BEMS Reports. 2017; 3(1): 17-20.
A Multifaceted Peer Reviewed Journal in the field of Biology, Medicine, Engineering and Science
www.bemsreports.org | www.phcog.net

Basics of Stem Cells and Preclinical Testing

K. Suryanarayana Rao

INTRODUCTION

What are Stem Cells

Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide to produce more stem cells. They are found in multicellular organisms. In mammals, there are two broad types of stem cells: embryonic stem cells, which are isolated from the inner cell mass of blastocysts, and adult stem cells, which are found in various tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues. In a developing embryo, stem cells can differentiate into all the specialized cells—ectoderm, endoderm and mesoderm—but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

Controversy on Stem Cell Research

Initially, the dream was to launch a medical revolution in which ailing organs and tissues might be repaired—not with crude mechanical devices like insulin pumps and titanium joints but with living, homegrown replacements. It would be the dawn of a new era of regenerative medicine, one of the holy grails of modern biology.

Revolutions, alas are almost always messy. So when James Thomson, a soft-spoken scientist at the University of Wisconsin in Madison, reported in November 1998 that he had succeeded in removing cells from spare embryos at fertility clinics and establishing the world's first human embryonic stem cell line, he and other scientists got a lot more than they bargained for. It was the kind of discovery that under most circumstances would have blossomed into a major federal research enterprise. Instead the discovery was quickly engulfed in the turbulent waters of religion and politics. In church pews, congressional hearing rooms, and finally the Oval Office, people wanted to know: Where were the needed embryos going to come from, and how many would have to be destroyed to treat the millions of patients who might be helped? Before long, countries around the world were embroiled in the debate.

Types of Adult Stem Cells

There are three known accessible sources of adult stem cells in humans:

1. Bone Marrow, which requires extraction by harvesting, that is, drilling into bone (typically the femur or iliac crest).
2. Adipose tissue (lipid cells), which requires extraction by liposuction.
3. Blood, is drawn from the donor, and passed through a machine that extracts the stem cells and returns other portions of the blood to the donor.
4. Stem cells can also be taken from umbilical cord blood after birth. Of all stem cell types, autologous harvesting involves the least risk. By definition, autologous cells are obtained from one's own body, just as one may bank his or her own blood for elective surgical procedures.

Application of Stem Cells

Adult stem cells are frequently used in various medical therapies (e.g., bone marrow transplantation. Stem cells can now be artificially grown and transformed (differentiated) into specialized cell types with characteristics consistent with cells of various tissues such as muscles or nerves. Embryonic cell lines and autologous embryonic stem cells generated through somatic cell nuclear transfer or differentiation have also been proposed as promising candidates for future therapies.

Properties of Stem Cells

All stem cells—regardless of their source—have three general properties: they are capable of dividing and renewing themselves for long periods; they are unspecialized; and they can give rise to specialized cell types.

1. Stem cells are capable of dividing and renewing themselves for long periods. Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times, or proliferate. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells.
2. Stem cells are unspecialized. One of the fundamental properties of a stem cell is that it does

K. Suryanarayana Rao
Advinus Therapeutics Ltd, 21 & 22, Phase II, Peenya Industrial Area, Bengaluru – 560 058, Karnataka, INDIA

Correspondence

Dr. K.S. Rao, M.V.Sc., Ph.D., DABT

Senior Director – Strategic Business Development, Advinus Therapeutics Ltd, 21 & 22, Phase II, Peenya Industrial Area, Bengaluru – 560 058, Karnataka, INDIA.

Email: ks.rao@advinus.com

Ph.no: +91-733-783-0074, 91-80-4090-1650;

DOI : 10.5530/bems.3.1.6

Article Available online
http://www.bemsreports.org

Cite this article : Rao KS. Basics of Stem Cells and Preclinical Testing. BEMS Reports, 2017;3(1):17-20.

not have any tissue-specific structures that allow it to perform specialized functions. For example, a stem cell cannot work with its neighbors to pump blood through the body (like a heart muscle cell), and it cannot carry oxygen molecules through the bloodstream (like a red blood cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

3. Stem cells can give rise to specialized cells. When unspecialized stem cells give rise to specialized cells, the process is called differentiation. While differentiating, the cell usually goes through several stages, becoming more specialized at each step. Scientists are just beginning to understand the signals inside and outside cells that trigger each step of the differentiation process. The internal signals are controlled by a cell's genes, which are interspersed across long strands of DNA and carry coded instructions for all cellular structures and functions. The external signals for cell differentiation include chemicals secreted by other cells, physical contact with neighboring cells, and certain molecules in the microenvironment. The interaction of signals during differentiation causes the cell's DNA to acquire epigenetic marks that restrict DNA expression in the cell and can be passed on through cell division.
4. Adult stem cells typically generate the cell types of the tissue in which they reside. For example, a blood-forming adult stem cell in the bone marrow normally gives rise to the many types of blood cells. It is generally accepted that a blood-forming cell in the bone marrow—which is called a hematopoietic stem cell —cannot give rise to the cells of a very different tissue, such as nerve cells in the brain. Experiments over the last several years have purported to show that stem cells from one tissue may give rise to cell types of a completely different tissue.

What are the similarities and differences between embryonic and adult stem cells?

1. Human embryonic and adult embryonic cells each have advantages and disadvantages regarding potential use for cell-based regenerative therapies. One major difference between adult and embryonic stem cells is their different abilities in the number and type of differentiated cell types they can become. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin.
2. Embryonic stem cells can be grown relatively easily in culture. Adult stem cells are rare in mature tissues, so isolating these cells from an adult tissue is challenging, This is an important distinction, as large numbers of cells are needed for stem cell replacement therapies.
3. Tissues derived from embryonic and adult stem cells may differ in the likelihood of being rejected after transplantation.
4. Adult stem cells, and tissues derived from them, are currently believed less likely to initiate rejection after transplantation. This is because a patient's own cells could be expanded in culture, coaxed into assuming a specific cell type (differentiation), and then reintroduced into the patient. The use of adult stem cells and tissues derived from the patient's own adult stem cells would mean that the cells are less likely to be rejected by the immune system. This represents a significant advantage, as immune rejection can be circumvented only by continuous administration of immunosuppressive drugs, and the drugs themselves may cause deleterious side effects.

Where are adult stem cells found, and what do they normally do?

Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis. They are thought to reside in a specific area of each tissue (called a "stem cell niche"). In many tissues, current evidence suggests that some types of stem cells are pericytes, cells that compose the outermost layer of small blood vessels. Stem cells may remain quiescent (non-dividing) for long periods of time until they are activated by a normal need for more cells to maintain tissues, or by disease or tissue injury.

Typically, there is a very small number of stem cells in each tissue and, once removed from the body, their capacity to divide is limited, making generation of large quantities of stem cells difficult. Scientists in many laboratories are trying to find better ways to grow large quantities of adult stem cells in cell culture and to manipulate them to generate specific cell types so they can be used to treat injury or disease. Some examples of potential treatments include regenerating bone using cells derived from bone marrow stroma, developing insulin-producing cells for type 1 diabetes, and repairing damaged heart muscle following a heart attack with cardiac muscle cells.

What tests are used to identify adult stem cells?

Scientists often use one or more of the following methods to identify adult stem cells:

1. label the cells in a living tissue with molecular markers and then determine the specialized cell types they generate;
2. Remove the cells from a living animal, label them in cell culture, and transplant them back into another animal to determine whether the cells replace (or "repopulate") their tissue of origin.

Importantly, scientists must demonstrate that a single adult stem cell can generate a line of genetically identical cells that then gives rise to all the appropriate differentiated cell types of the tissue. To confirm experimentally that a putative adult stem cell is indeed a stem cell, scientists tend to show either that the cell can give rise to these genetically identical cells in culture, and/or that a purified population of these candidate stem cells can repopulate or reform the tissue after transplant into an animal.

PRECLINICAL REGULATORY GUIDANCE'S

1. There are many guidance's (incomplete lists) which provide general framework, but do not offer specific direction. Most of them resort to case by case for preclinical assessment. Following are some of the salient ones:

- European Medical Agency Guidelines on Human Cell-Based Medicinal Products (2007). Guidance for Industry S6 Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (2012).
- FDA Guidance for Industry Preclinical Assessment of Investigational Cellular and Gene Therapy Products (2013).
- National Pharmaceutical Control Bureau Ministry of Health.
- Malaysia Guidance Document and Guidelines for Registration of Cell and Gene Therapy Products (CGTPs) in Malaysia (2016).

2. It is a standard practice in the industry to follow the recommendations of the ICH S6 (R1): Preclinical safety evaluation of biotechnology-derived pharmaceuticals (Revision 1). As per ICH S6 (R1), Toxicity studies are expected to be performed in compliance with GLP; however, it is recognized that some studies employing specialized test systems, may not be able to comply fully with GLP. Areas of noncompliance should be identified and their significance evaluated relative to the overall safety assessment.
3. In some cases, lack of full GLP compliance does not necessarily mean that the data from these studies cannot be used to support clinical trials and marketing authorizations". According to 21

CFR Part 58 (Part 58), all preclinical toxicology studies are to be conducted in compliance with GLP. For example, toxicology data for investigational CGT products are sometimes collected in POC studies that use an animal model of disease/injury, which may require unique animal care issues and technical expertise that may not be available at a GLP testing facility.

4. A critical step in the translational process is a thorough pre-clinical assessment of product safety, including local and systemic toxicities, dose-toxicity response, and onset and reversibility of any toxicity findings.
5. The use of multiple species (small and large animals – depending on aim of study) or animal models may be necessary to adequately model the functional aspects and potential toxicities of the investigational CGTP. Animal models of disease/injury may be preferable to healthy animals to assess the activity and safety of these products. Therefore, preclinical studies in disease/injury models are encouraged to better define the risk-benefit ratio associated with investigational CGT products. In addition, use of disease/injury models provides the opportunity for possible identification of activity-risk biomarkers that may be applicable for monitoring in clinical trials.
6. Responsible animal research should adhere to the principles of the three R″s – Reduce numbers, Refine protocols, and Replace animals with in vitro or non-animal platforms wherever possible. Further, animal models may not replicate the full range of human toxicities. Therefore, particular vigilance must be applied in pre-clinical analysis of the toxicities of cell based interventions. In any case, comparability of the product used in pre-clinical experiments to that intended to be used as clinical material should be ensured.
7. By and large most new guidelines refer to "EMA Guideline on human cell-based medicinal products/ US FDA Preclinical Assessment of Investigational Cellular and Gene Therapy Products" for the conduct of non-clinical studies.

Considerations prior to initiation of Preclinical Toxicology Studies

The need for toxicological studies depends on the product. Following general aspects need to be considered:

To identify risk factors associated with the quality and safety of the product

To determine the extent and focus of the data required during non-clinical and clinical development;

To establish the need for risk minimization activities,

To determine the post market risk management activities to be specified in the Pharmacovigilance plan.

Origin of stem cells (autologous-allogeneic)

Ability to proliferate and differentiate

Ability to initiate an immune response (as target or effector)

Level of cell manipulation (in vitro/ex vivo expansion/activation/genetic manipulation)

Mode of administration (ex vivo perfusion, local, systemic);

Duration of exposure (short to permanent);

Combination product (cells + bioactive molecules or structural materials)

Availability of clinical data on or experience with similar products.

Practical Suggestions for Conducting Preclinical Studies with Stem Cell

1. The objectives of the non-clinical studies are to demonstrate proof-of-principle, define the pharmacological and toxicological effects predictive of the human response, not only prior to initiation of clinical trials, but also throughout clinical development. The goals of these studies include the following: to provide information to select safe doses for clinical trials, to provide information to support the route of administration and the application schedule, to provide information to support the duration of exposure and the duration of the follow-up time to detect adverse reactions, to identify target organs for toxicity and parameters to monitor in patients receiving these therapies.
2. The non-clinical studies should be performed in relevant animal models. The rationale underpinning the non-clinical development, and the criteria used to choose a specific animal model must be justified. The inherent variability of some cell-based medicinal products should be reflected in the non-clinical studies.
3. Expression level of biologically active molecules, the route of administration and the dosages tested should reflect the intended clinical use in humans.
4. The number of animals, the genders and frequency and duration of monitoring should be appropriate to detect possible adverse effects.
5. The safety and suitability of all structural components for their intended function must be demonstrated, taking into account their physical, mechanical, chemical and biological properties.

One Paradigm for Preclinical Testing for Stem Cells

Use of SCID Mice

It is a standard practice to use SCID mice for toxicology studies with stem cell. Mice homozygous for the SCID mutation (SCID mice) are severely deficient in functional B and T lymphocytes. The mutation appears to impair the recombination of antigen receptor genes and thereby causes an arrest in the early development of B and T lineage-committed cells; other hematopoietic cell types appear to develop and function normally. SCID mice readily support normal lymphocyte differentiation and can be reconstituted with normal lymphocytes from other mice and even partially reconstituted with human lymphocytes. They also support the growth of allogeneic and xenogeneic tumors. Thus, SCID mice are of interest for studies of both normal and abnormal lymphocyte development and function.

Single Dose IV Toxicity Study in SCID MICE

First order of business is to establish the tolerance by SCID mice to stem cells. For this you conduct a typical single IV administration dose response study with increasing number of stem cells till you see toxicity. It is common to allow animals to observe for 14-days following single IV injection and sacrifice on Day 15. At necropsy conduct conventional hematology assessment, along with histologic examination of injection site and draining lymph node along with any observed lesions. At the end of this study, derive a dose or number of cells animals can tolerate without any adverse effects (NOAEL for Stem Cells).

Tissue Distribution of Stem Cells in SCID Mice

Using the above data, it is advisable to conduct a tissue distribution of stem cells. The fate of infused cells and tissue distribution profile of stem cell products need to be determined. This study is usually a stand-alone study using tagged cells. This will give an index of tissue distribution and potential fate of stem cells. This is a critical study that must be performed prior to initiating repeat dose toxicity studies.

Repeat Dose Toxicity Study in SCID Mice

Based on the nature of use, it is a standard practice to conduct a minimum of 28-day toxicity in SCID mice by the IV route. However, it is my preference to conduct a 13-week IV toxicity study, since the cells are long

living in the body. In the 13-week study, it is acceptable to inject stem cells once every 4-weeks and sacrifice mice 4-weeks after the last injection. In these studies, it is a customary practice to monitor all toxicology parameters including full histopathology.

Tumorigenicity Study

Uncontrolled proliferation of the administered cells and insertional mutagenesis following administration of integral viral vectors are risks unique to CGTPs. Thus, tumorigenicity study in single species is desirable for human safety. It is a usual practice to use BALB/c Nude mice for tumorigenicity study. In such a study, it is customary to inject IV a single dose in a dose response way (Low, Mid and Hid dose) and allow the animals to be observed for 26-weeks. It is also desirable to employ Vehicle (Saline), negative control using Human Lung Cells or Fibroblast Cells which do not induce tumors in nude mice. Along with these, it is essential to use a positive control group by employing A375 (human malignant melanoma cell line) which will induce tumors in nude mice within 26-weeks. This positive control group validates that the test system is working.

Some laboratories prefer to use PC3 or DU145 cell lines (Prostate Cancer cells)

DU145 cells could be cultured in MEM cell culture medium supplemented with 10% FBS and 1% penicillin-streptomycin. The cells could be harvested by trypsinization, when they reach 80% confluence. The cells should be re-suspended in serum free medium at a concentration of 5 x 106 cells/100 µL.

Tumor Cell Inoculation

Mice should be inoculated subcutaneously on the dorsal right flank. Prior to inoculation, the skin on the injection site should be swabbed with alcohol. Cells in serum free medium (5 x 106 /100 µL) should be mixed with matrigel at 1:1 and a total volume of 200 µL should be injected to each animal with a 1 mL BD syringe attached to a 231/2 gauge needle.

SUMMARY

There is no hard and fast guideline for preclinical assessment of stem cells. It is usually a case by case basis, following a thorough understanding of the risk factors. From a clinical perspective an acute study, Tissue distribution study, 13-week repeat dose toxicity study and a single dose tumorigenicity studies are generally adequate. However, every case is different. It is always prudent to consult an expert in the field including a buy in from the respective regulatory agencies prior to initiation of the program.

DISCLAIMER

This newsletter is solely intended for educational purpose. Some of the contents of this newsletter may have been adopted without or with modification from other published resources.

Cite this article : Rao KS. Basics of Stem Cells and Preclinical Testing. BEMS Reports, 2017;3(1):17-20.