a thrombolysis we get very completely fibrinogen depleted and also plasminogen, antiplasmin also depleted.

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

Our Favorite

at that time!

Smuggler!!

Thus in vivo experiments it is clear that t-PA is completely different in terms of thrombolysis in the circulation model.
So we move on the in vitro model, this is model with pulmonary embolisms.
We produced thrombosus with radiolabeled fibrinogen which is introduced to jugular vein to the lungs here is the radioactivities you see of 35,000 t-PA induced, the increased radioactivity.
But here 1,000,000 units UK decrease fibrinogen and also antiplasmin degraded greatly and plasminogen also degraded, but 100,000 units urokinase is almost same as saline infusions.
This is very typical experiment and we used the further experiments with a double doses of the previous slide.
70,000 t-PA was infused, you see this is thrombolysis in the serum the radioactivity is released, but in the urokinase 100,000 is almost same as the serum infusion, 1,0000,0000 unit of urokinase about 11,0000, but in case of the t-PA 35,000 it's about 17 %, 70, 000 units 23% thrombolysis.
During, these kind of experiments Désiré told me let's have dinner tonight, but I had to work until midnight to take a blood sample, but he took me to his house, so I carried my rabbit and rabbit sit, in his bathroom and I take blood samples during the drinking Smuggler.
This is our favourite at that time.

Thrombolytic Efficiency of PA

(IU/ml)	(A) Fibrinolysis	(B) Fibrinogenolysis	A/B
TA 100	49	30	1.63
UK 100	16	29	0.55
UK 260	66	100	0.66

$$= \frac{\text{Thrombolysis (lysis of fibrin)}}{\text{Fibrinogenolysis (lysis of fibrinogen)}}$$

$$= \frac{\text{Fibrinolysis on the Solid Phase (Thrombus)}}{\text{Fibrinolysis in the Circulating Plasma}}$$

Thrombolysis with human extrinsic (tissue-type) plasminogen activator in dogs with femoral vein thrombosis

Korninger C, Matsuo O, Suy R, Stassen JM, Collen D

J Clin Invest. 69:573-80, 1982

Before and after infusion of 100,000 IU of 2-chain t-PA

Higher specific thrombolytic effect of t-PA than UK was confirmed in canine thrombosis model.

We were Young!!

Our Hearts are still Young!!

I calculate these figures fibrinolysis ...
If this value is greater than the one it means it is very specific for thrombolysis, that means that fibrinolysis is induced on the surface of the fibrin, but if it is below 1, this is much greater fibrinolygenolysis induced.
So it is clear that t-PA is completed different in in vivo experiments.
And further we did a similar experiment with canine and during this kind of animal experiments I convinced that the t-PA is the ideal thrombolytic agent for the future.
At that time I was 1979 – 1980 here you will see I look very young, but Désiré also looks very young, but I believe our heart still young and I hope Désiré has another big story for, not only science and also for the management in the translational research work in the future.
Thank you very much for your attention.

tPA – Affinity to Fibrin – Localized Plasminogen Activation

- An affinity between tPA and fibrin was first suggested by Astrup and coworkers in the early seventies. However, no solid experimental data was presented.
- In the summer of 1975, I worked with the development of a caseinolytic assay for tPA, in collaboration with Per Wallen. Practically no activation of plasminogen occurred, in spite of high concentration of active tPA. However, by addition of a tiny amount of washed fibrin, a rapid caseinolysis occurred. Activation of plasminogen took place at the fibrin surface.
- In the autumn of 1977, as visiting professor in Leuven, I convinced Désiré Collen that tPA indeed was fibrin specific.
- A few weeks later, Désiré already had the goal to develop tPA as a new thrombolytic drug.

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

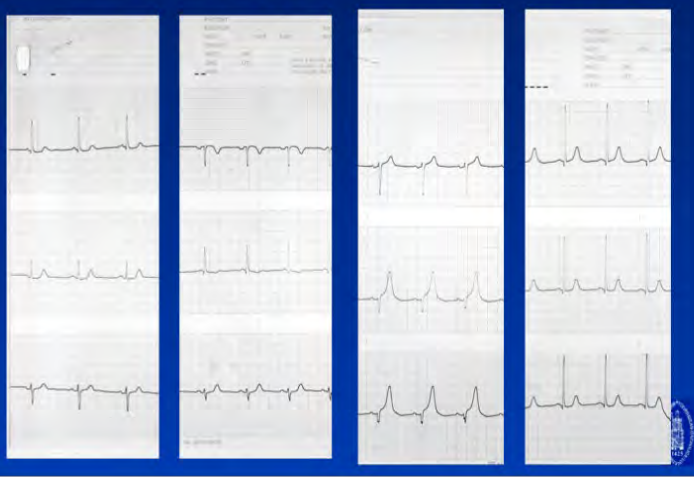
The (my) t-PA story

Frans Van de Werf, MD, PhD
University of Leuven, Belgium

t-PA : the first 7 patients

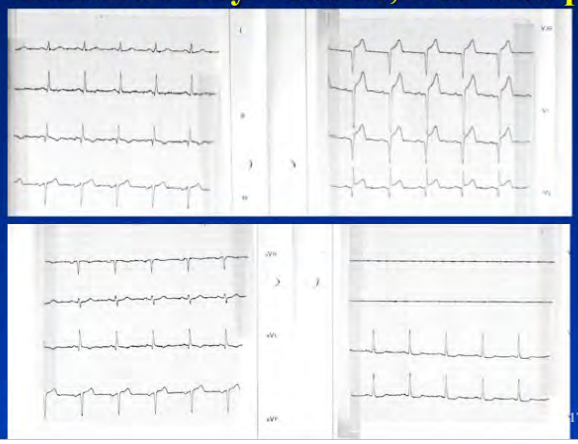
Duration of symptoms (hrs)	Dose IU	Response	Remarks
2.5	**t-PA: 6 x 10^5/30 min i.v. t-PA: 3 x 10^5/15 min i.c. SK: 250,000/60 min i.c.**	**No lysis**	**Transient hypotension during SK infusion**
2	t-PA: 6 x 10^5/30 min i.v. t-PA: 3.6 x 10^5/18 min i.c.	Lysis in 30 min, complete in 33	Successful CABG 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
B.R. male 58y Jan 20,1983 3.00 pm



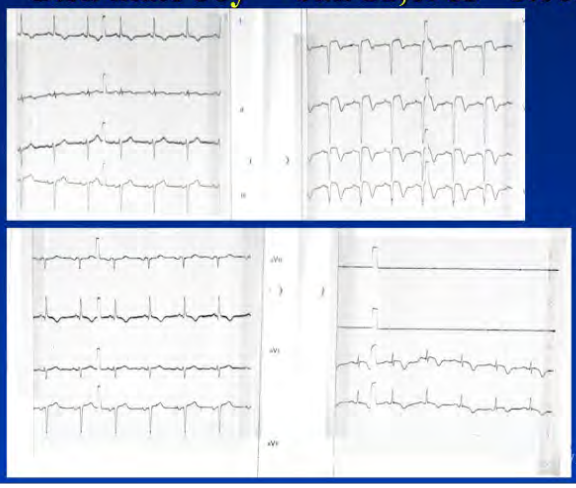
Ladies and gentlemen, Désiré, I got the great privilege to treat the five first patients in the world with an acute myocardial infarction.
Two other patients were treated by Burt Sobel and his collaborators in St. Louis.
All these patients were treated with t-PA from the BOWES melanoma cell lines, so purified in the lab of Désiré and if you would look at the doses, because at that time we didn't know what the right dose was for t-PA, I think it corresponds to a few milligrams and as you can see, we gave t-PA intravenously also intracoronarily and these doses were very small when you compare it now with the current dose of t-PA which is around 100 milligram.
The first patient, as you can see, was not successful, but I still remember the day when we did it, this was in January'83 and this was off course in the CAT lab, we had to check the occlusion first and then the recanalization process.
Désiré was off course there, but in addition I think 20 or 30 people attended this procedure.
I have seen this patient many times, and although it was not a success, I would like to show a few data from this patient.
For the cardiologists here in the room, they will recognize these very tall t-waves in the anterior leads and this was indeed a patient with an acute artery infarction.
And we started the procedure on January 20th at 3 pm.

The first patient
B.R. male 58y Jan 20,1983 9.00 pm



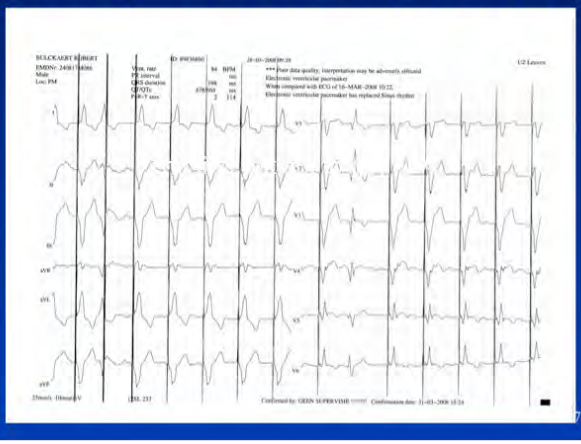
17/09

The first patient
B.R. male 58y Jan 22,1983 8.00am



17/09

The first patient
BR 83y March 28, 2009 9.30 am



7/09

And as I said, it was not successful and we took an ECG after the procedure in the evening and this showed the clear anteroseptal transmural myocardial infarction and then the patient was about to be discharged.
This ECG was taken and there was suggestion of the formation of an aneurysm so that was certainly not successful and we were concerned about the future of this patient.
But I've seen this patient many times and a couple of years ago, he got a pacemaker because he had some conduction disturbances and this ECG was taken this year so 25 years later after failed thrombolysis you see the pacemaker leads but he was still alive.

- **Patient died on July 25, 2008 in cardiogenic shock 25 years after t-PA administration at the age of 83 years**

11/17/09

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.)

"the copper coil"

11/17/09

And finally he died in July of this year, 25 years after the administration of t-PA at the age of 83.
Which means that you can survive an anterior infarction after failed reperfusion therapy for more than 25 years.
The other patients, the other six patients were successfully treated and this resulted in a publication in the New England in '84, so this was a publication on only seven patients and this is the smallest number of patients the New England has ever published.
And if you would calculate the number of citations per patient, this is absolutely a world record.
At that time I was also working with the copper coil and, off course in animals, and if you induce a copper coil in a coronary artery, you have a complete thrombotic occlusion after a couple of minutes.

“the copper coil”

11/17/09

“the copper coil”

11/17/09

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Heart Association
Learn and Live

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

FRANS VAN DE WERF, M.D., STEVEN R. BERGMANN, PH.D., KEITH A. A. FOX, M.B., CH.B., HILAIRE DE GEEST, M.D., CHARLES F. HOYNG, PH.D., BURTON E. SOBEL, M.D., AND DÉSIRÉ COLLEN, M.D., PH.D.

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.

11/17/09

And so this copper coil, here in detail, was advanced over a guidewire and so you could nicely position this copper coil into a coronary artery.
And then you could check the occlusion and the recanalization by repeat angiography.
And this we have done also in the same time, ’83, with a publication in ’84 in Circulation, but this time with recombinant t-PA made by Genentech and we treated nine dogs with t-PA and ten dogs with urokinase mentioned by the previous speaker and we could see a huge difference, not only in efficacy but also with regard to the fibrin specificity.
So this was a very successful study as well with recombinant DNA.

International Trials with rt-PA and its mutants

- GISSI-2 International
- GUSTO-I (accelerated infusion rt-PA
- COBALT (double bolus rt-PA)
- GUSTO-III (r-PA)
- ASSENT I (TNK-tPA)
- ASSENT II (TNK-tPA)
- ASSENT III (TNK-tPA)
- ASSENT-IV PCI (TNK-tPA)

11/17/09

11/17/09

11/17/09

And then afterwards, I got involved in many international studies here and our group has been able to coordinate many of these studies and all these studies have confirmed, not only the superiority of t-PA over streptokinase but also the tremendous importance of artery perfusion and in fact all these studies have paved the way for mechanical reperfusion which is now used in many hospitals with a CAT lab.
Because of the success of these papers, an academic session was organized by Dr. Boutelegier shortly after the first publications.
And this was organized in the Bordeaux region, more specifically in Pessac Leognan and I apologize for the quality of this picture because this was taken undercover.
You see here Désiré Collen and myself and we became there Doctor Honoris Causa, but there they call it slightly different.
They called us "Commandeurs d'honneurs dans l'ordre du bon temps" and of course we were very pleased by this nomination.
And shortly after the ceremony, this nice picture was taken from Desiré and Louisa in a local garden there and I'm not sure whether this will be a foretaste to what you and Louisa are going to do after your retirement, Désiré.
Anyhow, I would like to thank you for all the support I got during my 25 years of academic work here and all the support our department got from you and especially also all the friendship we got from you.
Thank you very much.

tPA – Affinity to Fibrin – Localized Plasminogen Activation

- An affinity between tPA and fibrin was first suggested by Astrup and coworkers in the early seventies. However, no solid experimental data was presented.
- In the summer of 1975, I worked with the development of a caseinolytic assay for tPA, in collaboration with Per Wallen. Practically no activation of plasminogen occurred, in spite of high concentration of active tPA. However, by addition of a tiny amount of washed fibrin, a rapid caseinolysis occurred. Activation of plasminogen took place at the fibrin surface.
- In the autumn of 1977, as visiting professor in Leuven, I convinced Désiré Collen that tPA indeed was fibrin specific.
- A few weeks later, Désiré already had the goal to develop tPA as a new thrombolytic drug.

Our next speaker is Diane Pennica, she is at Genentech at 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 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 and it is Diane Pennica who cloned the t-PA gene.

DIANE PENNICA

It's Great to Be Here Today to Honor Désiré and His Incredible Scientific Achievements

Instead of Showing a Lot of Data, I thought I'd tell you the Story of How Désiré & I Met

and

How Our Chance Meeting Led to a Very Exciting Collaboration Together

The Story Began in May 1980 When I was Hired by a Small, Unknown Biotech Company Called Genentech as Their 60th Employee

Genentech
Genentech
Genentech
Genentech

Today > 11,000 – One of the World's Most Successful Biotechs

- in Part Due to the Success of t-

PA

Genentech's Focus in 1980: Heart Disease

The Single Largest Killer of Men and Women in Both the U.S. & Europe

Every 20 Seconds Someone Has a Heart Attack

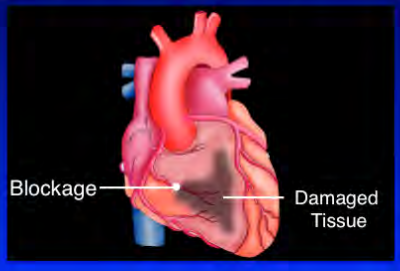
~ 1.5 Million People Have a Heart Attack Every Year

There was a Critical Need For a Drug for Heart Attacks

Thank you.
Désiré, it's such a pleasure and an honour to be here, to help celebrate your many accomplishments.
So instead of showing you a lot of data, I thought I would tell you the story of how Désiré and I met 28 years ago and how our chance meeting led to a very exciting collaboration together.
So the story began in May of 1980, when I was hired as my first job, by a small, very unknown biotech company called Genentech as their 60th employee.
Today, there's over 11000 people there.
It's one of the world's most successful biotech companies, in part due to the success of t-PA.
So Genentech focus back in 1980 was heart disease.
As you know, it's the single largest killer of men and women in both the US and Europe.
Every 20 seconds somebody has a heart attack and over a million and a half people have a heart attack every year.
So there was a real critical need for a drug for heart attacks.

My First Project in 1980

Try to Clone the Gene for a Protein that Would Quickly Dissolve Blood Clots in Heart Attack Patients



Rumors in 1980 About a Blood Clot Dissolving Protein called t-PA

Genentech Asked Me to Go to a Meeting in Sweden to Learn More About It

- I had been at Genentech *Only One Month*
- Barely Knew What Cloning Was

Young & Excited – Had a Passport

"Sure I'll Go"

June 12, 1980 in Malmo, Sweden

Ended Up Being in the Wrong Place at the Right Time

Met Désiré By Accident

World's Expert on t-PA

So my first project in 1980, when I got to the lab, was to try and clone a gene for a protein that would quickly dissolve blood clots in heart attack patients.
So there were rumours back in 1980 about a blood clot dissolving substance called t-PA and Genentech asked me to go to a meeting in Sweden to learn more about it.
I had been at Genentech only one month, I barely knew what cloning was but I was young, excited, I had a passport and I told my boss “Sure, I’ll go to Sweden”.
And on June 12 in 1980 in Malmo, Sweden, I ended up being in the wrong place at the right time and I happened to meet Désiré by accident, who was the world’s expert on t-PA.

An Accidental Meeting with Desiré is How Our Collaboration Began

Arrived in Sweden – Check Out Meeting Location a Day in Advance

Didn't Want to Get Lost & Be Late the Next Day

Hotel Clerk Mistake —

"Meeting of the Doctors — That Started Today!"

Ran to Meeting — Upset Thinking I Had Wrong Day

- Snuck in the Back of the Meeting Room…

Just as I Sat Down…

Désiré Walked Up to the Podium to Talk About t-PA

— During Break – Apologized for Being Late

Entered a *Private* Pre-Conference Session by Mistake of the Top Scientists in the Heart Disease Field

— I Wasn't Supposed to Be There —

If I hadn't Been so Time Conscious…
I May Never Have Met Désiré

They Didn't Ask Me to Leave When I Walked In Because…

They Thought I Was One of the Scientists Daughters …Waiting for Her Dad

- Invited For Dinner – I Met Désiré – We Talked About Trying to Clone t-PA

Wasn't Any Other Way to Get Enough Protein to Treat Patients

He Wondered Whether it Was Possible -

And I want to tell you about this accidental meeting, which is how our collaboration began.
So I arrived in Sweden to check out the meeting a day in advance because I didn't want to be late, I didn't want to get lost the next day.
My dad always said "don't be late, don't be late" so I wanted to check out the meeting and I got to the hotel to check in and the hotel clerk said, I asked her where this meeting was, like a walk over literally that day.
And she said "oh, the meeting of the doctors, that started today" and I got so upset because I thought I missed the first day of the meeting.
Genentech sent me halfway around the world, so I threw my suitcases in my room, I ran to the meeting and I peeked in the room and I saw about thirty guys, sitting around a big table and I was so upset because I thought I had the wrong day, I snuck into the room and set down.
And just as I sat down, Désiré had walked up to the podium and started to talk about t-PA.
And I got really excited because he said he had a cell line, he said he had an antibody and he had purified protein and this is exactly what I came here to hear.
During the break everybody kept looking back at me, they didn't know who I was.
Somebody came up to me and said "can I help you" and I said I'm Diane Pennica from Genentech.
I am so sorry I'm late for this meeting.
I missed the morning.
They said "oh this is not the real meeting, this is a pre-conference session".
The real meeting starts tomorrow and I had entered a private pre-conference session by mistake of all the top researchers in the heart disease field.
I was not supposed to be there.
And if I hadn't been so time-conscious, I may never have met Désiré.
And they told me later that they didn't asked me to leave when I walked in because they thought I was one of the scientist daughters waiting for her dad.
So I got really lucky.
So they invited me to dinner that evening, in a castle, a very beautiful dinner and I met Désiré at the time and we talked about trying to clone t-PA because there really wasn't any other way to get enough protein to treat patients.
And he wondered whether it was possible and because no gene that large had ever been cloned before, this was back in 1980 and I quickly told Désiré, being very naïve and excited, I said sure I can do it and I think because I was so enthusiastic, it helped convince him to collaborate with us to try and clone the t-PA gene.

I Quickly Told Désiré : "Sure I Can Do It"

I Think My Enthusiasm Helped Convince Désiré to Collaborate With us to Try and Clone the t-PA Gene

Should Admit → I Had *Never* Cloned Anything Before

I Had No Idea ***How*** to Do It or Even ***If*** it Could Be Done

Wasn't Going to Tell Désiré That...

Looking Back — Cloning t-PA was a Big Challenge

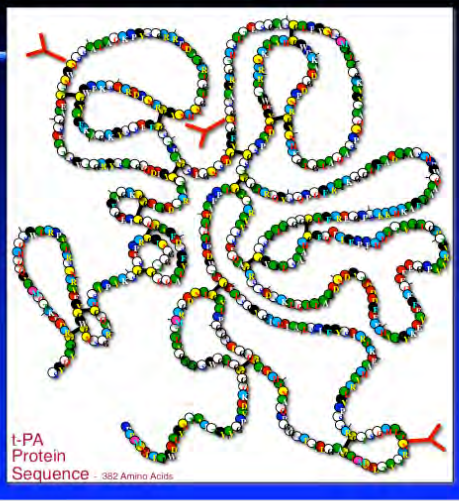
- ~~– Many Set Backs & Disappointments~~
  – A Lot of Competitors – A Race – Added to Stress & Excitement
- The Long Hours & Hard Work Paid Off – We Achieved Our Goal

Celebrating with My First Taste of Perrier-Jouet Champagne in our Clot Buster T-Shirts

1980: We Started Cloning Project

1982

We Determined the Structure of the Protein that Dissolves Blood Clots



And I have to admit, I had never cloned anything before.
I had no idea how to do it or even if it could be done, but I wasn't going to tell Désiré that.
I just, you know, I wanted to be enthusiastic.
And looking back, to make a long story short, it was a really huge challenge.
There were so many setbacks and disappointments.
We worked nonstop, I didn't take a day of for almost a year, there was a lot of competitors all around the world, which it actually became a race which added to all the stress and the excitement but the long hours and the hard work paid off because we achieved our goal.
And here you can see me celebrating with my very first taste of Perrier-Jouet Champagne in our clot buster T-shirts.
So 1980, we started the cloning project and in 1982 we determined the structure of the protein that dissolves blood clots and it's a very beautiful protein, shown here.
This was probably one of the most exciting times of my career.

1983: We Published Our Work in the Journal *Nature*

Reprinted from Nature, Vol. 301, No. 5897, pp. 214-221, 20 January 1983

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

Diane Pennica*, William E. Holmes*, William J. Kohr†, Richard N. Harkins†, Gordon A. Vehar†, Carole A. Ward†, William F. Bennett†, Elizabeth Yelverton*, Peter H. Seeburg*, Herbert L. Heyneker* & David V. Goeddel*

Departments of *Molecular Biology and †Protein Biochemistry, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA

Desire Collen

Center for Thrombosis and Vascular Research, Department of Medical Research, University of Leuven, B-3000 Leuven, Belgium

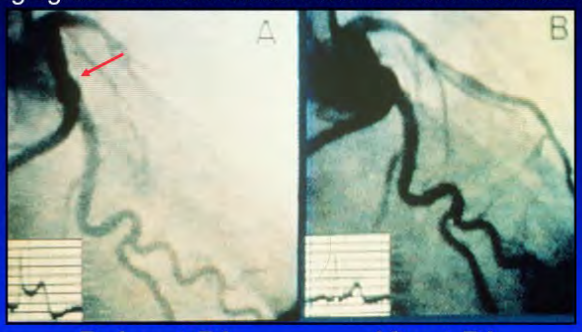
t-PA Appears as Refractile Particles in the Bacteria

1984: t-PA Was Produced in Large Quantities Genentech Named the Drug Activase

ACTIVASE

t-PA Dissolves Blood Clots in Blocked Arteries

Angiogram of Heart Attack Patient in the Clinical Trial



Before t-PA After t-PA

In 1983, we published our work in the journal Nature “Cloning and expression of human t-PA cDNA in the E.coli” and to this day, Désiré, I really don’t know how you got your name on a line by itself on a Nature paper, but that’s I think, a first. Shown here is the E.coli bacteria and these little refractile particles look like little headlights, that’s the t-PA being made in the bacteria.
And that was very exciting to see that too.
In 1984, t-PA was produced in large quantities and we named the drug Activase, we had a big contest to see what name we would give to the drug.
We found that t-PA dissolves blood clots in blocked arteries shown here, is an angiogram of a heart attack patient of the clinical trials, you can see the blood clot here, no blood flowing before t-PA and after t-PA, you can see with our recombinant material the blood is flowing freely.

November 13, 1987

7 Years After Désiré & I Met

It Was Approved by the FDA As a Drug For Heart Attack Patients

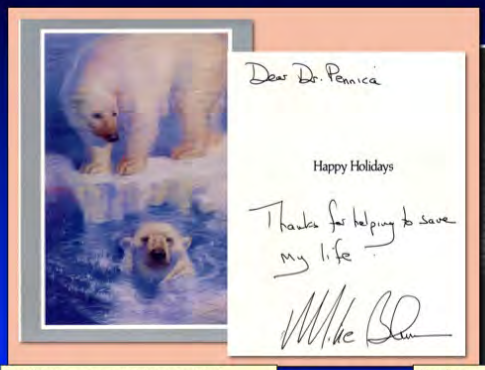
It Still Stands as one of the Fastest Drug Development Projects in History

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

Christmas Card From Heart Attack Victim --



Imagine How you would feel if you Got Something Like this ...

Needless to say-- This is what has kept me Excited about science for so many years

And November 13, 1987, another very exciting day for us, 7 years after Désiré and I met, it was approved by the FDA as a drug for heart attack patients and newspapers around the country broadcast us.
You can see the clot dissolving drug called a big step in coronary therapy.
It still stands as one of the fastest drug development projects in history.
And one of the highpoints in my career was meeting Steve Birnbaum, in the halls of Genentech.
I was walking down the hall from my lab one day and one of our marketing guys came up to me and said: “Diane, this is Steve Birnbaum”, he was the first heart attack patient treated with recombinant t-PA.
And my heart just leaped and then he said: Steve, this is Diane Pennica, she cloned t-PA and he just grabbed me and hugged me and said thank you, your drug saved my life.
Since 1987, close to 2 million heart attack victims have been treated with t-PA.
Here’s a Christmas card I have hanging in my office from one of the heart attack victims saying dear Dr. Pennica, and it should be Désiré and all the people here who contributed, happy holidays, thanks for helping to save my life.

1996: t-PA was Approved for the Treatment of Strokes

Every 45 Seconds Someone Has a Stroke
~700,000 People in Both the U.S & in Europe
Suffer From a Stroke Every Year

Since 1996:

> 150,000 Stroke Victims
Treated with t-PA

Francis Wagner
Stroke Survivor

Diane,

September 12, 2008

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 6th, 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'm 43 years old, I work at Genentech, I believe I'm having a stroke & I need t-PA...."

I would be honored to give you a tour of the Genentech Manufacturing Plant -or drive you to Belgium for that matter...

Rob

Rob Lippe
Stroke Survivor

"Thank you again so much from Catie, Britton and I"

Thank You Désiré … My First Project at Genentech
Was the Most Exciting Time of My Career

You Helped Made Our Dream of Developing
a Drug for Heart Attack and Stroke Victims Come True

I Wish You Happiness &
Exciting New Adventures in the Years to Come

Diane

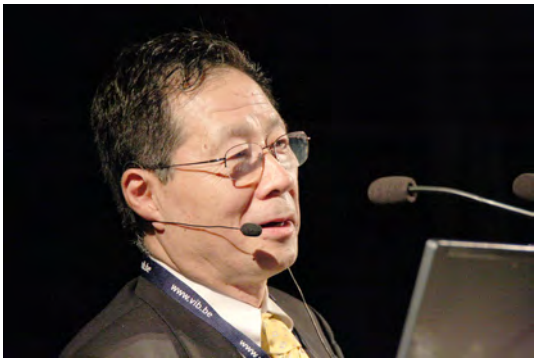
In 1996 t-PA was approved for the treatment of strokes. Every 45 seconds somebody has a stroke, over 700 000 people in both the US and in Europe suffer from a stroke every year and since 1996, over 150000 stroke victims have been treated with t-PA, our recombinant material.
And for my last slide I'd like to read an e-mail that I got just three weeks ago, and again it should be addressed to everybody here who contributed and the e-mail says:
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 October 6th, 2007 would have been a day where my life was significantly altered (or worse).
I can only imagine what the nurse must have thought when I stumbled into the emergency room (with one hand over my eye) and said: "Hi, my name is Rob Lippe, I'm 43 years old, I work at Genentech, I believe I'm having a stroke and I need t-PA.
I would be honored to give you the tour of the Genentech Manufacturing Plant – or drive you to Belgium for that matter."
So, thank you Désiré, my first project at Genentech was the most exciting time of my career.
You helped make our dream of developing a drug for heart attack and stroke victims come true and I wish you happiness, exciting new adventures and more discoveries in the years to come.
Thank you.

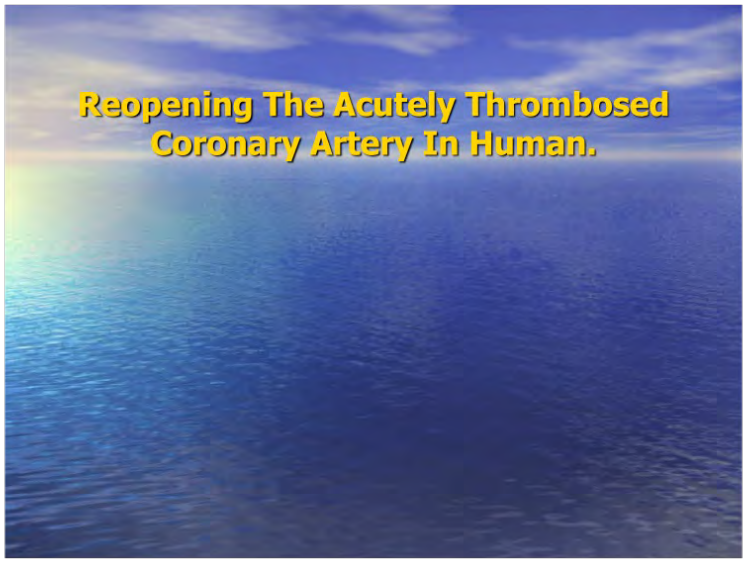
tPA – Affinity to Fibrin – Localized Plasminogen Activation

- An affinity between tPA and fibrin was first suggested by Astrup and coworkers in the early seventies. However, no solid experimental data was presented.
- In the summer of 1975, I worked with the development of a caseinolytic assay for tPA, in collaboration with Per Wallen. Practically no activation of plasminogen occurred, in spite of high concentration of active tPA. However, by addition of a tiny amount of washed fibrin, a rapid caseinolysis occurred. Activation of plasminogen took place at the fibrin surface.
- In the autumn of 1977, as visiting professor in Leuven, I convinced Désiré Collen that tPA indeed was fibrin specific.
- A few weeks later, Désiré already had the goal to develop tPA as a new thrombolytic drug.

Our next speaker is Hiro 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




Reopening The Acutely Thrombosed
Coronary Artery In Human.


Reopening The Acutely Thrombosed
Coronary Artery In Human.
A path to a new era of effective
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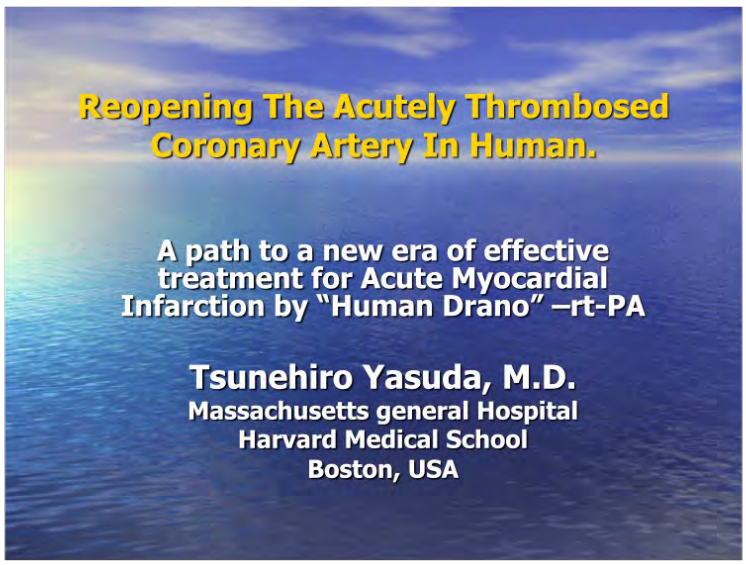
Infarction by "Human Drano" –rt-PA


Reopening The Acutely Thrombosed
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A path to a new era of effective
treatment for Acute Myocardial
Infarction by "Human Drano" –rt-PA
Tsunehiro Yasuda, M.D.
Massachusetts general Hospital
Harvard Medical School
Boston, USA

Good afternoon.
Thank you very much for this invitation.
My name is Dr. Yasuda.
I am from Boston and I like to share our t-PA story.

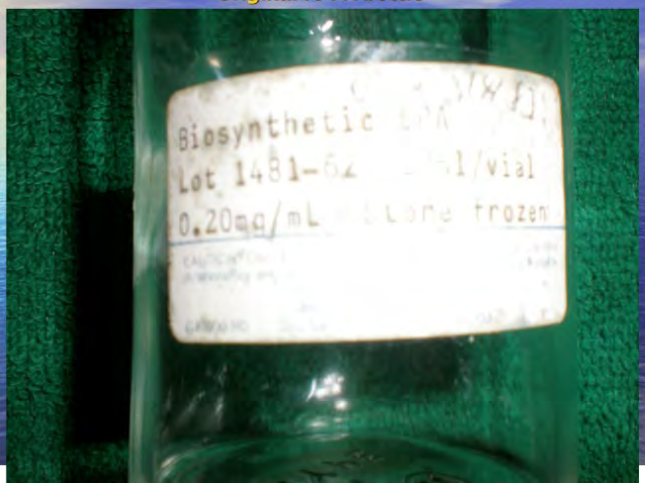
Herman K. Gold, M.D.

Massachusetts General Hospital
Harvard Medical School

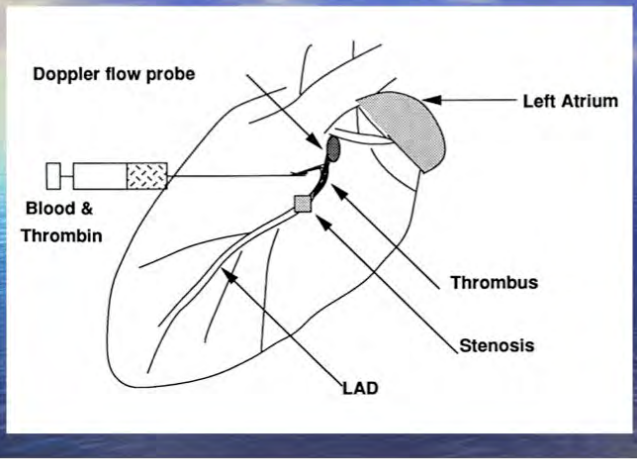
Associate Professor of Medicine in Cardiac Unit

Thrombolysis Treatment Era

Original rt-PA bottle

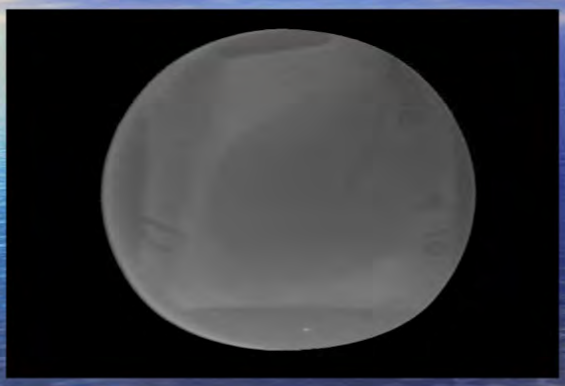


The LAD Dog Model



My talk can not be done without Dr. Gold, who unfortunately left us, several months ago.
He was the principal investigator.
I was the junior staff and worked with him and with Désiré.
We had a great time.
This is a bottle I think Diane's company made it and Diane cloned it, this is rt-PA, recombinant t-PA, we tested it in animals and later in human.
Well this is a dog model we concocted, this is an exposed dog heart and this is the LAD, this is a stenosis and we injected blood then thrombin in this section and this is a flow meter, continuously monitor the flow and also we confirm the finding with coronary angiogram,

Canine Coronary Angiogram



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)

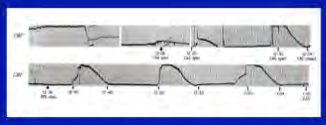
Human Acute Infarction Trial

Circulation 70, 1012-1017, 1984

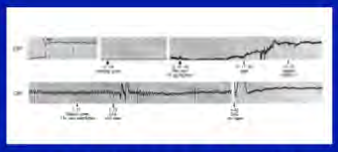
- 25 /33 Patients
  - with 0.5-0.75mg/kg of t-PA opened < 90min
- Only One patient /14-Control-saline patients
  - Opened, 13 remained closed
- The thirteen crossed over to t-PA,
  - 9/13 opened <45 min
- Circulating fibrinogen decreased by only 8% of the baseline
- Confirms the dose, safety and efficacy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

This is the LAD, this is occluded position right there and LAD goes this way will come up.
After 20 or 30 I can't see where and in this area begun to show some fuzz, showed something showing up, but still I couldn't see the artery very clearly.
And this is the thirty, something there, but again oh I can see something and I think probably be perfused and the flow meter shows clear flow and now it is very clear and we are very excited and this is a sixty minutes and then a very clear still maintain the perfusion, so we know that this really worked and we are excited.
Well this is a dog model.
This is a dose response, this is 4.3 μg per kg per minute, this is a 10, this is a 15, this is a 25 and Désiré quickly pointed out the reperfusion time is really getting better.
So I think this is a great dose and then we moved on to dog randomization trial.
We are blinded and we give 1 dog saline, an other 1 dog t-PA and t-PA opened in about 30 minutes.
Obviously saline opened only 1 and rest of them didn't do anything.
So we are stunned.
Now we expected, but this was fantastic data and we had to do human.
Dogs are fine, but I really wanted to try in human.
Look-alike but a little different.
And basically we did many patients, but same results.
Again randomized and one group was saline group which crossed over, didn't open but again 9 out of 13 open.
This was published in Circulation 1984 acute myocardial infarction with t-PA treatment was very successful.
One I have to tell you, we really wanted to do this, because we had to go fast.
Désiré was saying, never seen Désiré slow, always fast.
So like you had to go fast ... so obvious Dr. Gold said we have to go fast, so comes down to meet to get the patient.

Cyclical occlusion and reperfusion

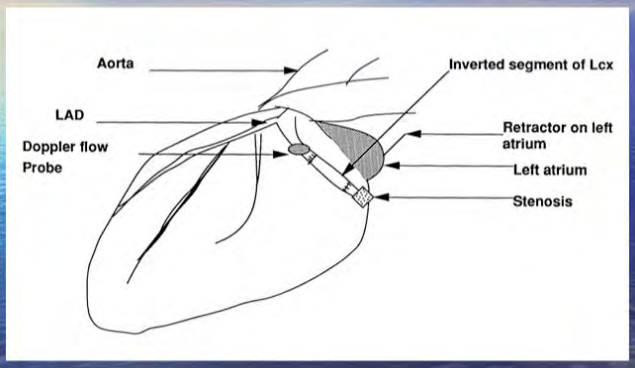


rt-PA and heparin

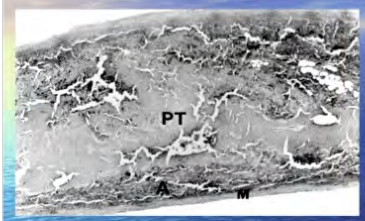


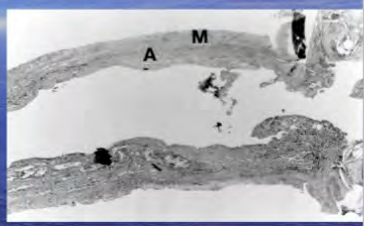
rt-PA, Heparin & GpIIb/ IIIa Blocker

The Circum Flex Artery Dog model



Pathology



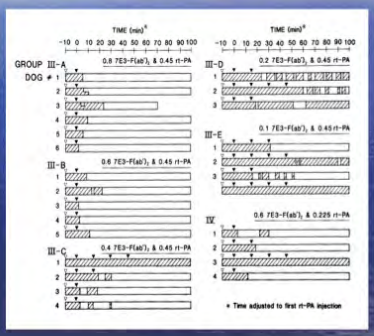


- Top Left_ Thronbosed LCx
- Top Right: Opened, Clean Lcx
- PT:Platelet rich Thronbus
- A: Advetitia, M: Media

Patients, don't come by appointment, they have to have a heart attack first and have to come to our hospital.
So how do we found out?
You know, it is very difficult and they come to emergency room, next thing you know they go CCU and then you missed it, so how do I find this patient?
And our ex-chief resident, currently Novartis' chairman, director of research and development, Mark Fisherman recommended just to give pizza.
What do you mean "pizza"?
You know, if you call just give us a pizza.
No, they are smart doctors from Harvard we buy with pizza?
You know, so I decided try it so you know I can't go at the pizza shop and calling in the middle of a heart attack, that is a very stressful, so I decided give us a pizza chit, you know the little card, give one they can order by telephone, give us a pizza card to the pizza delivery man.
So that is how we did it.
So that is how we got one acute MI patient, perfectly fit to the t-PA study.
Otherwise, we would have taken much longer time.
This is an important thing that we realize once open the heart, coronary artery, but occlude again a couple of minutes to hours, so we even make the effort to open.
We could not keep it open for a long time
And this is a dog experiment we confirmed.
This is a normal flow we occluded it and watch this is all closed given t-PA and some opening and a perfect opening, this is a flow meter's recording and this is closed completely, popped open again and closed, popped open and closed.
So this is really not helpful, but later we give the pre-treatment of the antithrombotic agent slowly opens it doesn't pop open and stays open.
So this is called a cyclical occlusion of flow and we have to try this kind of treatment to the human.
So to do that we developed this special model called circum flex model, because a dog does not have right coronary to talk about and LAD is not easy to do.
A dogs circum flex are big, so we segmented this part to removed inverted it inside out these stitched so that the adventitia inside of the arterial lumen and give a stenosis in the distal portion and the flow probe here to monitor the flow and it did the same thing, but to do this we have to close here cramp off and the cramp off so that the blood would be shut off for a while.
So we had to operate very quickly.
And when you open this segment, release the clamp, blood clots develop in the matter of 2 minutes, complete occlusion just incredible and incredible part is that clot is platelet rich, white clot and it was just remarkable and this kind of clot sits there and t-PA wouldn't work very well.
So this is a pathology, this is a just I talked about this a complete open occlusion by platelet rich white clot here and this is a pretreated with antiplatelet agent a platelet GPIIb/IIIa blocker completely patent after 3 hours of observation.
M means media and A means adventitia this is an inverted segment of the circumflex.

Rapid and Sustained Coronary Artery Recanalization

- rt-PA only
  - **33±15** min
  - Reocclusion cyclical
- rt-PA and 7E3-$(Fab')^2$
  - **6±3 min**
  - No reocclusion



Thank You

And this is the data.
Rapid and sustained coronoray artery recanalization.
I think it is important open fast when the blood arteries are closed and also we have to keep it open.
So when you use two together reperfusion time is much shorter and remained open.
And this is a graph that published, this is reperfusion time, so very quickly reperfusion in this area and never closed.
This is never open.
The doses gradually get smaller and GPIIb/IIIa reperfusion becoming problematic and the cyclical flow appears.
So apparently in the United States often, routine that the patient comes with loaded with GPIIb/IIIa then come to hospital give t-PA.
I don't know why they don't give it over their, but they come like this and we see like one or two a week and as Diane mentioned we have lots of t-PA for the ischemic stroke patients.
And thank you and Désiré happy many years and productive second heart career.
Thank you.

tPA – Affinity to Fibrin – Localized Plasminogen Activation

- An affinity between tPA and fibrin was first suggested by Astrup and coworkers in the early seventies. However, no solid experimental data was presented.
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- In the autumn of 1977, as visiting professor in Leuven, I convinced Désiré Collen that tPA indeed was fibrin specific.
- A few weeks later, Désiré already had the goal to develop tPA as a new thrombolytic drug.

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 actually coordinated the European clinical studies with recombinant t-PA and that is what he will tell us about.

MARC VERSTRAETE

European Cooperative Study Group on alteplase (rt-PA)

E.C.S.G.
1985-1994
Chairman: M. VERSTRAETE

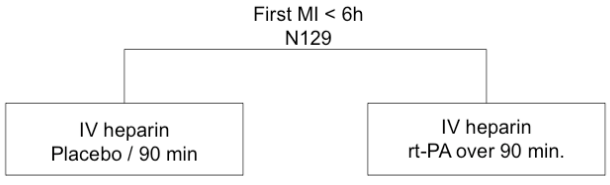
European Working Party on Streptokinase (SK) 3 Mortality trials

EWP-1: 167 patients with MI < 72h. SK/72h VS heparin / 72h

EWP-2: 764 patients with MI < 24h. SK/24h. VS heparin / 24h mortality at 40d: 30%

EWP-3: 514 patients with MI < 12h. SK/24h. VS glucose / 24h mortality at 6 mths: 49%

M. Verstraete J.Intern.Cardiol. 1995; 8:611-621

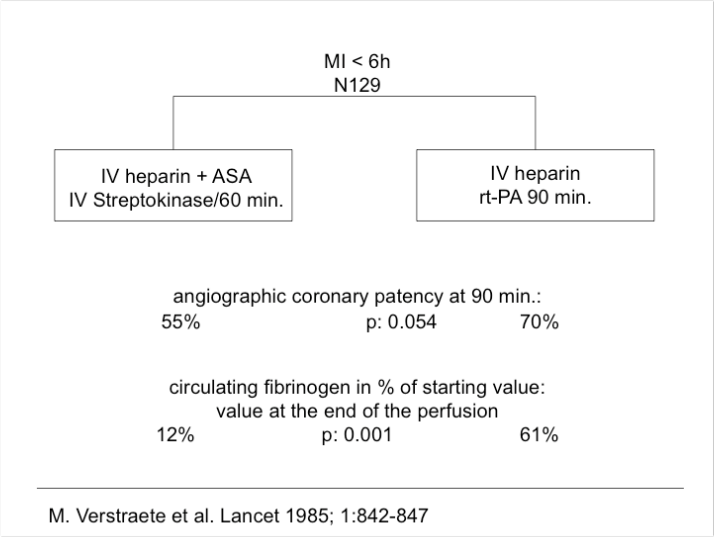


coronary patency
at 90 min.:

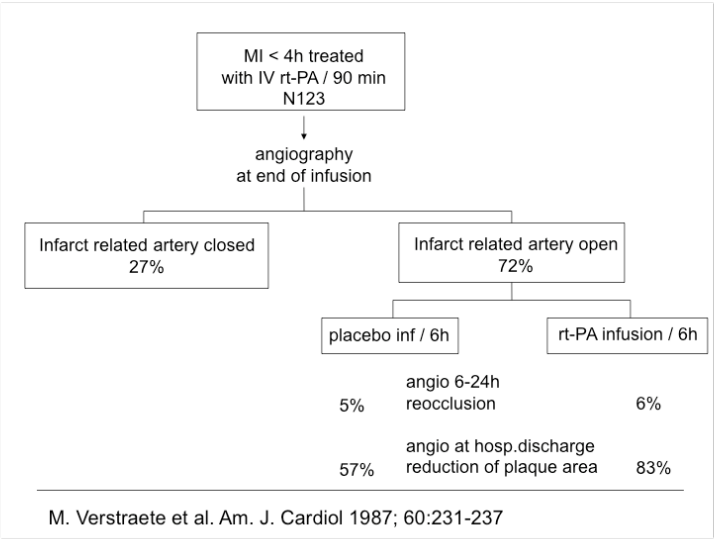
21% 2p: 0.0001 61%

M. Verstraete et al. Lancet 1985; 2:965-969

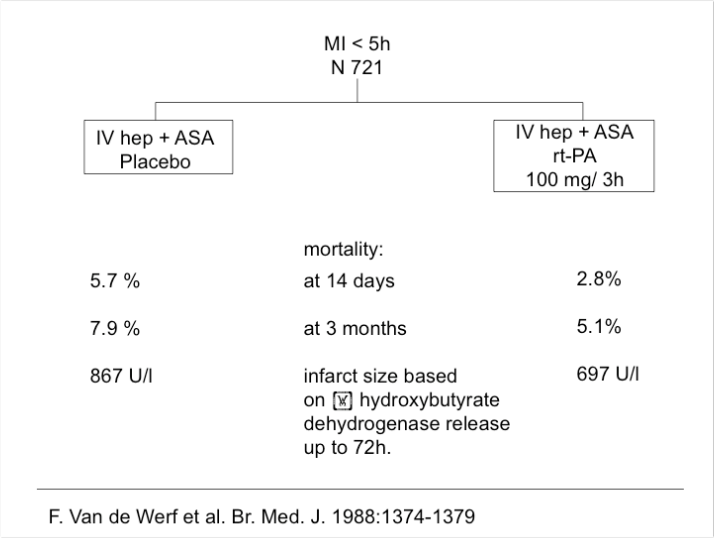
Désiré, ladies and gentlemen, thank you very much for suggesting my presence here.
Now, when recombinant t-PA became available in Europe, I started to call on a number of friends who have been joining me for another series of trials, not on t-PA but on streptokinase and you see here, that we could collect a certain number of patients who were treated 72 hours after myocardial infarction with streptokinase, administered during 72 hours versus heparin.
Now that trial did not show a difference, in the two groups.
When we took patients who had a myocardial infarction of less then 24 hours and gave them streptokinase, then there was a decrease of mortality, 24-day mortality of 30%.
Our next trial was still more remarkable because we could select patients of less than 12 hours duration.
They got streptokinase or glucose and the 6-month mortality was close to 50%.
This is a remarkable decrease in mortality and although this trial was published in a decent journal, actually The New England Journal of Medicine, it did not attract the interest of cardiologists, very much to our surprise.
Now, when recombinant t-PA became available in Europe, we continued the European cooperative study group and the very first trial was on the limited number of patients, 129, who were randomized either to t-PA, given over 90 minutes or to placebo.
And at 90 minutes after beginning of the infusion, an angiogram was made and you see a remarkable difference between the patency at that time in the control group 21% versus 61% in the group receiving t-PA.
Of course, that was a remarkable statistical difference.



M. Verstraete et al. Lancet 1985; 1:842-847

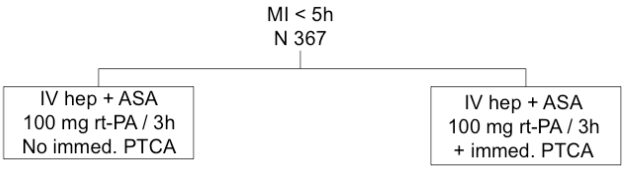


M. Verstraete et al. Am. J. Cardiol 1987; 60:231-237



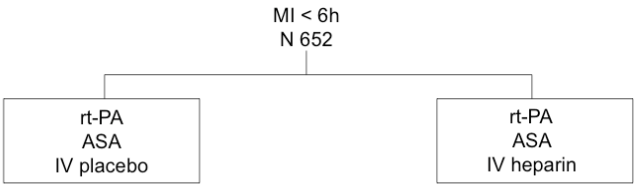
F. Van de Werf et al. Br. Med. J. 1988:1374-1379

So, we moved to the second European trial, which was a comparison between t-PA plus heparin but no aspirin versus streptokinase plus heparin and aspirin.
Actually at that time, we did not dare to add aspirin to this combination and again we were making angiograms at 90 minutes and you see that also here we had a remarkable difference, although less great than in previous trials, in the t-PA group.
And also what we noted, was that there was a saving of circulating fibrinogen.
In the streptokinase group the circulating fibrinogen fell to 12% of the original value or pre-treatment value.
But 61% of the pre-treatment fibrinogen level was maintained in the t-PA group.
So, t-PA was indeed safer than streptokinase and also more active.
The third question that we wanted to solve was whether we have hit the right dose of t-PA or whether a prolonged infusion of t-PA would be better than the single infusion.
To this end we have collected 123 patients who had an angiogram at the end of t-PA infusion.
Based on the angiogram, we found that 27% had still a closed coronary artery, but that 72% had an open coronary artery.
Then we gave a second infusion of t-PA to those with an open coronary artery and found that with this second infusion, the re-occlusion rate at between 6 and 24 hours was practically the same.
So the conclusion was that the prolongation or the addition of a further dose of t-PA to avoid the occlusion was not, demonstrated.
It is important of course to reopen a closed coronary artery, but still more important is to decrease mortality.
In this trial we have used t-PA heparin and aspirin compared to placebo instead of t-PA, but presence of heparin and aspirin and you see that at 14 days, the overall, the total mortality was half in the t-PA group compared to the control group.
There was also saving of lives at three months, in the t-PA group compared to the control group.
And also the infarct size was smaller in the t-PA group compared to the control group.



13%	angiographic re-occlusion 6 -165 min after end of infusion	18%
3%	recurrent ischemia during the first 24h	17%
11%	occluded IRA at end of hospital stay	13%
3%	hospital mortality at 14 days	7%
3%	hospital mortality at 3 months	8%

M.L. Simoons et al. Lancet 1988; 1:197-202



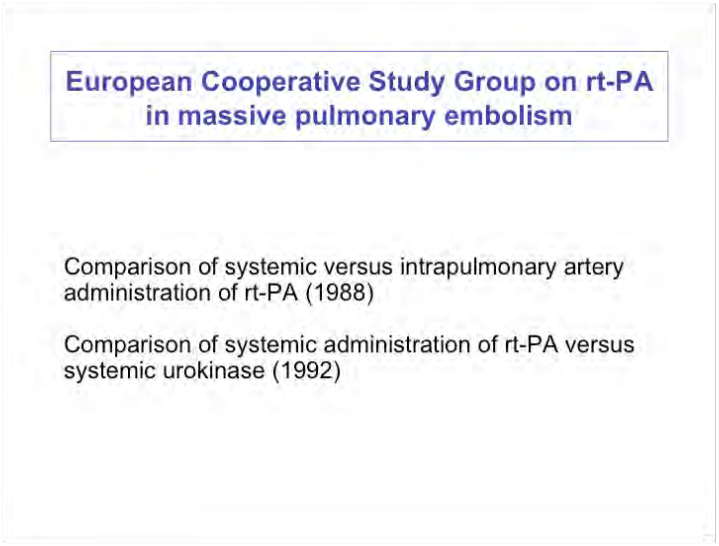
75%	coronary patency at 48 - 120h after randomisation	84%
672 U	enzymatic infarct size (Q_{72} HBDH)	646 U
2.9%	hospital mortality	1.6%

D.P. de Bono et al. Br. Heart J. 1992; 67:122-128

The six trials of the ECSG enrolled 2121 patients with acute MI including 1 to 3 coronary angiograms.

Six princeps plus 18 ancillary publications.

Then, we moved to the next trial where we gave everyone heparin and aspirin and then 100 mg of t-PA in three hours in this group and we also had an immediate, PTCA.
This group was randomized not to receive or to have immediate PTCA.
You can see that the angiographic occlusion between 6 and 165 minutes after the beginning of the infusion was no different between the two groups.
Also ischemia during the first 24 hours, was actually more frequent in the t-PA group than in the control group.
The mortality was 50% higher in the t-PA with immediate infusion compared to the control group.
So the conclusion was that there was no gain when you use IV heparin, aspirin and t-PA to have immediate PTCA indeed, that the situation could even be worse.
And then, the next trial we tried to solve the question how the coronary patency would be between 48 hours and 120 hours after randomization to t-PA, aspirin and heparin, or t-PA, aspirin and placebo instead of heparin.
You see that the incidence of coronary patency is practically similar, although slightly higher in the heparin group.
And that the infarct size also was smaller in the IV heparin group plus t-PA plus aspirin.
The hospital mortality was also lower in this combination group compared to this group where heparin was replaced by placebo.


European Cooperative Study Group on rt-PA in massive pulmonary embolism
Comparison of systemic versus intrapulmonary artery administration of rt-PA (1988)
Comparison of systemic administration of rt-PA versus systemic urokinase (1992)

A further endeavour, but I will not extend on that, was on pulmonary embolism where we made an European trial comparing the systemic versus the intrapulmonary administration of t-PA and the conclusion was that systemic administration was as good as intrapulmonary administration of t-PA.
The second trial was in patients with a severe pulmonary embolism where rt-PA was compared to systemic urokinase and there was no significant difference between these two groups.
Of course, this indifference where European endeavours on a limited number of patients and could not compete with the American trial, the TIMI trial, the TAMI trials which were conducted on very large battalions.
But certainly the two sets of trials were comparable, lead in general to the same conclusion and had put the whole t-PA story at the clinical level in a minimum of time.
Thank you very much.

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- In the autumn of 1977, as visiting professor in Leuven, I convinced Désiré Collen that tPA indeed was fibrin specific.
- A few weeks later, Désiré already had the goal to develop tPA as a new thrombolytic drug.

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

The t-PA story

Overview of US Studies with rt-PA in AMI

- a brief account

David Stump

0

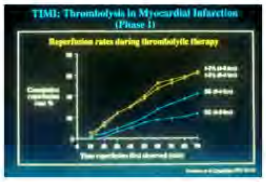
The TIMI Trials

- Phase 1: Angiographic findings

  The New England Journal of Medicine Volume 312, pp. 932-936, 1985

- Phase 2: Manufacturing process change dose finding and testing adjunctive benefit of early versus late angioplasty and beta-blockers

  The New England Journal of Medicine Volume 320, pp. 618-627, 1989



rt-PA is superior to streptokinase in opening previously occluded coronary arteries.

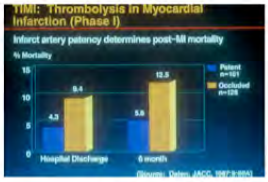
Intracranial hemorrhage rates unacceptably high with rt-PA at 150 mg dose. Rates at 100 mg are acceptably lower and accompanied by favorable overall mortality rate.

1

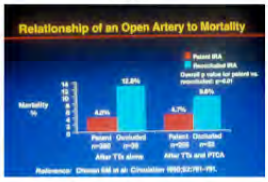
Thank you very much Roger.
What a pleasure to be here on this great occasion as I continue on with the theme that was started by professor Verstraete.
I would like to now talk about what was a little bit of parallel universe that was ongoing in North America at this time and then come back with what turned out to be the ultimate moment, I think in the story where not the North American trial was done, but truly international effort was undertaken to really establish the potential of t-PA.
My return to the US in 1986, following my two years here in Leuven, I began my academic career at the University of Vermont and in fact my collaboration with Désiré continued there as we became closely involved with two very active US clinical research group, one, the TIMI investigators led by Eugene Braunwald. This was an NIH sponsored group and the TAMI investigators led by at that time the very young Eric Topol and Rob Califf a private non-government sponsored academic clinical trial consortium.
We also, from Vermont, did support studies that were ongoing as described earlier with Chip Gold.
Désiré pretty much took the lead and managing a collaboration.
I took the lead in working with Rob and Eric and together we tried to figure out how we were going to work with TIMI and it turned out to being important piece of work when all was said and done.
This was a really interesting advantage point that we observed clinical trial results emerging from.
From the TIMI trials there were two key findings.
In the intent of time, I will not take you through every single piece of data.
First in a relatively small randomized trial which compared t-PA to streptokinase, reperfusion of occluded coronary arteries was superior with our t-PA and very interestingly relatively independent of the age of the occluding thrombus.
Second a much larger outcome study was proceeded by a dose finding phase. This was TIMI II.
This had been necessitated by a manufacturing process change for rt-PA at Genentech and during this time it was observed that a dose of 150 mg actually appeared to offer more efficacious thrombolysis so in fact this larger second phase of the study which was not a comparative thrombolysis study, but was a study to test the impact of early versus late intervention with angioplasty on beta-blockers, but after about 900 patients that actually have been treated it became clear that the risk of intracranial haemorrhage at a 150 mg was unacceptably high.
As a consequence the dose for further study was lowered to a 100 mg resulting in an acceptably lower risk of 0.5 % and a very attractive overall mortality of 4.5 %.
As a result the enthusiasm for t-PA became very high among the many TIMI investigators and spread similar among practising cardiologists in the United States after its approval by the FDA for use in 1987.
The beneficial effects of rapidly opening an occluded coronary with t-PA were felt to be obvious.
An exploratory research on characterizing and optimizing coronary patency ensued.

The Nature of the Open Artery with rt-PA

TIMI

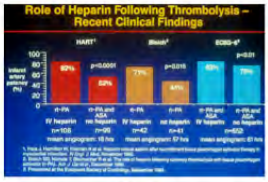


TAMI



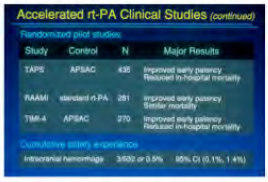
Long term mortality improved with achieving and sustaining coronary patency.

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.

2

Crisis in 1990

- Two large multinational mortality trials either completed or nearing completion (GISSI-2 and ISIS-3)
- Compared rt-PA to streptokinase in acute myocardial infarction
- Used minimal concomitant heparin anticoagulation
- Did not utilize new accelerated dosing with rt-PA
- Showed no incremental mortality benefit for rt-PA at 10 times the cost

3

Results from these studies, shown in this slide, were interesting, in fact would have great importance in shaping advance that were to follow, though we didn't understand that fully at the time.
First the predictive value of an open artery seem consistently positive based on exploratory analysis of both the TIMI and TAMI data.
The magnitude of the effect were comparable with the sustained reperfusion was approximately twice the mortality for initial reperfusion followed by re-occlusion and approximately three to four times the mortality for failed reperfusion.
Second the risk of re-occlusion following successful reperfusion was shown in three independent studies.
One done here in Europe to be significantly higher when intravenous heparin was not utilized concomitantly with t-PA.
Third and maybe most importantly accelerating the administration time of a 100 mg of t-PA from the standard 3 hours by 1 half to 90 minutes was shown in three different studies to improve early coronary patency rates especially those measured at 60 minutes of infusion time.
There did remain concern about the potential enhancement of risk of intracranial haemorrhage with this approach, while the observe rate in the pooled the experiments with these small patency showed only a 0.5 percent risk. The relatively small sample size could not allow the excess risk seen in TAMI II at an 150 mg to be comfortably excluded.
Further research to confirm and expand on these interesting findings then waned as the field awaited the first reporting of data in early 1990 of large multinational mortality trials comparing our t-PA either alteplase or duteplase to the much less expensive streptokinase.
Importantly both the GISSI-2 and ISIS-3 trials used minimal concomitant heparin anticoagulation as well as the standard non accelerated dosing of our t-PA.
When results of both studies showed no incremental mortality benefit for rt-PA and those of you who were with me at that time will I think agree this represented a true crises for the future of rt-PA and I might say for Genentech absent further clarifying clinical research.
While sharing in the disappointment at this unexpected turn of events for a concept in which I was certainly scientifically vested, I found myself confronting a rather personal dimension of the situation since I had in fact moved to Genentech in 1989 to lead the further clinical development of t-PA.
As a trained hematologist hoping to study new thrombolytic approaches to other thrombotic diseases in addition to acute myocardial infarction, I suddenly found myself facilitating a discussion among most influential positions in the world of cardiology, such as our colleague Frans Van de Werf who you have heard from earlier along with some of the keenest business minds in the world of science and technology all focused on what exactly to do next.
There quickly emerged the consensus that absent a more definitive study, rt-PA would be relegated to afterthought status, given the impossibility of a reduction in its price.

The GUSTO Trial
Efficacy

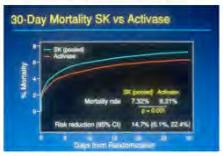
- Mortality

The New England Journal of Medicine Volume 329, pp 673-682, 1993

- Angiographic Substudy

The New England Journal of Medicine Volume 329, pp 1615-1622, 1993

Four arm study in 41,021 patients primary end point all cause mortality.



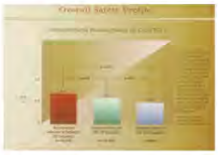
Mortality significantly reduced by accelerated rt-PA versus streptokinase.

Early coronary patency higher with accelerated rt-PA. Measures of LV function paralleled early patency. Mortality lower with early patency

4

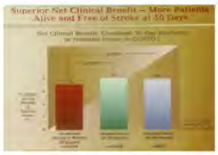
The GUSTO Trial
Safety

Intracranial Hemorrage



Risk of intracranial hemorrhage was significantly higher with accelerated rt-PA.

Net Clinical Benefit



Despite the increased risk of bleeding the chance of being a stroke-free survivor was significantly greater with rt-PA.

5

The t-PA Story

GUSTO trial implications

- rt-PA established as the leading thrombolytic for treatment of acute mycardial infarction
- Timely coronary reperfusion established as the mechanism for benefit
- An innovative premium-cost therapy shown to be cost-effective
- "Cutting edge" science and technology prevailed
- Genentech (and biotechnology) thrived

6

Shortly there are after the design of the GUSTO trial was conceived in the substantial effort for its implementation was initiated.
The design and results of the trial had become a landmark.
Undoubtly the most difficult decision I personally had to make for the trial was to support the useful accelerated dosing regimen for t-PA.
My own quantity modelling of the relationship of coronary artery patency status and mortality suggested that in fact both the use of accelerated t-PA and concomitant heparin would likely be required to achieve our target of an absolute 1% mortality reduction for t-PA.
This 1% benefit was critical as it represented the magnitude of benefit that would be minimally clinical meaningful to practising cardiologists.
However the uncertainty around the safety risk of accelerated t-PA wouldn't be comfortably addressed until nearly half of the GUSTO trial patients had been treated.
I anxiously watched the trial unfold over the next three years until results from the 41,021 patient study emerged in early 1993.
The results showed that all cause mortality was significantly reduced for t-PA versus streptokinase followed by either intravenous or subcutaneous heparin.
Furthermore a nested cohort angiographic sub-study confirmed higher coronary patency with accelerated t-PA and parallel improvement in measures of left ventricular function and mortality.
The safety results did show a small but significantly increase risk of intracranial haemorrhage with accelerated t-PA.
But when integrated into a combined net clinical benefit endpoint of stroke-free survival, the benefit of t-PA was still statistically significant.
The implications of the GUSTO trial results were far reaching.
rt-PA was established as the leading thrombolytic for the treatment of acute myocardial infarction.
This was clear.
It's mechanism of benefit was demonstrated to be timely coronary reperfusion.
An innovative premium-cost therapy was shown to be cost-effective.
In a sense of more broadly cutting edge science and technology had prevailed as a basis for translational medicine.
Finally and importantly for some of us Genentech and in a way biotechnology itself would survive if not thrive.
So let me now say way the what is perhaps a bit more difficult challenge describing history is one thing, interpreting it is quite another.

The t-PA story

Reflections on translational research between academia and industry

Relationship to the development of rt-PA

David Stump

0

The t-PA Story

Four key players

- Science
- Medicine
- Industry
- Government

Questions

- Could it have happened without all of the first three?
- Is this a basis to call for more such collaborations?

1

The t-PA Story

If there were no science

- Streptokinase and urokinase would be the available thrombolytics
- Mortality of patients with acute MI would be lower but still at a residual cost of 1 life per hundred patients treated
- Thrombolytic therapy would not be available for patients with early acute ischemic stroke at a cost of 10 disabilities per hundred patients treated

2

The t-PA Story

If there were no medicine

- rt-PA would be a niche thrombolytic for a few patients with deep venous thrombosis and pulmonary embolism
- Reperfusion therapy would not be widely applied for acute MI at a cost of 3.5 lives per hundred patients treated
- Reperfusion therapy would not be applied for early acute ischemic stroke at a cost of 10 disabilities per hundred patients treated

3

I've been asked though to reflect on translational research, how it takes place between academia and industry, and related specifically to the development of the t-PA story.
It is a story that we have chronicled here today and in fact it has been widely discussed by many in academic science and medicine as well as industry.
This is not the first time the story of t-PA has come up.
Although I most certainly was not there from the very start, my years in Leuven, at the University of Vermont and finally at Genentech have together offered me a rather unique perspective developed from these somewhat diversed vantage points.
It's one of the few who engaged the story from both the academic and industry sides.
I have on occasion been asked to reflect on it.
Actually the last such time was the gathering of chairs of US biochemistry departments, invited by our colleague and friend Ken Mann at the University of Vermont.
For whom I used what I think was an interesting way of get out the question of whether the t-PA story is in fact so compelling as to represent a basis for a call to such collaborations as a standard, in other words do we really have a whole which is greater than a sum of its parts?
Very simply I've asked what would have been the outcome had anyone of the key contributors not been part of the story?
A theoretical knock-out experiment if you will.
So could it have happened as it did without the involvement of science, medicine or industry?
I will leave the topic of government to another time, but it is certainly had its moments in the story.
We consider first the scenario of the absence of science.
What if there had been no science and in this case by science I'm talking about the key biochemical and biological contributions made by many in this room to the full understanding of fibrinolysis and thrombolysis.
Streptokinase and urokinase would be the currently available thrombolytic agents.
The mortality of patients with acute myocardial infarction would be lower than without them but still at a residual cost of 1 live per 100 patients treated.
And thrombolytic therapy would not be available for patients with early acute ischemic stroke at a cost of 10 disabilities per 100 patients treated.
Consider if you will the scenario of the absence of medicine.
In this case I am talking about invasive cardiology and neurology which elucidated the key role of arterial thrombosis in the pathogenesis of both acute myocardial infarction and ischemic stroke.
Today rt-PA would been a niche thrombolytic for a few patients with deep venous thrombosis and pulmonary embolism.
Reperfusion therapy would not be widely applied for acute myocardial infarction at a cost of 3.5 lives per 100 patients treated.
And reperfusion therapy would not be applied for early acute ischemic stroke at a cost of 10 disabilities per 100 patients treated.

The t-PA Story

If there were no industry

- t-PA would be a novel scientific discovery of perhaps some pathophysiologic relevance
- The cost to patients would be as if t-PA had never been discovered at all
- The effort to discover and develop additional innovative therapies by Genentech would have had to be underwritten by other sources

4

The t-PA Story

Conclusions

- No, it could not have happened as it did without all three
- Yes, it is a model which can work well provided the nature of relationships is very clear and conflict of interest is avoided

5

The t-PA Story

If there were no Désiré Collen

- Would not have happened with the urgency and efficiency that it did, if at all
- My own life and career would have been much less interesting and gratifying over the last 25 years
- Fewer patients with acute MI and stroke would be alive and well

6

Lastly let's consider the scenario the absence of industry.
Specifically the contribution of biotechnology to the cloning, expression and production of an otherwise relatively unavailable trace protein in nature.
t-PA would be a novel scientific discovery of some pathophysiologic relevance, but the cost to patients would be as if t-PA had never been discovered at all.
And consequently the effort to discover and develop additional innovative therapies by Genentech would have had to be underwritten by other sources.
And in order to put some context on this, let me just remind you that this would have been perhaps realised at a cost of not having drugs like Ritaxin, Herceptin, Avastin truly cutting edge therapeutics in medicine today.
Taking all together my own conclusion that it most certainly could not have happened as it did without the contributions of all three.
And yes it is a model which can work well provided the nature of relationships is very clear and conflict of interest is avoided.
But finally because we are gathered here today to celebrate the career of our teacher, collaborator and friend Désiré, allow me to consider the most unfortunate scenario without Désiré Collen.
One last theoretical knock-out if you will.
I am absolutely convinced that the t-PA story would not have happened with anywhere near the urgency and efficiency that it did if it all.
I am even more convinced that my own life, and no doubt many of you will echo this, would have been much less interesting and gratifying over the last 25 years.
But most critically fewer patients with acute MI stroke would be alive and well today.
It is most fortunate that this last scenario will never materialize beyond the hypothetical and for that we on behalf of these patients are grateful and do celebrate here today.
Désiré our congratulations, our thanks, well done, it's a great story, I'm proud to have been a part of it.
Thank you for your attention.

ROGER LIJNEN

It is my privilege to invite a very special young lady to the stage, which is Juliette, the youngest of Désiré's grand children.

tPA – Affinity to Fibrin – Localized Plasminogen Activation

- An affinity between tPA and fibrin was first suggested by Astrup and coworkers in the early seventies. However, no solid experimental data was presented.
- In the summer of 1975, I worked with the development of a caseinolytic assay for tPA, in collaboration with Per Wallen. Practically no activation of plasminogen occurred, in spite of high concentration of active tPA. However, by addition of a tiny amount of washed fibrin, a rapid caseinolysis occurred. Activation of plasminogen took place at the fibrin surface.
- In the autumn of 1977, as visiting professor in Leuven, I convinced Désiré Collen that tPA indeed was fibrin specific.
- A few weeks later, Désiré already had the goal to develop tPA as a new thrombolytic drug.

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

MARC VERSTRAETE

Clones ...

Well, when you are requested to come to conclusions after a remarkable day one is really a little bit uncertain.
Because conclusions suggest the end of an event or the completion of an endeavour and this is exactly what it should not be.
Although today is a milestone in the life of Désiré Collen, it is certainly not the end, by far, of a scientist.
We badly need the inspiration, the help, the stimulation, the support of Désiré Collen and not only at our laboratory, or what used to be my laboratory, but also at our Faculty of Medicine in Leuven and at the University at large.
Now, how to reach the goal, to maintain and to continue to have the benefit of a Désiré Collen?
I think one suggestion is to make a number of clones of Désiré Collen so that he would survive himself forever.
And once we have obtained the clones, I think we should take a patent on the product.
Thank you very much Desiré for having inspired so many people, for having being an example for many under us, for having really worked very hard, but always on the very amicable way.
You have been, for me personally, a friend, an advisor and a loyal man and therefore I would suggest and ask the audience to give this gentle giant, Désiré Collen, a standing ovation.

tPA – Affinity to Fibrin – Localized Plasminogen Activation

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We still have a surprise on behalf of the Flemish Institute of Biotechnology.

JO BURY

Well Désiré, it's my pleasure to speak, in front of the audience and in the name of all the VIB-scientists.
The scientists of VIB want to thank you for infecting us with your enthusiasm on basic science, on tech transfer, on translational research and we would like to thank you to inspire us with your vision and to enlight us with your vision which is centered around focus on excellence, on giving young talent the potential to grow up in an environment which you made very stimulating for the scientists to excel and these are now the cornerstones of the VIB policy.
We thank you for that.
And we have brought you a little present from the VIB scientists.
We have found a good bottle of Leoville Poyferré of 99, which people tell me, is a very good year and of course for a giant like you a simple bottle wouldn't be good enough.

A big bottle for a big man.

Thank you all very much