



Changing the Paradigm

WHITE PAPER

Cell Potency Assays for the 21st Century Stem Cell Transplantation and Cord Blood Storage Processing Laboratory

Executive Summary

The number of patients requiring a stem cell transplant increases annually. In recent years the trend has been to use mobilized peripheral blood and umbilical cord blood as a source of stem cells to repopulate the patient's blood-forming system. With the use of umbilical cord blood, both for children and adult transplantation purposes, the inventory of units collected and stored by non-profit and commercial cord blood banks continues to increase every year. Cord blood units are processed and cryopreserved. They can remain in the frozen state for 10 years or more. Prior to the product being released for transplantation, it is necessary to ensure that the cells being transplanted exhibit the potential to proliferate, since without proliferation, the cells will not engraft and reconstitute the patient. Cell potency and release assays should be used to compare the processed product with a reference standard and determine whether the product is capable of proliferation greater than an arbitrary set limit. Unfortunately, neither the assays presently employed nor the standards that requires "appropriate" and "validated" assays to measure these parameters, have kept pace with the growing need for quality control. This White Paper discusses the shortfalls presently associated with this vitally important aspect of the stem cell transplantation process and how new technology can obviate these shortfalls to provide the physician with more quantitative information and increased safety to the patient.

Introduction

In 2007, it is estimated that in the United States alone, about 116,000 people will be diagnosed with leukemia or lymphoma¹. Many of these patients will receive either a bone marrow, mobilized peripheral blood or umbilical cord blood transplant. If a patient does not have an identical sibling, there are approximately 11.5 million potential stem cell donors that can be matched with a patient. This information is contained in 59 stem cell donor registries in 43 countries and 37 cord blood banks in 21 countries around the world². To date, there have been more than 5,500 cord blood stem cell transplants¹. Since 1988, there have been approximately 25,000 stem cell transplants with an increasing trend every year. In 2006 alone, more than 3,000 transplants were performed. There has been a decreasing trend using bone marrow as a transplant source, but an increasing trend using mobilized peripheral blood and umbilical cord blood³.

The purpose of the transplant is to provide the patient with a new set of stem cells that will produce a new blood-forming (hematopoietic) system. Regardless of whether the cells are from the bone marrow, mobilized peripheral blood or umbilical cord blood, they must be processed and/or manipulated prior to transplantation. Whenever a patient receives a transplant, a number of parameters need to be measured to help ensure that engraftment of the transplant will be successful. However, other conditions can complicate the prediction as to whether the stem cell transplantation will be a success to enable the patient to enter remission and disease-free survival. Tests or assays performed on the stem cell product prior to and after transplantation may help predict the response in the patient.

The Problem

Regardless of whether a patient is transplanted with bone marrow, mobilized peripheral blood or umbilical cord blood, the tissue must be processed and/or manipulated so that the correct cells are in a form that can be infused. Besides the tests for tissue/cell typing, sterility etc., there are three measurements of the product that are normally required. These are the cell number, the viability of the cells and the number of cells expressing a specific membrane surface antigen (e.g. CD34) that is indicative of the cells necessary for transplant. For bone marrow and mobilized peripheral blood, these three parameters are considered sufficient to decide whether a product can be transplanted. Prior to transplanting umbilical cord blood, the cells are cryopreserved until required. As described in the next section, it is mandatory for the cell unit(s) to undergo a fourth test that is supposed to provide an indication of

growth potential and cell potency, in order for it to be released as a transplantation product. This test or assay, known as the Colony-Forming Cell (CFC) assay, is the problem. First published in 1966^{4,5}, it was never developed or designed as a cell potency assay, even by those who modified it for use with human cells⁶. For the stem cell processing laboratory, the CFC assay also suffers from numerous other problems and deficiencies.

- It takes 14 days to obtain a result, which is approximately the time it takes for a patient to engraft. In other words, it does not provide any predictive information⁷.
- It is subjective, requiring manual enumeration.
- The assay cannot be calibrated or standardized because no external standard exists.
- Despite numerous attempts, the assay has never been properly validated between laboratories for this particular application.
- For the reasons mentioned above, proficiency testing is problematic.
- The assay is considered an “all-or-nothing”, “growth – no growth” assay and provides no definitive or quantitative information on stem cell potency so that it can be used as a release assay.
- Finally, the assay is a functional differentiation assay and therefore does not measure the proliferation status of the cells in question.

Regulatory Issues

There are a number of organizations that are responsible for drawing up standards required for cell and tissue processing and manipulation. These are the Foundation for Accreditation of Cellular Therapy (FACT)⁸, the Joint Accreditation Committee of the International Society for Cellular Therapy (ISCT), the AABB (American Association of Blood Banks), and JACIE⁹, a joint accreditation committee consisting of the European Group for Blood and Marrow Transplantation (EBMT) and FACT Europe. The suggestions from these organizations are used in Guidance Documents by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA).

The latest editions of national and international standards^{10,11,12} call for “appropriate” and “validated” laboratory processes and assays. Specifically, the International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release by Netcord-FACT¹¹ require “adequate provisions for monitoring the reliability, accuracy, precision and performance of CBB (Cord Blood Bank) Processing Facility test procedures and instruments”. These standards and associated Guidance Documents mandate not only that the CFC assay has to be performed, but that it should be used to detect the “total colony forming units granulocyte macrophage (CFU-GM)” and, if applicable, total burst-forming unit-erythroid (BFU-E) and total colony for unit-granulocyte erythrocyte monocyte macrophage (CFU-GEMM). Exactly when it is appropriate to determine the BFU-E and CFC-GEMM content is not given, but since the CFC-GEMM cell population is an *in vitro* multipotential stem cell population, it might have been expected that this, rather than the GM-CFC cell population, would have been more pertinent and would have taken greater priority for a procedure that relies on the transplantation of stem cells.

However, it is obvious from the previous section and from the definitions provided by the Code of Federal Regulations (CFR) for “standards” (21 CFR 600.3), “potency” (21 CFR 600.3 and 610.10) and “validation” (21 CFR 820.3) that the CFC assay does not meet the necessary requirements.

The Solution to the Underlying Problem.

In all fairness, it should be emphasized that until recently, the CFC assay was the only assay available that could provide some indication that the cells present in a processed product could grow and differentiate. But this should not distract from the fact that proliferation and differentiation cannot be detected in the same assay using the same measurement procedure or readout. Certainly proliferation and differentiation are, from a biological viewpoint, intricately intertwined. From an assay viewpoint however, they could not be further apart.

The CFC assay is a functional differentiation assay whereby an unidentifiable and/or undifferentiated cell, e.g. a stem cell (by definition), acquires the features of a specialized cell. In this way, the colonies that are counted after 14 days in culture can be identified. Although proliferation is an inherent part of the process to produce colonies of cells, the CFC assay does not directly measure proliferation and is, therefore, not a proliferation assay.

Proliferation is the expansion of cells by continuous division into initially two identical daughter cells. Proliferation therefore, occurs prior to differentiation or a differentiation step. Without proliferation, differentiation would not

occur. Differentiation is a default program requiring prior proliferation. The key to ascertaining whether a processed stem cell product for transplantation will have the capability to exhibit growth, short- and long-term engraftment and reconstitution, is whether that product has the potential to proliferate.

Even though proliferation and differentiation are part and parcel of the lympho-hematopoietic system, these two processes have to be considered as separate entities for a cell potency assay to comply with the ever-increasing need for standardized and validated regulatory requirements. Once proliferation and differentiation are considered completely separate entities as far as assay measurement is concerned, it is then possible to understand and address the solution.

The 21st Century Solution to Standardized and Validated Stem Cell Potency and Release Assays for Transplantation and Storage Quality Control.

The basis of a cell potency assay is to quantify, in a cell dose-dependent manner, the horizontal displacement of the parallel response to the left or right of a reference standard. A displacement to the right indicates a decreased, while a displacement to the left indicates a greater potency compared to the reference standard. Furthermore, by comparing the proliferation response to a historical background control, it is also possible to arbitrarily set the lower limits above which a processed cellular product is acceptable for transplantation purposes.

Taking the arguments discussed in the previous sections and the requirements to measure potency and acceptability criteria, HemoGenix[®] has designed and developed a suite of three *in vitro* assay platforms that can be demonstrated to conform to specifications (accuracy, sensitivity, specificity, reliability, relevance and robustness) allowing validation as potency and release assays.

All of the assay platforms rely on the direct correlation between a biochemical marker, namely the intracellular ATP (iATP) concentration and the proliferation status of the target cells. As a biochemical marker, the concentration of iATP can be calibrated against an external ATP standard. After incubation of the cells in the normal manner, iATP is released from the cells by lysis and acts as a limiting substrate, for a highly sensitive luciferin/luciferase reaction to produce bioluminescence in the form of light that can be measured in a plate luminometer. The amount of bioluminescence produced is directly proportional to the proliferation status of the cells.

The standardized, instrument-based proliferation Stem and Progenitor Cell – Quality Control (SPC-QC) potency assays developed by HemoGenix[®] are summarized in the table below.

Name of assay platform:	CAMEO™-96 STD	HALO[®]-96 MeC^{14,15,16}	HALO[®]-96 SEC
Type of assay:	Proliferation and differentiation	Proliferation	Proliferation
Type of culture:	Methylcellulose	Methylcellulose	Suspension
Cell growth:	Clonal	Clonal	Expansion
Parameters measured:	Colonies and iATP	iATP	iATP
Assay readout type:	Manual colony counts + luminometer	Luminometer	Luminometer
Calibration and standardization:	Yes	Yes	Yes
Reference standard:	Yes	Yes	Yes
Format:	96-well plate	96-well plate	96-well plate
Total Cells required:	500,000	500,000	500,000
Total assay volume/well:	100µl	100µl	100µl
No. of replicate:	6	6	6
Incubation time:	14 days	7 days	5 days
Cell potency measurement:	Yes	Yes	Yes

Conclusion

“Growth – no growth” assays are unacceptable in an environment where a patient is the ultimate beneficiary of a cell therapy product. The HemoGenix[®] SPC-QC suite of assays provides the user with all the necessary requirements to measure and quantify the potency and release criteria for processed cell therapy products utilizing fully standardized, reference- and instrument-based technology. Change your paradigm today!

Literature Cited

1. The Leukemia and Lymphoma Society at <http://www.leukemia.org>.
2. Bone Marrow Donors Worldwide at <http://www.bmdw.org>.
3. National Marrow Donor Program (NMDP) at <http://www.nmdp.org>.
4. Bradley, TR and Metcalf D. The growth of mouse bone marrow cells *in vitro*. Aust J Exp Biol Med. 1966;44:287-299.
5. Pluznik DH and Sachs L. The induction of clones of normal mast cells by a substance from conditioned medium. Exp Cell Res 1966;43:553-563.
6. Pike BL and Robinson WA. Human bone marrow colony growth in agar-gel. J Cell Physiol. 1970;76:77-84.
7. Cottler-Fox M. Hematopoietic progenitor stem cells. In “On Call In Bone Marrow Transplantation” Ed. Burt RK, Deeg HJ, Lothian ST and Santos GW. Landes Company, p69, 1966
8. Foundation for the Accreditation of Cellular Therapy (FACT) at <http://www.factwebsite.org>.
9. Joint Accreditation Committee of the International Society for Cellular Therapy (ISCT) and the European Group for Blood and Marrow Transplantation (EBMT) at <http://www.jacie.org>.
10. FACT-JACIE International Standards for Cellular Therapy Production Collection, Processing and Administration, 3rd Edition, 2007.
11. NetCord-FACT International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release, 3rd Edition, 2006.
12. Standards for Cellular Therapy Product Services, 2nd Edition. AABB.
13. Rich IN and Hall KM. Validation and development of a predictive paradigm for hemotoxicology using a multifunctional bioluminescence colony-forming proliferation assay. Tox Sci. 2005;87:427-441. [Click here](#) to download this article.
14. Rich IN. High-throughput *in vitro* hemotoxicity testing and *in vitro* cross-platform comparative toxicity. Expert Opin. Drug Metab. Toxicol. 2007;3:295-307. [Click here](#) to download article.
15. Reems J, Hall KM, Gebru LH, Taber, G and Rich IN. Development of a novel assay to evaluate the functional potential of umbilical cord blood progenitors. Transfusion (Accepted for publication).

About HemoGenix[®], Inc

HemoGenix[®] is a privately-held Contract Research Service and Assay Development Laboratory that produces and sells its services and assay kits in the U.S.A. and many other countries throughout the world. HemoGenix[®] specializes in developing predictive *in vitro* assay platforms for primary human and animal target cells. The assays have been specifically developed for Contract Research Services and as assay kits for in-house use by our customers. HemoGenix[®] is responsible for changing the paradigm and bringing stem cell hemotoxicity testing into the 21st century, by developing the HALO[®] Platforms that allows biotechnology and pharmaceutical companies to detect and predict the effects of large numbers of compounds on up to 14 different cell populations from 5 different species simultaneously. HemoGenix[®] is also changing the paradigm by providing standardized, instrument-based stem cell quality control potency assays for transplantation and umbilical cord blood processing laboratories. HemoGenix[®] prides itself on bringing the best possible *in vitro* assay tools to its clients and customers.