20 post-transplant showed a mean engraftment 87% +/- 9.6% vs 71% +/- 9.5% (p=0.027) in spleen and 76% +/- 5.3% vs 78% +/- 4% (NS) in BM respectively). Spleen and BM results from the 2nd experiment are still pending. Our results show that CD34+HSC from cadaveric donors can achieve long term multilineage engraftment in vivo following primary and secondary transplantation. CD34+ HSC from CD demonstrated to be at least equipotent to LD regarding engraftment capacity. Our results suggest that CBMC can be considered as a new HSC source for HSCT.

408

Hematopoietic Stem/Progenitor Cells and Engineering

AUTOLOGOUS EXPANDED PERIPHERAL BLOOD CD34+ STEM CELLS FOR THE TREATMENT OF ACUTE MYOCARDIAL INFARCTION

I. Garitaonandia[1], C. Vignon[1], G. Trébuchet[1], M. Destalminil[1], M. Kowalczyk[1], M. de Kalbermatten[1], H. Streefkerk[1], P. Henon[1,2]
[1]CellProthera, Mulhouse, France; [2]Institute of Research in Hematology and Transplantation, Mulhouse, France.

Abstract withdrawn.

409

Hematopoietic Stem/Progenitor Cells and Engineering

EVALUATION OF CELL CONCENTRATION, TRANSIT TIME AND CRYOPRESERVATION OF UNRELATED DONOR PRODUCTS ON ENGRAFTMENT

J. Triana Dopico[1], J. Bonilla-Baker[1], **L. Feehery**[1], S. Malakian[1], M. Gustafson[1], J. Adamski[1]
[1]Laboratory Medicine and Pathology, Mayo Clinic Arizona, Scottsdale, AZ, United States.

Keywords: cryopreservation, viability, engraftment.

Background & Aim: Prior to the COVID-19 pandemic, allogeneic transplants were typically performed with fresh hematopoietic stem cell (HPSC) products. Unrelated donor (UD) cells are obtained through the National Marrow Donor Program (NMDP). The logistics for coordinating collection, transport, and delivery of fresh products with preconditioning of recipients is complicated under the best circumstances. The pandemic created uncertainty and disruptions in the UD HPSC process. In March 2020 the NMDP required cryopreservation of UD HPSC products, with rare exceptions, prior to patient conditioning. The impacts of cryopreservation on allogeneic HPSC engraftment are not well defined and conflicting outcomes based on transport time and cell concentration have been published. We aimed to determine if cryopreservation, transport time and pre-processing cell concentration negatively impacted patient engraftment.
Methods, Results & Conclusion: Methods: Between July 2021 and January 2022, we analyzed UD HPSC products from 24 patients for CD34+ pre- and post-thaw cell recovery and viability based on transit time and pre-processing cell concentration. Transit time, defined as the interval from end of collection to start of processing, was divided into 3 cohorts: 1-20 h, 21-40 h, and >40 h. Pre-processing nucleated cell counts were divided into 2 cohorts: <200 x10^6 cells/mL and >200 x10^6 cells/mL. Neutrophil and platelet engraftment data were obtained from the patients' medical record. Medians for 2 unpaired groups were compared by using Mann-Whitney U test. Three or more unpaired groups were compared using one-way ANOVA with Tukey's multiple comparisons or Kruskal Wallis non-parametric test with Dunn's test for post hoc analysis, as appropriate. For paired data a mixed model ANOVA with Geisser-Greenhouse correction was applied. Results: Information regarding patient diseases and product characteristics are shown in Table I. There were no statistically significant differences between the nucleated cell count in the product bag reported by the collection center and those measured at the time of processing. When these parameters were evaluated based on transit time and pre-processing cell concentration, no statistically significant differences were observed. Conclusion: Although our data set is small, the results suggest that transit time and cell concentration of the HPSC product bag does not negatively impact allograft quality and engraftment.

Table 1. UD HPSC Product and Engraftment Analysis

Characteristic	Median	Range
Transit time (hours)	37.3	7-60
Nucleated cell concentration (cells x10^6/mL)	175.3	136.7-313.0
CD34+ cell viability-prefreeze (%)	98.8	96.4-99.9
CD34+ cell viability-postthaw (%)	85.9	41.7-98.1
CD34+ viable cell recovery (%)	42.4	12.2-151.7
Neutrophil engraftment (days)*	14.5	11-21
Platelet engraftment (days)*	18	15-31

*Engraftment goals based on historical data for unrelated allogenic transplant: Neutrophils by day 19; Platelets by day 36.

Table 1 (abstract 409).

410

Hematopoietic Stem/Progenitor Cells and Engineering

WASHING OF ABO INCOMPATIBLE ALLOGENEIC PERIPHERAL BLOOD STEM CELL PRODUCTS IS ASSOCIATED WITH LESS INFUSION REACTIONS BUT SIMILAR TRANSFUSION BURDEN AND TRANSPLANT OUTCOMES

S. Clugston[1], E. Leung[1], M. Wright[1], J. Cooney[1], P. Cannell[1], P. Chiappini[2], D. Purtill[1]
[1]Haematology, Fiona Stanley Hosptial, Perth, WA, Australia; [2]Fiona Stanley Hospital, Murdoch, WA, Australia.

Abstract withdrawn.

411

Hematopoietic Stem/Progenitor Cells and Engineering

EVALUATION OF FACTORS THAT EFFECT MOBILIZATION AND COLLECTION OF HEMATOPOIETIC STEM CELLS FROM PERIPHERAL BLOOD

R. Grubovic Rastvorceva[1,2]
[1]Institute for Transfusion Medicine of RNM, Skopje, Macedonia (the former Yugoslav Republic of); [2]Faculty for Medical Sciences, University Goce Delcev, Stip, Macedonia (the former Yugoslav Republic of).

Keywords: hematopoietic stem cell transplantation, apheresis collection, mobilization.

Background & Aim: Mobilization and collection of hematopoietic stem cells (HSC) are the crucial part in the transplantation procedure, with large variations in clinical practice. The aim of the study is to analyze factors that influence mobilization and collection of HSC from peripheral blood (PBSC).
Methods, Results & Conclusion: This study was performed in the Institute for Transfusion Medicine of RNM and the University Clinic of Hematology in Skopje. There were 30 allogeneic and 90 autologous donors that underwent mobilization and collection of PBSC. Factors that could influence mobilization and collection of PBSC were analyzed, such as demographic characteristics, laboratory parameters and collection parameters in both groups, and mobilization strategy and clinical characteristics in autologous donors. Results: There was a statistically significant correlation between the total number of collected MNC in autologous donors and platelet count before mobilization, the number of cycles in one apheresis procedure, quantity of collected graft and the number of collected MNC and CD34+ cells on the first day of harvestration. There was a statistically significant correlation between the total number of collected MNC in allogeneic donors and platelet count before mobilization, the number of cycles in one apheresis procedure, quantity of collected graft and number of MNC

on first day of harvestration. There was a strong correlation between the number of collected MNC and CD34+ cells on the first harvest and the total number of collected MNC and CD34+ cells in poor mobilizers, and inverse correlation with the number of apheresis procedures. Donors who donated MNC ≤ $0.7x10^8$/kg and/or ≤ $0.7x10^6$/kg CD34+ cells on the first harvest (84.6%) were strong predictors of poor mobilizers. Conclusion: Baseline platelet count is a sensitive indicator of PBSC mobilization in autologous and allogeneic donors. Development of universal guidelines for initiation of apheresis procedure is needed for optimization of mobilization and collection of HSC, as well as for early intervention in poor mobilizers.

412

Hematopoietic Stem/Progenitor Cells and Engineering

ALLOGENIC BONE MARROW DERIVED HEPATOCYTE INFUSION IN CRIGGLER NAJJAR PATIENTS

W. M. Abo Elkheir[2], **H. Gabr**[1]

[1]Clinical Pathology, Cairo University, Cairo, Egypt; [2]Immunology, Military Medical Academy, Cairo, Egypt.

Keywords: Hemopoeitic stem cells, Hepatocytes, crigler najjar.

Background & Aim: Crigler–Najjar syndrome is a rare autosomal recessive inherited disorder with an annual incidence of 1 in 1 000 000. CN syndrome is caused by genetic defect in the UGT1A1 gene resulting in absence of the enzyme UDP-glucuronosyltransferase. This enzyme defect affects bilirubin metabolism causing non hemolytic jaundice. High levels of unconjugated bilirubin can lead to brain damage in affected infants. The only treatment available for CN cases is phototherapy; while the only curative measure is liver transplantation. Liver transplantation is a major procedure with potential hazards for both donor and recipient. Isolated hepatocyte transplantation is a practical alternative. Bone marrow derived hemopoeitic stem cells can be transdifferentiated into hepatocytes as bone marrow aspiration is less invasive than liver biopsy.

Methods, Results & Conclusion: Two brothers suffering from Crigler-Najjar syndrome were subjected to hepatocyte transplantation from their father. Hepatocytes were obtained by transdifferentiation of bone marrow stem cells and infused to the portal vein in a dose of 5 X 109 cells. After two weeks, the patients received phenobarbitone to enhance the enzymatic activity of UDPGT. The patients were followed up using total bilirubin estimation. Results: The procedure was well tolerated, with no complications. Serum bilirubin showed a significant decrease over the 4 weeks follow up period with no relapse. After 4 weeks, the serum bilirubin showed steady figures. The patient is scheduled for another hepatocyte infusion.

413

Hematopoietic Stem/Progenitor Cells and Engineering

A NEW METHOD FOR THE AUTOMATED ENUMERATION AND ANALYSIS OF CD34+ HEMATOPOIETIC STEM AND PROGENITOR CELLS BY FLOW CYTOMETRY

A. Boehmler[1], L. Barrientos[2], D. Faye[2], D. Flagler[1], E. Flores-Fuentes[1], J. Bower[2], **E. Holl**[1]

[1]Beckman Coulter, Inc., Miami, FL, United States; [2]Beckman Coulter Immunotech, Marseille, France.

Keywords: CD34+, Automation, Enumeration.

Background & Aim: Clinical laboratories rely on commercially available IVD solutions for CD34+ cell enumeration in order to avoid time- and resource-consuming validation of user-defined tests. Most reagent kits and software packages used in flow cytometry were developed as a response to the 1996 and 1998 ISHAGE Guidelines, but have not been updated since then to meet the growing demands of diagnostic laboratories, in terms of automation, data traceability, and the ability to adapt acquisition and analysis panels to evolving regulations and guidelines.

Methods, Results & Conclusion: We have developed* an automated solution for CD34+ cell enumeration by flow cytometry, consisting of hardware, software, and reagents, that streamlines operations by incorporating automated sample loading, sample preparation, reagent management, and barcode scanning, as well as data analysis and bidirectional LIS connectivity in one compact device platform. Samples are just loaded on the system by the operator, and sample preparation and data analysis are performed automatically by the analyzer. The acquisition and analysis software follow the sequential gating strategy of the ISHAGE Guidelines and provide panel options that allow the running of tests in duplicate plus negative control, without the use of a negative control, or as a single test. Antibody and reagent vials include a unique barcode identity for tracking the expiration date, on-board expiration, manufacturing lot and container numbers, supporting Quality Management system data traceability requirements. Our newly developed system for CD34+ cell enumeration by flow cytometry provides a comprehensive solution for automated CD34+ cell enumeration that minimizes the need for human intervention, and potentially enables laboratories to offer CD34+ enumeration outside regulatory lab office hours. Both innovative aspects may increase the level of patient care and reduce time-to-result for this time-critical application. * Pending submission and clearance by the United States Food and Drug Administration; not yet available for in vitro diagnostic use in the US. For Investigational Use Only. The performance characteristics of this product have not been established. Patent pending.

414

Hematopoietic Stem/Progenitor Cells and Engineering

IT'S TIME TO EXCLUDE HEMATOGONES FROM THE RESULT OF CD34+ HEMATOPOIETIC STEM CELL ENUMERATION IN HPC PRODUCT

M. Sun[1], S. G. Schwister[2]

[1]Cell Therapy and Stem Cell Graft Engineering Facility, Rush University Medical Center, Chicago, IL, United States; [2]Clinical Flow Cytometry Laboratory, Rush University Medical Center, Chicago, IL, United States.

Keywords: stem cell, Hematogone, flow cytometry.

Background & Aim: Bone marrow transplant is the dominant treatment for many hematological malignant diseases even though Cellular Therapy has made significant progress. With years of hand-on experience and observation in FACT inspections, corresponding author believes the complexity of CD34+ hematopoietic stem cell enumeration was overlooked commonly and had submitted a poster in ISCT 2020 virtual annual meeting on viable CD34+ cell testing in post-thaw HPC product. This abstract focuses on improving the accuracy of CD34+ stem cell enumeration for another common scenario. ISHAGE protocol is widely, if not exclusively, used for the enumeration in clinical laboratories. However, it was known that the gating strategy can't separate young hematogones from true CD34+ stem cells. As a result, the reported cell counts were overestimated for some patients. Technology has changed since ISHAGE in 1996, many clinical laboratories are equipped with 10 or more-color flow cytometers. It's time to fine-tune the protocol to report true CD34+ stem cell in HPC product.

Methods, Results & Conclusion: A patient with DLBCL was mobilized with Mozobil and G-CSF. HPC-Apheresis was tested with ISHAGE as routine. Two clusters were identified (Fig. 1). The cluster with lower SSC/dimmer CD45 was noticed because it comprised more than 50% of the events (total 4.9 x 10^6 /kg). Eventually, the cluster was excluded from final report (2.2 x10^6 CD34/kg) because of similar to characteristic of hematogone (B-lym precursors). To confirm our hypothesis, CD10/CD19/CD38 were added and thawed QC vial from the same collection was tested. On Fig. 2, the two clusters (brown and green) and