# Plasma Derived Versus Biotechnological Manufactured Medicine

*Paul F. W. Strengers MD, FFPM.*
*Sanquin Blood Supply, Ams terdam, The Netherlands ,Email: p.strengers@sanquin.nl*

## Introduction

The research on alternatives for plasma products, whether biotechnological developed medicinal products or recombinant alternatives to plasma products, with the development of recombinant factor VIII started at the end of the 1980s. There was considerable interest in the possibility of producing a 'synthetic' form of factor VIII and factor IX in view of the high rate of transmission of blood-borne viruses by plasma derived factor concentrates in the 1970s and early 1980s,.

This objective became reality when scientists at the Genetics Institute (Cambridge, Mass., US) and Genetech (Berkeley, Ca.,US) simultaneously announced the successful cloning of Factor VIII gene. In a landmark series of articles in a single issue of Nature in 1984, the two groups of scientists described the structure of the Factor VIII gene, the isolation of cDNA clones encoding the complete factor VIII sequence, and the in vitro expression of human factor VIII in tissue culture (1, 2, 3, 4). In collaboration with scientists of these two institutes, two US based pharmaceutical companies accomplished scale-up, purification and standardization of two recombinant factor VIII products for clinical use.

## Recombinant technology

The first recombinant factor VIII products, produced by cell culture, were licensed in 1992-1993 and induced a great change in the treatment of patients with haemophilia A. The high costs of haemophilia treatment and the unavailability of clotting factor products for developing countries were stimuli for researchers to study the basics of factor proteins. The expectations and beliefs in new biotech products at that time were sky-high. While many research groups focussed on recombinant factor VIII and IX, only a few products reached the market. In 1997, recombinant factor IX was licensed, and in the same year recombinant factor VIIa. The current products are similar to natural coagulation factors, but recombinant technology also allows for the design of bio-engineered proteins with

improved biological properties. This is therefore one of the most promising areas of research in haemophilia is related to factor VIII protein. Also biotechnological research on other plasma proteins started and resulted in 2001 in the licensing of activated protein C. Since then however, on recombinant technology, no new developments in plasma products have reached the market. What has changed is the formulation of the recombinant products by deleting human plasma proteins in the formulations and as stabilisers. The newest recombinant products lack any human or veterinarian protein.

The research developments in this area are however still on going. Recombinant thrombin is in phase III pre-registration phase, recombinant factor XIII in phase I, and recombinant von Willebrand factor in preclinical phase. Factor VII, protein C and fibrinogen are expressed in cell culture.

On recombinant human albumin two companies has formed a joint venture to expand the commercial development opportunities of a recombinant human albumin program. The joint venture will manage development of the product for both the blood expander market and for the use in the excipient market. Recombinant human alpha-1 antitrypsin is a recombinant form of the blood protein alpha-1 antitrypsin and is also in the development phase. The company has established founder transgenic animals that express recombinant human alpha-1 antitrypsin . The plasma form of this product has been used as a treatment for certain forms of emphysema caused by a congenital deficiency of plasma alpha-1 antitrypsin. Recombinant human alpha-1 antitrypsin may also be developed as an effective treatment for other diseases, potentially including cystic fibrosis, chronic obstructive pulmonary diseases, acute respiratory syndrome, and severe asthma.

## Monoclonal antibody based immunoglobulins

Monoclonal antibodies are antibody proteins that bind to a specific target molecule (antigen) at one specific site (antigenic site). In response to either infection of immunization with a foreign agent, the immune system generates many different antibodies that bind to the foreign molecules. Individual antibodies within this polyclonal antibody pool bind to specific sites on a target molecule known as epitopes. Isolation of an individual antibody within the polyclonal antibody pool would allow biochemical and biological characterization of a highly specific molecular entity targeting only a sin-

gle epitope. Realization of the therapeutic potential of such specificity launched research into the development of methods to isolate and continuously generate a supply of a single lineage of antibody, a monoclonal antibody (mAb). Disease areas that currently are especially amenable to antibody-based treatments include cancer, immune dysregulation, and infection. Depending upon the disease and the biology of the target, therapeutic monoclonal antibodies can have different mechanisms of action. A therapeutic monoclonal antibody may bind and neutralize the normal function of a target. For example, a monoclonal antibody that blocks the activity of the protein needed for the survival of a cancer cell causes the cell's death. Another therapeutic monoclonal antibody may bind and activate the normal function of a target. For example, a monoclonal antibody can bind to a protein on a cell and trigger an apoptosis signal. Finally, if a monoclonal antibody binds to a target expressed only on diseased tissue, conjugation of a toxic payload (effective agent), such as a chemotherapeutic or radioactive agent, to the monoclonal antibody can create a guided missile for specific delivery of the toxic payload to the diseased tissue, reducing harm to healthy tissue.

At the present time, there are at least 213 companies active in various aspects of protein-based therapeutics development. Immunotherapeutic drugs divide broadly into two groups: vaccines that elicit an active immune response (generating antibodies that target the disease), and therapeutic antibodies that bypass the immune system and directly target the disease (so-called passive immune treatment).

By nature, the polyvalent immunoglobulins (Immunoglobulin IM, SC and IV) are considered as not being replaced by monoclonal antibody products. The polyvalent immunoglobulins derived from plasma of at least 1,000 donations consist of a great number and variety of antibodies representing the normal physiological status of the human body. Plasma derived specific or hyper immune immunoglobulins however, could theoretically be replaced by monoclonal antibody products because these products have a titre of one specific antibody. Research on monoclonal anti Rhesus (D) immunoglobulin is an example of these efforts. Monoclonal anti-Rhesus (D) antibodies (MAbs) are all derived from human anti-D producing B cells from immunised donors, as mice do not recognise the D antigen. The first mAbs were produced by immortalizing B cells with Epstein-Barr virus then cloning and

selecting the anti-D secreting EBV-B lymphoblastoid cell lines. Although a huge amount of information has been collected on the biological activity of antibodies directed to blood cells, no products have reached the market due to break troughs during clinical trials (5).

## Transgenic technology

The current scarcity of factor products is caused by the low concentration of factor VII and factor IX in human plasma. Also in a bioreactor cultures of genetically engineered animal cells (Chinese Hamster Ovary -CHO- cells) only limited concentrations can be produced. In contrast, milk of transgenic livestock is a prodigious and expedient source of complex therapeutic proteins such as factor VIII or factor IX. In 2004, Van Cott et al calculated that 1.2 million litres of plasma donations per year are needed to meet the needs of prophylactic therapy of all haemophilia B patients n the US (6). For recombinant factor IX production the equivalent is 600.000 litres of CJO Cell Supernatant per year. An equivalent amount of factor IX can be produced from no more that 12,000 litres milk of transgenic pigs, the total milk yield from an average sow being 100-300 litres a year. This enables the production of large quantities of factor IX, even more than the amounts needed for patients' use. Van Cott suggests in his article that this excess volume can be used for another administration route for factor VIII and factor IX, for instance oral administration even though this is less efficient than the traditional intravenous route (7). For transgenic production, admission to the market by European and American registration authorities will be of utmost importance. The first medicine produced using genetically engineered animals was Atryn, which is the brand name of the anticoagulant antithrombin manufactured from the milk of goats that have been genetically modified to produce human antithrombin, a plasma protein with anticoagulant properties. According to the manufacturing company one genetically modified goat can produce the same amount of antithrombin in a year as 90,000 blood donations. Goats were selected for the process because they reproduce more rapidly than cattle and produce more protein than rabbits or mice. In 2009, ATryn was approved in the U.S. for treatment of patients with hereditary antithrombin deficiency who are undergoing surgical or childbirth procedures. In 2006, the European Medicines Agency (EMEA) initially rejected and, after an appeal from GTC, approved the drug for use in the European

Union countries.

A second transgenic product is C1-inhibitor. C1-inhibitor a protease inhibitor belonging to the serpin superfamily is also produced from plasma. Deficiency of this protein is associated with hereditary angioedema ("hereditary angioneurotic edema"), or swelling due to leakage of fluid from blood vessels into connective tissue. Its main function is the inhibition of the complement system to prevent spontaneous activation.Transgenic C1-esterase inhibitor is produced in glycosylated form using transgenic rabbits. The product is licensed in Europe, but not in the US.

Transgenic haemophilia products are still in an early research phase so the products cannot be expected within ten years. Nevertheless, this timeframe implicates that transgenic haemophilia products could mean serious competition for research on gene therapy. Factor VIII is expressed in genetically-modified tilapia, a fresh fish farmed for food, and in transgenic sheep, factor IX in transgenic sheep and in preclinical phase, and with factor VIIa the developments have been started. On thrombin, factor XIII, factor VII, protein C, fibrinogen, the protein involved has been expressed in transgenic animals, while with fibrin sealant this product is in phase II research. Such developments may lead to production of cheaper plasma products on a larger scale in the future.

## Pro and Cons

The main advantage of biotechnological products is viral safety. While the current plasma-derived plasma products are safe and transmission of blood borne infections have not occurred since 1996, any discovery of an emergent virus that potentially can be transmitted by blood requires evidence by validation studies and risk assessment analyses that the plasma product is regarding the potential transmission of transfusions transmittable infections. Maximum safety is the objective. The transmission of viruses which are potentially present in cell lines which produce the recombinant proteins has never been reported.

The disadvantages of these products are the development of antibodies against the biotechnological produced proteins since these proteins are not identical to the self protein. Currently a prospective study is ongoing where the incidence of antibody formation against recombinant factor VIII is compared to the antibody formation against plasma derived factor VIII. Another disadvan-

tage of these products is the high costs. Also with transgenic produced proteins the occurrence of antibody formation is a matter of concern. In particular because of the treatment with these products of chronic diseases, a life long concern on antibody formation and thus neutralisation of the administered protein ask for intensive post marketing follow up. Although biotechnological manufactured proteins inherently have the promise that abundant production implies low costs, this promise had not become truth over the last years.

Recombinant factor VIIa has been proposed as alternative to prothrombin complex concentrates for reversal the effects of vitamin K antagonists such as warfarin or cumadin. However, its short half life may result in a need for repeated doses, increasing the risk of thrombosis. Given recent reports of its usefulness in vitamin K antagonist reversal, trials assessing safety are needed.

## Future

The developments on new plasma products manufactured by biotechnological production techniques will continue and will result in new recombinant substitutes. In particular efforts will be made to manufacture a new generation of improved recombinant products with extended half-life, less immunogenic, and more resistant to inactivation. The question whether the new transgenic substitutes will be less expensive and enable larger supplies is not solved. At the same time efforts will be made to get new indications for existing products, and new therapies (e.g. gene therapy).

For the hyper-immune immunoglobulins substitution developments are ongoing to manufacture new vaccines, new anti-virals, and new monoclonals.

## References

*1. Gotschier J, Wood WI, Goralka TM et al. Characterization of the human factor VIII gene. Nature 1984; 312:326-330.*

*2. Toole JT, Knopf JL, Wozney JM et al. Molecular cloning of a cDNA encoding human aanti-hemphilic factor. Nature 1984; 312:342-347.*

*3. Vehar GA, Keyt B, Eaton D et al. Structure of human factor VIII. Nature 1984; 312:337-342.*

*4. Wood WI, Capon DJ, Simonsen CC et al. Expression of active human factor VIII from recombinant DNA clones. Nature 1984; 312:342-347.*

*5. Kumpel BM. Efficacy of RhD monoclonal antbodies in clinicval trials as replacement therapy for prophylactic anti-D immunoglobulin: more questions than answers Vox Sanquinis 2007;93:99-111.*

*6. Van Cott KE, Monahan PE, Nichols TC, Velander WH. Haemophilic factor produced by transgenic livestock: abundance*

that can enable alternative therapies worldwide. Haemophilia 2004; 10 Suppl 4:70-76.
7. Hemker HC, Hermens WT, Muller AD, Zwaan RF. Oral treatment of haemophilia A by gastro-intestinal absorption of factor VIII entrapped in liposomes. Lancet 1980; 1(8159):70-71.