than targeting either alone. Erythropoietin-producing hepatocellular carcinoma-A2 (EphA2)-specific CAR T cells were used to target the A549 tumor cells. EphA2-specific T cells when administered together with FAP-specific T cells, resulted in a significant decrease in tumor growth and increased survival compared to mice that received either EphA2- or FAP-specific T cells alone. Our study underscores the value of co-targeting both CAFs and cancer cells to increase the benefits of T-cell immunotherapy for solid tumors.

POSTER SESSION 2: STEM CELL BIOLOGY

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Fully Automated Clinical-Scale Separation of CD133$^+$ Cells From Bone Marrow Aspirate

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There is a growing interest in CD133 antigen expressing stem cell in the field of regenerative medicine such as cardiovascular, peripheral artery, and liver disease. Investigators in particular concentrate on bone marrow-derived stem cells for these applications. Today the clinical scale enrichment of CD133$^+$ cells has to be performed as a complex procedure involving numerous manual handling steps.

We have developed a fully automated clinical scale process within a closed sterile system to purify CD133$^+$ cells from human bone marrow aspirates. In this context, erythrocyte reduction, generation of autologous plasma, labeling time and the conditions for immunomagnetic separation were optimized.

To determine the process performance, CD133$^+$ cells were separated from human bone marrow aspirates with an initial volume of about 60 mL (n=10). We performed colony-forming unit (CFU) assays, which allowed us to evaluate the differentiation potential of the enriched cells.

The total processing time was reduced from about 4.5 h (previous manual process) to 2.5 h. The number of enriched CD133$^+$ cells was $7.9x10^5$ (range: $3.7x10^5$ to $1.9x10^6$). The average yield was 47% and the average viability of the separated CD133$^+$ cells achieved 90% (range: 69.9% to 96.9%). The depletion of CD133 negative cells was >99.9%. CFU assays perfomed after the fully automated enrichment process showed that the CD133$^+$ cell fraction contained primitive and multipotent progenitor cells, such as CFU-GEMM and CFU-GM. The cell separation system described provides a safe and easy way to purify CD133$^+$ cells from bone marrow aspirates within 2.5 h without any intermediate manual steps. The cell preparation in a closed sterile system facilitates a fast and robust enrichment of CD133$^+$ cells. The cells are eluted in a small volume (6 mL) and can be used directly for further applications according to requirements e.g. for use in regenerative medicine.

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Promotion of Wound Healing by Cord Blood Derived Unrestricted Somatic Stem Cells (USSCs) in a Murine Wound Healing Model and Analysis on Their Bio-Distribution by In Vivo Bioluminescent Imaging (BLI)

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Background: Delayed healing of skin wounds is a major morbidity. Repeated wounding is characteristic in patients with recessive dystrophic epidermolysis bullosa (RDEB), caused by mutations in *COL7A1* gene. Stem cell therapy offers an option in treating this disease (Kiru/Cairo et al. PNAS, 2011). Recently, cord blood (CB) derived pluripotent stem cells, USSCs, have been applied in several animal models of degenerative diseases with beneficial outcomes.

Goal: To determine the potential of USSCs in the treatment of RDEB and its associated wounding phenotype.

Method: CB-USSCs were characterized for genetic and functional properties. Their in vivo functions were evaluated in a murine full-thickness excisional wound healing model and by bioluminescent imaging (BLI), using USSCs modified with a luciferase reporter gene.

Results: CB-USSCs share several embryonic stem cell properties and could be induced to express hallmark genes of keratinocyte differentiation. USSCs constitutively express Col7A1, supporting their therapeutic potential in the treatment of patients with RDEB. In the wounding model, a single USSC intradermal injection promoted epithelialization and facilitated formation and remodeling of epidermis, accompanied by a significantly accelerated rate of wound healing on days 6-10 post wounding ($F_{(1,168)}=50.8$ $P<.01$). In vivo BLI revealed specific migration of USSCs from a distant intradermal injection site toward the wound, as well as following systemic injection. Temporal quantification on the total bioluminescence indicated an overall 59.9% signal loss over 3 days followed by a 95.06% loss at 1 week. The bioluminescence in the area of wound was then maintained at ~0.5-1% level till the end of the experiment (3 month). USSCs express several chemokine receptors that may mediate their migration to the wound, including CXCR4 (for SDF1), CCR7 (for CCL21) and PDGFRα (for HMGB1). In vitro chemotaxis assays indicated that SDF-1 significantly enhanced USSC migration at a concentration of 100ng/ml, while neither CCL21 nor HMGB1 showed significance even at a concentration of 10μg/ml. The effects of such chemokine/receptor interactions on USSC recruitment in vivo are now being investigated.

Conclusion: These results suggest significant beneficial effects of CB-USSCs on wound healing and raised the possibility of USSC's therapeutic beneifit in the treatment of patients with RDEB.

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Another Method for Thawing Hematopoietic Stem Cells and its Impact in the Recovery of the Transplanted Hematological Patient

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Introduction: There are several hematopoietic-stem cells (HSCs) thawing methods for bone marrow reconstitution. They intend to avoid cell death and patient's side effects due to the dimethyl sulfoxide (DMSO). We propose another thawing method that diminishes cell death and therefore a more rapid hematological recovery.

Material and Methods: The standard thawing-removing DMSO method for cord blood units was described by

Rubinstein in 1995 (method 1). Ours (method 2) pretend to increase more than 10 fold the dilution of cryopreserved HSCs in the standard washing solution (5% albumin + dextran 40). Methods 1 and 2 were compared to determine viability by means of total-nucleated-cell (TNC) count by trypan blue and flow cytometry at the time of collection, cryopreservation, thawing, and after removing the DMSO, as well as the patient's day of engraftment.

Results: Results are shown in Tables 1 and 2.

Conclusions: A greater dilution of cryopreserved HSCs in the washing solution, as a new thawing method, may decrease cell death; therefore a greater number of HSCs will be infused to the patient. Studies with a larger number of thawing procedures are needed to make this assertion.

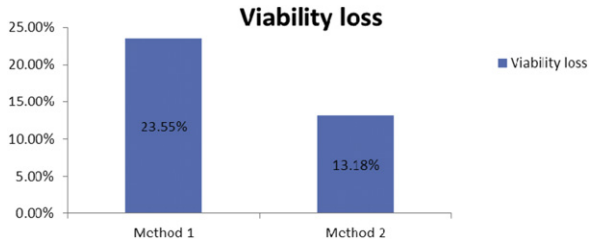

Figure 1. Viability loss

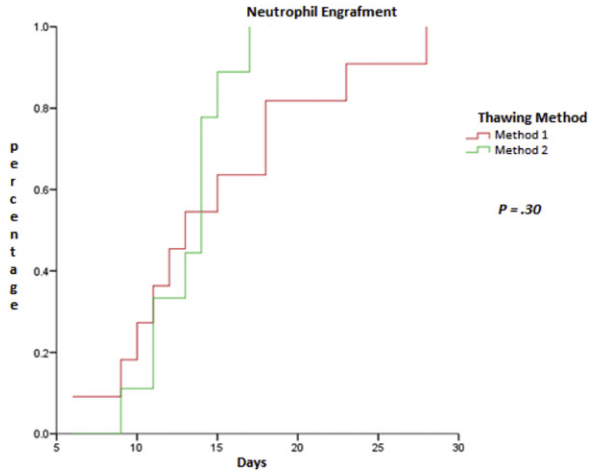

Figure 2. Neutrophil engrafment

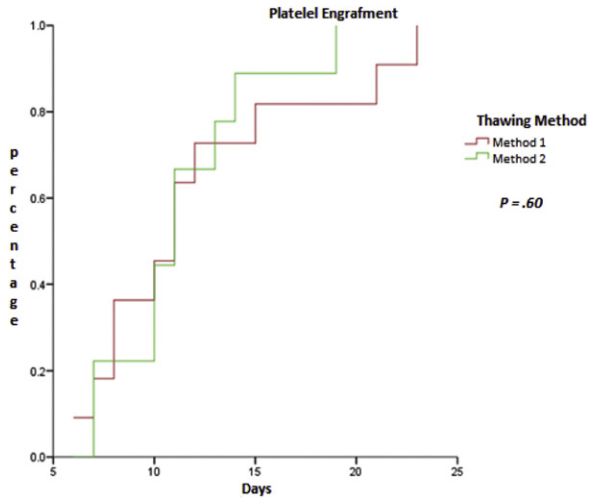

Figure 3. Platelel engrafment

Table 1
Patient's demographic data

	Global	Method 1	Method 2	P
Total (n)	26	13	13	
Transplant (n)				.43
Autologous	12	5	7	
Allogeneic	14	8	6	
Receptor age (years)				.75
Median (range)	26 (3-56)	27 (5-55)	25 (3-56)	

Table 2
Overall results in the study

	Global	Rubinstein's Method	New Method	P
Viability before thawing. Mean (SD)	97.28% (6.55)	99.01% (1.08)	95.56% (9.04)	.30
Viability after thawing. Mean (SD)	78.92% (11.69)	75.46% (12.5)	82.39% (10.12)	.15
Viability loss. Mean (SD)	18.36% (12.11)	23.55% (12.74)	13.18% (9.21)	.02
Engrafment days >20,000 Platelets. Median (range)	11 (6-23)	11 (6-23)	11 (7-19)	.60
Engrafment days >500 Neutrophils. Median (range)	13.5 (6-28)	13 (6-28)	14 (9-17)	.30

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Dissection of the Human Multipotent Adult Progenitor Cell (MAPC) Secretome by Proteomic Analysis

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Multipotent adult progenitor cells (MAPC; Multistem®) are adult adherent stromal stem cells currently being assessed in acute GVHD clinical trials with demonstrated immunomodulatory capabilities and the potential to ameliorate detrimental autoimmune and inflammation-related processes. Our previous studies documented that MAPC secrete factors that play a role in regulating T cell activity. Here we expand our studies using a proteomics approach to characterize and quantify MAPC secretome components secreted over 72 hours in vitro under steady-state conditions and in the presence of inflammatory triggers IFN-gamma and LPS, or a tolerogenic CD74 ligand, RTL1000. MAPC differentially respond to each of the tested stimuli, secreting molecules that regulate the biological activity of the extra-cellular matrix (ECM), including proteins that comprise the ECM itself, proteins that regulate its construction/de-construction, and proteins that serve to attach and de-attach growth factors from ECM components for redistribution upon appropriate stimulation. MAPC secrete a wide array of proteases, some detectable in their zymogen forms, as well as protease inhibitors that serve to poise the ECM in a state of repose, ready to respond