Cytotherapy, 2011; 13: 848–855

informa
healthcare

Guidelines for the development and validation of new potency assays for the evaluation of umbilical cord blood

STEPHEN SPELLMAN[1], CAROLYN K. HURLEY[2], COLLEEN BRADY[1], LISA PHILLIPS-JOHNSON[10], ROBERT CHOW[3], MARY LAUGHLIN[4], JOHN MCMANNIS[5], JO-ANNA REEMS[6], DONNA REGAN[7], PABLO RUBINSTEIN[8], JOANNE KURTZBERG[9]

[1]*Center for International Blood and Marrow Transplantation Research, Minneapolis, Minnesota, USA,* [2]*Georgetown University Medical Center, Washington, District of Columbia, USA,* [3]*StemCyte, Covina, California, USA,* [4]*University of Virginia, Charlottesville, VA, USA,* [5]*The University of Texas MD Anderson Cancer Center, Houston, Texas, USA,* [6]*Puget Sound Blood Center, Seattle, Washington, USA,* [7]*SSM Cardinal Glennon Children's Medical Center, St Louis, Missouri, USA,* [8]*New York Blood Center, New York, New York, USA, and* [9]*Carolinas Cord Blood Bank, Durham, North Carolina, USA,* [10]*National Marrow Donor Program, Minneapolis, Minnesota, USA*

Abstract

The following commentary was developed by the National Marrow Donor Program Cord Blood Advisory Group and is intended to provide an overview of umbilical cord blood (UCB) processing, summarize the current state of potency assays used to characterize UCB, and define limitations of the assays and future needs of the cord blood banking and transplant community. The UCB banking industry is eager to participate in the development of standardized assays to uniformly characterize cellular therapy products that are manufactured in a variety of ways. This paper describes the desired qualities of these assays and how the industry proposes to co-operate with developers to bring relevant assays to market. To that end, the National Marrow Donor Program (NMDP) Cord Blood Bank Network is available to serve as a resource for UCB testing material, research and development consulting, and product/assay testing in an accredited UCB manufacturing environment.

Key Words: *umbilical cord blood banking, umbilical cord blood potency assessment, umbilical cord blood processing, umbilical cord blood transplantation*

Introduction

As the umbilical cord blood (UCB) banking community moves towards United States Food and Drug Administration (FDA) licensure, it is critical to define standardized testing methodologies that can be employed to characterize UCB, facilitate comparison of UCB between UCB banks, and assess potency prior to clinical use. The methodologies used currently have strong intralaboratory correlation, i.e. UCB evaluated within a UCB bank are tested in a consistent fashion and yield consistent results. However, the methodologies and assays can yield highly variable results in interlaboratory testing settings on identical material. The UCB banking community could benefit from the development of new potency assays that are highly reproducible and correlate with the outcome of interest, predominately hematopoietic reconstitution.

Potency is defined as the specific capacity of a cellular product to affect a given result. In the case of UCB, the community is interested in the capacity to engraft cells in a transplant recipient or to produce viable cells (e.g. viral or tumor-specific cytotoxic T lymphocytes or natural killer cells) for manipulation and administration for a desired effect. The UCB banking industry is eager to participate in the development of standardized assays to uniformly characterize cellular therapy products that are manufactured in a variety of ways. This paper describes the desired qualities of these assays and how the industry proposes to co-operate with developers to bring relevant assays to market.

History of clinical UCB transplantation

The first successful UCB transplantation (UCBTx) was performed in 1988 by Gluckman *et al.* (1) in a patient with Fanconi's anemia. The patient achieved stable engraftment of donor hematopoiesis

Correspondence: **Stephen Spellman,** Center for International Blood and Marrow Transplant Research, 3001 Broadway St. N.E., Minneapolis, Minnesota, USA, MN 55413. E-mail: sspellma@nmdp.org

(Received 19 October 2010; accepted 4 March 2011)

ISSN 1465-3249 print/ISSN 1477-2566 online
DOI: 10.3109/14653249.2011.571249

and survived without disease relapse (1). Because of the concern that UCB might not contain sufficient numbers of cells to engraft larger children or adults reliably, initial studies were limited to children. Three simultaneous studies that extended UCBTx to adults were reported in 1996 suggesting that cord blood transplantation (CBT) can be used successfully for this population (2–4). Following these early reports, there have been a number of studies using UCB for transplantation in adults. As of 2009, it is estimated that more than 20 000 UCBTx have been performed world-wide. In 2009, more UCBTx were performed than bone marrow transplants (5).

Public banking of UCB was initiated in 1992 at the New York Blood Center (New York, NY, USA) (6–9). Because of the establishment of more public banks, the number of UCB units available for transplantation climbed from 44 000 in 1999 to 452 000 in 2008 (10). Early on, standards for defining the quality of a UCB unit began to evolve (11). This included not only defining standards for donor eligibility, but also for the collection, processing, storage, transport and transplantation of UCB. Over the past 18 years, the standards have undergone continuous modification as new technologies have been introduced and new information has been collected to improve the process. However, approximately 15–24% of patients receiving an unrelated UCBTx do not engraft (12,13). Although there are many clinical reasons why a patient may not engraft, the quality of the UCB unit, including potency, is likely to play a significant role. The potency of a UCB unit can be negatively impacted at several points in the manufacturing process, as discussed below.

Key steps in cord blood product manufacturing that could affect unit potency

While donor-specific factors probably impact on the potency of a UCB unit (14), the collection, processing, distribution and infusion stages may also affect the potency of this stem cell product, as described in Table I. Because of the central importance of cell dose in UCBTx, whatever processing technology a laboratory chooses, the goal is to achieve maximal recovery of nucleated cells, mononuclear cells, $CD34^+$ cells, colony-forming units (CFU), progenitor cells and stem cells. Typical UCB processing methods are described in Table II. In the future, other cell subsets, for example aldehyde dehydrogenase (ALDH)-bright or very small embryonic-like stem cells (VSEL) (15,16), may be determined as important too, and this development may require routine enumeration by processing laboratories. UCB units may be stored for an indefinite amount of time before use. Studies have demonstrated that cryopreserved UCB retrieved after $>$ 15 years of storage can reconstitute hematopoiesis in NOD/SCID mice in a quantitative and qualitative manner consistent with freshly isolated UCB (17). While the overall cellular recovery declines as a result of the freezing process, the overall potency may be preserved for more than 15 years if the UCB is maintained below –150°C (17).

Testing of potency at the cord blood bank

The FDA Guidance for Industry (18) defines UCB potency by parameters measured on the UCB after processing but prior to cryopreservation. Currently the FDA recommends evaluation of potency through assessment of the following *in vitro* assays: total

Table I. Stages at which potency might be lost.

Stage	Process	Primary causes of loss of potency
Collection	*Ex utero* or *in utero* collection UCB (31)	Extremes in temperature during shipment
	Addition of anticoagulant[a]	
	Shipment to processing laboratory in validated container with temperature-monitoring equipment	
Processing	Volume reduction as described in Table II (32,33)	Reduction in cell number during volume reduction (36)
	Cryopreservation with 10% dimethyl sulfoxide (DMSO)	Temperature fluctuations during controlled-rate freezing, causing intracellular ice crystals (37)
	Liquid nitrogen storage (34,35)	Transient warming during storage
	Removal of samples for additional testing needed for unit selection	Transient warming during removal of samples
Distribution	Shipment to transplant center in validated dry shipper with temperature monitor	Complete or partial thawing during shipment; exposure to X-rays during shipment
	Temporary liquid nitrogen storage at transplant center	Transient warming during transfer to temporary storage
Infusion	Thawing of unit in 37°C water bath (27)	Exposure to high concentrations of DMSO over extended times during thawing (42)
	Dilution of product depending on protocol (32,33,38–41)	
	Infusion into patient	

[a]Anticoagulants commonly used include citrate phosphate dextrose, with or without adenosine (CPD or CPDA-1) or lyophilized heparin citrate phosphate dextrose, with or without adenosine (CPD or CPDA-1) or lyophilized heparin.