Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Somatic Stem Cells: Hematopoietic Stem/Progenitor Cells

ENDOTHELIAL PROGENITOR CELLS OVEREXPRESSING ENDOTHELIAL NO-SYNTHASE MAY IMPROVE INFARCT HEALING: RESULTS FROM THE ENHANCED ANGIOGENIC CELL THERAPY – ACUTE MYOCARDIAL INFARCTION (ENACT-AMI) TRIAL

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Keywords: Heart Failure, Cell-based gene therapy, Angiogenesis.

Background & Aim: Introduction: The goal of the Enhanced Angiogenic Cell Therapy-AMI (ENACT-AMI) trial was to determine whether intracoronary delivery of early outgrowth endothelial progenitor cells (EPCs) engineered to overexpress endothelial NO-synthase (eNOS) would improve global left ventricular ejection fraction (LVEF) (primary outcome) and infarct size as assessed by cardiac MRI (CMR) in patients with anterior wall acute myocardial infarction (AMI) treated with evidence-based therapies.

Methods, Results & Conclusion: ENACT-AMI (NCT00936819) was a double-blind placebo-controlled trial in which participants were randomized to one of 3 arms: 1) saline placebo; 2) EPCs; or 3) eNOS-transfected EPCs using a plasmid DNA vector (pVax) containing the human eNOS sequence combined with JetPEI (Polyplus). Target sample size was 100 participants. Groups were compared using analysis of covariance (ANCOVA).

The trial was terminated due to slow recruitment after 47 patients were enrolled at three Canadian sites over six years (n=18 placebo, n=15 EPCs; n=14 eNOS-transfected EPCs). The groups were comparable with respect to demographic variables including age (56.1±9.7 years), cardiac risk factors, pre-existing cardiac disease, peak CK and troponin values and baseline LVEF by cardiac magnetic resonance imaging (40.7±9.3). Intracoronary cell delivery (20M cells; 20±5 days post-AMI) was well tolerated. At 6 months, there were no significant differences in the primary endpoint of LVEF between groups (p=0.30, mean difference between the average of the two EPC groups vs. placebo: 0.5% [95% Confidence Interval (CI) -2.9% to 3.9%). The secondary outcome of left ventricular infarct mass indexed to LV mass at 6 months demonstrated no significant difference between the average of the two EPC groups versus placebo (p=0.72); however, a significant difference was seen in those receiving eNOS-transfected EPCs compared to EPCs (-6.6; CI -12.0 to -1.1, p=0.02). Only four major cardiovascular events were observed over an average follow up of 4.1±1.6 years, and these were equally distributed across groups.

While there were no significant differences in LVEF, the results of the ENACT-AMI trial suggest that intracoronary delivery of gene-enhanced EPCs reduced infarct size and LV diastolic diameter in patients with large anterior wall AMI consistent with improved scar healing. These findings have important implications for remodelling and require confirmation in larger clinical trials.

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CRYOPRESERVATION OF ALLOGENEIC STEM CELL COLLECTIONS DURING COVID-19 PANDEMIC: PRODUCTS CHARACTERIZATION AND ENGRAFTMENT OUTCOME

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Keywords: Allogeneic Stem and Progenitor Cells, Cryopreservation, Engraftment.

Background & Aim: The COVID19 pandemic has affected the practice of allogeneic hematopoietic stem cell transplantation at multiple levels including donor selection, evaluation and stem cells collection and processing. Due to the risk of SARS-CoV2 infection and the impact of pandemic-related restrictions on transportation and handling, on March 13 2020, the National Marrow Donor Program/Be the Match recommended cryopreservation of allogeneic stem grafts which have been traditionally infused fresh. Here we report a single center experience on cryopreservation of allogeneic products and engraftment outcome after their infusion.

Methods, Results & Conclusion: A total of 35 products [graft sources, 13 bone marrow (HPC-BM) and 22 apheresis (HPC-A)] were cryopreserved and infused at Mount Sinai Hospital between March and December 2020. The median dose of $CD34^+$ cells was 1.57×10^6 cells/Kg and 4.46×10^6 cells/Kg for thawed BM and HPC-A (Table 1) as calculated based on the post-thaw total nucleated cells recovery of 88% and 96%, respectively. The median time between collection and freezing was 24 hours and storage time ranged from 7 to 35 for HPC-BM and 7-262 days for HPC-A products. The time to absolute neutrophil count (ANC) and platelet (PLT) engraftment in patients receiving HPC-BM was 16 and 26 days, respectively. When compared to the engraftment times observed after infusion of fresh products during 2019, there was no significant difference for either ANC or PLT (Table 1). Importantly, none of the patients receiving cryopreserved

Table 1 (abstract 303)
Comparison between fresh and cryopreserved grafts

	HPC, Bone Marrow		HPC, Apheresis	
	Year 2019	Year 2020 (April-Dec)	Year 2019	Year 2020 (April-Dec)
# of patients	10	13	28	22
CD34+ cells/kg (fresh)				
Average	2.54E+06	1.86E+06	8.06E+06	5.31E+06
Median	1.94E+06	1.37E+06	8.11E+06	5.03E+06
Range	8.9E+05-6.87E+06	5.3E+05-4.31E+06	3.79E+06-l.07E+07	1.32E+05-l.08E+07
CD34+ cells/kg (cryopreserved)				
Average	N/A	1.57E+06	N/A	5.05E+06
Median	N/A	l.21E+06	N/A	4.46E+06
Range	N/A	4.3E+05-3.51E+06	N/A	1.27E+06-9.16E+06
Time to ANC engraftment*				
Average	19	16	13	15
Median	18	16	11	14
Range	11-32 days	12-22 days	9-22 days	10-24 days
p value		1		0.073
Time to PLT engraftment**				
Average	36	27	23	28
Median	31	26	19	23
Range	14-107 days	15-39 days	11-55 days	17-57 days
p value		0.933		0.154

*Neutrophils $\geq0.5\times10^9$/L the first of 3 consecutive days

**Platelets $\geq20\times10^9$/L the first of 3 consecutive days 7 days after the last platelet transfusion

HPC-BM experienced primary graft failure. The time to engraftment in patients receiving HPC-A was 14 days for ANC and 23 days for PLT. While there was no significant difference between the times to ANC or PLT engraftment in 2020 vs. 2019 (Table 1), comprehensive analyses including long-term clinical outcomes are required to determine the impact of cryopreserved allografts on transplantation. Of note, 3 of the patients receiving cryopreserved HPC, A died prior to engraftment due to severe infections at days 12, 22 and 32. In summary, we report that cryopreservation resulted in acceptable TNC recovery and CD34+ cell doses and infusion of thawed products did not significantly affect the time to ANC or PTL engraftment when compared to that observed after transplant of fresh products. Our results, corroborated with those of others, support the implementation of cryopreservation of allogeneic products as an adequate approach in the management of HSCT during challenging times and beyond.

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CONVERTING A LEUKEMIC TRANSCRIPTION FACTOR INTO A POWERFUL TOOL FOR LARGE-SCALE EX VIVO PRODUCTION OF HUMAN PHAGOCYTES

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Keywords: Expansion, Differentiation, Macrophages.

Background & Aim: The key hurdle for ex vivo expansion of human CD34+ hematopoietic stem and progenitor cells (HSPCs) represents the rapid cellular differentiation after detachment from the supporting bone marrow stem cell niche. However, a few leukemia-related chimeric transcription factors, including MLL fusion proteins, are able to circumvent this issue.

Methods, Results & Conclusion: Here, we fused the coding sequence of an FKBP12-derived destabilization domain (DD) to the fusion gene MLL-ENL and subsequently expressed the protein switch (DD-ME) in human CD34+ progenitors derived from healthy donors. The DD-specific ligand Shield1 was added to regulate DD-ME protein turnover yielding massive and long-term expansion of HSPC-derived late monocytic precursors without additional driver mutations and with normal karyotype preserved. Upon removal of Shield1, the cells completely lost self-renewal and colony-forming properties and spontaneously differentiated, even after 12 months of ex vivo expansion. In the absence of Shield1, a six-day stimulation with IFN-γ, LPS, and GM-CSF triggered robust monocytic differentiation.

Immunophenotypic characterization revealed upregulation of the surface markers CD14, CD68, CD80, CD163 and MHC class I and II, concordant with monocytic morphology as judged by cytospin preparations. Upregulation of inflammatory markers such as IL-6, IL-10 and CCL2 on mRNA level was detected by qRT-PCR. Furthermore, nCounter gene expression analysis covering a total of 770 myeloid specific genes revealed the cells' identity as differentiated phagocytes. In functional assays, we demonstrated the ability of the obtained cells to migrate towards the chemokine CCL2, attach to VCAM-1 under flow and shear stress, to produce reactive oxygen species and engulf both bacterial particles and apoptotic cells. Finally, we demonstrated IgG Fc domain binding and phagocytosis of lymphoblastic tumor cells, including Daudi, Raji and patient-derived MCL cells in an antibody-dependent manner.

Taken together, we demonstrate the conversion of a leukemia-associated transcription factor to a useful tool for ex vivo blood cell production. Using this engineered protein switch, we were able to obtain HSPC-derived monocytic progenitors in large-scale and differentiate those towards functional monocytes under well-defined ex vivo conditions, both efficiently and quantitatively. This controllable system could set the stage for directed generation of patient-derived monocytes for cell-based immunotherapeutic approaches.

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IMPACT OF FANCONI ANEMIA GENOTYPE ON OUTCOME AFTER HEMATOPOIETIC STEM CELL TRANSPLANT: A SINGLE CENTRE EXPERIENCE OF 20 YEARS

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Keywords: Fanconi anemia, Genotype, Transplant.

Background & Aim: The effect of Fanconi anemia (FA) genotype on hematopoietic stem cell transplant (HSCT) outcome is not well elucidated in literature. Our aim was to compare HSCT outcome of patients with FANCA vs non- FANCA mutations.

Methods, Results & Conclusion: Retrospective data from hospital electronic records of FA patients who underwent HSCT from April 1998 to April 2019 were retrieved. FANCA mutation group was compared with non- FANCA for demographics, physical abnormalities, HSCT characteristics and outcome. Fisher's exact, Wilcoxon and log rank tests, and Kaplan-Meier were used for analysis. Event was defined as primary graft failure, relapse or death.

Data on complementation group was available for 50 out of 53 patients. FANCA mutations were found in 38/50 (76%) patients; FANCG in 5/50 (10%); FANCC in 4/50 (8%); FANCD1, FANCD2 and FANCL each in 1/50 (2%) patients. Most common physical anomaly was pigmentary abnormalities in 37(74%). Microcephaly was higher in the non-FANCA group (5/12 vs 5/38, p = 0.05). In both FANCA and non-FANCA patients, half underwent HSCT for severe aplastic anemia (SAA) and half for MDS/AML. The HSCT characteristics were similar between the two groups except donor population(Table 1).