A Cord Blood Potency Assay that Accurately Predicts Engraftment Potential

INTRODUCTION

The Stem Cell Therapeutic and Research Act, 2005, 2010, states: “To provide for the collection and maintenance of cord blood stem cells for the treatment of patients and research.” To authorize the C.W. Bill Young Cell Transplantation Program. Throughout this statute, the statement “high quality cord blood units” refers to the stem cells that have to be of “high quality” in the units as required in the first sentence of the statute.

The Goal
The goal is to transplant the patient with sufficient stem cells of high enough potency and “quality” that allows engraftment and reconstitution.

All potency assays used for release testing of licensed biological drug products must comply with applicable biologics and cGMP regulations including:
- Indication of biological activity specific to the product.
- Results that allow release of the product.
- Provide quantitative data.
- Meet pre-defined acceptance/rejection criteria.
- Include reference materials, standards and controls.
- Demonstrate validation.
- Measure the identity and activity of the “active” ingredients or components.

Definition of Potency for Hematopoietic Stem Cell Therapy Products
Stem cell potency is the quantitative and validated measurement of biological activity of the “active” stem cell components that are responsible for and result in an engraftment response.

Requirements for a Stem Cell Potency Assay
1. A quantitative and validated readout system that measures biological activity.
2. The ability to identify and specifically measure the “active” stem cell components in a dose-dependent manner.
3. A reference standard of the same source so that the response of the sample can be compared and the potency ratio evaluated.
4. The assay must be highly sensitive and accurate. This sensitivity and accuracy can only be achieved using a specific biological activity that is a property or characteristic of the cells for which potency is to be measured. Since the assay must be validated according to FDA guidelines. The readout of the assay must be able to quantitatively measure a ratio evaluated.
5. The ability to identify and specifically measure the “active” stem cell components in a dose-dependent manner.
6. The purity of the Cord Blood Sample Affects both Stem Cell Potency and “Quality”

The First Requirement for a Stem Cell Potency Assay
A quantitative and validated readout system that measures biological activity

To meet the specific requirements for a potency assay it is necessary to develop an assay specific for the product that can be validated according to FDA guidelines. This makes of this assay must be able to quantitatively measure specific biological activity that is a property or characteristic of the cells for which potency is to be measured. Since the goal of the assay is to measure rare populations of stem cells that are responsible for the engraftment process, the assay readout must be highly sensitive and accurate. This sensitivity and accuracy can only be achieved using a luminescence readout.

Adenosine triphosphate (ATP) is produced in the mitochondria (the cell’s chemical energy plant). The concentration of ATP correlates directly with the state of proliferation or inhibition (cytotoxicity), viability, cell number and apoptosis. The ATP concentration of cells can be quantitatively measured using a luminescence plate reader.

Prior to processing and measuring the samples, the assay is calibrated and standardized:
- The controls included with the assay calibrate the instrument.
- The ATP standard curve standardizes the assay and allows the output of the instrument in Relative Luminescence Units (RLU) to be interpreted into standardized ATP concentrations.
- The process ensures that all the reagents are working correctly prior to measuring the samples.
- Reproduces and improves precision by comparing results with expected results and ranges.
- It allows results to be directly compared over time without normalization.

The Principle of ATP Bioluminescence Proliferation Assays
Using the Cell’s Chemical Energy (Adenosine Triphosphate, ATP) as a Biochemical Marker for Multiple Readouts

Changes in ATP correlate directly with:
- Proliferation
- Cytotoxicity
- Viability
- Cell Number
- Apoptosis

Calibrated, Standardized & Validated Assays for Specific Applications

ATP Bioluminescence Assay Characteristics
The following assay characteristics must be obtained prior to sample processing and measurement:
- Standard curve correlation coefficient (R²) > 0.997
- Standard curve slope = 0.937 (Range 0.0 - 0.17)
- Low control = 0.05µM (Range 0.04µM - 0.25µM)
- High control = 0.7µM (Range 0.5µM - 0.85µM)
- Acceptance/rejection criteria = 0.14µM (Range 0.13µM - 0.24µM)
- Non-viability cutoff = 0.10µM

Release Criteria for a Umbilical Cord Blood Stem Cell Product

A cord blood unit can be released for use if:
1. Stem cell “quality” (proliferation ability) for both stem cell populations > 0.4µM ATP, or the pre-determined acceptance/rejection criteria.
2. The cumulative potency ratio for both stem cell populations > 1 (the potency of a reference standard).

CONCLUSIONS

1. The stem cells are the “active” components required for engraftment.
2. Present cord blood tests and assays do not measure the stem cells required by U.S. statutes.
3. To measure potency a standardized and validated assay is an absolute requirement.
4. To measure both potency and “quality”, a MNC fraction (not a TNC fraction) must be prepared.
5. Measuring 2 stem cell populations using HALO®-96 PQR can measure both potency and “quality” in a single assay with an accuracy of >90%.
6. HALO®-96 PQR should be used by cord blood banks prior to shipment of the cord blood unit to the transplant center.

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