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Discovery by Prof Désiré Collen and his team

Alteplase, an ‘essential medicine’ with Flemish roots

A blockbuster, a long time in the making

An addendum to the book Désiré Collen, Biotech Pioneer, Lannoo 2018

Book is available on Google Play

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t-PA, a blockbuster medicine, anno 2024

Sometimes events take decades to become relevant and thereby tend to be overlooked. A striking example is the discovery in the 1980s by prof Désiré Collen and his team of the clinical potential of the protein t-PA *(tissue plasminogen activator, generic name Alteplase)* for the dissolution of blood clots in thrombo-embolic diseases, such as heart attacks and strokes.

Following the FDA regulatory approval of the use of rt-PA (recombinant t-PA) as a treatment for acute heart attack in 1987, for ischemic stroke in 1996, and later for pulmonary embolism, rt-PA was recommended by the World Health Organization, WHO, as ‘essential medicine’ for the treatment of acute ischemic strokes in 2019 (1).

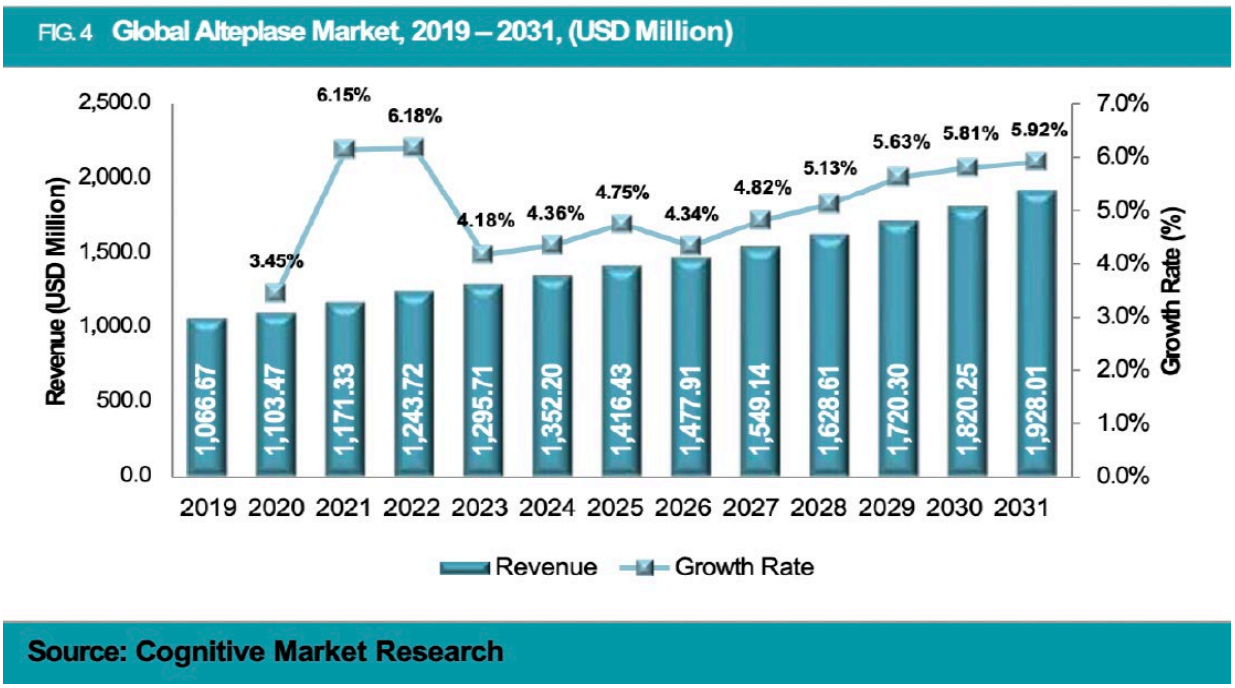
The global sales of medicines based on rt-PA, mainly Alteplase (Activase®) and its derivatives Tenecteplase and Reteplase, exceed the two-billion-dollar threshold and thus became a 'blockbuster', an achievement Flanders and KU Leuven can be proud of. Furthermore, its financial turnover is anticipated to increase at a stronger pace than the overall medicines market. This represents millions of lives saved or ameliorated for victims of an ischemic stroke, a heart attack or a pulmonary embolism.

It is today generally accepted that the benefits of t-PA far outweigh the risks. But an uphill battle it was! For a long time, Alteplase met with opposition and scepticism.

Those days are over now, although it is still not easy to map out the track of t-PA’s progressive global success. Pharmaceutical companies involved in the production and marketing of t-PA-based medicines, do not disclose quantities, prices or turnover figures.

Recently several market research companies published comprehensive reports on t-PA and Alteplase (2). Our analysis of these reports concludes that PA-based medicines, including Alteplase, Tenecteplase and Reteplase, generate today over $2 billion global turnover. A medical ‘blockbuster’ stands for a $1 billion figure. And most researchers predict an annual growth rate of about 5 % until 2032. The figures provided by the different market research companies differ significantly, mainly because only one report covers all t-PA brands, as can be seen in the following two graphs.

Source: Coherent GLOBAL TISSUE PLASMINOGEN ACTIVATOR MARKET, Feb 2025



In American community hospitals in 2017, Alteplase was by far the medicine most money was spent on (3)(4). According to Coherent the entire t-PA turnover in North America will be $1.8 billion this year and will grow to $2.6 billion in 2032. North America stands for 61% of the total market, Europe for 19.4%

Source: www.statista.com/statistics/994392/

What is t-PA used for?

t-PA is used against three main syndromes: acute myocardial infarction (AMI) or heart attack, acute ischemic stroke (AIS) and pulmonary embolism (PE) – which are among the leading causes of death in the world. (5)

1. Acute Myocardial Infarction (AMI) is according to the WHO the world's largest killer, responsible for 13% of the 60 million world's total deaths per year. In 2019, there were an estimated 5.8 million new cases of ischaemic heart disease in the 57 countries member of the European Society of Cardiology (6).

2. Acute Ischemic Stroke (AIS) is according to the WHO and the Global Stroke Factsheet 2012, the second leading cause of death and disability. In 2019, there were worldwide 12.2 million new cases of stroke and 3.3 million deaths associated with stroke. Globally one in four people over age 25 have a stroke in their lifetime (5). In the United States alone, stroke affects about 700,000 persons each year.

3. Acute Pulmonary Embolism (PE) is the third most common cause of cardiovascular morbidity and mortality. The incidence of PE is reported to be between 39 and 115 cases per 100,000 people annually.

4. Alteplase is also used to treat blood clots that form in central venous catheters (central line-associated thrombosis). In these cases, Alteplase can be infused directly into the catheter to restore proper flow (7).

What is specific about rt-PA based medicines?

Alteplase and other rt-PA derived drugs, primarily Tenecteplase and Reteplase, help break down blood clots by targeting the fibrin component, with much less disruption to overall blood clotting than the first generation thrombolytics Streptokinase and Urokinase.

T-PA binds to fibrin, locally converting plasminogen into plasmin, the active enzyme that dissolves the fibrin in the clot.

This helps restore blood flow, resulting in a time dependent reduction of the damage to brain tissue or heart muscle.

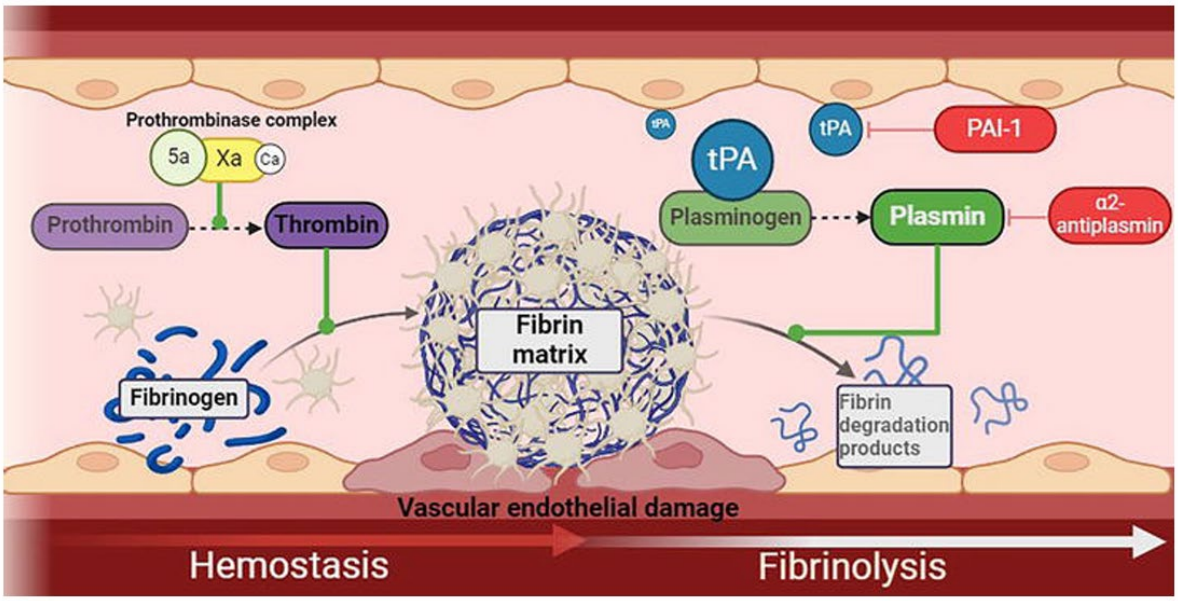
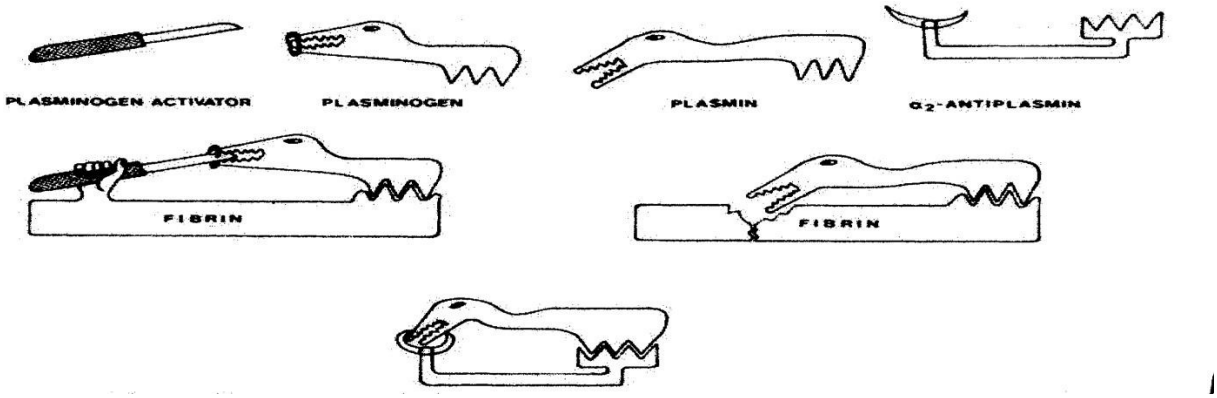


Figure 1.
The process of hemostasis leading to the formation of the fibrin matrix, followed by fibrinolysis mediated by tissue plasminogen activators.

Source: Rebecca S.Y. Teng, (based on Dr David Waisman and Dr Alamelu Bharadwaj), Fibrinolysis, Past and Future, July 24, 2024.

Regulation of fibrinolysis



Source: prof Désiré Collen and prof Roger Lijnen.

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.

Genentech clones t-PA

In August 1980, KU Leuven licensed the patent on the preparation and clinical use of the protein t-PA, discovered by professor Désiré Collen and his collaborators, for further development to the very promising American biotech company Genentech, founded in 1976.

In San Francisco, USA, the DNA encoding t-PA would be cloned, and this recombinant DNA (rDNA) would be used to produce the protein t-PA (rt-PA). That cloning succeeded in May 1982.

Two-dimensional representation of the t-PA protein

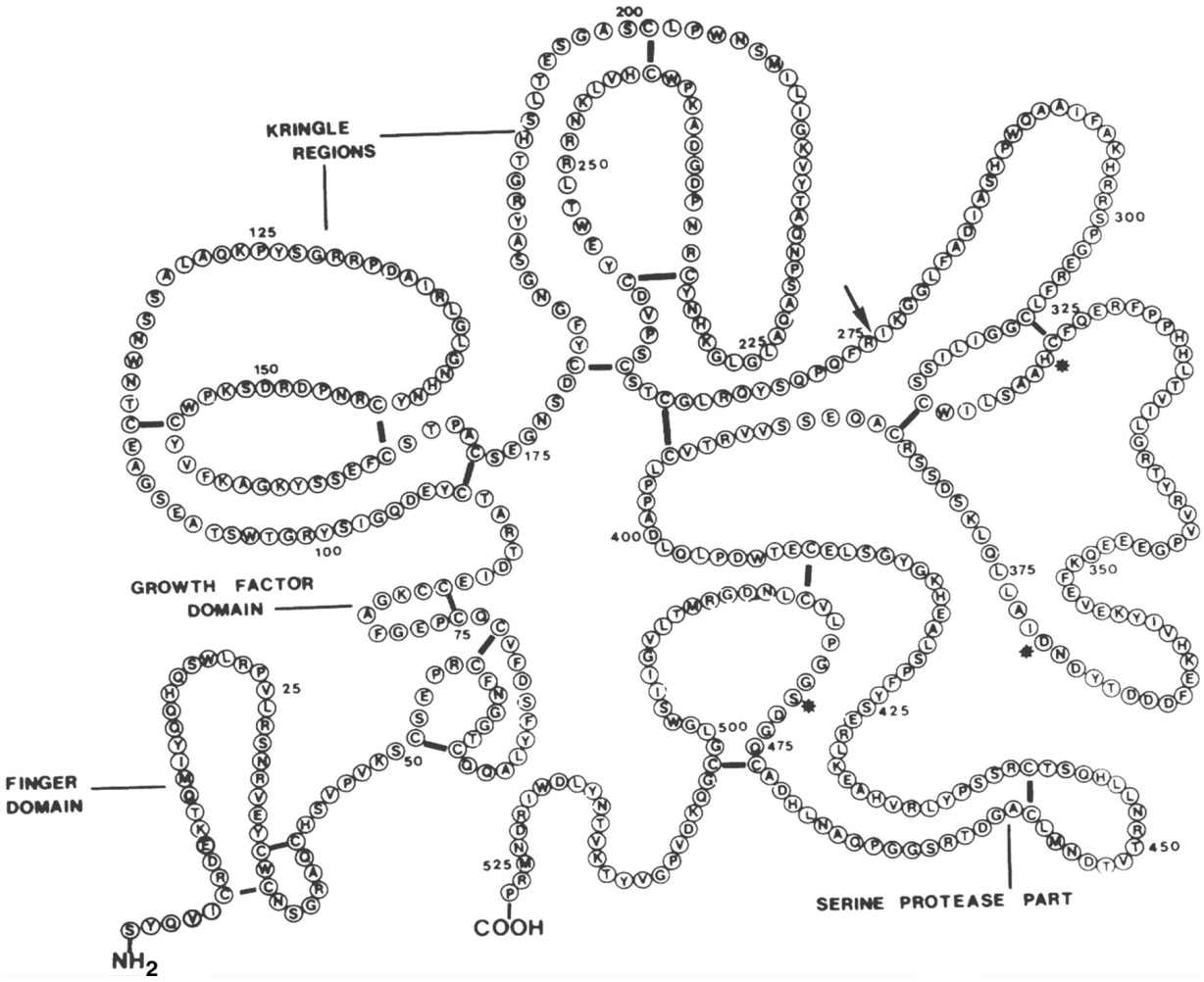


Figure 2. Schematic representation of the primary structure of t-PA. The amino acids are represented by their single letter symbols, and black bars indicate disulfide bonds. The active site residues His322, Asp371, and Ser478 are marked by asterisks. The arrow indicates the cleavage site for conversion of single-chain to two-chain t-PA (modified from reference 11).

Source: AHA/ASA Journal, Aug 1, 2009, D.Collen and H.R.Lijnen, The Tissue-Type Plasminogen Activator Story

A remarkable medical story

That was the beginning of a truly remarkable medical story.

Let's examine the main blood supply problems relieved by rt-PA medicines.

1. Acute Myocardial Infarction (AMI) or Heart Attack

In 1984, the American National Heart, Lung, and Blood Institute (NHLBI) supported the first multicentre, randomized clinical trial using rt-PA from Genentech in heart attack patients. This trial showed successful coronary artery opening in 75% of patients with limited bleeding complications. Based on positive results in additional large, randomized trials, the American regulatory agency FDA approved t-PA for treating heart attack on 13th November 1987.

That clear 'yes' from the FDA was the coronation of seven years arduous work by Désiré Collen and his KU Leuven colleagues, the Genentech biotechnology team in South San Francisco and initial clinical trial groups both in the USA and Europe. From then on and until 2006 when the t-PA patent expired, the KU Leuven and Désiré Collen would receive nearly $145 million as license fees. This unique entrepreneurial success of a then small Flemish university became trendsetting in Europe. Almost all universities and even colleges are nowadays 'entrepreneurial'. The managerial knowhow developed by KU Leuven Research and Development has been replicated in many places.

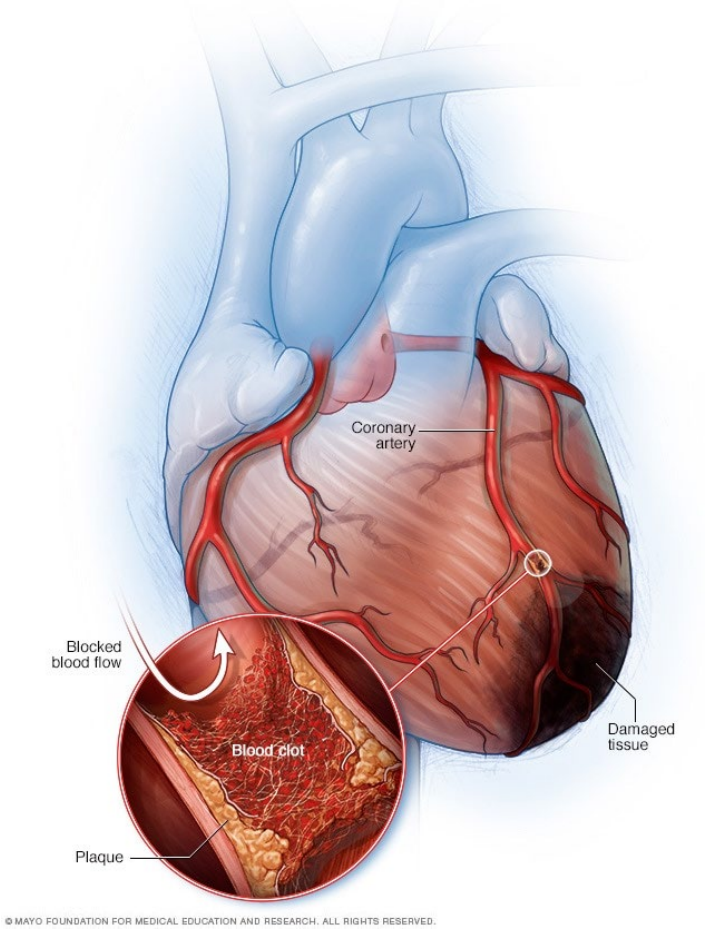
But rt-PA and Genentech faced many headwinds and in February 1990 the company was reconstituted as part of the Swiss F.Hoffmann - La Roche group. A substantial number of cardiologists continued to use as 'clot buster' the less effective but much cheaper Streptokinase which in addition also activates plasminogen in the circulating blood and causes more extensive haemostatic breakdown.

The resistance by many authoritative members of the medical community may explain why the European Medicines Agency (EMA) approved the use of t-PA for the treatment of heart attack only on August 29, 1996, nine years after the USA!

The preferred treatment of AMI today comprises thrombectomy, angioplasty and stenting, which were developed in the 1990s and revolutionized the treatment of coronary artery diseases.

In cases where percutaneous coronary interventions *(definition at the end)* are not available or feasible, Alteplase can still be a life-saving option. It is typically most effective when administered within 6 but occasionally up to 12 hours of the onset of symptoms. According to market research AMI represents a stable 22% of the t-PA turnover.

Heart Attack



Source: Mayo Clinic, Heart Attack, Symptoms and Causes, Dec 29 2014

The European Society of Cardiology Guidelines (6) state for the management of acute coronary syndromes, “fibrinolytic therapy is an important reperfusion strategy for STEMI patients *(definition at the end)* presenting within 12 h of symptom onset when primary percutaneous coronary intervention, PPCI, cannot be performed in a timely manner. It prevents 30 early deaths per 1000 patients treated within 6 h of symptom onset.

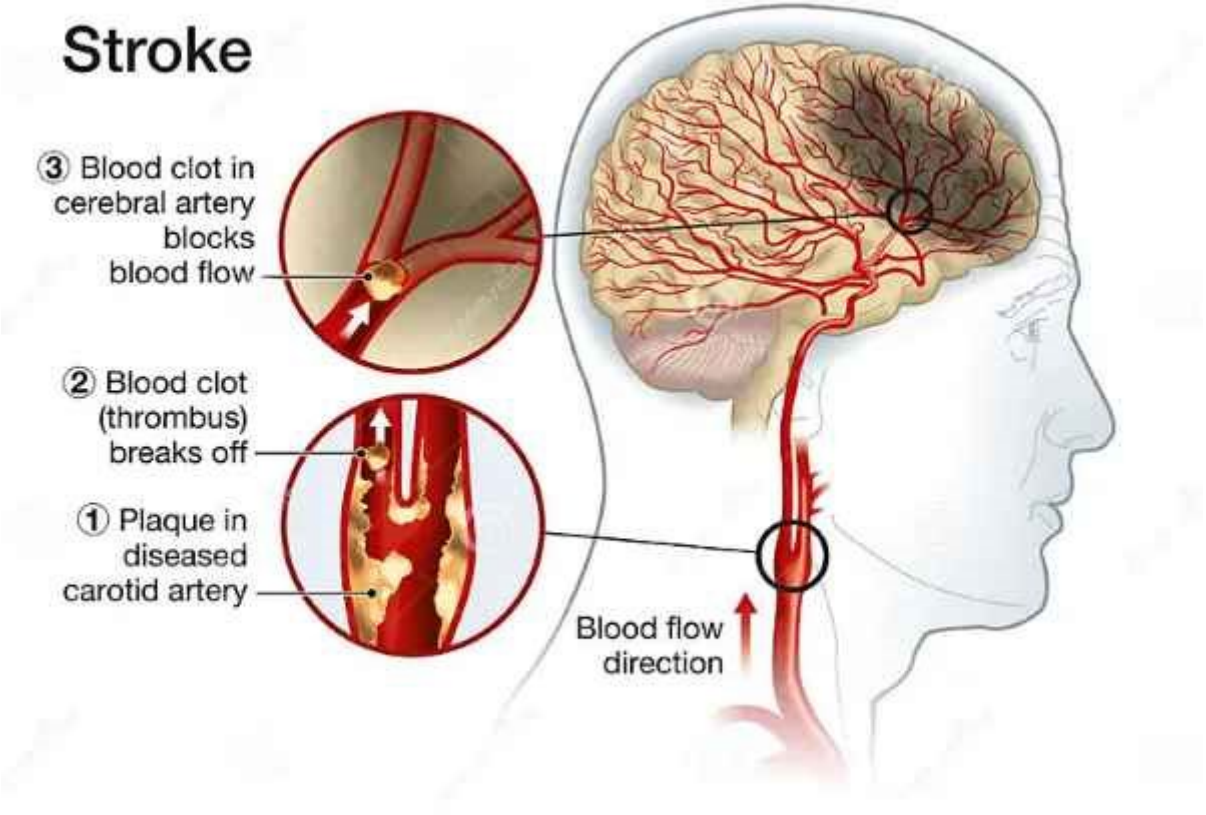
The largest absolute treatment benefit is seen among those patients at the highest risk, including the elderly (…) If trained medical or allied health staff can interpret the electrocardiogram, ECG, on site, or transmit the ECG for remote interpretation, it is recommended to initiate fibrinolytic therapy in the pre-hospital setting. A fibrin-specific agent (i.e. Tenecteplase, Alteplase, or Reteplase) is the preferred agent.”

2. Acute Ischemic Stroke (AIS)

In 1995, the results of a landmark clinical trial by the American National Institute of Neurological Disorders and Stroke (NINDS) caused a paradigm shift in managing acute ischemic stroke (AIS) patients.

“A paradigm shift!”, professor Yuanmei Pan of the Ren Ji Hospital in Shanghai, PRC, stressed in an article on the ‘silver jubilee’ (25 years) of t-PA for stroke in 2021. The NINDS-study demonstrated the efficacy of rt-PA (Alteplase) in improving neurological and functional outcome in AIS patients when administered within 3 hours of stroke onset.

Later the therapeutic window was extended to 4.5 hours, which still represents a major logistic issue, depriving many AIS patients from the potential benefits of rt-PA therapy. (8)



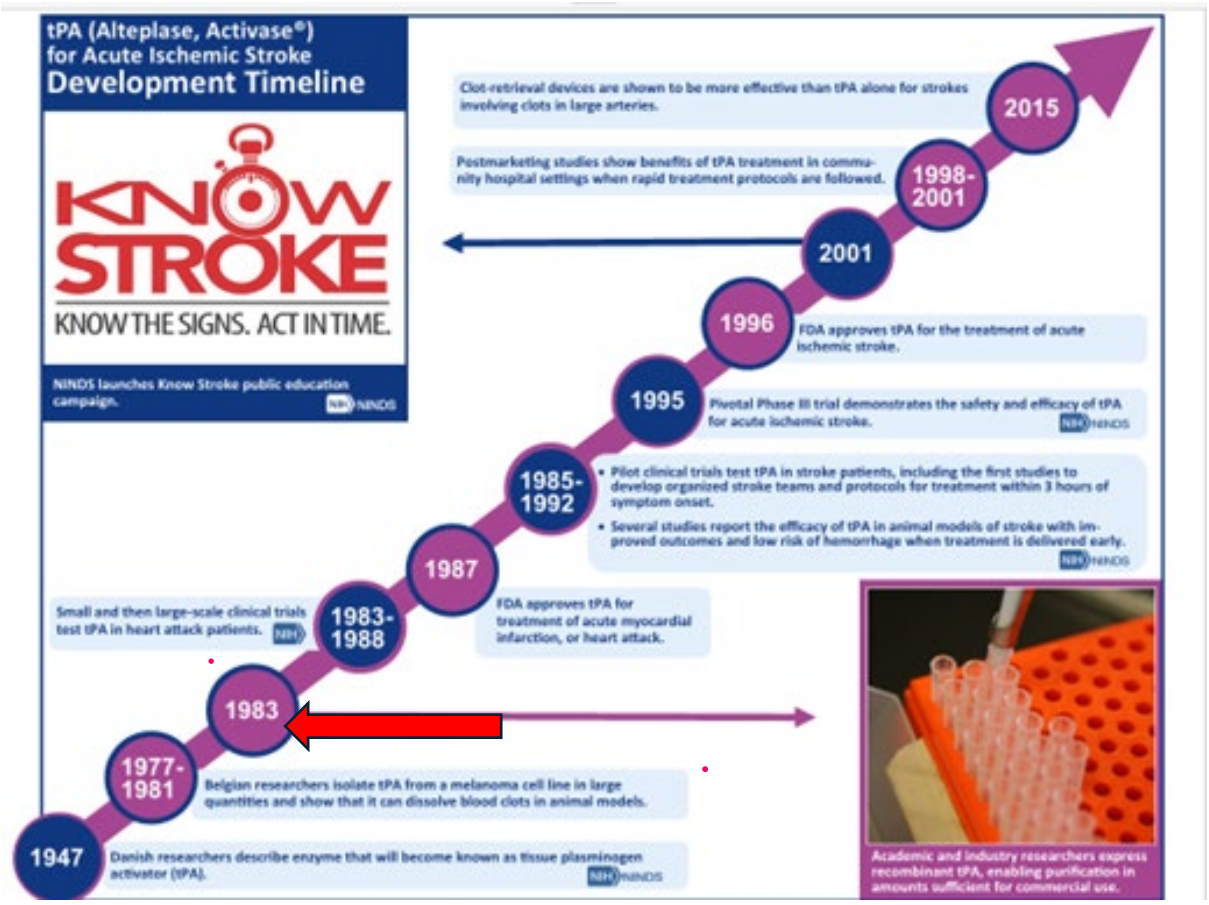
Source: Dreamstime, Axel Kock, Medically Accurate Illustration, June 10, 2009

Nine years after the approval for AMI the American regulator FDA approved in 1996 rt-PA for the treatment of AIS. The European Medicines Agency (EMA) approved t-PA for AIS on April 1, 2002. Parallel studies with Streptokinase for the treatment of AIS failed and its further development was halted.

The present Guidelines for the Early Management of Patients with Acute Ischemic Stroke of the American Stroke Association recommend Alteplase within 4.5 hours of stroke onset as "Standard of Care" in eligible patients, resulting in a 1.9 times higher likelihood to have a favourable outcome.

The Lancet confirmed in 2012 (9) that "the evidence indicates that intravenous *(definition at the end)* rt-PA increased the proportion of patients who were alive with favourable outcome and alive and independent at final follow-up. The data strengthen previous evidence to treat patients as early as possible after acute ischemic stroke, although some patients might benefit up to 6 h after stroke."

Despite this beneficial effect on brain damage, Alteplase remains much underused in low- and middle-income countries. To increase access and use of thrombolysis around the globe, the World Stroke Organization (WSO) assembled 13 stroke experts from five continents. This group submitted a 30-page application to the World Health Organization (WHO) for Alteplase to be included in the Essential Medicines List (10). In 2019 the WHO accepted this proposal.



This treatment for AIS became the primary use (60% according to Coherent) of Alteplase. In this condition, a clot obstructs a cerebral artery, leading to a lack of blood flow and oxygen to part of the brain. If administered within a specific time window, usually within 3 to 4.5 hours from the onset of symptoms, Alteplase can help dissolve the clot. The circulation to the affected brain tissue is restored and the long-term neurological deficits can be significantly reduced. Alteplase significantly improves outcomes for stroke patients if given promptly. It however carries risks, especially if administered too late or inappropriately. The most serious

risks of thrombolytic therapy are intracranial haemorrhage (ICH, affecting up to 6% with variable trial-dependent definitions), and major extracranial haemorrhage.

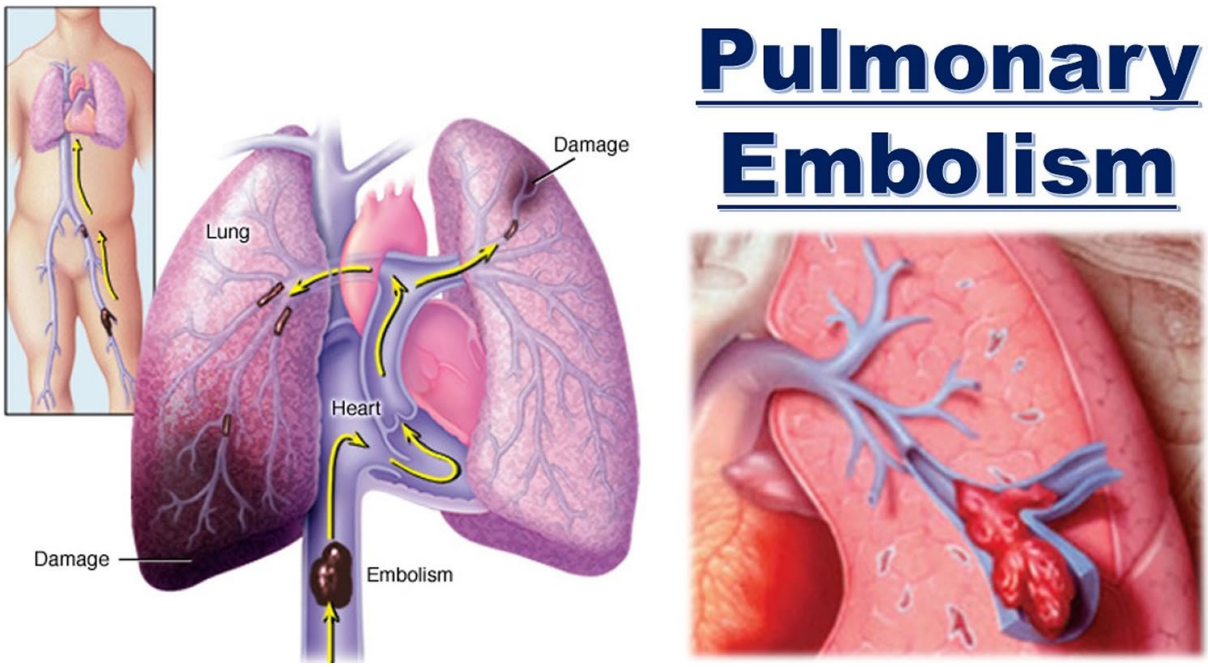
To denote the urgency required in the treatment of stroke patients neurologist Camilo Ramiro Gomez coined in the early nineties the phrase “Time is Brain!”. Time had already been identified as crucial in the treatment of AMI two decades earlier. Then the cardiologist Eugene Braunwald coined the phrase : “Time is Muscle!“. It was demonstrated that the severity and extent of AMI resulting from coronary occlusion could be radically altered by an adequate intervention as late as 3 hours after the coronary occlusion. (11).

During an ischemic stroke, the extent of damage to the brain depends on how long the brain tissue is deprived of oxygen and nutrients. For every minute that blood flow is disrupted, approximately 1.9 million brain cells die. If the ischemia persists for more than five minutes with very low blood flow (less than 5% of normal), some neurons will begin to die. The severity of the damage increases with the duration of the ischemia. If the ischemia is mild, the damage can progress more slowly, potentially taking up to six hours to destroy the brain tissue. (12)

Because a stroke is a result of a brain haemorrhage (and not a clot) in some 15% of cases, Alteplase can only be used after a CT scan of the brain has shown that the stroke is indeed caused by a clot. Of course, the hospital must then have a CT (computed tomography) scanner as part of a stroke unit. The analysis of the CT scan images remains work for an experienced neuroradiologist, especially to determine when the stroke took place. With the development of artificial intelligence (13) technology improved rapid diagnosis may be expected.

3. Acute Pulmonary Embolism (PE)

The FDA approved the use of tissue plasminogen activator (t-PA) for the treatment of acute pulmonary embolism on June 2, 1990. In Europe EMA approved t-PA on September 30, 2002. According to the market research reports PE represents between 10 and 20% of the financial turnover of Alteplase, Tenecteplase and Reteplase.



Source: Myrightsspot, March 19,2018

Alteplase is used in the management of severe PE, where a blood clot obstructs one or more arteries in the lungs. Alteplase can help dissolve the clot, restoring blood flow to the lungs and improving oxygenation.

Although multiple schemes have been developed, it is still unclear which advanced PE therapy should be used for the individual patient and within what optimal timing.

t-PA derived medicines

The rt-PA market remains dominated by the pharmaceutical companies Genentech/Roche and Boehringer Ingelheim (brands Activase and Actilyse) with an estimated 45% market share (14).

Following the expiration of the Alteplase patents, several other pharmaceutical companies began producing and marketing the drug. According to Coherent, Abbott holds end 2024 some 19% of the market, while Merck KGaA's market share is estimated at 11%. Coherent reports a significant 21% turnover in India, Russia and Iran, sometimes involving biosimilars *(definition at the end)* of t-PA.

As an alternative for intravenous infusion Alteplase, bolus **Tenecteplase** (TNKase or Metalyse) is also marketed by Genentech/Roche and Boehringer Ingelheim. It's Alteplase but with some changes in 7 of its 527 (530) amino acids that primarily reduce its elimination rate from the blood.

End 2024 The Lancet (15) published the results of a large Chinese trial comparing Tenecteplase and Alteplase in the treatment of 1430 stroke patients. Tenecteplase was found to be non-inferior to alteplase.

In July 2024 NICE, the organisation advising the British National Health Service, published a guideline stating Tenecteplase was “as effective as Alteplase, which NICE also recommends, in breaking up blood clots or preventing new blood clots from forming after an acute ischemic stroke. Because Tenecteplase costs less than Alteplase its use could potentially save the NHS millions of pounds” (16).

Tenecteplase continues to undergo clinical trials. According to the market research company Global Data (17) there are 9 new Tenecteplase studies ongoing with 4 in phase 3 and 2 in phase 2. The area covered is cardiovascular with 3 in AIS and 1 in PE.

Another t-PA based medicine is **Reteplase**. It is a recombinant non-glycosylated derivative form of human t-PA, which has been modified to contain 357 of the 527 amino acids of the original protein. The FDA approved Reteplase for AMI in 1996, and the EMA granted its approval in 1992.

Few reliable figures about the usage and turnover of Reteplase are available. According to the market research by Coherent Reteplase covers 7.6% of the total market with $232 million turnover expected for 2025. In 2018, the Italian pharmaceutical company Chiesi acquired Reteplase, which is primarily produced by the German Wacker Biotech under the Retavase brand.

Recently several studies comparing Alteplase and Reteplase took place, primarily in China. In the New England Journal of Medicine (18) a group of Chinese researchers published the results of a randomized trial on 1412 patients and concluded: “Among patients with ischemic stroke within 4.5 hours after symptom onset, Reteplase was more likely to result in an excellent functional outcome than Alteplase.” To be continued.

Further growth of rt-PA use

The market research companies all expect a continuing growth for Alteplase in three of the four different market segments we described. Overall, the global growth might reach an annual 5.2%. AMI usage of Alteplase is expected to remain stable.

In an aging North America and Europe the incidence of blood clots in arteries and veins will predictably increase. In more countries nearby hospitals with the necessary diagnostic tools and competent specialists are available. And for AIS, as the WHO states, the ‘essential medicine’ is Alteplase.

The strongest growth, 9% per year, learns CMR, is expected in the Asia Pacific region. According to several statistics China alone accounts for over a third of the global stroke burden.

The main explanation for that continuous growth might be that there still is no comparable alternative to Alteplase in AIS. Surgical interventions like stenting and other forms of thrombectomy are available since the nineties and will continue to be used, but access is limited to larger arteries and it is regularly used in combination with Alteplase.

The limited time scope of a treatment with Alteplase and other t-PA based medicines remains a major problem because many people with stroke arrive at hospital after the crucial time

window. Only an estimated five per cent of AIS people currently receive this therapy in most countries.

For strokes that major time handicap is coped with using mobile stroke units and e-medicine. An example is Icliniq in India. In a company brochure from Icliniq dated Jan 4, 2024, the Indian pneumologist Dr. Kaushai Bhavsar states with an enthusiasm most doctors abhor: “With its amazing capacity to dissolve blood clots, t-PA has completely changed how thromboembolic disorders are treated. Its discovery and practical application mark a crucial turning point in medical history, improving patient outcomes and saving many lives.” (19)

Collen states however: “t-PA does work, but under very specific conditions and with an absolute limitation in the treatment time. A miracle medicine it is not! “

The pricing of Alteplase

The pricing of Alteplase by Genentech/Roche and Boehringer Ingelheim has been an issue since the launch of the medicine in November 1987. Alteplase was priced more than 10 times the only alternative Streptokinase.

In the United States prescription drug prices are more than 2.5 times higher than those in similar high-income nations, so pricing is probably a real problem there (20).

Prices can vary significantly depending on the country and the specific hospital. According to the WHO the price for a single dose of 63 mg Alteplase for a 70 kg patient varies from $260 (Brazil, public hospital) to $6400 (average billing amount in the United States). On the American web shop Pharma Checker a vial Alteplase of 20 mg costs $718 dollars. Also on American Drugs.com the cost for an intravenous powder recipient for injection 50 mg is $4,643. In the Netherlands a vial of 50 mg Actilyse (Alteplase) costs €532 and 50 mg Metalyse (Tenecteplase) €1002 (website Pharmacotherapeutisch Kompas). The American and Dutch prices were registered on Feb 10, 2025. In Belgium the cost of medicines like Alteplase is a hidden component of the daily residence cost in hospitals.

The price of the drug is part of a much broader treatment cost, be it for heart attacks, lung emboli or strokes. In the case of stroke, a CT scan is necessary beforehand. Implementing and administering Alteplase within the recommended 4.5 hours requires some initial investments in pre-hospital and intrahospital services. These additional costs have to be balanced by generally shorter hospital stays, reduced rehabilitation needs and reduced long-term care (including nursing homes and home care), given the reduction of handicap.

Few studies on the costs of medicines take a societal perspective (21) emphasising the importance of the burden stroke poses on patient/family costs, especially for those patients with high disability, and among younger adults due to productivity loss.

In 2017, the cost of stroke in 32 European countries was estimated at a total of €60 billion of which €27 billion were healthcare costs, €5 billion were community costs, and €28 billion were patient/family costs (of these, €16 billion were caregiving and €12 billion were patient productivity loss). In 2016, a Spanish study concluded that the average cost per patient was €27,711 during the first year, of which more than two-thirds corresponded to patient/family costs (caregiving).

Conclusion: the price of Alteplase and Tenecteplase is acceptable as a component of a set of medical and human costs, but probably high enough to generate more competition e.g. with Reteplase and its Chinese and Indian biosimulars, and in the future possibly with Staphylokinase. With some 30 years delay Collen might one day welcome his 'poor man's t-PA'.

Staphylokinase, an alternative to t-PA?

With his KU Leuven colleagues Collen developed in the nineties an alternative to Alteplase. As Genentech priced its medicine at a level which was unaffordable to less affluent countries, Collen believed that a ‘poor man’s t-PA’ might suit their needs. In 1988 an American patient paid 200 dollars for a treatment with Streptokinase and more than 2000 dollar for a treatment with 100 mg Alteplase! (22).

That was the start of Staphylokinase (SAK), a plasminogen activator derived from the staphylococcus aureus bacteria. Extensive basic and experimental animal work, much of it directed by Collens’ longtime collaborator prof Roger Lijnen, resulted in over 50 peer reviewed publications. The development and the tests on patients in seven Belgian hospitals delivered excellent results, but to be accepted by the regulators the new and cheaper medicine had to be tested against the expensive Alteplase. The costs of these large-scale comparative trials were in the 10s of million dollars, way too high for the companies Collen managed at that time.

In 2006, Collen founded the company ThromboGenics to develop Staphylokinase and other potential medicines. But the company dropped the inexpensive thrombolytic alternative to focus on more lucrative ophthalmology. Indian Bharat Biotech and Egyptian Rhein Mina Pharm took over the Staphylokinase know how and acquired a licence. Bharat later requested support for a broader trial then the one already successfully finished, but ThromboGenics declined. After leaving as chairman of ThromboGenics on Nov 1, 2013 Collen tried to reclaim the unused patents, but unreasonable financial conditions led to patent expiration. As a company ThromboGenics, renamed Oxurion, ultimately failed in 2024.

The patents on Staphylokinase owned by the KU Leuven R&D and Collen expired in 2014, 2017 and 2019 but the extensive preclinical results remain available to the worldwide scientific community. Recently scientists in China, Russia, South Korea and the Czech Republic picked up the possibility to compare Staphylokinase with rt-PA based treatments.

In Russia Fortelyzin® (recombinant staphylokinase) is a potential medicine developed by the company Supergene LLC, Moscow. In 2014-2016 tests called Fridom1, showed in AMI cases survival rates of 94.1% versus 93.5 for Metalyse (Tenecteplase). It restored blood flow in 80% of the cases. Fortelyzin® is currently in Phase III clinical trials for AIS. In Jan 2025 the company was recruiting patients for several trials.

Staphylokinase was approved for AMI by China Food and Drug Administration (CFDA) in September 2, 2010. It is developed and marketed as 施爱克® (Shiake) by Shanghai Tonghuayujin and as 依力通®(Yilitong) by Diao. The medicine is not approved yet for strokes. Alteplase remains the standard treatment for AIS in Russia and China, as it is in Western countries.

Collen himself published a summary of the KU Leuven research on Staphylokinase in Nature Medicine in March 1998. The follow-up in medical research was limited. Only recently, in August 2022, the specialized publication Stroke ran an article on "the hidden potential of highly efficient and widely accessible thrombolytic Staphylokinase", written by researchers from the Masaryk University in Brno, Czeck Republic. (24) Conclusion: "Staphylokinase nonimmunogenic variant was proven noninferior to Alteplase in a clinical trial, with decreased risk of intracranial haemorrhage and the advantage of single bolus administration. (…) Staphylokinase has a significantly smaller and simpler structure, lower production costs, higher fibrin selectivity, and no inhibition compared with Alteplase." The BRNO article was based on research and on Russian work by Professor Eugene Gusev and colleagues of the Russian Academy of Science, which was published in The Lancet Neurology in 2021. Between 2017 to 2019 the Russian scientists evaluated a nonimmunogenic Staphylokinase variant against Alteplase on 336 AIS patients. Intracranial hemorrhage developed in only 3% of nonimmunogenic staphylokinase-treated patients compared with 8% of patients treated with alteplase. Within 90 days, 4% more people died in the Alteplase group.

The leading researcher in Brno, Dr. Martin Toul, is now working in Ghent for VIB (Vlaams Instituut voor Biotechnologie). He confirmed: "My former colleagues from Czechia are still actively working on this project and I am in live contact with them. So hopefully, we will reach the point of commercializing it as an approved thrombolytic at some point in the future. But now, it is still in the development phase." Prof Collen is sceptical about the future of Staphylokinase in Western research and medical practice but is happy with the developments occurring in Russia and China.

Staphylokinase is not the only potential rival for Alteplase. With his investment company Fund+ professor Collen participates in a Dutch biotechnological start-up called TargED Biopharmaceuticals B.V. This 'targeted' thrombolysis works by binding its TGD001 to the platelets in a platelet-rich arterial blood clot. Platelets, also known as thrombocytes, are tiny, disc-shaped cell fragments in the blood that play a crucial role in clotting. Similar to the action of rt-PA the targeted activator locally converts plasminogen into enzymatically active plasmin. TGD001 just entered the phase of initial clinical testing.

TGD001 is one of several start-up companies seeking breakthroughs in thrombolysis.

A further search for t-PA alternatives learns that there's a discouraging collection of medical literature documenting stroke drugs that have failed in clinical trials.

Even mid-term there are no alternatives for t-PA in sight. Moreover, the now tested advanced drug delivery systems, ADDS, might even accelerate the expected growth of the existing t-PA based medicines.

The winding road of t-PA to its present 'blockbuster' status

So, that's what has happened during the last 45 years to Collen's t-PA. But how did it start?

It started with a series of serendipitous events, as recounted in the book "Désiré Collen, Biotech Pioneer" which we published in 2018. These comprise a.o.:

- The fortuitous visit in 1964 of the then 20 years old student Désiré Collen to prof Marc Verstraete's (1925-2018) laboratory for a blood donation. There he met Guido Tytgat, the later renowned gastroenterologist, who inspired him to reorient his career towards research.

- The fortuitous discovery, during his studies with radio-labelled plasminogen for his thesis work, of alpha2-antiplasmin, the primary inhibitor of plasmin in circulating blood. This led to a collaboration with Björn Wiman of the Swedish Karolinska Institute and with the then newly recruited Belgian biochemist Roger Lijnen by Verstraete's laboratory, on the purification and characterization of alpha2-antiplasmin, resulting in a molecular model for the regulation of physiological fibrinolysis, requiring assembly on the blood clot of both plasminogen activator and plasminogen, allowing local activation to plasmin causing breakdown of the blood clot.

- The collaboration with Fons Billiau (KULeuven) on the role of fibrinolysis in the phenotype of malignant cells, which led to the acquisition from Dan Rifkin New York University), of the Bowes melanoma cell line as a known source of plasminogen activator, which early 1979 Collen found out to bind to fibrin.

- The collaboration with Dick Rijken (Leiden), who following his PhD thesis on the purification of tissue plasminogen activator from human uterine tissue, joined Verstraete's laboratory for a post-doc in 1979, but could be convinced to collaborate with Collen on the purification and characterisation of the melanoma activator, proving that both were identical.

- The collaboration with Osamu Matsuo (Japan) who joined Verstraete's laboratory for a post doc in 1979 but early 1980 joined the t-PA group to demonstrate its thrombolytic properties in experimental animal models.

- The serendipitous meeting with Diane Pennica from Genentech during the first presentation of our results with t-PA, one day after submission of the patent application of KU Leuven on the preparation and use of t-PA.

- The cloning and production of recombinant t-PA by Pennica within 2 years and the first use of melanoma-cell-derived t-PA rapidly followed by recombinant t-PA in experimental animals and patients, resulting in FDA approval of the use of rt-PA in AMI patients in 1987.

- The rest is history as recounted and commented by Huybrechts and Van Wijck in 2018.

What were, prof Collen, the most difficult episodes in your scientific life?

Collen: *"Science is an eliminative process. Theories and discoveries survive until they are refuted. We're on a playing field of scientists defending ideas and promoting hypotheses. And it's also a competition for financial support. Most research in Life Sciences is very expensive. In my professional life I lost some battles, but with Alteplase my collaborators and I survived twice confrontations with sceptical opponents. "*

Until 1980 there has been an endless discussion among physicians about the sequence of events in a heart attack. Was cardiac ischemia leading to coronary artery thrombosis? Or was a blockage of a coronary artery causing cardiac ischemia?

Collen's mentor Marc Verstraete was convinced that the blockage of a coronary artery caused a heart attack ('*open artery hypothesis'*). In 1979 a European study group led by Verstraete demonstrated that streptokinase reduced mortality in heart attack patients, presumably by reopening the blocked artery, but other similar trials were inconclusive.

During the first half of the 1980s several megatrials in tens of thousands of patients (GISSI, ISIS, TIMI etc.) established "*beyond a reasonable doubt"* that thrombolytic therapy reduces mortality in AMI. The effect is time dependent, establishing the *"time is muscle"* hypothesis.

Once the open artery hypothesis was confirmed, during the second half of the 1980s and into the early 1990s, the focus moved towards the optimal thrombolytic therapy scheme, with protagonists of the inexpensive *"streptokinase plus aspirin scheme"* versus the defendants of the more expensive *"t-PA plus intravenous heparin anticoagulation"*.

The t-PA scheme gradually moved from an intravenous infusion of t-PA over 3 hours to the more effective front-loaded t-PA with intensive heparin anticoagulation.

In 1993, the Gusto trial in over 41,000 patients with STEMI (ST Elevated Myocardial Infarction) with its Gusto Angiographic sub study in 2,431 patients concluded that more rapid and complete restoration of coronary flow through the infarct-related artery results in improved ventricular performance and lower mortality among patients with myocardial infarction. This would appear to be the mechanism by which accelerated t-PA therapy produced the most favourable outcome in the GUSTO trial.

However, in 1997, Collins et al reported a meta-analysis *(definition at the end)* of the GISSI-2, ISIS-3 and GUSTO megatrials (together over 90,000 AMI patients treated with either t-`PA or streptokinase) and concluded that "consideration of all the evidence does not demonstrate any clear difference in net clinical outcome (in terms of death or stroke within 30 days) between these different fibrinolytic regimens". They concluded that "the standard one-hour regimen of 1.5 million units of streptokinase would generally be the fibrinolytic treatment of choice". (23)

Collen: *"For my colleagues and I having worked for more than two decades on the basis of the hypothesis that a heart attack is caused by an occluding coronary thrombus and that*

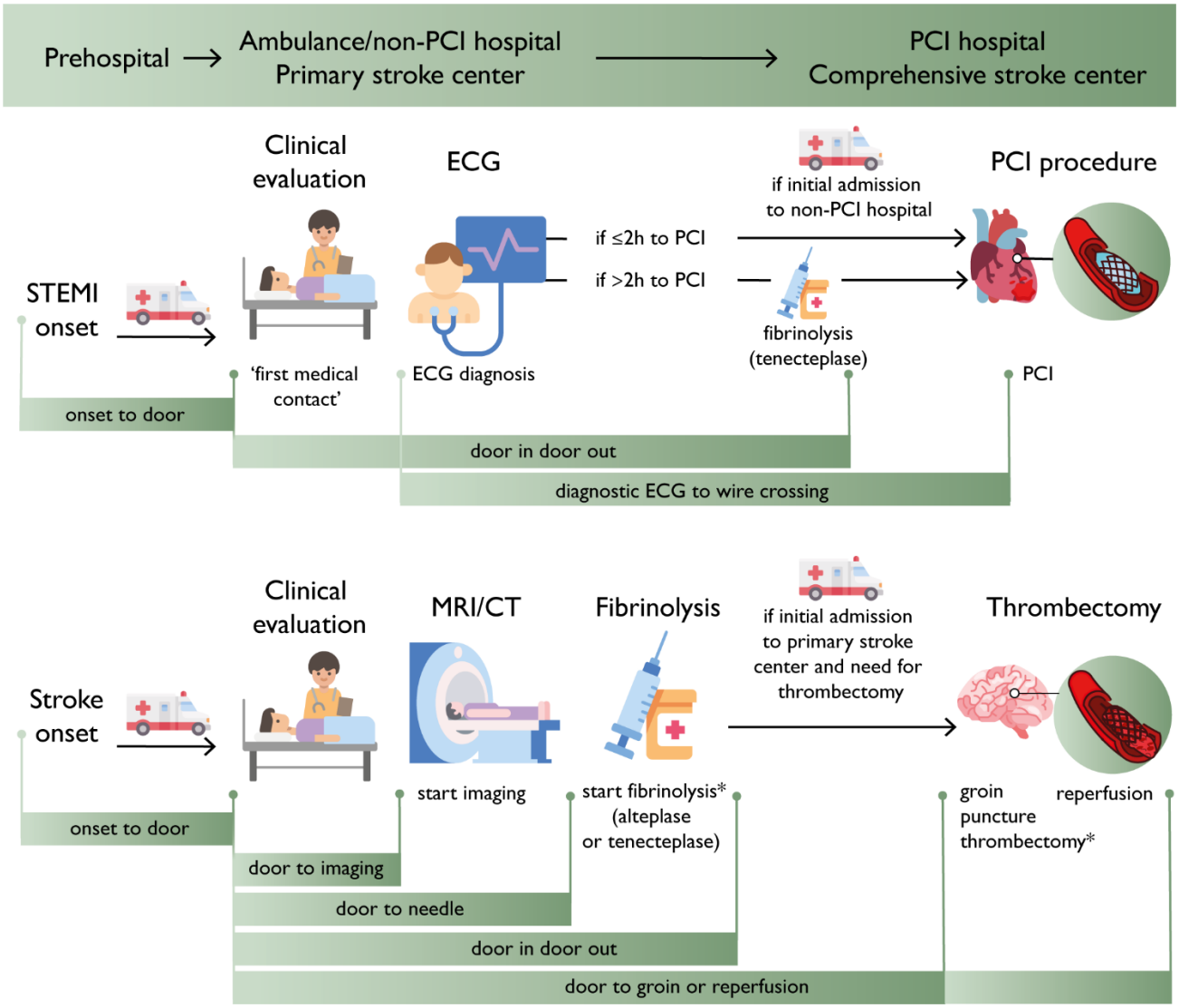
timely recanalization was the mechanism behind the clinical benefit, this was a sobering experience."

But wrong our opponents turned out to be! Meta-analysis, says Collen, is a powerful tool to generate "evidence based" guidelines for new treatments gathered from multiple randomised trials. The analysis of Collins et al however disregarded differences in patient selection ("suspected" infarction not requiring electrocardiographic evidence in ISIS 3 versus STEMI in GISSI 2 and GUSTO), slow administration of t-PA (3 or4 hour intravenous infusion in GISSI 2 and ISIS 3) versus front-loaded in GUSTO) and concommitant administration of heparin (delayed subcutaneous in GISSI 2 and ISIS 3 versus concommitant intravenous in GUSTO). Furthermore Collins et al compared the combined endpoint of death or stroke within 30 days, disregarding the fact that 1/3 of these stroke patients fully recovered.

Beyond the controversies on the use of t-PA or Streptokinase in AMI, the superiority of t-PA over Streptokinase is overwhelmingly established in Acute Ischemic Stroke where, in eligible patients (up to 20% with adapted logistics), t-PA has become the standard of care, projecting it to a blockbuster status, whereas all large trials with Streptokinase in AIS have failed.

Collen: *"But it took a long time, almost 40 years, before 'our clot buster' became a worldwide lifesaving blockbuster, not only against heart attacks but also against stroke and possibly pulmonary embolism. As a convinced supporter of the 'open artery hypothesis' throughout my career, I feel vindicated that it was not all futile!"*

P.S.: Diagnostic and therapeutic workflow in ST-elevation myocardial infarction and acute ischaemic stroke



Patients with ST-elevation myocardial infarction are transferred to either a hospital with or without facilities for percutaneous coronary intervention. After clinical evaluation and electrocardiogram diagnosis of ST-elevation myocardial infarction, patients will receive immediate treatment with percutaneous coronary intervention if time from electrocardiogram diagnosis to percutaneous coronary intervention initiation is 120 min or shorter. If a longer delay (>120 min) is expected (e.g. long transfer to percutaneous coronary intervention hospital), patients should receive fibrinolytic treatment and are scheduled to undergo per cutaneous coronary intervention within 2–24 h after administration of fibrinolytic (pharmaco-invasive strategy) or rescue percutaneous coronary intervention if needed. Patients with acute ischaemic stroke are transferred to either a primary or comprehensive stroke centre. After a clinical assessment, patients undergo brain imaging (computed tomography or magnetic resonance), and based on time criteria and imaging findings, the decision to fibrinolytic treatment is made. If there is evidence for a large-vessel occlusion, and if other time and imaging criteria are fulfilled, the patient will undergo thrombectomy treatment (after transfer to the comprehensive stroke centre if first admitted to a primary stroke centre).

Source: European Society of Cardiology, Acute myocardial infarction and ischaemic stroke: differences and similarities in reperfusion therapies—a review, European Heart Journal (2024) 45, 2735–2747 Authors: Lauranne Scheldeman, Peter Sinnaeve, Gregory W.Albers, Robin Lemmens and Frans Van de Werf, all UZ KU Leuven.

Footnote and References

Alteplase an 'essential medicine' with Flemish roots

(1) WHO Model List of Essential Medicines – 23rd List (2023), page 40.
International Journal of Stroke : Alteplase (rtPA) now included on the WHO's List of Essential Medicines (EML) for acute ischemic stroke (July, 11 2019) OR eEML - Electronic Essential Medicines List.

Alteplase is not on the essential medicines list for acute myocardial infarction. The WHO maintains streptokinase as a treatment. Pulmonary Emboly is not on the list of covered diseases.

(2) Global Alteplase Market Report, oct 2024, published by Cognitive Market Research. AND Global Tissue Plasminogen Activator Market, Feb 2025, published by Coherent MR.
As the companies involved in t-PA medicines do not provide detailed information on usage or turnover, the turnover figures are a result of estimated quantities and prices. How reliable are the turnover figures provided by market research companies? Without entering the complexities of market research, the figures used are as reliable as the research company collecting them is. The predicted future turnover depends on numerous parameters and are an educated guess. Moreover, most research companies limit themselves to one of the three marketed brands. The Coherent figures include all t-PA variants. However, we chose not to follow either the high Coherent figures or the lower Cognitive figures. We project an estimated turnover of $2 billion in 2025.

We extended an opportunity to one of the pharmaceutical companies producing Alteplase to rectify the figures.

(3) AHA.org American Hospital Association, Recent Trends in Hospital Drug Spending and Manufacturer Shortages - GPO Hospital Spending (in $Millions) for Drugs with the Highest Hospital Spending in 2017.

(4) Lifesaving as It undoubtedly is, this rt-PA market is only a relatively small part of the huge cardiovascular diseases (CVDs) market which encompasses a range of conditions impacting the heart and blood vessels, including coronary artery disease, heart failure, stroke, high blood pressure, and congenital heart abnormalities. According to the market research company Precedence Research the global cardiovascular drugs market size was valued at $144 billion in 2023 and is expected to reach around $208 billion by 2033, poised to grow at a compound annual growth rate of 4%. The total turnover value of pharmaceuticals in the world is estimated at some $1,200 billion.

(5) WHO, The top ten causes of death, 2021. In June 2022, according to a report published by the National Center for Biotechnology information, across European Union countries, stroke accounted for approximately 465,000 deaths in 2020, and the number is expected to rise by one-third by 2035 due to population ageing and increases in some risk factors. Among all strokes, the ischemic subtype is the most common, representing approximately 80% of cases in Europe.

(6) European Society of Cardiology, ESC, European Heart Journal, 2023.

(7) Alteplase is occasionally used for thromboembolisms beyond the three commonly mentioned. Coherent states: “The application of tissue plasminogen activator (tPA) in thrombolysis has contributed significantly to the market growth, due to its role in effectively treating conditions like venous thromboembolism (VTE) and improving outcomes in patients with large vessel clots. For instance, a study published by the Centers for Disease Control and Prevention (CDC) on January 27, 2025, revealed that venous thromboembolism (VTE) affects up to 900,000 people in the U.S. annually, highlighting the large patient population in need of effective thrombolytic therapy . This growing incidence of VTE has spurred greater demand for treatments like tPA to address such critical conditions.” (Coherent report p 95)

(8) Yuanmei Pan, Guowen Shi, Silver Jubilee of Stroke Thrombolysis With Alteplase: Evolution of the Therapeutic Window - PMC
Frontiers of Neurology, March 01 2021.

(9) The Lancet, June 23, 2012

(10) See also World Stroke Organization, 2012, Global_Stroke_Guidelines_and_Action_Plan_All_in_one.pdf

(11) Wikipedia AND Luis Maria Abreu ABC Cardiol, apr 2019

(12) Health Partners.com, Why time is critical after a stroke, July 2024; UMMS.org, St.Louis Medical Center, University of Maryland, 2025 AND Wikipedia, Stroke., Feb 2025.

(13) Medplace, Revolutionizing Healthcare: The Impact of AI on Patient Care in Hospitals, Dec 12, 2024.
AND: This AI reads stroke patients’ brain scans to decide how best to treat them | World Economic Forum

(14)

Producers of t-PA based medicines (2024 estimates)

Company name	Region	Product name	Market Share
Hoffmann-la Roche Genentech	Global USA	Activase	33,40%
Boehringer Ingelheim	Gobal Germany	Actilyse	12,90%
Merck KGaA	Global Germany	t-PA	10,60%
Abbott	Global USA	Retelex	19,40%
Chiesi Pharma	USA Italy	Retavase	1,50%
Others			21,80%
Emcure	India	Enlaxim Tenectase (*)	
Reliance	India	TenecteRel MiRel (**)	
Petrovax	Russia	Metalyse	
Generium	Russia	Revelise (*)	
Arena	Iran	Altelyse (*)	
Saintroy	India	Alteplase	
TAJ Pharma	India	t-PA	

(*) biosimulars (**) probably only AMI

Source: Coherent Marketing Research, 2025

(15) The Lancet Nov 24, 2024. Tenecteplase versus Alteplase for acute stroke within 4·5 h of onset (ATTEST-2): a randomised, parallel group, open-label trial

(16) National Institute on Heath and Care Excellence, NICE, Tenecteplase for treating acute ischaemic stroke, July 24, 2024.

(17) GlobalMarkets Direct report, pag 47

(18) New England Journal of Medicine, June 14, 2024.

(19) Icliniq, the virtual hospital, Blood Health, Jan 4 2024. Icliniq is a quite successful Indian web based e-medicine initiative.

(20) ASPE, Issue Brief Sept 2022, Trends in Prescription Drugs Spending 2016-2021.

(21) Costs during the first year after stroke, A societal perspective, Mercé Soler Font et al, European Stroke Journal, Nov 30, 2024

(22) John Diebold, The Innovators, A Plume Book, 1990, page 243

(23) Stroke, Hidden Potential of Highly Efficient and Widely Accessible Thrombolytic Staphylokinase, *Aug 2022 AND*
Gusev EI, Martynov MY, Nikonov AA, Shamalov NA, Semenov MP, Gerasimets EA, Yarovaya EB, Semenov AM, Archakov AI, Markin SS; FRIDA Study Group. Non-immunogenic recombinant staphylokinase versus alteplase for patients with acute ischaemic stroke 4·5 h after symptom onset in Russia (FRIDA): a randomised, open label, multicentre, parallel-group, non-inferiority trial. *Lancet Neurol*. 2021;20:721–728.

(24) Key graphs in the March 20, 1997 study mentioned:

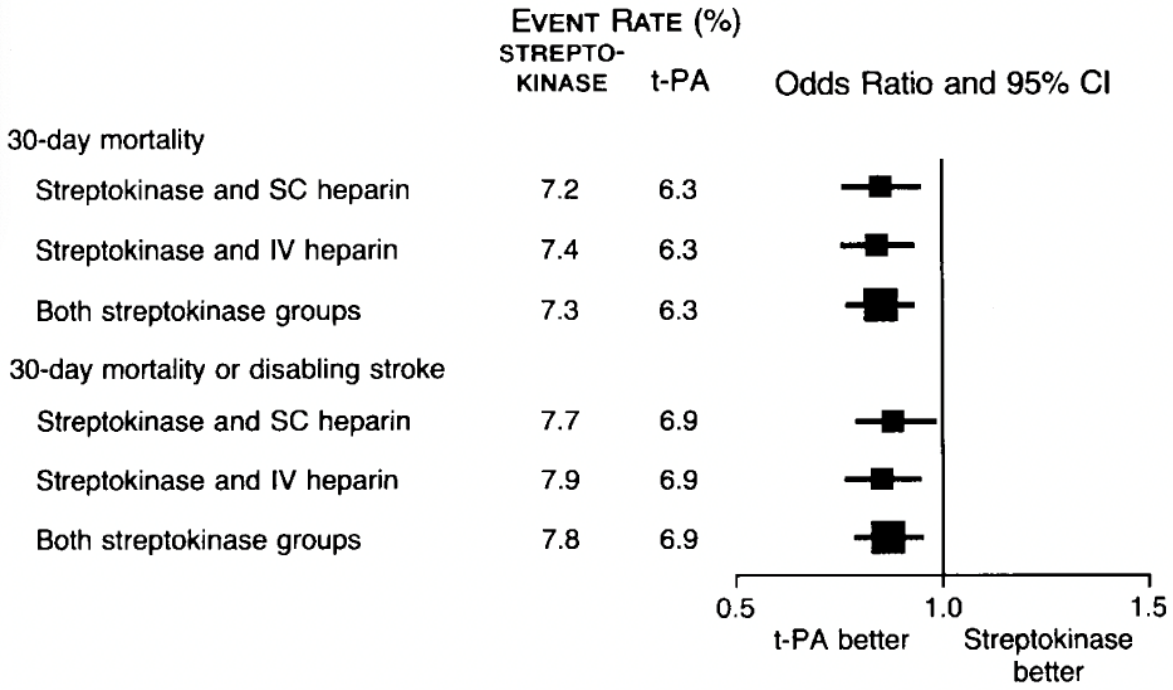


Figure 2. Odds Ratios and 95 Percent Confidence Intervals (CI) for Reduction in Mortality and Net Benefit, Defined as Reduction in Mortality and Disabling Stroke, in the Group Assigned to Accelerated t-PA as Compared with the Streptokinase Groups.

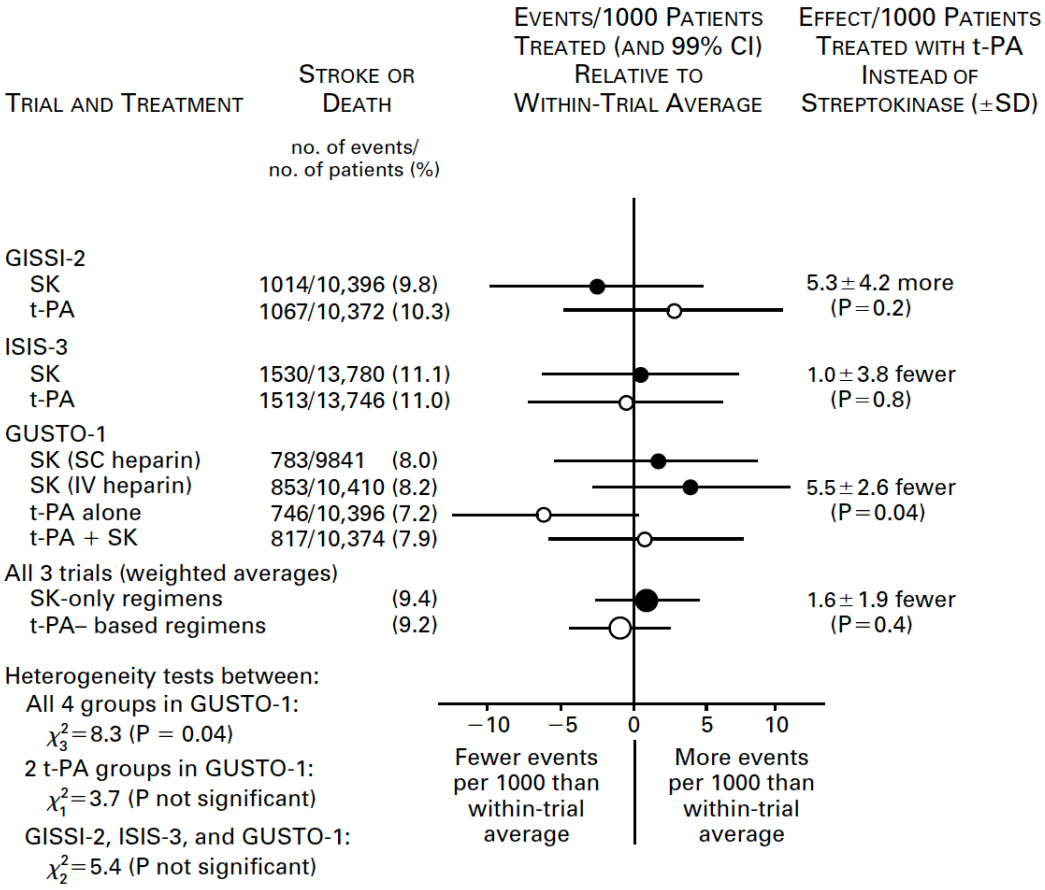


Figure 3. Stroke or Death in the Three Large, Directly Randomized Comparisons (GISSI-2, ISIS-3, and GUSTO-1) of the Standard Streptokinase Regimen with More Intensive t-PA–Based Fibrinolytic Regimens.

Source: Alastair J.J.Wood, Roby Collins et al, Aspirin, Heparin, and fibrinolytic Therapy Iin Suspected Acute Myocardial Infection, 1997.

Abbreviations and definitions

AIS: Acute Ischemic Stroke

AMI: Acute Myocardial Infarction

ANGIOPLASTY: During angioplasty, a thin tube called a catheter with a small balloon at its tip is inserted into an artery, usually in the groin or wrist, and guided to the blocked coronary artery. Once in place, the balloon is inflated to widen the artery, improving blood flow to the heart. In many cases, a stent (a small wire mesh tube) is placed in the artery to help keep it open.

BIOSIMILARS: Biosimilars are essentially "generic" versions of biologic drugs, designed to be very similar or identical to the original brand-name product. Biosimilars must show they have no clinically meaningful differences in terms of safety, potency, and purity compared to the original biologic reference product. Regulatory agencies like the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) have stringent guidelines for approving biosimilars. Biosimilars can offer significant cost savings and improve patient access to essential medications by providing more affordable alternatives to expensive biologic drugs.

CFDA: China Food and Drug Administration

CMR: Cognitive Market Research

Coherent: market research company

CT: Computed Tomography

ECG: Electrocardiogram

INTRAVENOUS AND BOLUS: "intravenous" and "bolus" refer to two different methods. The intravenous method involves delivering medication directly into a vein over a longer period, usually through a drip. The medication is administered slowly and steadily, allowing for continuous and controlled delivery. “Intravenous Bolus “or Push involves injecting a single, concentrated dose of medication directly into a vein over a short period, often within a few minutes. Bolus is often used in emergency situations or when a rapid response is needed, such as administering a clot-busting drug for a stroke.

EMA: European Medicines Agency

FDA: Federal Drug Administration, American regulatory agency

META-ANALYSIS: Meta-analysis is a statistical technique used to synthesize evidence from different studies, increasing the statistical power and reliability of the findings. It can provide a clearer picture of the effectiveness of an intervention or the strength of an association.

MRI: Magnetic Resonance Imaging

NHLBI: American National Heart, Lung, and Blood Institute

NINDS: American National Institute of Neurological Disorders and Stroke

PE: Acute Pulmonary Embolism

PCI: percutaneous coronary intervention

rDNA: recombinant DNA

rt-PA recombinant t-PA

SAK: staphylokinase

STEMI: ST Elevation Myocardial Infarction. STEMI stands for ST-Elevation myocardial infarction. It is a type of heart attack that is characterized by an elevation of the ST-segment on an electrocardiogram, ECG. The elevation indicates that a significant portion of the heart muscle is not receiving blood due to a complete blockage of a coronary artery.

THROMBECTOMY: A thrombectomy is a surgical procedure used to remove a blood clot (thrombus) from a blood vessel. A catheter (a thin, flexible tube) is inserted into a blood vessel and then guided to the location of the blood clot. Various techniques can be used to remove the clot. The goal is to physically extract the clot from the vessel.

t-PA: (tissue type plasminogen activator, generic name Alteplase)

WHO: World Health Organization

WSO: World Stroke Organization