the 7AAD permeability marker in conjunction with CD45 and CD34 gating [1]. CFU-GM assays are also performed on at least 10% of all frozen vials for QC purposes. No additional assays are performed at the time of thawing for transplant.

Question 4

We perform CFU-GM assays on 10% of our cryopreserved material for quality assurance purposes [2]. We also perform these assays on cellular products in long-term storage under consideration for transplantation, for collections with low CD34 doses and on harvest material where there was delayed engraftment after use for transplant.

Question 5

We aim to collect a minimum CD34 + cell dose for transplant of 2×10^6/kg with an ideal dose of $4–5 \times 10^6$/kg for PBSC and BM. The minimum cell dose for a cord blood transplant depends on the degree of HLA match: where there are two or less HLA disparities a TNC count of at least $2{\cdot}5 \times 10^7$/kg and CD34 count of greater than 2×10^5/kg are sought. Higher doses may be sought where there is greater HLA mismatch or in non-malignant disease where the risk of rejection is higher. For all three sources of HPCs, cell doses below these thresholds may be transplanted on the basis of clinical need as determined by the referring clinician.

We do not use the viable CD34 + cell dose calculated from a frozen sample as a measure of the acceptable dose for transplant. We have seen some variation in this number versus that found in the fresh samples, irrespective of the fresh or frozen cell viability but have not seen any correlation with times to engraftment. Our minimum for CD34 + cell viability on cryopreserved cells is 75% although it is unusual to see results below 80%. For the CFU-GM assay, our recommendation is a dose of $> 1 \times 10^5$ CFUs/kg as determined on a frozen vial.

Question 6

We have developed guideline thresholds for cell dose and viability in fresh and/or frozen stem cell collections based on collation of our data versus engraftment times and by comparison with international standards [3, 4].

Question 7

If either viability or CFU-GM results are below threshold, the referring physician will be informed. If an alternative stem cell donation is sourced, e.g. a second autologous donation or from an unrelated donor, then the stored cells may be discarded after successful engraftment as long as there is written authorisation from the referring physician. Should cells be found to have viability below our threshold further tests will be done and a decision made on clinical need. Products with reduced viability or CFU dose will then be issued under concession with acceptance recorded from the referring physician.

Question 8

Our laboratories participate in the UK National External Quality Assurance System (UK NEQAS) for CD34 + stem cell enumeration (every two months), for a full blood count (every month) and for the automated differential leucocyte count (every three months). We also participate once a year in the international CFU-GM QA scheme run by Stem Cell Technologies.

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Question 1

At Puget Sound Blood Center (PSBC) in Seattle, umbilical cord blood (UCB) products are frozen in cryopreservation bags with two attached integral segments containing aliquots of cryopreserved final product. Two cryovials containing 2–3 drops of final product are also frozen along with each UCB product. The bag containing UCB with attached segments and cryovials are stored frozen in liquid nitrogen in different locations. Other stored samples include one vial of red cell sediment from the UCB, two leukopoor plasma aliquots stored at −80 °C and one filter card spotted with red cell sediment that is stored at room temperature. Attached UCB segments are used for confirmatory typing and other relevant testing that is needed before a unit is selected for transplantation. If attached segments are no longer available, cryovials containing final product are used for confirmatory typing. All other UCB samples are available for quality control testing and to the transplant site for testing. Maternal samples that are also archived include two-1 ml serum samples and one buffy coat aliquot. Under some circumstances additional serum and/or

plasma maternal samples are stored frozen at −80 °C. Maternal back-up samples are sent to transplant sites upon request and are also used for process improvement.

Peripheral Blood Stem cell products collected by apheresis (HPC(A)) are stored frozen in cryopreservation bags with multiple attached integral segments containing aliquots of cryopreserved final product. Using a control rate freezer, two cryovials containing 1ml of HPC(A) are also frozen along side the product bag. The cryovials and the HPC(A) product are stored in separate places. Segments and cryovials are used as back-up samples for sterility testing, CD34+ cell analysis and for any other relevant assays.

Question 2

To evaluate UCB storage stability, selected units along with their samples are set-aside for quality control testing on an annual basis. Units that range from one day in storage to the length of operation that the UCB program at PSBC has been in operation are evaluated.

Question 3

UCB storage stability testing includes the performance of post-thaw total nucleated cells, cell viability using acridine orange/propidium iodide, viable CD34+ cell analysis by flow cytometry, colony forming unit (CFU) analysis and sterility testing.

Question 4

Using a commercially available methylcellulose based medium impregnated with cytokines, stem/progenitor CFU assays are routinely performed on UCB products (StemCell Technologies, Vancouver, Canada).

Question 5

For a unit of UCB to qualify for banking, total nucleated cells (TNC threshold values have been defined. However, TNC threshold values have not been established for the release of an UCB unit for transplantation. Although thresholds values for the number of stem/progenitor cells in a unit of UCB (i.e. CFU, CD34+ cells) are yet to be defined, an UCB unit is required to show CFU growth to qualify for banking and for transplantation.

For an autologous collection of peripheral blood stem cells to qualify for transplantation, the usual request is for a standard dose of 5×10^6 viable CD34+ cells/kg. However, there are situations in which it is not possible to obtain 5×10^6 viable CD34+ cells/kg from autologous transplant donors. In these cases, conversations are held between the Medical Director at the PSBC and the patient's oncologist to determine what is an acceptable collection for transplantation.

Question 6

The TNC and CFU threshold values to bank an UCB unit at PSBC were established based on criteria set by members of the National Marrow Donor Program Umbilical Cord Blood Advisory Board Committee. These banking threshold values were set by the committee based on the literature and the recommendations of committee members who are nationally recognized for UCB transplantation. Threshold values for selecting an UCB unit for transplantation are at the discretion of the transplant physician.

The recommended target threshold value of 5×10^6 CD34+ cells/kg of recipient weight for the transplantation of autologous peripheral blood stem cell products is based on the literature [1]. However, in the end, due to variability associated with mobilizing autologous hematopoietic stem/progenitor cells from patients, it is at the discretion of the patient's physician along with PSBC's Medical Director to define acceptable cell doses for an autologous stem cell transplant.

Question 7

There is a written policy indicating that if specific product endpoints are not met that the patient's physician and oncologist will be notified. There is also a policy which requires that the Medical Director discuss with the recipient's physician any deviation from established processes and procedures that may affect the safety and efficacy of the unit.

Question 8

PSBC participates in proficiency testing for CD34 and CFU assays twice per year. For CD34+ cell proficiency testing, we use surveys distributed by the College of American Pathologists (CAP). CFU proficiency assessments are conducted under the auspices of StemCell Technologies (Vancouver, Canada).

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