Erythrocytes as a Drug Delivery System

The encapsulation of drugs and other therapeutic agents inside red blood cells represents a natural and unique delivery system, and addresses the need for new administration forms to improve the therapeutic index of existing therapies.

THE TECHNOLOGY
Erythrocytes are the most frequently injected cells in the world and are subject to very strict rules. The encapsulation process is performed in accordance with cell therapy and good transfusion practices. Encapsulation of therapeutic molecules into erythrocytes is performed using the patient’s own blood, or donor blood when a patient suffers from anaemia. Regardless of the source, all blood is qualified by the national blood bank to ensure compliance with transfusion regulations.

Why use red blood cells as a vehicle for therapeutic agents? Red blood cells offer a natural and unique vehicle for drugs for a number of reasons:
- The absence of a nucleus, thereby avoiding cell division
- Natural and total biocompatibility
- Complete and well-known biodegradability
- An adjustable in vivo half-life
- The ability to encapsulate molecules or enzymes

THE ENCAPSULATION PROCESS
The encapsulation process relies on reversible osmotic stress by dialysis:
1. Red blood cells are first submitted to a hypotonic medium, which makes them swell. Pores appear on the surface of the erythrocyte membrane
2. The membrane becomes permeable to the therapeutic molecule, which enters the erythrocyte through the pores
3. Once the molecule has entered the cell, the erythrocyte is put into an iso-osmotic medium where it comes back to its initial shape and size, and the pores disappear
4. The therapeutic molecule is definitively encapsulated inside the red blood cell

Protected by an international patent, the technology makes it possible to produce, at an industrial scale, a supply of consistently high quality erythrocytes loaded with drugs – at a constant incorporation rate and at a constant cell rate – whatever their initial characteristics are and whether or not they come from the patient being treated (see Figure 1). The process – which takes only two hours – is innovative, practicable, reproducible and safe. It enables full control of all parameters – such as red blood cell quality, duration of the process, the size of molecule to be encapsulated and process temperature – throughout the process.

APPLICATIONS
All our research projects are focused on exploiting the physiological parameters of red blood cells and share several common goals: to increase treatment efficacy, lower the risk of complications or side effects, reduce the need for aggressive intensive therapy for patients and improve patients’ quality of life and comfort.

Applications of the technology can be divided into two types:
- Type 1 (such as Cleav’ERY and Oxygen’ERY) which has a long circulating action with no release of the compound and specific characteristics
- Type 2 (such as Deliv’ERY and Vaccin’ERY) with modification of the red cell surface in order to target rapidly specific cells such as dendritic cells and macrophages

Figure 1: Stages in the encapsulation process

Figure 2: The encapsulation of therapeutic enzymes (Cleav’ERY system™)
Therapeutic Enzymes (Cleav'ERY System®)

The encapsulation of therapeutic enzymes offers a new solution for their use by increasing their half-life and reducing their toxicity (see Figure 2). Therapeutic enzymes are very often characterised by toxicity. Thanks to their encapsulation in red blood cells, enzymes are active only inside the erythrocyte. It is important to note that there is no release of the enzyme; it is thus protected by the red cell membrane from degradation and contact with the plasma environment. Consequently, hypersensitivity reactions – which decrease the effectiveness of the treatment – are prevented. Moreover, enzymes have a very short half-life ranging from a few minutes to a maximum of a few days. Encapsulated enzymes’ half-life is increased up to that of the erythrocyte; they become active for longer than in their free form – up to one month – and dosages are greatly reduced.

Therapeutic enzymes find application in cancer therapy. Some tumour cells are auxotrophic – that is, they feed on proteins contained in the plasma that are essential to their development and proliferation. Thus cleaving these proteins with specific therapeutic enzymes leads to tumour regression.

The Cleav'ERY system® also finds application in some rare genetic diseases caused by enzyme deficiency and further build-up of undigested macromolecules. The accumulation of macromolecules can affect tissues and organs, leading to deterioration and possibly death. The Cleav'ERY system® can be loaded with enzymes that are known to be efficient but normally have a very short half-life, require repetitive injections and provoke allergic reactions. This new approach strongly increases the therapeutic index of treatment.

Oxygenation (Oxygen'ERY System®)

We have used one of the main functions of red blood cells, namely to bring oxygen to tissues in order to develop a two-fold R&D programme – tissue oxygenation and tumour oxygenation – based on the encapsulation of a haemoglobin allosteric effector into red blood cells. Delivery of oxygen into tissues involves two processes: transport to the microcirculation and diffusion from capillaries into cells. The physiological release of oxygen to cells is, however, only of the order of 25 per cent. By encapsulating a haemoglobin allosteric effector into red blood cells, the haemoglobin’s affinity for oxygen is lowered and oxygen extraction is increased. Tissue oxygen consumption can thus be increased by two to three times the physiological value.

One of the major limitations of radiotherapy is that, as solid tumours outgrow their blood supply, they become deficient in oxygen causing hypoxia. Oxygen is a potent radiosensitiser, increasing the effectiveness of a given dose of radiation by forming DNA-damaging free radicals. Tumour cells in a hypoxic environment may be as much as two to three times more resistant to radiation damage than those in a normal oxygen environment. By better oxygenating these tumours, the Oxygen'ERY system® thus makes it possible to improve the efficiency of radiotherapy. Two types of particularly hypoxic tumours will initially be targeted in our research programme: cerebral tumours, such as glioblastoma, and head and neck cancer. Other tumours treated by radiotherapy, such as cervix and bladder cancer, could also be treated by this new approach.

The oxygenation-based programme is also expected to bring important developments in the treatment of chronic and genetic diseases characterised by hypoxia and anaemia, such as inherited blood diseases – sickle cell anaemia or thalassaemia. Another application can also be found in the fields of surgery – notably liver and heart surgery – and transplantation to prevent ischaemia/reperfusion side effects.

Drug Delivery (Deliv'ERY System®)

We have developed a programme that enables the use of the red blood cell as a delivery system for therapeutic drugs to specific targeted organs. Packed red blood cells have a physiological half-life of about 27 days; they are destroyed by phagocytosis in the liver, spleen, bone marrow and, more extensively, by organs of the reticulo-endothelial system, such as lungs. The technology makes it possible to reduce the red blood cell life span by altering the membrane so as to activate phagocytosis more quickly. The kinetics of drug delivery can be modulated according to the particular process applied to the red blood cell membrane.

Depending on the therapeutic strategy, specific types of delivery are possible: either a progressive and targeted release or an accelerated release, depending on which organ is targeted. Also, the dosage is significantly reduced. It is possible to encapsulate molecules with high systemic toxicity, such as cardiotoxicity or neurotoxicity; these molecules are selected because of their potential for delivery to the organs of the reticuloendothelial system.
Vaccine Delivery (Vaccin’ERY System®)

This technology consists of loading red blood cells with tumour antigens to specifically target and activate immune cells – such as dendritic cells and macrophages – in vivo to induce an anti-tumour response. These cells catch the red cells, engulf them via phagocytosis and then ingest the tumour antigens. The antigens are then presented to the lymphocytes, which are activated and able to destroy specifically the tumour cells. Entrapment inside red blood cells is not limited by the size or nature of the tumour antigens, and adjuvants can also be entrapped to target intracellular receptors making them much more effective. Product manufacturing requires only two hours thanks to an industrial process. It also offers technical and economic advantages by avoiding the complex and laborious manipulations of dendritic cells or macrophages, usually performed ex vivo. Animal studies have confirmed that the system is very effective and the co-development of anti-cancer vaccines with pharma or biotech partners that have different tumour antigens or adjuvants is being strongly considered.

PRODUCT PIPELINE

We are currently in the process of developing a pipeline of innovative therapeutic solutions based on these four main applications (see Figure 4).

GRASPA® is L-asparaginase encapsulated inside homologous red blood cells. The loading of L-asparaginase into the red cells is very reproducible and is completed within two hours in a cGMP facility. The red cell membrane protects the enzyme against antibodies, allowing it to remain in circulation within the body for several weeks, as opposed to just a few days, as is the case with injection of the free form. Consequently, the dosage and number of injections is significantly reduced, leading to an equivalent reduction in side effects and particularly allergic reactions.

Success with this lead compound was demonstrated by positive results in Phase II trials in acute lymphoblastic leukaemia, and an excellent safety profile compared with the control treatment. Phase III trials in leukaemia will start in 2009 and a programme in other indications is ongoing.

GR-ARA1 is a medicinal product developed for use in the fields of tissue and tumour oxygenation. As mentioned previously, in tissue oxygenation it is aimed at chronic and genetic diseases characterised by hypoxia and anaemia, such as sickle cell anaemia or thalassaemia. GRARA is designed to have a preventive/curative effect in sickle cell disease, as well as to greatly replace or reduce the need for classical red cell transfusions.

With regard to tumour oxygenation, two types of tumour particularly prone to tumour hypoxia will initially be targeted: cerebral tumours, such as glioblastoma, and head and neck cancers. In these indications, GRARA will be used to increase the responsiveness of tumours to radiotherapy. GR-ARA1 has already completed the in vitro and in vivo proof-of-concept in both applications. Preclinical studies will be done this year and we expect to initiate clinical studies in 2010.

Other Molecules

In each application field, we are currently encapsulating other molecules, while for the encapsulation of certain molecules we are involved in European collaboration. GR-DD201 is a new formulation of glucocerebrosidase encapsulated in red blood cells with specific delivery to bone marrow macrophages (Deliv’ERY system®) for the treatment of skeletal manifestations in Gaucher’s disease. GR-EGG1 consists of encapsulation of an enzyme into red blood cells for the treatment of hepatocellular carcinoma and melanoma; it has successfully completed in vitro and in vivo proof-of-concept. GR-EGG1, like GRASPA®, relies on the Cleav’ERY System®.

THE FUTURE

The opportunity to develop high-value speciality therapeutics has never been so promising, first, to accelerate clinical development of the GRASPA® programme in acute lymphoblastic leukaemia and solid tumours, and second, to accelerate the GRARA programme. We are also open to discussing new co-development projects with companies interested in testing new approaches for their drugs, which could fit with the Deliv’ERY system or Vaccin’ERY system. Co-development partnership agreements are also being sought on antigens/adjuvants to develop active immunotherapies with the Vaccin’ERY System®, and on new molecules for liver or bone marrow disease with the Deliv’ERY System®.

Dr Yann Godfrin, PhD, and Biomedical Engineer is the Co-founder, Chief Executive Officer and Chief Scientific Officer of ERYtech Pharma. He was also former Project Manager of BioAlliance Pharma, Hemosystem, and R&D Director of Hemosystme Europe. Email: contact@erytech.com

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