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Therapeutic plasma proteins: their incredible potential as a source of health

From the ancient Greeks and Arabs to Leonardo da Vinci in the Renaissance, scholars have been fascinated by the function of blood — and this interest has only grown as medical science has progressed. For more than 75 years, companies such as Grifols have been developing research projects with plasma proteins — so-called haemoderivatives, which include albumin, immunoglobulins, coagulation factors and α1 antitrypsin, among others. These proteins are used to treat rare diseases such as haemophilia, primary immunodeficiencies and α1 antitrypsin deficiency, but they have even greater potential. Grifols is trialling the use of plasma-derived products to treat disorders such as Alzheimer's disease, cirrhosis of the liver and myasthenia gravis. Constant innovation is needed in the process of obtaining plasma proteins to improve already sophisticated production methods and guarantee the highest levels of patient safety.

Since its foundation in 1940, Grifols has played a decisive part in the development of the plasma industry by combining research and development with innovation (R&D+i). This commitment to science and research remains central to the company's mission. Grifols' R&D+i strategy seeks to leverage internal capacity together with external investments and partnerships, with the aim of optimizing the development of innovative therapies.

Plasma proteins have long been a focus of great interest because an excess of or deficit in these substances is frequently a direct cause of illness. Of the many thousands of proteins that circulate in our plasma, we currently only know of therapeutic uses for a few tens of them. The most important proteins are albumin, immunoglobulins, coagulation factors (such as factor VIII and factor IX), antithrombin and α1 antitrypsin (**Fig. 1**). Generally, they are used for the treatment of rare diseases that are linked to a deficit of the protein in question. But now, there is a growing research effort focused on identifying the relationship between such proteins and 'non-plasma-related' pathologies, and working out what the many blood proteins of unknown function actually do. Because plasma is the vehicle used by a vast number of molecules to reach their target organs, it is hardly surprising that plasma is starting to be seen as an 'organ' in its own right, as it is capable of influencing almost every system in the body.

Over the past decade, the therapeutic possibilities of plasma-derived products have expanded dramatically as a result of the identification of relationships between some plasma proteins and certain diseases that have not traditionally been linked to plasma. For example, Grifols is studying the use of plasma proteins, either individually or in combination with others, in the treatment of neurodegenerative disorders such as Alzheimer's disease. One example of this is the Alzheimer Management by Albumin Replacement (AMBAR) project, which explores the use of plasma exchange with albumin, an approach based on the plasmapheresis technique developed during the 1950s in Barcelona, Spain, by José Antonio Grífols Lucas[1] (see below).

Grifols has been a major contributor to the development of the plasma products industry

Plasma fractionation can be traced back to the treatment of soldiers who suffered burns and other injuries during the Second World War[2]. At first, the only treatment available

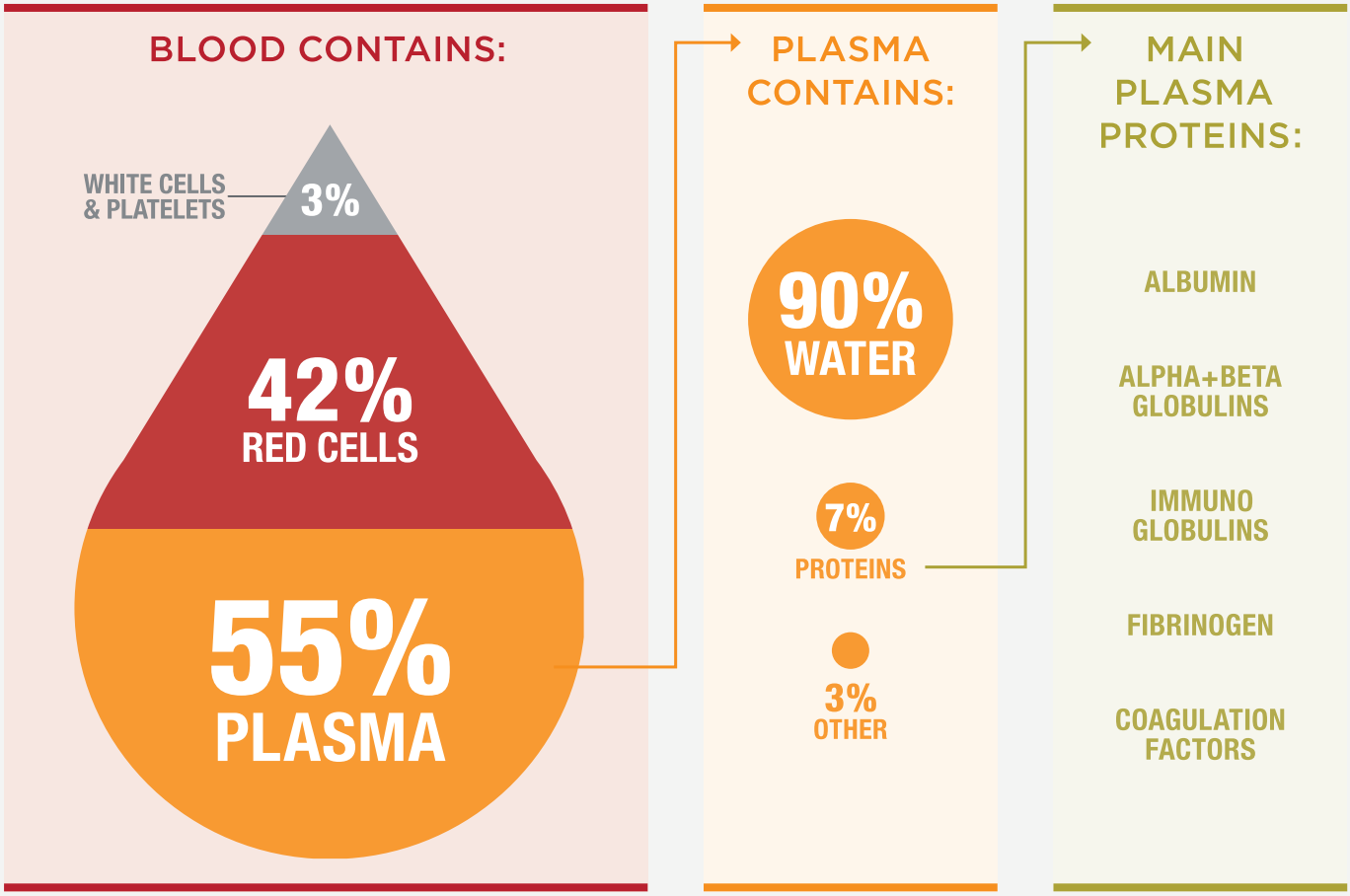

Figure 1. Blood composition diagram.

was fresh plasma, but later, after Edwin J. Cohn from Harvard University in Cambridge, Massachusetts, introduced his fractionation method[3], the focus was on obtaining albumin. Albumin accounts for 55–60% of the total protein volume of plasma and was used to treat heavy bleeding due to war injuries, a common occurrence on the battlefield. Unlike plasma, albumin did not require blood typing to be administered allowing a much faster intervention. A further step was the development of donor plasmapheresis[1], a technique that made it possible to separate plasma from the other blood components (red blood cells, platelets and other cells), which are injected back into the donor during the donation process. As a result, plasma could be collected from donors without extracting other blood components, which take much longer to regenerate than plasma, thus allowing more frequent successive donations.

Plasmapheresis was developed by José Antonio Grífols Lucas in 1951 in Barcelona, Spain. Grífols Lucas's work was presented at the *Fourth International Congress of Blood Transfusion* in Lisbon, Portugal, and published in the *British Medical Journal* in 1952 (ref. 1). The development of plasmapheresis was fundamental to the ability to produce medicines from human plasma, and it is currently the most widely used technique for collecting plasma from donors for therapeutic purposes.

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However, the interest in blood disorders and the therapeutic properties of blood is far older. Although the ancient Greeks made the first attempts to control haemorrhage, it was not until the eighteenth century that John Conrad Otto began to study a disease related to excessive bleeding, which afflicted male members of the same family[4]. In 1828, Friedrich Hopff used the term haemophilia to describe this disease for the first time, thus naming one of the first identified blood-related illnesses[5]. The earliest treatment of the condition can be traced back to 1900, when the first blood transfusions were performed. In 1950, plasma was used as a treatment, and in the 1970s, cryoprecipitate was introduced as a therapy. The production of coagulation factor concentrates (primarily factor VIII) began in the 1980s (ref. 5).

The interest in the immunological properties of plasma grew in parallel with the development of modern immunology. For example, the understanding of primary immunodeficiencies (PIDs) began to develop with Ogden Bruton's articles in the journal *Pediatrics* in 1952 (ref. 6).

PIDs are a group of diseases related to immunoglobulin deficiencies that cause serious, recurring infections. To date, more than 150 types have been identified.

In the 1950s, intramuscular injection of immunoglobulin was developed as a therapy to treat these diseases[7]. However, because

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intramuscular injections are painful, only small volumes could be administered, which did not allow for adequate dosing. In the 1980s, purification methods had sufficiently improved to enable the production of 'intact' molecules (similar to those found in human plasma) with full biological efficacy, allowing the development of commercial preparations of intravenous immunoglobulin (IVIG). Grifols launched its first IVIG in 1992.

Grifols also produces a range of hyperimmune immunoglobulins that provide rapid, temporary immunity against a series of infections such as rabies, tetanus, hepatitis B and rhesus disease (also known as Rh incompatibility), some of which are potentially fatal.

α1 antitrypsin deficiency (AATD) was first described in 1963 by Carl-Bertil Laurell and Sten Eriksson, who observed the deficiency in this plasma protein in people who developed pulmonary emphysema and liver disease[8]. During the 1980s, α1 antitrypsin was purified for the first time, and in 1987, Grifols obtained a license for its use as replacement therapy to treat severe congenital deficiency and impaired lung function. Today, Grifols is the world's leading producer of α1 antitrypsin for the treatment of AATD in patients with pulmonary emphysema.

Antithrombin, which belongs to the same biological family as α1 antitrypsin, is another protein manufactured by Grifols and is used in the treatment of congenital antithrombin deficiency. A deficit of this protein has the opposite effect to that of haemophilia: a tendency toward thrombosis or the formation of a clot inside a blood vessel, particularly when the sufferer is undergoing surgery or during childbirth.

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The discovery of diseases linked to plasma-protein deficiencies and the interest in both blood-related and plasma-protein-related disorders helped to drive an expansion in the technological capacity of the plasma industry, which developed the technology required to fractionate, purify and prepare plasma proteins for use as medicines.

From donor to patient: the role of plasma donors in the production of plasma-derived medicines

Grifols follows the US Food and Drug Administration (FDA)'s Code of Federal Regulations, European Medicines Agency (EMA) regulations, Plasma Protein Therapeutics Association International (PPTA) Quality Plasma Program guidelines, and World Health Organization and European Pharmacopoeia regulations with regard to plasma collection. All of the plasma used to produce its plasma-derived medicines comes from registered donors, who donate at centres approved by the FDA and European Union authorities.

Thanks to plasmapheresis, it is possible today to produce plasma-derived medicines on a large scale. Owing to the rapid regeneration of plasma compared with that of blood cells, a person can donate plasma by plasmapheresis up to twice a week. Plasma donors are paid for their time and for their commitment to making regular donations to ensure a sustainable plasma supply. Plasma production makes exclusive use of plasma from regular donors, and the donor remuneration policy guarantees the availability of plasma to treat people who require these life-saving medicines.

Testing laboratories use techniques that have been approved and validated by the FDA in the United States and by the authorities in the European Union.

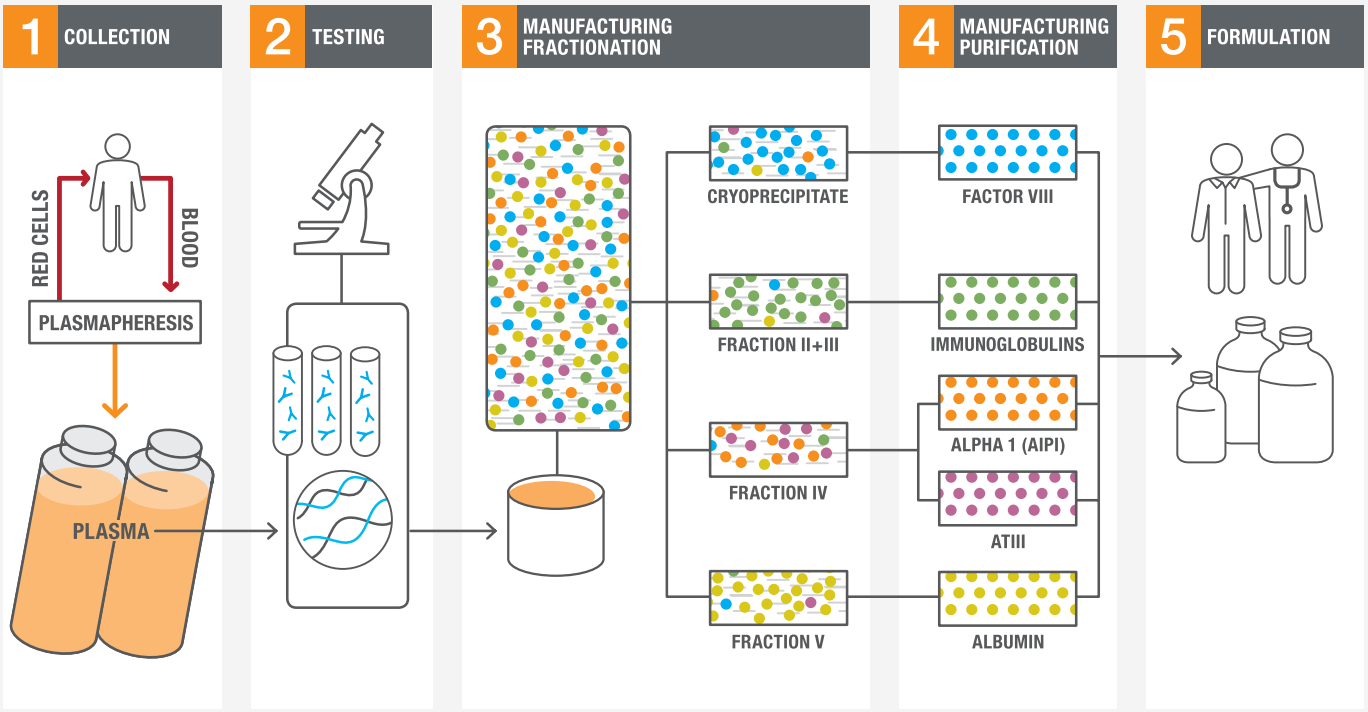

Figure 2. Manufacturing process for plasma-derived medicines.

Every single unit of donated plasma is analysed in FDA-licensed laboratories to guarantee the safety and quality of the source plasma. Plasma samples from different donor centres are delivered to testing laboratories on a daily basis for classification. Immunological analyses (for example, ELISA) and NAT (nucleic acid testing) are routinely performed to guarantee the safety of donated plasma.

All units of plasma that pass the initial viral analysis tests are stored for at least 60 days at −30 °C before being fractionated. This waiting period, or inventory hold period, allows the donor to make a repeat donation. The results of the sample are verified again to confirm that tests for viruses and other pathogens are negative (**Fig. 2**). In addition to all these analyses, different methods of prevention against potential emergent pathogens are applied during the manufacturing process (for example, pasteurization and nanofiltration).

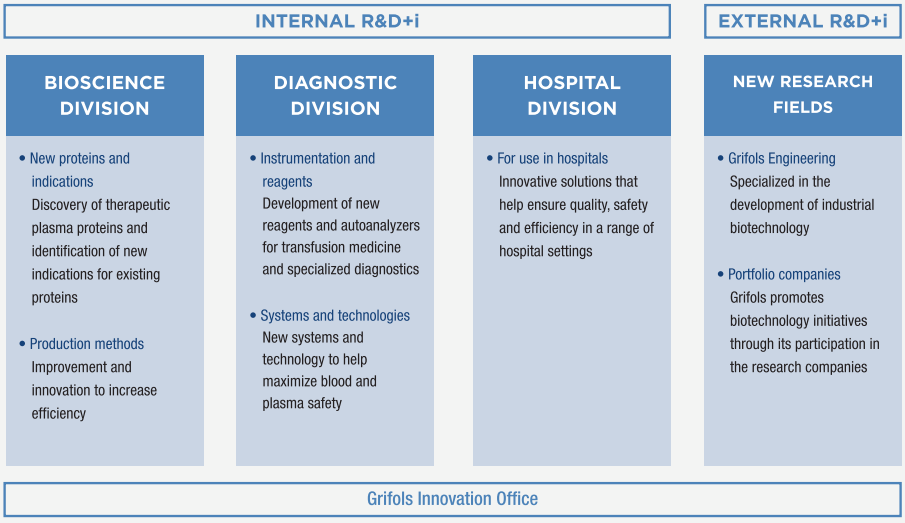

Figure 3. Grifols' internal and external research and development with innovation (R&D+i) projects.

Every single unit of donated plasma is analysed in FDA-licensed laboratories to guarantee the safety and quality of the source plasma.

The future of plasma protein treatments: new therapeutic opportunities

One of Grifols' most ambitious clinical trials is the AMBAR study, which aims to stabilize the progress of Alzheimer's disease through the use of plasma exchange with albumin. This involves extracting plasma from the patient through plasmapheresis and replacing it with albumin. This treatment is based on the hypothesis that most amyloid-β, one of the proteins that accumulates in the brains of people with Alzheimer's disease, is bound to albumin and circulates in plasma. Grifols started its investigations into Alzheimer's disease in 2004. Before starting the AMBAR trial, the company performed several preclinical studies, two pilot clinical studies and a phase 2 clinical trial.

In 2013, the first patients were recruited to the AMBAR trial, and in November 2015, Grifols presented intermediate results of the study, demonstrating the safety and tolerability of this treatment. In December 2016, the last of almost 500 patients were included in the study in 40 hospitals in Spain and the United States. The trial is scheduled to end in 2018, with the efficacy results to be released shortly after completion.

Other studies in progress include the treatment of myasthenia gravis and post-polio syndrome with immunoglobulin, the use of albumin in liver conditions and amyotrophic lateral sclerosis (Lou Gehrig's disease), and the administration of antithrombin in cardiac surgery.

There is particularly exciting research into the use of immunoglobulins in diseases that are not linked to deficits. These include myasthenia gravis, a chronic, autoimmune neuromuscular disease characterized by weakness of the body's skeletal muscles. Grifols is conducting clinical trials into both the maintenance and exacerbation stages of the disease.

Grifols is also trialling an immunoglobulin treatment for post-polio syndrome, a debilitating and painful muscle condition that occurs many years after the patient has suffered polio.

In the field of hepatology, there is a programme researching the use of albumin in cirrhosis of the liver, which will culminate in two large phase 3 clinical trials. The first is the Acute-on-Chronic Liver Failure Plasma Exchange (APACHE) study, which, like the AMBAR study, trials plasma exchange with albumin in the treatment of multi-organ failure associated with cirrhosis (acute-on-chronic liver failure). The second is the Prevention of Mortality with Long-Term Administration of Human Albumin in Subjects with Decompensated Cirrhosis and Ascites (PRECIOSA) study, which uses the intravenous infusion of albumin in the treatment and prevention of the complications of cirrhosis. Both trials will be conducted in Europe and the United States, with between 35 and 40 hospitals participating in each.

One of Grifols' most ambitious clinical trials is the AMBAR study, which aims to stabilize the progress of Alzheimer's disease.

In addition to clinical trials, Grifols is heavily involved in supporting the international hepatology community through its involvement in the European Foundation for the Study of Chronic Liver Failure (EF-CLIF) and the CLIF Consortium, which has almost 100 European member hospitals. There is also a Grifols Chair for the Study of Cirrhosis that is hosted by EF-CLIF. The CLIF Consortium is performing a series of studies to investigate the functions of albumin — particularly its anti-inflammatory properties — and to explain the favourable clinical outcomes observed in

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clinical trials of albumin. These investigations may give rise to a totally new era for albumin in which specific indications beyond 'plasma expansion' can become a reality.

Grifols also actively supports the study of cirrhosis of the liver in the United States.

In terms of increasing our knowledge of plasma proteins, Alkahest, in partnership with Grifols (see below), is studying the relationship of ageing with plasma protein content and the therapeutic implications that this may have. Alkahest has also begun a large study with the aim of elucidating the human plasma proteome.

There is no doubt, however, that we are still very far from completely understanding the biological mechanisms that human evolution has selected over millions of years, and the plasma-protein universe is no exception.

Integrated strategy and long-term vision for R&D+i at Grifols

R&D+i is one of the key foundations of Grifols' success and is designed to accelerate the development of innovative therapies, products and services whose end users are patients. This strategy seeks to leverage internal capacity together with external investment and partnerships.

Within the sphere of plasma proteins, Grifols focuses its internal R&D projects on discovering and developing proteins, investigating therapeutic applications for existing proteins, and improving production processes to increase efficiency, safety and efficacy.

In addition, through the group GIANT (Grifols Innovation and New Technologies) — which was created in 2016 with the objective of boosting innovation — the company promotes other research projects by acquiring stakes in research companies with projects that complement Grifols' core activities. These include Araclon Biotech (Zaragoza, Spain), a firm specializing in the research and development of treatments and diagnostic methods for Alzheimer's disease and other neurodegenerative diseases; Aradigm (Hayward, California), which develops and markets drugs administered by inhalation for the treatment and prevention of serious respiratory diseases; Alkahest (California), which focuses on the R&D of therapeutic applications of age-related plasma proteins to treat illnesses associated with ageing, including neurodegenerative disorders such as Alzheimer's disease; AlbaJuna Therapeutics (Barcelona, Spain), which is developing a treatment based on monoclonal antibodies with great potential to neutralize HIV; Access Biologics (Vista, California), which is dedicated to the manufacture of biological products such as specific sera and plasma-derived reagents used by biotechnology and biopharmaceutical companies for *in vitro* diagnosis, cell cultivation and R&D in diagnostics; and GigaGen (San Francisco, California), which specializes in biotherapeutic drugs to treat serious diseases using antibodies derived from human B cells*.

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In addition, through its Investigator Sponsored Research (ISR) system, Grifols helps researchers to expand scientific knowledge in key therapeutic areas such as immune deficiencies, neurological diseases sensitive to treatment with immunoglobulins, chronic obstructive pulmonary disease, AATD, coagulation and anti-coagulation, shock and trauma, cirrhosis, ascites, and the inflammatory response.

Grifols also has in-house research projects in its Diagnostic and Hospital divisions, which deal with technologies to help clinicians with diagnosis (for example, ELISA and blood typing tests) and with fluid therapy and nutrition, respectively (**Fig. 3**). Through its three divisions — Bioscience, Diagnostic and Hospital — Grifols aims to continue increasing our knowledge of plasma-related molecules and their potential applications.

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Type of white blood cell that produces antibodies. B cells are part of the immune system and are formed from stem cells in the bone marrow. They are also known as B lymphocytes.