

Tissue plasminogen activator and Granulocyte-macrophage colony-stimulating factor. Made by genetic engineering, No. 7

Recombinant tissue plasminogen activator (rt-PA) in fibrinolytic therapy

For decades conventional biotechnology has been of major importance in enriching lifestyles, as well as in human health care. The major milestones of biotechnology have been conservation or taste improvement of food, production of alcoholic beverages and cheese, manufacturing of primary and secondary metabolic products of micro-organisms such as vitamins, amino acids, nucleotides, antibiotics, cytostatic or immunomodulating compounds and enzyme inhibitors. In the field of biotransformation, stereospecific modification of steroids or specific cleavages of penicillin or cephalosporin were carried out, yielding 6-aminopenicillanic acid or 7-amino cephalosporanic acid for further chemical modifications.

The new biotechnology breakthrough in the pharmaceutical field is the production of endogenous human proteins which guarantee life processes in the human organism and which can be administered in order to substitute the missing or defective protein responsible for the pathological status in patients. Several human proteins can be isolated in small amounts from body fluids, but they cannot be obtained in sufficient amounts for the treatment of patients in the clinic and without the hazard of human viral contaminants.

It is obvious that before starting clinical trials it has to be demonstrated by basic research that a defined protein is in strong correlation

with a pathological status. Subsequently, by means of genetic engineering, based on the knowledge of molecular biology, the specific genetic information for the synthesis of the desired protein has to be isolated, integrated into an appropriate vector and transferred into a suitable single-cell organism for production of the envisaged protein.

Tissue plasminogen activator, a fibrin specific thrombolytic agent for the therapy of thromboembolic diseases such as myocardial infarction (AMI), pulmonary embolism and deep venous thrombosis was genetically engineered by Genentech and jointly developed with Boehringer Ingelheim (Table 1). It is marketed world-wide by Genentech, Boehringer Ingelheim, Mitsubishi and Kyowa Hakko. Due to its clear and well-understood mode of action, tissue plasminogen activator could be developed straight forwardly from biotechnical production through clinical investigations and international registration to marketing within 4 years until its launch in 1987.

Although fibrinolytic therapy has been available since streptokinase reached the market in 1962, only a few studies showing the benefit of this therapy had been performed until 1985. Due to the development of Actilyse® (Alteplase) the recombinant DNA-derived tissue plasminogen activator (rt-PA, CAS, W5857-23-6), there was growing interest in fibrinolytic treatment of myocardial infarction and other thromboembolic diseases.

Within a short period, 5–7 years, more than 100000 patients with AMI have been studied in prospective clinical trials showing a significant reduction in short term as well as in long term mortality with fibrinolytic therapy compared to controls.

It has also been clearly shown, that rt-PA is superior to other thrombolytic substances reducing mortality in patients with myocardial infarction. This superiority is related to the faster mode of action and less side effects of rt-PA. So far more than a million patients with AMI have been treated world-wide with rt-PA.

Besides the registered indications myocardial infarction and pulmonary embolism ongoing clinical development of rt-PA hopefully will offer new therapeutic chances in thromboembolic diseases like stroke, peripheral arterial disease and deep venous thrombosis. The first clinical results are available and look very promising.

The quality standard for such products as rt-PA derived from recombinant DNA technology is very high and can only be achieved by controlled and validated biotechnical production processes with corresponding in-process controls and tight specifications for the final product. Moreover, recombinant DNA derived proteins are produced by well-characterized cells in defined media with tested raw materials, thereby avoiding potential risks of viral contamination. This guarantees a consistent high reliability for the therapeutic use.

The combination of an efficient fermentation process in serum-free medium with sophisticated protein purification methods leads to a high yield of active protein with a purity greater than 99.9%. Dedicated and sensitive analytical methods are necessary to detect minor protein contamination in the ppm range. The final product is tested to be free of any bacterial or viral contaminants. The DNA level in the final product is below the limit which is specified by the WHO (100 pg/dose) and FDA (10 pg/dose).

Biotechnical processes offer the possibility of synthesizing complicated low molecular weight molecules with stereochemical problems and complex protein structures on an economical basis. In addition, biotechnology has further en-

vironmental advantages. The reactions are carried out under low energy consumption, with low pressures, moderate temperatures, physiological pH values, using biodegradable raw materials, producing biodegradable products, providing low emission, without dangerous reactions and in predominantly aqueous systems.

In summary, genetic engineering is of great benefit for patients who require protein substitution therapies. In connection with modern biotechnology these substances can be produced by reduced hazards for the environment.

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GM-CSF: more than a growth factor

Until recently, cancer patients undergoing chemotherapy or radiation treatments had few options when their white blood cell counts sank to dangerously low levels, leaving them at risk of life-threatening infections: they could receive preventive antibiotics and/or their treatments could be reduced or delayed until the blood counts came back up to safe levels. Unfortunately, treatment delays and dosage reductions may compromise the effectiveness of the therapy, reducing the probability of remission or cure. Now, thanks to genetic engineering, there is another option for cancer patients.

It has long been known that there are substances called 'growth factors' in the blood of healthy individuals. These growth factors are proteins that can regulate the production and function of blood cells, including the white blood cells, which are important components of the immune system.

Unfortunately, it was not possible to isolate these growth factors, in sufficient numbers for medical research and use until the development of recombinant DNA technology.

One of the growth factors is called granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF was named for its ability to stimulate the growth in cell culture of two types of white blood cells—granulocytes and macrophages—that are critical for fighting infection. It has since been discovered that GM-CSF also stimulate the functional activity of mature granulocytes and macrophages, increasing their ability to combat infections and destroy tumour cells. GM-CSF acts at a relatively early stage in the development of blood cells, before they are fully differentiated and also stimulates the growth and maturation of other blood cell types.

LEUCOMAX[®] Molgramostim (GM-CSF) is the tradename for the GM-CSF developed cooperatively by Sandoz Pharma and Schering-Plough (Table 1). The human gene for GM-CSF was identified and isolated. Using the techniques of recombinant DNA technology, the gene was inserted into a common type of bacterium, *Escherichia coli*, which reproduces rapidly and is easy to grow commercially. The genetically engineered *E. coli* thus yields GM-CSF in the large amounts required for general medical use.

LEUCOMAX[®] Molgramostim (GM-CSF) is widely used to help prevent the decrease in white blood cells that is associated with the administration of cancer chemotherapy. It is also utilised to restore normal levels of fully functional white blood cells after depletion or damage by chemotherapy or underlying diseases such as myelodysplastic syndromes and after bone marrow transplantation. Benefits to the patient include a reduction in the number of infections, days of hospitalisation and antibiotics, as well as fewer treatment delays and dosage reductions. Less commonly, LEUCOMAX[®] Molgramostim (GM-CSF) is also used to allow the administration of full dosages of an immunosuppressive drug in patients with AIDS-related CMV retinitis, a viral eye infection.

Future applications of LEUCOMAX[®] Molgramostim (GM-CSF) will include special situations within the general areas of current use, but will also take completely new directions.

Of great interest in the medical community is the ability of GM-CSF, particularly in combination with interleukin-3 (IL-3)—another recombinant growth factor from Sandoz research—to mobilise early progenitor cells to leave their usual growth sites within the bone marrow and enter the bloodstream. These peripheral blood progenitor cells are easier to collect than are bone marrow cells and they may be used to replace or to supplement bone marrow for transplantation.

In addition, adding GM-CSF to the harvested progenitor cells may greatly enhance their numbers prior to transplantation. In the cancer area, GM-CSF is also being evaluated for its ability to activate tumour-killing white blood cells as well as its ability to induce malignant cells into a phase of their growth cycle during which they are more sensitive to being killed by many chemotherapeutic agents.

The antimicrobial potential of LEUCOMAX[®] Molgramostim (GM-CSF) is being further investigated for use against fungal infections and parasites and for patients who are not immunosuppressed but have infections and may benefit from increased stimulation of the immune system.

LEUCOMAX[®] Molgramostim (GM-CSF) is administered systemically by subcutaneous injection or intravenous infusion for most uses, but it is also being studied for local application to accelerate wound healing (including post-surgical wound closure, leprosy, diabetic ulcers, radiation wounds and skin grafts) and as a mouthwash for the sores associated with cancer chemotherapy. Both the wound healing and anti-tumour activities of GM-CSF may be responsible for its observed activity in the treatment of Kaposi's sarcoma.

An exciting new direction for GM-CSF is research into its potential use as a vaccine adjuvant. Because of its ability to stimulate immunological mechanisms, GM-CSF may be used to ensure the effectiveness of antimicrobial vaccines. This could be important for patients with immune deficiencies who are particularly susceptible to infections but for whom vaccinations may be ineffective. Future appli-

cations in the vaccine area may include inserting the GM-CSF gene into tumour cells and reinfusing these genetically engineered tumour cells into the patient to induce a strong anti-tumour response.

The production of recombinant GM-CSF by genetic engineering has made it possible to decrease cancer treatment-related side effects and may allow patients to receive full doses or higher doses of chemotherapy. Recombinant GM-CSF

also holds great promise in other clinical applications.

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The products published in this series so far are summarised in Table 2.

Table 1
Made by genetic engineering No. 7

Product	Tissue plasminogen activator (rt-PA)	Granulocyte-macrophage colony-stimulating factor (GM-CSF)
Principal trade names	Actilyse® Alteplase	Leucomax® Molgramostim (GM-CSF)
Principal uses	Therapy of thromboembolic diseases	Supporting treatment in anticancer chemotherapy and immuno-suppressive drug therapy
Manufacturer	Dr Karl Thomae Boehringer Ingelheim Pharma	Sandoz Pharma Schering Plough International
Donor organism	Human gene	Human gene
Host organism	Mammalian cells	<i>Escherichia coli</i>
Advantages	Production by well characterized cells No potential risks of viral contamination Less side effects than other thrombolytic substances Well documented successful and safe use (more than a million patients with myocardial infarction have been treated)	High-yield production by easy bacterial fermentation Wide use in preventing decrease of white blood cells associated with anticancer therapy Further potential applications under investigation (release of early progenitor cells, activation of tumor-killing white blood cells, stimulation of the immune system, wound healing)

Table 2
Summary of products published in the series made by Genetic Engineering

Product:	Lipase	Hepatitis B vaccine
Author/manufacturers	B. Diderichsen, Novo Nordisk, Denmark	P. Crooy, SmithKline Beecham Biologicals, Belgium
Made by genetic engineering No. 1	Biotech Forum Europe 8, 246–247, 1991	
Product:	Human insulin	Human growth hormone
Author/manufacturers	E. Rasmussen, Novo Nordisk, Denmark	L. Fryklund, Kabipharmacia, Sweden
Made by genetic engineering No. 2	Biotech Forum Europe 9, 144–145, 1992	
Product:	Protein G	Interferon alpha-2a
Author/manufacturers	R. Hjorth, Pharmacia LKB Biotechnology, Sweden	S. Ryser, F. Hoffmann-La Roche AG, Switzerland
Made by genetic engineering No. 3	Biotech Forum Europe 9, 641–642, 1992	

Table 2 (Continued)

Product:	AIDS test	α-Amylase
Author/manufacturers	E. Baumann, F. Hoffmann-La Roche AG, Switzerland	B. Diderichsen, Novo Nordisk, Denmark
Made by genetic engineering No. 4	J. Biotechnol. 38, 193–197, 1995	
Product:	Erythropoietin	Interferon beta-1b
Author/manufacturers	C. Kionka, Boehringer Mannheim, Germany	T. Petri, Schering AG, Germany
Made by genetic engineering No. 5	J. Biotechnol. 43, 73–77, 1995	
Product:	Interferon gamma	Rabies vaccine
Author/manufacturers	E. Falkner and I. Maurer-Fogy, Bender & Co GesmbH/Boehringer Ingelheim Vienna, Austria	J. Terré, G. Chappuis, M, Lombard and P. Desmettre, Rhône Mérieux, France
Made by genetic engineering No. 6	J. Biotechnol. 46, 155–159, 1996	
Product:	Tissue plasminogen activator (rt-PA)	Granulocyte-macrophage colony-stimulating factor (GM-CSF)
Author/manufacturers	W. Werz and R.G. Werner, Dr Karl Thomae GmbH/Boehringer Ingelheim Pharma, Germany	R. Till, Novartis / Schering Plough International, Switzerland
Made by genetic engineering No. 7	J. Biotechnol. 61, 157–161, 1998	