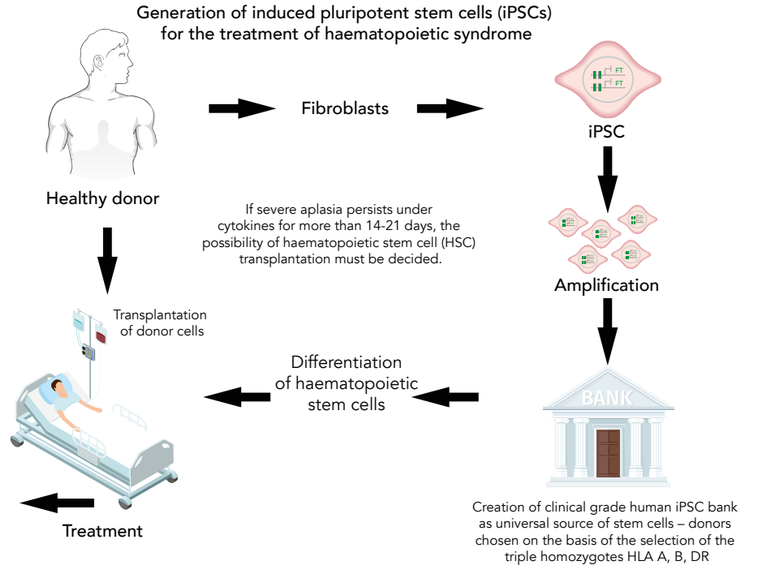
Stem cell therapy – the answer to radiation damage

The team of scientists at the Institute for Radiological Protection and Nuclear Safety (IRSN) and National Institute of Health and Medical Research (INSERM) aim to develop novel cell therapies for tissue damage caused by radiation. In their latest research, the GIPSIS project, the team investigates the potential of using cutting-edge stem cell therapy to develop a revolutionary approach for the treatment of haematopoietic syndrome – a potentially fatal symptom of acute radiation syndrome (ARS).



Haematopoietic stem cells (HSCs) are produced from fibroblasts taken from a healthy donor. These are then treated to make induced pluripotent stem cells (iPSCs) - these are cells that can become any cell in the body. The iPSCs are then caused to multiply to create a bank of iPSCs. These are then differentiated into HSCs to be used in transplants.

Acute radiation syndrome (ARS) is the result of a radiation overdose to the body – a particularly common after-effect of nuclear war, terrorist attacks or nuclear accidents. It can lead to the life-threatening destruction of stem cells in the bone marrow which play a vital role in producing important blood cells. The last two decades have seen breakthroughs in the field of stem cell research and, as such, stem cells can now be generated artificially in the laboratory. The next big challenge is to leverage existing knowledge to develop stem cell-based therapies for diseases caused by abnormalities in the bone marrow. The consortium from the Institute for Radiological Protection and Nuclear Safety (IRSN) and National Institute of Health and Medical Research (INSERM) is striving to achieve just that, having already made great advances in producing blood artificially – this team were the first to produce functional human blood from skin cells *in vivo*.

SOCIAL, ECONOMIC, ENVIRONMENTAL, AND INDUSTRIAL CHALLENGES

ARS is often a result of one of five key events. These include: exposure to a nuclear explosion, a nuclear reactor accident, an accident when handling fissile material, exposure to a powerful beamwidth, or an act of terrorism. ARS was responsible for 180 deaths between 1945 and 2004 from 600 identified radiological accidents – excluding Hiroshima and Nagasaki. A nuclear accident in Chernobyl in 1986 caused over two hundred workers and firefighters to suffer ARS. This event proves that, despite nuclear safety precautions being put in place, nuclear accidents can still happen – and they can be devastating when they do. At present, 442 nuclear power reactors are operating in 31 countries, with sixty-five further nuclear reactors under construction.

Not only that, but numerous nuclear reactors can also be found on military warships – once again demonstrating why the risk of nuclear accidents occurring remains such a major concern for military agencies. Developing and implementing a therapeutic strategy capable of dealing with the radiative effects of nuclear accidents or terrorist attacks, is therefore vital for both military operatives and civilian populations.

ACUTE RADIATION SYNDROME

The European Commission has formed a consortium of experts to develop a manual of medical care following accidental irradiation. This will, in effect, develop a system for assessing organ damage in relation to the time following the accident and classifying this using prognostic codes for the neuro-vascular, haematopoietic, cutaneous and gastrointestinal systems.

The extent of this accidental irradiation can often be diverse but in the most severe cases stem cell transplants should be considered as a therapeutic option. Currently however, and due to the nature of the incident in which the irradiation occurred (i.e., nuclear accident or terrorist attack), accessing large stocks of stem cells for victims proves difficult. As such, there remains a gap in the management of acute radiation syndrome. The consortium are investigating a new therapeutic approach to bridge this gap and ensure help is available when needed.

Acute radiation syndrome is the result of a radiation overdose to the body – a particularly common after effect of nuclear war, terrorist attacks or nuclear accidents

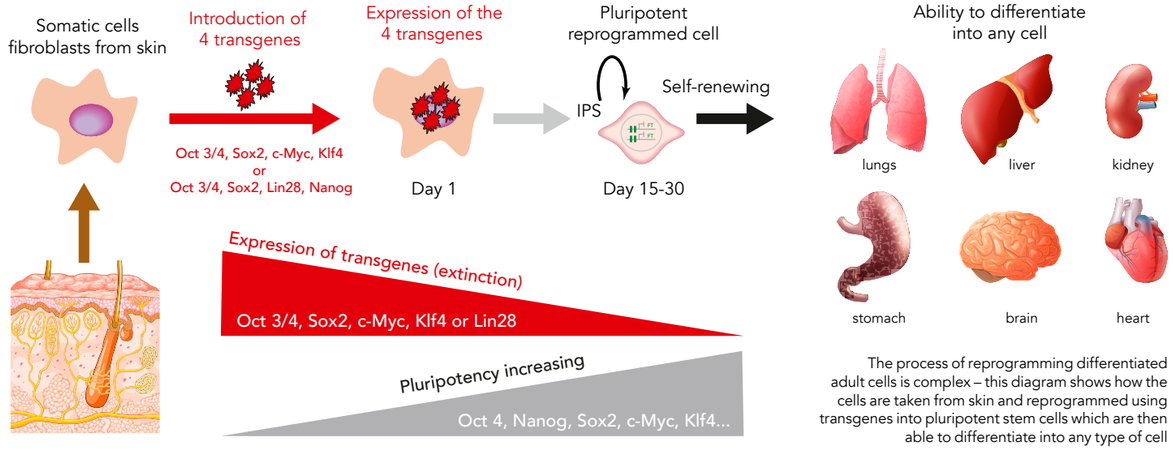
BEYOND ACUTE RADIATION SYNDROME

Chemotherapy in leukaemia patients is, in a way, a voluntary exposure to damaging doses of radiation. The aim is the destruction of cancerous cells in the patient's bone marrow to subsequently replace them with healthy hematopoietic (blood-cell producing) stem cells (HSC), either from the patient themselves or from a healthy donor.

Haematopoietic stem cell transplantation has become the main treatment used in the management of various haematologic malignancies, but it is not without its issues. In the European Union alone, over 5000 individuals per year receive HSC transplants for haematological diseases and malignancies. However, significant numbers of patients (20-30%) cannot receive the life-saving treatment they require because they cannot access sufficient numbers of HLA-matched HSCs (this kind of matching uses a protein, human leukocyte antigen, located on the body's cells – it acts as an indicator to your immune system that your cells belong). Not only that, but treatments often fail due to the matched donor cell not being well-enough suited, resulting in graft vs host disease (GvHD). In other words, even though donors are carefully matched to maximise the chances of acceptance, graft rejection is common when using donor cells: GvHD contributes substantially to transplant-related morbidity and mortality.

A perfect donor match can only be achieved by using the patient's own stem cells. This bears the risk that the disease returns because of residual diseased cells in the graft. Nonetheless, haematopoietic stem and progenitor cells, generated from patient-derived induced pluripotent stem cells (iPSC), could provide an unlimited supply of HLA-matched transplantable cells capable of treating disease. In 2006, researchers at Kyoto University in Japan identified conditions that would allow specialised adult cells to be genetically "reprogrammed" to assume a stem cell-like state. These adult cells, induced pluripotent stem cells (iPSCs), were reprogrammed to an embryonic stem cell-like state by introducing genes important for maintaining the essential properties of embryonic stem cells (ESCs). Since this initial discovery, researchers have rapidly improved the techniques to generate iPSCs, creating

Reprogramming of adult differentiated cells



The process of reprogramming differentiated adult cells is complex – this diagram shows how the cells are taken from skin and reprogrammed using transgenes into pluripotent stem cells which are then able to differentiate into any type of cell

a powerful new way to "de-differentiate" cells whose developmental fates had been previously assumed to be determined.

iPSCs built a medical revolution. Like naturally occurring stem cells, such artificially induced cells can self-renew and develop into almost any cell in the body (pluripotency). Human iPSCs (hiPSCs) share this pluripotency with embryonic stem cells but do not cause the same ethical debate because they are derived from adults instead of embryos. They hold tremendous promise for regenerative medicine: researchers might take a person's skin, blood or other cells, reprogramme them into iPSCs, and then use those to grow liver cells, neurons etc. while side-stepping the risk of immune rejection.

TURNING SKIN CELLS INTO BONE MARROW

While there is considerable evidence that hiPSCs can form haematopoietic precursor cells in the laboratory, it is less clear under which conditions such cells survive and fulfil their function of forming blood cells in a living organism. Despite high hopes for regenerative therapy, these cells have barely made it into clinical trials so far. Dr Chapel and his team endeavour to change that as their work on creating blood artificially from fibroblasts shows.

This consortium are the first scientists to have obtained a full reconstitution of blood components from iPSCs *in vivo* in order to develop human blood from adult human skin. More specifically, they have uncovered a way to convert fibroblasts, taken from skin, into human blood. The discovery could mean that, in the foreseeable future, people who need blood for surgeries, cancer treatments or other blood conditions, such as anaemia, will be able to have blood created from a patch of their own skin.

THE QUEST AHEAD

Although further additional basic research will be required before iPSCs can be applied in the clinic, these cells represent multi-purpose tools for medical research. The team are embarking on a preclinical quest to establish hiPSCs suitable for therapeutic use in humans.

So far, all clinical attempts to do this have failed. However, the team hypothesise that obtaining a less mature cell type might have several advantages over producing an adult HSC. They believe this cell type would display more plasticity in response to changing microenvironments, particularly when reaching the adult bone marrow.

To generate cells capable of this, the team have designed a dedicated protocol that can be adapted to hiPSCs of diverse origins. This will enable cells to be produced *in vitro* which display all the features of progenitors capable of endothelial to haematopoietic transition.

This process will evolve in three main stages. First, the team must demonstrate that their hiPSC-derived haematopoietic cells from healthy donors can form blood cells in a radiation-damaged living organism. To do this, Dr Chapel's team used a one-step, GMP-grade, vector-free and stromal-free system to produce a cell population capable of reconstituting human haematopoiesis in immunocompromised mice, from hiPSCs. Second, they then must show that hiPSCs from radiation-damaged patients can perform the same function. The final step involves the development of an injectable cell preparation to treat acute radiation syndrome. One major consideration is the safety of this cell-based therapy with regards to recurrence of the patient's leukaemia or the development of another cancer.

If this team's proposed therapy passes all its pre-clinical tests, there will be huge potential for entering the clinical trial stage and, eventually, the creation of a patient-specific treatment of ARS and other haematopoietic diseases. However, success in this will depend on the ability to produce clinical grade hiPSCs capable of generating either primitive multipotent stem cells, which will restore damaged organs or replace perfectly mature and functional cells in a substitutive aim. As a consequence of this, the concept of universal iPSC banking makes sense for future development.

BANKING ON STEM CELLS

If it is possible to produce clinical-grade hiPSCs, which can then be manipulated to form almost any cell in the body, it is envisaged that a library of hiPSCs from healthy donors could be established. Such donors would need to represent a large part of the population.

Among the tools of regenerative medicine, induced pluripotent stem cells (iPSCs) are interesting because the donor genotype (the type of genes the donor has) can be selected. One proposal to maximise the number of people served by the banks is to create the banks using cells from HLA-homozygous donors. These donors have two identical genes coding for HLA rather than two different ones. However, the creation of this is only achievable through a large-scale concerted worldwide collaboration.

A consortium of 26 partners has already been formed to establish the "European Bank for induced pluripotent Stem Cells" (EBiSC) with support from the Innovative Medicines Initiative (IMI). The EBiSC will act as a central storage and distribution facility for hiPSCs from healthy individuals and patients suffering from various inherited diseases, to be used by researchers across academia and industry in the study of disease and the development of new treatments. Furthermore, a Bank of iPS GMP HLA homozygous is under development for future clinical trials in an international consortium involving several countries. This consortium "Global Alliance for IPS Therapies" (GAIT) will establish standardised procedures.

The medical significance of such a cell library would be enormous. It would not only provide doctors with on-demand cell-based therapy for some of the most severe diseases, but it would reduce the need for time-consuming searching and testing of potential donors. It also reduces the risk of the original disease reoccurring.

Current cell-based regenerative therapies have already delivered substantial advances in medicine. However, this is no reason to be complacent. Existing strategies have considerable limitations we cannot ignore. In addition, there are several reasons to be watchful of the nuclear risk to human health. Identifying scalable treatment solutions now may be decisive should a catastrophe strike. However, even though Dr Chapel's research currently focuses on ARS, its outcome, if successful, could revolutionise the way clinicians treat any disease caused by cellular abnormalities in the bone marrow.

The team's discovery could mean that people who need blood for surgeries, cancer treatments or other blood conditions, will be able to have blood created from a patch of their own skin

Q&A

Why are patient fibroblasts not damaged by acute radiation?
Because radiation may damage DNA introducing mutations. In cases of accidental irradiation, there is a small part of the exposed victim which is not irradiated. So, first we determine which part of the body has not been irradiated, then we take a small biopsy of skin in order to produce iPSCs after carefully checking that there is no DNA damage.

How long will the GIPSIS project take?
Three years (2014–2017) it will be followed by another project (2017–2020) in order to produce clinical grade cells. The next project will run from 2017 to 2020.

What are the most important quality criteria for a clinical-grade cell therapy?
We are establishing protocols for generation of human induced pluripotent stem cells (hiPSCs) that would not involve viral vector integration, and that are compatible with Good Manufacturing Practices (GMP) standards: no integrative reprogramming, clone selection, absence of mutations on 50 hot spots, GMP, graft without contamination of IPSC, biodistribution to long-term in animal and absence of teratomas, production of cells by a labelled cell therapy unit.

Detail

RESEARCH OBJECTIVES

IRSN's research looks at using haematopoietic stem cell therapy to improve the medical management of acute radiation syndrome (ARS) for members of the military and the public who have been victims of nuclear exposures and accidents.

FUNDING

- Direction Générale de l'Armement (ASTRID/ANR program (ANR-13-ASTR-0009))
- Etablissement Français du Sang (EFS) (APR 2013 & Combattre La Leucémie)

COLLABORATORS

Laurence Guyonneau-Harmand, Loïc Garçon, Hélène Lapillonne (CRSA, EFS, INSERM); Bruno L'Homme Marc Benderitter (IRSN); Brigitte Birebent, Nathalie Chevallier, Luc Douay (Unité d'Ingénierie et de Thérapie Cellulaire, EFS); Christophe Desterke (INSERM); Thierry Jaffredo (Sorbonne Universités, UPMC Univ Paris 06, CNRS, INSERM, Laboratoire de Biologie du Développement)

BIO

For 25 years, Dr Alain Chapel has been developing gene and cell therapy using non-human primates, immune-tolerant mice and rats to protect against the side effects of radiation. He collaborates with clinicians to develop strategies for treatment of patients after radiotherapy overexposures.

CONTACT

Dr Alain Chapel
Laboratory of Research on Irradiated Healthy Tissues Regeneration (LR2I)
Institute for Radiological Protection and Nuclear Safety (IRPNS)
F-92260, Fontenay-aux-Roses
France

E: alain.chapel@irsn.fr
T: +33 615 401931