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Oral Abstract Presentation Listing

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3:30pm - 3:40pm	Ex-vivo expansion and prophylactic infusion of CMV-PP65 specific t-lymphocytes following allogeneic haemopoietic stem cell transplantation	Kenneth	Micklethwaite	241
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4:00pm - 4:10pm	Direct surgical implantation of autologous bone marrow stem cells in spinal cord injury: preliminary report.	Luis	Geffner	232
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4:15pm - 4:25pm	Fibronectin facilitates ex vivo expansion and maturation of megakaryocyte progenitors (MK-P) from cord blood.	Varda	Deutsch	95

Plenary Abstract Session Tuesday, June 26 (Bayside Auditorium A)

9:30am - 9:40am	PR1-specific T cell responses in the first months following T-cell depleted allogeneic stem cell transplantation (SCT) occur in both myeloid and non-myeloid malignancies but are only associated with a graft-versus-leukemia (GVL) effect in myeloid leukemias.	John	Barrett	242
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10:00am-10:10am	Autologous bone marrow mononuclear cell (ABMMC) transplantation in Type 1 and Type 2 insulin dependent diabetes mellitus (IDM)	Jorge	Tuma-Mubarack	238
10:15am-10:25am	Gene modification at clinical scale: engineering resistance to HIV infection via targeted disruption of the HIV co-receptor CCR5 gene in CD4+ T cells using modified zinc finger protein nucleases	Bruce	Levine	230

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Oral Presentations 4: Cell & Tissue Evaluation, Legal & Regulatory Affairs & Gene Therapy Tuesday, June 26 (Bayside Aud. A)

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5:45pm - 5:55pm	Matrix-induced autologous chondrocyte implantation (MACI®): biological and histological assessment.	Ming Hao	Zheng	228
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5:45pm - 5:55pm	Microchimeric fetal cells contribute to postreproductive maternal tissue repair in murine and human models.	Nicholas	Fisk	247
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5:45pm - 5:55pm	Glycogen synthase kinase-3 β inhibitors regulate normal human haematopoiesis and leukaemia cell growth.	Tiffany	Holmes	233
6:00pm - 6:10pm	A novel strategy of <i>ex vivo</i> expansion of cord blood cells for potential clinical applications	Nadim	Mahmud	235
6:15pm - 6:25pm	Automated rapid microbial detection for an autologous cell therapy: a twelve month review of sterility testing data	John	Duguid	244

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CYTOKINES STIMULATE GROWTH OF HUMAN ARTICULAR CHONDROCYTES IN THE ABSENCE OF SERUM**S. J. Duguay¹**, A. Parker¹, M. Zhang², S. Madden², B. Seymour¹; ¹Genzyme, Cambridge, MA, ²Genzyme, Framingham, MA.

Autologous chondrocyte implantation (ACI) is a successful treatment for the repair of articular cartilage defects. Current methods for manufacturing chondrocytes for clinical use rely on fetal bovine serum (FBS) as a component of the culture medium. In order to eliminate the risk of disease transmission from FBS to the patient, we have developed a serum-free medium (SFM) for chondrocyte culture. Microarray analysis was employed to compare gene expression of cells grown in SFM containing PDGF and bFGF versus cells grown in 10% FBS. More than 3,500 genes were found to be differentially expressed. Key word searching of Gene Ontology annotation identified 178 growth factors, hormones and other secreted factors as potential candidates for stimulating growth in serum-free medium. Testing identified the cytokines IL-6 and OSM as potent stimulators of chondrocyte growth in the absence of serum. A serum-free medium containing IL-6, OSM, PDGF and bFGF was compared directly to medium with FBS for the ability to stimulate growth of primary human chondrocytes through three passages, and the potential of the cells to redifferentiate was examined. Growth rate and total cell yield for cells grown in SFM was equal to or greater than that for cells cultured in medium with FBS. Cells grown in SFM formed proteoglycan-positive colonies in agarose with the same frequency as cells grown with FBS. Expression of collagen 2 and aggrecan in alginate cultures was detected at lower levels in SFM-derived cells than cells cultured in FBS. In addition, cells grown in SFM senesced and displayed normal karyotypes. These data indicate that SFM containing IL-6 and OSM is suitable for culture of human chondrocytes.

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RNA BASED CANCER VACCINES - CLINICAL TRIALS IN PATIENTS WITH PROSTATE CANCER AND MALIGNANT MELANOMA**G. Kvalheim**, S. Aamdal, G. Gaudernack; *Rikshospitalet-Radiumhospitalet HF, Oslo, Oslo, NORWAY.*

Anti-tumour vaccines targeting the entire tumour antigen repertoire represent an attractive immunotherapeutic approach. This repertoire is present in the total mRNA isolated from the tumour. The mRNA from each cancer patient may be amplified and thus overcome the problem of limitation of material that has hampered the development of individualized vaccines. We have developed immuno-gene-therapy for malignant melanoma and prostate cancer. Monocyte-derived dendritic cells are transfected with autologous melanoma-mRNA or mRNA from three prostate cancer cell lines (DU-145, LN-CaP and PC-3). The vaccines may generate T cell responses against a broad repertoire of tumor-associated epitopes, and the melanoma approach moreover target patient-specific tumor antigens. Effective protocols were established for mRNA-transfection by square wave electroporation and for generation of clinical grade dendritic cells. A full scale preclinical evaluation demonstrated *in vitro* T cell responses in 6/6 advanced melanoma patients. The responses were specific to antigens encoded by the transfected tumor-mRNA. Recently, we have conducted two phase I/II trials, in advanced malignant melanoma and androgen-resistant prostate cancer. Successful vaccine preparations were obtained for all 41 patients elected. No serious adverse effects were observed. Specific T cell responses (T cell proliferation and/or IFN γ ELISPOT) were demonstrated in 9/19 evaluable melanoma patients and in 12/19 prostate cancer patients. The response rates were higher for patients receiving intradermal vaccination, compared to intranodal injection. Thirteen prostate cancer patients developed a decrease in log-slope PSA. The PSA-response was significantly related to the T cell response ($p=0.002$). We conclude that the tumour mRNA based DC-vaccine is feasible and safe, and that T cell responses are elicited in about 50% of patients. In the next generation clinical protocols, patients will undergo Treg depletion by chemotherapy prior to DC vaccination. Subsequently, T cells will be expanded *ex vivo* using the Dynabeads Clin Ex Vivo system before re-infusion into the patients.

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MATRIX-INDUCED AUTOLOGOUS CHONDROCYTE IMPLANTATION (MACI[®]): BIOLOGICAL AND HISTOLOGICAL ASSESSMENT.**M. Zheng¹**, C. Willers¹, L. Kirilak¹, P. Yates¹, D. J. Wood¹, A. Shimmin²; ¹University of Western Australia, Nedlands, AUSTRALIA, ²Melbourne Orthopaedic Group, Melbourne, AUSTRALIA.

Matrix-induced autologous chondrocyte implantation (MACI[®]) has been a treatment of cartilage injury since 2000, but little is known of the histological paradigm of tissue regeneration after implantation. MACI[®] is a stable cell-based delivery system that enables the regeneration of hyaline-like cartilage. From a cohort of 56 MACI[®] patients, we examined the phenotype of chondrocytes seeded on type I/III collagen scaffold, and conducted progressive histologic assessment over a period of six months. Chondrocyte-seeded collagen scaffolds from patient implants were analyzed by electron microscopy, immunohistochemistry (type II collagen and S-100), and reverse transcription polymerase chain reaction (aggrecan and type II collagen). Coincidental cartilage biopsies were obtained at 48 hours, 21 days, 6 months, 8 months, 12 months, 18 months, and 24 months. Our data showed that chondrocytes on the collagen scaffold appeared spherical, well integrated into the matrix, and maintained the chondrocyte phenotype as evidenced by aggrecan, type II collagen, and S-100 expression. Progressive histologic evaluation of the biopsies showed the formation of cartilage-like tissue as early as 21 days, and 75% hyaline-like cartilage regeneration after 6 months. This preliminary study has suggested that MACI[®] may offer an improved alternative to traditional treatments for cartilage injury by regenerating hyaline-like cartilage as early as 6 months after surgery.

GENE MODIFICATION AT CLINICAL SCALE: ENGINEERING RESISTANCE TO HIV INFECTION VIA TARGETED DISRUPTION OF THE HIV CO-RECEPTOR CCR5 GENE IN CD4+ T CELLS USING MODIFIED ZINC FINGER PROTEIN NUCLEASES**B. L. Levine¹**, M. DeCaul¹, O. Liu¹, J. Wang², G. Lee², K. Kim², N. Wang², D. Ando², P. D. Gregory², J. L. Riley¹, M. C. Holmes², C. H. June¹, E. E. Perez¹; ¹University of Pennsylvania, Philadelphia, PA, ²Sangamo BioSciences, Richmond, CA.

Background: HIV requires the CD4 receptor and the co-receptors CXCR4, or more commonly CCR5, to infect its target cells. A naturally occurring mutation of CCR5 (delta 32) renders homozygous carriers highly resistant to infection with CCR5-tropic HIV. Loss of HIV co-receptors is therefore an attractive therapy for HIV patients, yet no current methods permit efficient therapeutic disruption of a chosen gene in the human genome. Transient delivery of zinc-finger protein nucleases (ZFNs) can be used to target the CCR5 gene and create a double strand break (DSB) at predetermined sequences. Natural DNA repair pathways can subsequently be usurped to imperfectly repair the DSB resulting in the permanent disruption of the target gene. Methods: Leukapheresis units were depleted of monocytes and CD8 cells. The resulting CD4-enriched fraction was transduced with an adenoviral vector encoding CCR5 targeted ZFNs and expanded >100-fold via CD3/CD28 co-stimulation. Functional disruption was measured by a PCR-based assay to quantify the frequency of Non Homologous End Joining-based mutations in CCR5 and by HIV challenge assays. Results: Cell-based assays revealed the CCR5-ZFNs generated DSBs *in vitro* leading to high efficiency targeted gene disruption (>30%) in transduced cells. Extensive off-target analysis has shown the gene disruption to be highly specific for the CCR5 gene. Modified primary CD4+ T cells treated with CCR5-ZFNs were also shown to be resistant to HIV challenge, and resulted in an enrichment of ZFN generated CCR5-/- cells. Conclusion: These data demonstrate that ZFN-treated cells can be permanently modified at clinical scale to prevent CCR5 dependent HIV infection. A clinical trial is planned using this approach for the generation of HIV resistant CD4+ T cell populations *ex vivo* and subsequent infusion as a potential therapeutic intervention in the treatment of HIV/AIDS.

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FIBRONECTIN FACILITATES EX VIVO EXPANSION AND MATURATION OF MEGAKARYOCYTE PROGENITORS (MK-P) FROM CORD BLOOD.V. R. Deutsch, E. Hubel, S. Kay, A. Many, E. Naparstek, D. Grisaru; *Tel Aviv Medical Center, Tel Aviv, ISRAEL.*

Protracted thrombocytopenia remains a serious clinical problem following cord blood transplant (CBT). Thrombopoietin (TPO), has no clinical impact on platelet production in patients post BMT due to the paucity of MK-p in the grafts. If expanded, MK-p could facilitate platelet engraftment. MK-p can be expanded from CB-CD34+ cells, however, the limited number of stem and progenitor cells in the CB units renders removal of stem cells from the fresh CB impossible. Additionally, MK expansion from stem cells requires a long time. We propose a novel strategy to enhance thrombopoiesis, by expanding MK-p from mononuclear cells (MNC) from a small aliquot of CB in short term cultures. CB MNC were progenitor enriched by separation on gelatin/ficolin (1). Growth conditions included fibronectin (FN) coated dishes in the presence new cytokine combinations; r-hu-TPO(10 ng/ml), r-hu-SCF(50 ng/ml), β -FGF(10 ng/ml) and ARP a peptide derived from acetylcholinesterase recently discovered to have potent stem cell and MK-p expansion activity (2). MK-p were characterized by high resolution flow cytometry. True MK expansion was assessed by appropriate elimination of granulocyte and monocytes, which acquired CD41+ adherent platelets in culture. FN alone increased viability and expansion of MK-p (CD41high, SSClow and FSClow). FN+TPO increased the number of MK-p by 4.8+2.7 fold and the addition of either SCF, β -FGF or ARP further expanded MK-p, all producing a similar 4-6 fold increase. FN+TPO in the presence of other growth factors expanded the CFU-MK by 5-35 fold. FN+TPO increased Mk ploidy, which was further incremented with ARP or β -FGF. MK maturation was confirmed by elevated expression levels of GPIIb/III using qRT-PCR. We describe an easy method to expand MK-p from an aliquot of the CB which can comply with GTP regulations. This may facilitate developing more effective cellular therapeutic modalities to reduce the time of thrombocytopenia post CBT. 1. Pick M, Eldor A, Grisaru D, Zander AR, Shenhav M, Deutsch VR. Ex vivo expansion of megakaryocyte progenitors from cryopreserved umbilical cord blood. A potential source of megakaryocytes for transplantation. *Exp Hematol.* 2002 Sep;30(9):1079-87 2. Pick M, Perry C, Lapidot T, Guimaraes-Sternberg C, Naparstek E, Deutsch V, Soreq H. Stress-induced cholinergic signaling promotes inflammation-associated thrombopoiesis. *Blood.* 2006 Apr 15;107(8):3397-406

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DIRECT SURGICAL IMPLANTATION OF AUTOLOGOUS BONE MARROW STEM CELLS IN SPINAL CORD INJURY: PRELIMINARY REPORT.L. F. Geffner¹, M. Izurieta¹, B. Maldonado², P. Santacruz¹, L. Flor¹, A. Aua¹, A. Aua¹, B. Cardenas¹, B. Zurita¹, M. Razuri¹, A. Romero², X. Garcia², E. Landivar¹, E. Salazar¹, R. Gonzalez², F. Silva³; ¹Hospital Luis Vernaza, JBGYE, Guayaquil, ECUADOR, ²SOLCA, Guayaquil, ECUADOR, ³Primecell Therapeutics, Irvine, CA.

Background: Presently, there is no cure to spinal cord injury (SCI) which affects millions of patients. There is evidence in neural tissue demonstrating nerve growth in mice hippocampus; human neural stem cells that migrate, differentiate, and graft into rats brain. Moreover, human neural stem cells can be differentiated into oligodendrocytes leading to remyelination of demyelinated axons. Studies in a SCI model have demonstrated that embryonic stem cells injected in the spinal cord cavity of mice sprouted axons 1 cm long and after 1 month some mice recovered their motility. In this study, we designed a protocol to assess safety, feasibility and efficacy of autologous bone marrow stem cells (BMSC) implanted into spinal cord injury patients. Methods: Twenty five patients with SCI were treated from May 2006 to January 2007 with autologous BMSC. The average time of being paraplegic was 48.28 +/- 56.92 months (0.5 to 264 months). BMSC suspensions with 1.23 +/- 0.44 x 10⁶/kg CD34+ cells that were 89.01 +/- 5.94 % viable were implanted. Electromyography, evoked somatosensory potentials, urodynamic studies, spinal cord MRI, ASIA, Asworth, Frankel and Barthel data and scores were collected. A strict rehabilitation protocol was started 4-7 days after surgery to be followed along 1 year. Results: Patients demonstrated improvements in sensitivity; motility; bladder sensation even controlling sphincters; erection and ejaculation. ASIA score increase from 0.4 +/- 0.17 to 0.84 +/- 0.20 (p<0.05). Fifteen patients (60%) could stand up, 10 (40%) could walk on the parallels with braces, 7 (28%) could walk without braces, and 4 (16%) could walk with crutches. No adverse event was observed. Conclusion: Increasing strong evidence demonstrates spinal cord regeneration and clinical improvement after stem cells implantation. There were no patient's conditions which degenerated. However, longer follow-up and a larger amount of patients must be studied before coming to a definitive conclusion.

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GLYCOGEN SYNTHASE KINASE-3 β INHIBITORS REGULATE NORMAL HUMAN HAEMATOPOIESIS AND LEUKAEMIA CELL GROWTH.T. Holmes^{1,2}, T. A. O'Brien^{1,3}, R. Knight^{1,2}, A. Dolnikov¹; ¹Sydney Cord and Marrow Transplant Facility, Sydney, AUSTRALIA, ²The University of New South Wales, Sydney, AUSTRALIA, ³Centre for Children's Cancer and Blood Disorders, Sydney, AUSTRALIA, ⁴Prince of Wales Hospital, Sydney, AUSTRALIA.

Glycogen synthase kinase-3 beta (GSK-3 β) is involved in the regulation of many cellular events including Wnt signaling. Wnt signaling was previously shown to regulate haematopoiesis. GSK-3 β acts to suppress Wnt, and GSK-3 β inhibitors were shown to activate Wnt. The effect of GSK-3 β inhibition on human haematopoiesis was not examined before. Here we show that a novel GSK-3 β inhibitor BIO supports *ex vivo* expansion of human primitive cord blood-derived haematopoietic progenitor cells (HPC). This was translated *in vivo* with the promotion of human cell engraftment in the NOD/SCID mouse model. An increased frequency of the most primitive SCID Repopulating Cells was seen in the BIO cohort. The effect of BIO was improved by co-culture with murine stroma MS5 cells. BIO induced the intracellular accumulation of β -catenin and its translocation from the cytoplasm to the nucleus. Up-regulation of the transcription of c-myc, HoxB4, p21^{Waf1} and cyclin D1 was observed. The effect of BIO was negated by IL-3 withdrawal from the culture: in these conditions BIO acted to suppress haematopoiesis. Interleukin-3 withdrawal resulted in the complete abrogation of cyclin D1 up-regulation and reduced the expression of HoxB4 but not c-myc. In addition, BIO suppressed the growth of leukaemia TF-1, K562, U937 and HL-60 cells by inducing p53-independent apoptosis. The latter was accompanied by the up-regulation of β -catenin and down-regulation of survivin, both highly expressed in leukaemic blasts. To the best of our knowledge, the direct suppression of leukaemia cell growth by GSK-3 β inhibitors has not previously been reported. Collectively, our results show that modulators of GSK-3 β may be used in the expansion of primitive human haematopoietic progenitor cells and increase the range of novel anti-cancer tools.

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PHASE I CLINICAL RESULTS USING SELECTIVELY AMPLIFIED HEMATOPOIETIC STEM/PROGENITOR CELLS (HPC) MEETS PRIMARY ENDPOINT FOR SAFETY IN 10 PATIENT TRIALM. Kraus¹, K. Gunter², J. Laning¹, R. Abonour³, M. Laughlin⁴, S. Rowley⁵, J. E. Wagner⁶; ¹ViaCell, Inc., Cambridge, MA, ²Hospira Inc., Lake Forest, IL, ³Indiana University School of Medicine, Indianapolis, IN, ⁴Case Western Reserve University, Cleveland, OH, ⁵Hackensack University Medical Center, Hackensack, NJ, ⁶University of Minnesota School of Medicine, Minneapolis, MN.

Using a lineage depletion strategy to selectively amplify HPCs [Mackin et al.] umbilical cord blood (UCB) units obtained from public UCB banks were thawed, expanded and administered to patients undergoing treatment for high risk hematological malignancies. Patients and Methods: Ten patients diagnosed with ALL (n=3), AML (n=6) and MDS (n=1) were treated with a myeloablative regimen consisting of fludarabine 75 mg/m², cyclophosphamide 120 mg/kg and fractionated 1320 cGy TBI. Prophylaxis of GVHD consisted of cyclosporine A and mycophenolate mofetil. Patients received two partially HLA matched UCB units, including one thawed and unmanipulated and one thawed and placed in *ex vivo* expansion culture prior to infusion. 80% of patients received two units that were both 4/6 HLA matched with the recipient. The median infused cell dose of the combined units was 26 x 10⁶ nucleated cells per kilogram. Results: No infusional toxicities were observed. For 8 evaluable patients, the incidence of neutrophil recovery (ANC >500/uL) was 80% (95% CI: 55-100) at a median of 22 (range: 17-37) days. The incidence of platelet recovery (50,000/uL) was 54% (95% CI:28-77) at 6 months. Presence of the expanded product was detected in 3/9 evaluable patients in the peripheral blood on day 7. However, chimerism assays after day 21 only revealed cells derived from the unmanipulated unit. 4/10 patients experienced Grade III-IV acute GVHD with 8/10 patients alive at day 100 after transplant. Conclusions: Infusion of selectively amplified HPCs was not associated with any infusional toxicity. While lack of chimerism from the expanded product after day 21 suggests an alteration in long term engraftment potential after the selective amplification procedure in the context of 'double UCB' transplantation (either due to T cell depletion or effect on long term engrafting cells), additional studies are needed to evaluate efficacy in terms of rate of hematopoietic recovery.

Oral Abstracts

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A NOVEL STRATEGY OF EX VIVO EXPANSION OF CORD BLOOD CELLS FOR POTENTIAL CLINICAL APPLICATIONS

H. Araki¹, K. Yoshinaga¹, S. Petiwala¹, U. Nawaz¹, X. Li², N. Mahmud^{1,3}; ¹University of Illinois at Chicago, Chicago, IL, ²University of Chicago, Functional Genomics Facility, Chicago, IL, ³University of Illinois Cancer Center, Chicago, IL.

Cord blood (CB) transplantation in adults is associated with prolonged neutropenia and often graft failure, limiting its use to treat hematologic malignancies. It is widely accepted that the time to engraftment following transplantation is associated with the number of hematopoietic stem cells (HSC) infused. Efforts to promote the number of HSC ex vivo have failed primarily due to loss of HSC. We have previously demonstrated that culture conditions result in the silencing of genes which can be circumvented by treating CB cells with chromatin modifying agents, 5-aza-2'-deoxycytidine (5azaD) and trichostatin A (TSA) resulting in 9 fold increase in the number of SCID mouse repopulating cells (SRC). In our current studies, the mechanism as well as signature genes associated with HSC self-renewal/expansion was investigated. Our results show that CD34+CD90+ cells treated with 5azaD/TSA divide at a slower rate than cells exposed to cytokines alone. When CD34+CD90+ cells that have undergone 5-10 cell divisions in the presence of cytokines alone were transplanted into immunodeficient mice, donor cell chimerism was not detectable. By contrast, 5azaD/TSA treated cells still retained their marrow repopulating potential. Furthermore, bone marrow cells from the mice engrafted with 5azaD/TSA treated cells were capable of engrafting secondary hosts indicating their self-renewal potential. The transcription levels of several genes and their products (i.e., HOXB4, Bmi-1 and P21) previously implicated in self-renewal, were up-regulated in cells treated with 5azaD/TSA. The efficacy of another chromatin modifying agent, valproic acid (VPA) was also examined; VPA did not result in expansion, but maintained SRC numbers. Global microarray analysis was performed using CD34+ cells exposed to various chromatin modifying agents with known in vivo function (expansion, maintenance or loss of SRC). This strategy has the potential to identify signature genes linked with HSC expansion to serve as potency-markers for expanded CB grafts for therapeutic applications.

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SUCCESSFUL TREATMENT OF REFRACTORY ANGINA (RA) BY MINIMALLY INVASIVE DELIVERY OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS (ABMMC). MID-TERM FOLLOW-UP

J. Tuma-Mubarack¹, R. Fernández-Viña², A. A. Carrasco-Yalán³, J. Castillo-Aguirre³, H. Rios-Diaz³, R. de Moura⁴, C. Cruz¹, M. Vargas¹, A. Carrillo¹, J. Ercilla¹, C. Yarleque¹, C. Cunza¹, N. Gómez¹, S. Chirinos¹, M. Aranda¹, M. Arroyo¹, J. Rafael¹, A. N. Patel⁵; ¹Division of Interventional Cardiology and Regenerative Medicine, Clínica San Felipe, Clínica Ricardo Palma, Clínica Maison de Santé, Lima, PERU, ²Centro Cardiovascular San Nicolás, Don Roberto Fernandez-Viña Foundation, San Nicolas, ARGENTINA, ³Instituto de Criopreservación y Terapia Celular, Lima, PERU, ⁴Universidad Fluminense, Rio de Janeiro, BRAZIL, ⁵University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: ABMMC have been shown to be safe in early clinical trials for cardiac cell therapy. The goal of this study was to evaluate the use of ABMMC delivered via percutaneous retrograde coronary sinus technique (PRCST) to treat chronic RA. Methods: Twenty-six patients were evaluated from May 2005 to October 2006, 12 completed enrollment and follow-up. Median follow-up was 16 months, median age was 68 years old, M/F ratio was 10/2, all of them with RA and ischemic stress-induced myocardial segments assessed by SPECT. Median number of mononuclear and CD34+ cells infused were 7.88×10^8 and 1.206×10^7 . They were implanted by PRCST in selected veins using balloon occlusion "over wire" for 8 to 10 minutes. Results: During the study period, no arrhythmias or increase in enzymes were observed. After a median time of 21 days, the ABMMC transplantation led to significant relief of angina symptoms and improvement in functional class. All but one evaluable patient improved Canadian Cardiovascular Society class by one (p<0.001) when compared at baseline and last follow-up. SPECT evaluation of ischemic myocardium percentage, median baseline was 38.2% and at one year follow-up this was reduced to 26.5% (p=0.002). Rest LVEF by SPECT at baseline median: 31.6% and one year follow-up was median: 34.8%, p=0.066; while stress LVEF did show improvement between baseline (median: 31.8%) and follow-up (median 38.6%, p=0.001). Median number of ischemic myocardium segments by SPECT were reduced from a baseline of 6.5 to 4.5 (p=0.002). Conclusions: The treatment of RA using ABMMC transplantation delivered by PRCST is safe. The PRCST can be used when antegrade access is limited or not possible due to severe coronary disease. This study suggests improvement, clinical benefit and potential outcome durability of angina symptoms relief demonstrated by better functional class, myocardial perfusion and contractility.

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IMMUNE RECONSTITUTION AFTER UNRELATED UMBILICAL CORD BLOOD TRANSPLANTATION - ELEVEN YEARS EXPERIENCE AT CHILDRENS HOSPITAL LOS ANGELES

E. M. Smogorzewska¹, L. Dukes¹, L. Kuo¹, G. Crooks¹, D. B. Kohn¹, A. Shah¹, R. Parkman¹, K. I. Weinberg², H. Abdel-Azim¹, N. Kapoor¹; ¹Childrens Hospital Los Angeles, Los Angeles, CA, ²Stanford University Medical Center, Palo Alto, CA.

Forty nine umbilical cord blood (UCB) transplantations were performed in pediatric patients at CHLA between 1995 and 2006: 23 for ALL, 6 AML, 1 CML, 17 primary immunodeficiency [7 SCID, 4 WAS, 2 Hyper IgM, 2 XLP, 2 FEL] 2 aplastic anemia. The mean age of the patients was 3.5 years, ranging from 4 months to 11 years. The mean weight was 16 kg [5.9 - 40.6]. UCB were obtained from 5 different Cord Blood Banks, 3 registered with NMDP. All but 3 products were RBC depleted prior to cryopreservation; all but one unit was Dextran/Albumin washed post thawing. Patients received a mean dose of TNC/kg of 7.1×10^7 /kg [2.8 - 15.1], and 7×10^5 /kg [0.7 - 28] CD34+/kg post thawing. Twenty six patients are alive [53%]. Forty patients achieved hematological recovery ANC > 500 within 36 days [range 13 - 406], and 26 recovered platelets > 20k within 73 [range 31 - 195] days post transplantation. Immunological recovery: on average there were 71 [13 - 240] days to achieve 100 T cells/mm³, and 104 days to reach 10 CD4+CD45RA+ naïve T cells/mm³ [range 2-1051]. One year post transplantation the average number of peripheral T cells was 1284/mm³ [range 71 - 3702], and 301 naïve T cells/mm³. Hematological recovery and immune reconstitution were compared to those in related and unrelated marrow transplantations. Table 1. The comparison of immune reconstitution matched by diagnosis will be presented on the poster.

	Unrelated UCB n=40	Unrelated Marrow n=40	Sibling's Marrow n=35
ANC >500/uL [days]	36	23	24
Platelets >20k/uL [days]	73	54	33
Days to reach 100 T cells/mm ³	71	82	38
Days to reach 10 CD4+CD45RA+ cells/mm ³	104	127	37
Absolute T cells/mm ³ 1 year post transplant	1284	938	1018
Absolute CD4+CD45RA+ cells/mm ³ 1 year post	301	174	332

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AUTOLOGOUS BONE MARROW MONONUCLEAR CELL (ABMMC) TRANSPLANTATION IN TYPE 1 AND TYPE 2 INSULIN DEPENDENT DIABETES MELLITUS (IDM)

J. Tuma-Mubarack¹, R. Fernandez-Viña², A. A. Carrasco-Yalán³, J. F. Castillo-Aguirre³, H. N. Rios-Diaz³, L. More¹, N. Gómez¹, S. Chirinos¹, M. Aranda¹, M. Arroyo¹, J. Rafael¹, A. N. Patel⁵; ¹Clínica San Felipe, Clínica Ricardo Palma, Clínica Maison de Santé, Fundación Peruana de Terapia Regenerativa y Centro de Diagnóstico y Tratamiento del Corazón CENCOR, Lima, PERU, ²Centro Cardiovascular San Nicolás, Don Roberto Fernandez-Viña Foundation, San Nicolás, ARGENTINA, ³Instituto de Criopreservación y Terapia Celular, Lima, PERU, ⁴University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: Recent reports have shown that bone marrow-derived stem cell may contribute to islet regeneration. The goal of our study was to evaluate the safety and efficacy of ABMMC transplantation for patients with IDM. Methods: From June 2005 to January 2007, 28 consecutive patients: 8 Type 1 IDM (T1DM) and 20 Type 2 IDM (T2DM); who were receiving maximal medical therapy including insulin treatment for 5 years before enrollment. Median time of disease for T2DM patients was 13 years, without pancreatic islet auto-antibodies. After IRB approval and signed informed consent, bone marrow was harvested and ABMMC were isolated and infused directly into the pancreas via splenic artery using endovascular catheters. Glucose, glycosylated HbA1c and C peptide were measure before and after transplantation. HOMA2 Calculator v2.2 was used to calculated IR and % B ("if Glucose : 3.0 to 25.0 mmol/L and C-peptide : 0.2 to 3.5 nmol/L). Results: There were no study related complications. At 1 year follow-up, mean daily insulin requirement was the same in group T1DM and significantly reduced in group T2DM, from 42.5 to 4.5 U/d (t=7.94, p<0.001). Ten of the twenty (50%) T2DM established complete insulin independence. Data in table 1. Conclusions: The use of ABMMC transplantation for T1DM and T2DM is safe. In this pilot study, only T2DM patients have significant improvement in pancreatic function demonstrated by better glycemic and HbA1c control, and are associated with a significant independence of the insulin. This has formed for a randomized multi-center study which is currently in progress. Table 1. Median values

	Pre	Post	t	p
T2DM (n=20)				
Fasting Glucose (mmol/L)	10.8	6.6	3.98	0.01
Glycosylated HbA1c (%)	9.6	8.1	3.98	0.01
C Peptide (nmol/L)	0.5	0.84	5.11	<0.01
HOMA 2 IR (n=17)*	2.2	2.26	0.94	0.92
HOMA 2 % B (n=17)*	42.4	130.2	4.9	<0.01
T1DM (n=8)				
Fasting Glucose (mmol/L)	10.1	11.1	1.382	0.21
Glycosylated HbA1c (%)	8.7	8.7	0.45	0.66
C Peptide (nmol/L)	0.17	0.16	1.00	0.35

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VACCINATION OF ACUTE MYELOID LEUKEMIA (AML) PATIENTS WITH CLINICAL GRADE MATURE DENDRITIC CELLS (DC) ELECTROTRANSFECTED WITH MESSENGER RNA ENCODING THE WILMS' TUMOR PROTEIN WT1: A PHASE I/II TRIAL

Z. N. Berneman¹, A. Van Driessche¹, A. Van de Velde¹, G. Nijs¹, A. P. Gadisseur¹, W. A. Schroyens¹, I. J. de Vries², V. F. Van Tendeloo¹; ¹Division of Hematology, Laboratory of Experimental Hematology and Center for Cell Therapy and Regenerative Medicine, Antwerp University, Antwerp University Hospital, Edegem, BELGIUM, ²Radboud Universiteit Nijmegen, Molecular Immunology, Centre for Molecular Life Sciences, Nijmegen, THE NETHERLANDS.

WT1 is a promising target for immunotherapeutic interventions of WT1-overexpressing tumors such as AML. We conducted a phase I/II clinical immunotherapy trial in AML remission patients to evaluate a clinical grade DC vaccine loaded with WT1-encoding mRNA. Five patients with AML in remission were included, each receiving four biweekly vaccines and a delayed-type hypersensitivity (DTH) test 2 weeks following the last vaccination. Monocytes were labeled with CD14 microbeads and positively selected with the CliniMACS⁺ plus device. Mature DC (mDC) were generated in CellGRO medium supplemented with 1% human serum with GM-CSF and IL-4 followed by maturation with TNF- α and prostaglandin E2 (and KLH as helper antigen). mDC were then transfected with GMP-grade mRNA encoding WT1 and given intradermally as a first vaccine. The remainder of the cells was frozen in aliquots for subsequent vaccinations. Flow cytometry confirmed dendritic cell viability and phenotype. WT1 tumor marker RNA levels in peripheral blood were monitored by quantitative RT-PCR for minimal residual disease (MRD) monitoring. We obtained successful vaccine production for all patients selected. DC injections were well tolerated and no serious adverse effects were observed. A vaccine-specific immune response was demonstrated in 5/5 patients evaluable by an *in vivo* DTH reaction both to KLH as well as to WT1. A drop in WT1 RNA levels was observed in 4/5 patients suggesting an anticancer effect, and one patient remains in complete remission although follow-up is relatively short (14 months). *In vivo* and *in vitro* analysis of WT1-specific T cell responses in pre- and post-vaccination T cell samples will be performed as follow-up of the vaccinated patients. We conclude that immunogene therapy with the described DC vaccine is feasible and safe, and that the vaccine can elicit anti-vaccine T-cell responses *in vivo* and a decrease in WT1 expression levels in MRD monitoring in all vaccinated patients.

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EX-VIVO EXPANSION AND PROPHYLACTIC INFUSION OF CMV-PP65 SPECIFIC T-LYMPHOCYTES FOLLOWING ALLOGENEIC HAEMOPOIETIC STEM CELL TRANSPLANTATION

K. P. Micklethwaite¹, A. Hansen², A. Foster¹, L. Clancy², E. Snape³, V. Antonenas², M. Sartor³, P. Shaw⁴, K. Bradstock³, D. Gottlieb^{1,2}; ¹Westmead Millennium Institute, Sydney, AUSTRALIA, ²Sydney Cellular Therapies Laboratory, Westmead Hospital, Sydney, AUSTRALIA, ³Blood and Marrow Transplant Service, Westmead Hospital, Sydney, AUSTRALIA, ⁴Blood and Marrow Transplant Service, Children's Hospital Westmead, Sydney, AUSTRALIA.

Cytomegalovirus (CMV) reactivation and infection post-allogeneic haemopoietic stem cell transplant (HSCT) continue to cause morbidity and mortality. Current pharmacological therapies are limited by side effects. Adoptive transfer of ex-vivo generated CMV-specific T-cells has the potential to restore immunity, prevent CMV and circumvent the need for pharmacological therapies. We have administered donor-derived CMV-specific cytotoxic T-cells to 11 mostly non-T-cell depleted HSCT recipients. These have been produced by one of two methods. Using dendritic cells (DC) pulsed with the HLA-A2 restricted nonapeptide NLVPMVATV (NLV) derived from the CMV-pp65 protein, we have generated T-cells which have been given prophylactically to 9 recipients aged 4 to 65 years on or after day 28 post-allogeneic HSCT. We have also generated CMV-specific T-cells for 4 recipients using DC transduced with an adenoviral vector encoding the entire pp65 protein. These have been given prophylactically to 2 HSCT recipients. Recipients were monitored for adverse reactions, CMV reactivation and immune reconstitution. There were no immediate adverse reactions to the infusions. During 97-798 days of follow-up of those receiving NLV-specific T-cells, 2 recipients developed cytomegalovirus reactivation; neither developed cytomegalovirus disease or required pharmacotherapy. Three recipients developed acute graft versus host disease (GVHD) after infusion. Two recipients died, 1 from thrombotic thrombocytopenia purpura secondary to cyclosporine, 1 from complications of graft GVHD. A transient increase in numbers of CMV-specific T-cells demonstrated by NLV-tetramer binding was seen in 6 recipients. The two patients who have received T-cells generated using the Adenovirus vector have had no adverse reactions, no CMV infection and no acute GVHD to date. One recipient had a positive qualitative CMV PCR 24 hours post infusion below the level of detection by qualitative PCR. This did not require therapy. Prophylactic adoptive transfer of CMV-specific T-cells is safe and may be effective in preventing CMV reactivation in allogeneic HSCT recipients.

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TARGETING LEWIS Y POSITIVE MULTIPLE MYELOMA WITH GENE-MODIFIED T CELLS

D. Honemann^{1,2}, P. Guru¹, J. A. Westwood¹, M. H. Kershaw¹, M. J. Smyth², J. A. Trapani², A. M. Scott³, F. E. Smyth³, G. A. Cartwright³, B. E. Power⁴, P. K. Darcy², S. Peinert^{1,2}, D. Westerman¹, P. Gambelli¹, M. H. Prince^{1,2}; ¹Peter MacCallum Cancer Centre, Melbourne, AUSTRALIA, ²Cancer Immunology Program, Peter MacCallum Cancer Centre, Melbourne, AUSTRALIA, ³Ludwig Institute for Cancer Research, Austin Hospital, Heidelberg, AUSTRALIA, ⁴Commonwealth Scientific and Industrial Research Organization Health Sciences and Nutrition, Parkville, AUSTRALIA.

Aim/Background: Haematological neoplasias including multiple myeloma (MM) are considered suitable targets for immunotherapy as evidenced by the success of allogeneic stem cell transplant and other immunotherapy approaches. Recently, adoptive T cell based immunotherapy with tumour infiltrating lymphocytes (TIL) or gene modified T cells has shown clinical activity in solid tumours. We have been examining the expression of the carbohydrate antigen Lewis^y (Le^y) on MM with the aim of testing its suitability as a target for T cell mediated immunotherapy with T cells expressing a chimeric anti-Le^y receptor. Methods/Results: We developed a construct of a chimeric T-cell receptor which recognises the Le^y antigen in an MHC-independent manner, activates T-cells and confers additional co-stimulatory signals. We have shown efficient retroviral transduction of this construct into human T-cells with a transduction efficacy of up to 65% in a GMP-conform protocol. Functional analysis of transduced T-cells showed specific interferon gamma secretion in response to a co-culture with Le^y target cells. Further, we demonstrated cytotoxicity of transduced T-cells against target cells with up to 89% specific lysis. Finally, *in-vivo* activity of gene-modified T cells was demonstrated in delaying tumour growth of Le^y pos. myeloma xenografts in NOD/Scid mice in three independent experiments. Mice receiving T cells transduced with the Le^y vector (Le) had significant advantages compared with mice receiving primary unmanipulated T cells (T) regarding disease free survival (DFS, 71% vs. 0%, day 56 exp. 1) as well as overall survival (OS, 80% vs. 0%, day 43 exp. 2). Conclusion: Le^y is present in a subset of MM with high expression levels in individual patients. Preclinical studies with transduced T-cells targeting Le^y are very promising. Consequently, we are soon to undertake a clinical phase I study using this transduction system for modification of autologous T-cells from patients with Le^y positive MM.

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PR1-SPECIFIC T CELL RESPONSES IN THE FIRST MONTHS FOLLOWING T-CELL DEPLETED ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) OCCUR IN BOTH MYELOID AND NON-MYELOID MALIGNANCIES BUT ARE ONLY ASSOCIATED WITH A GRAFT-VERSUS-LEUKEMIA (GVL) EFFECT IN MYELOID LEUKEMIAS.

K. Rezvani¹, A. Yong¹, S. Mielke¹, R. Eniafe¹, B. Savani¹, D. Price¹, D. Douek¹, J. Goldman², A. J. Barrett¹; ¹National Institutes of Health, Bethesda, MD, ²Imperial College School of Medicine, London, UNITED KINGDOM.

Lymphopenia-driven homeostasis early post-SCT allows exaggerated expansions of donor T-cells against antigens. Leukemia-associated-antigens including proteinase 3 (PR3) and elastase (ELA2) are self-antigens which induce low frequency autoreactive T-cells in normal individuals. PR1, an HLA-A*0201 epitope shared by PR3 and ELA2, is expressed in normal neutrophils and overexpressed in myeloid (but not lymphoid) leukemias. T-cells against PR1 have been linked to GVL. We looked for PR1-specific CD8+ T-cells in 28 patients (13 CML, 10 ALL, 5 solid tumors) in the first 30-120 days following T-cell depleted SCT, using PR1/HLA-A2 tetramers and intracellular interferon-gamma staining, and correlated these with ELA2 and PR3 expression (using qRT-PCR) and GVL. Ten CML, 6 ALL and 3 solid tumor patients had detectable PR1 responses post-SCT. PR3 and ELA2 expression was strongly associated with emergence of PR1-specific T-cells. Disappearance of PR3 and ELA2 expression coincided with disappearance of PR1-specific T-cells ($P < 0.001$). The *in-vivo* anti-leukemia effect of the PR1 response was assessed in CML patients by BCR-ABL qRT-PCR. Nine of ten patients with early PR1 responses were BCR-ABL negative at day 90 post-SCT compared to 0/3 without ($P < 0.001$). This GVL association was restricted to CML. In ALL, using WT1 qRT-PCR to measure minimal residual disease (MRD), 2/5 patients with PR1 responses and 3/5 patients without were MRD- on day 90 ($P = 0.36$). Since PR1 responses were not CML restricted and all patients had 100% donor myeloid chimerism by day 30, the recovering donor marrow is the likely antigenic source of PR3 and ELA2 driving the PR1 response. Our findings suggest that the post-SCT milieu is favorable for exaggerating weak autoimmune responses to self-antigens such as PR1. GVL may follow if the self-antigen is expressed on the leukemia as in CML. These results suggest that vaccination together with induction of T-cell homeostatic proliferation is likely to enhance anti-leukemia responses of transplantation.

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CELLULAR IMMUNOTHERAPY WITH ALLOREACTIVE NK CELLS AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION

L. Uharek¹, C. Gentilini¹, C. Loddenkemper¹, A. Muessig¹, T. Lange², N. Basara², C. Kliem², D. Niederwieser², E. Thiel¹; ¹Charité, Berlin, GERMANY, ²University Hospital, Leipzig, GERMANY.

NK cell alloreactivity can mediate strong graft versus leukemia effects following haploidentical hematopoietic stem cell transplantation. In an attempt to further improve the antileukemic effectiveness of this approach, we have adoptively transferred donor NK cells during the early phase after transplantation. Seventeen patients with very high risk or refractory haematological malignancies lacking an HLA identical donor received purified NK cells from their haploidentical donors at day +2 after hematopoietic stem cell transplantation. Conditioning consisted of TBI, thiotepa, fludarabine and OKT3. NK cells were isolated from the CD34⁻ fraction using an automated two-step procedure of CD3⁺ depletion and subsequent CD56⁺ selection. No severe technical problems occurred and a mean of 9.11×10^9 CD34⁻ cells was selected in high purity. A mean of 6.0×10^9 CD56⁺CD3⁻ NK cells was transferred. The mean number of contaminating CD3⁺ cells was 3.1×10^4 /kg. No severe acute toxicity attributable to NK cell infusion was observed. All patients developed early GvHD of the skin which promptly resolved after treatment with CSA and steroids. Nine of seventeen patients are alive and in CCR with a median follow up of 609 days. Only one patient relapsed. Our data show for the first time that the early adoptive transfer of high numbers of HLA-mismatched NK cells is safe and feasible. The low relapse rate in this very high risk patient population is encouraging and will justify to evaluate NK cell therapy in a larger patient cohort.

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AUTOMATED RAPID MICROBIAL DETECTION FOR AN AUTOLOGOUS CELL THERAPY: A TWELVE MONTH REVIEW OF STERILITY TESTING DATA

J. Duguid, S. Prinzi, G. Kielbinski, M. Aranedá, G. Galvez, T. Ostrovskaya, G. C. du Moulin; Genzyme, Cambridge, MA.

Introduction: Rapid detection of microbial contaminants is an essential component of quality control testing for cell therapy and tissue-engineered products with abbreviated shelf lives as short as 72 hours. Methods: After extensive validation, FDA approved the BacT/ALERT Microbial Detection System (bioMerieux, Durham, NC) as an alternate procedure to the compendial sterility test for Carticel® (autologous cultured chondrocytes). Between February 2005 and February 2006, we tested 5899 sterility samples using the newly validated and approved system. Results: We detected 5 positives having detection times ranging from 6.9 to 32.1 hours. Our investigation concluded that one of the 5 positive results was a false positive due to *Staphylococcus epidermidis*. We determined that the remaining 4 positive results were true positives; fluid waste containers contaminated with *Novosphingobium capsulatum* and *Comamonas testosteroni* were the source of the contamination. Rapid detection of product contaminants now allows us to detect and reject contaminated product lots during the transport of Carticel to the orthopedic surgeon. Based on our confidence in the ability of BacT/ALERT to rapidly identify positive sterility samples, we now require a minimum of 48 hours of sample incubation before surgical implantation of Carticel, which is sufficient to detect the majority of potential contaminants. Automation of microbial detection methods not only improved the detection technology, but also decreased material and labor costs significantly. We achieved the majority of our cost savings by reducing the number of disposable materials used in the test. The decreased labor also resulted in less sample handling, which dramatically improved the rate of false positive sterility tests. Our false positive rate decreased from 0.08% using compendial membrane filtration methodology to 0.01% after implementation of automation. Conclusion: Implementation of rapid microbiological testing systems plays an important role in improving the safety of cell therapy and tissue-engineered products.

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CONTINUOUS QUALITY IMPROVEMENT USING THE BALDRIGE MODEL FACILITATES FACT REACCREDITATION

J. Schriber¹, E. Simpson², G. Majkowski¹, S. Kendrick², S. Opie², C. Sarkodee-Adoo², A. Briggs², S. Forman³, J. Alvarnas²; ¹City of Hope Banner BMT Unit, Phoenix, AZ, ²City of Hope Banner BMT Unit, Phoenix, AZ, ³City of Hope, Duarte, CA.

FACT is the standard accreditation body used for transplant centers in the United States and increasingly in other countries. Centers must become reaccredited on a regular basis. Typically this process involves a reevaluation and reorganization of SOP's as well as involvement with multidisciplinary BMT staff to facilitate these changes. Since 2005, our program has used the Malcolm Baldrige model to facilitate ongoing continuous improvement. This model looks at the processes in 6 key areas: Leadership, Strategic Planning, Patients, Customers and Markets, Measurement Analysis and Knowledge Management, Human Resources, Process Management. Metrics are applied to ensure that the processes are meeting the desired goals. By having the BMT team "buy into" the Baldrige philosophy, we encourage ongoing refinements to improve the transplant experience for patients, staff and referring physicians. It also ensures that processes are continually examined by the very staff that utilize them on an ongoing basis, as they have the best opportunity to recognize Opportunities For Improvement (OFI's) that may exist within their spectrum of activity. Between our FACT inspections using this model we significantly modified 23 % of the 378 SOP's for our clinical, laboratory, apheresis, administrative and quality manual. Less than 1 percent of SOP's required significant changes in the 6 months prior to inspection. The Baldrige philosophy significantly shifted the time and effort needed for reaccreditation. Rather than most changes being performed in the months immediately prior to a reaccreditation this allows for ongoing and incremental changes when appropriate. Such changes may be implemented rapidly as new information is available. An additional benefit is improved staff satisfaction by allowing staff to define how better to improve their jobs.

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T REGULATORY CELLS RESPOND TO MESENCHYMAL CELLS REGULATION

M. Di Ianni, M. De Ioanni, B. Del Papa, D. Cecchini, L. Moretti, E. Bonifacio, P. Sportoletti, F. Falzetti, A. Tabilio; Hematology Section, Perugia, ITALY.

In the present study we investigated the effects of human mesenchymal cells (hMSC) on regulatory T cells expressing a memory (CD4/CD25/CD45RO) or naive (CD4/CD25/CD45RA) phenotype. T cells from healthy subjects were enriched by immunoselection to provide populations of CD45RA⁺ cells (95 % ± 2.9) and CD45RO⁺ cells (97 % ± 0.25). Naive and memory cells were cultured in presence of human mesenchymal cells (hMSC) (ratio 5:1). After 7 days' culture, in the naive population the T reg starting fraction of 0.05 % ± 0.01 of CD4/CD25 positive cells, rose to 0.2 % ± 0.14 in presence of MSC. In the memory population the T reg starting fraction of 0.3 % ± 0.05 of CD4/CD25 positive cells, rose to 1.5 % ± 0.9 in the presence of MSC. FoxP3 and CD127 expressions were measured by real time PCR in sort-purified subsets of peripheral blood, identified by staining with a combination of CD4, CD25, CD45RA or CD45RO. Sorted Tregs exert potent immunosuppressive activity on CD4⁺/CD25⁻ cell populations with a percentage of inhibition of 82±10 when CD4/CD25⁺ cells were used, of 96±6 when CD4/CD25⁺/CD45RA⁺ cells were used and of 93±4 when CD4/CD25⁺/CD45RO⁺ cells were used. After culture with MSC the inhibition was 90±4 when CD4/CD25⁺ cells were used, 85±6 when CD4/CD25⁺/CD45RA⁺ cells were used and 50±12 when CD4/CD25⁺/CD45RO⁺ cells were used. FoxP3 expression increased 5.38 fold in the presence of MSC in naive T reg and 7.98 fold in memory T reg. CD127 expression decreased 216 fold in the presence of MSC in naive T reg and 71.95 fold in memory T reg. Observing that naive and memory T regulatory cells respond to MSC regulation opens new perspectives for clinical use. Post transplant infusion of MSC and/or donor regulatory cells after co-culture with MSC might be a new option for GvHD treatment.

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MICROCHIMERIC FETAL CELLS CONTRIBUTE TO POSTREPRODUCTIVE MATERNAL TISSUE REPAIR IN MURINE AND HUMAN MODELS.

N. Fisk¹, K. O'Donoghue¹, F. Amin¹, H. Sultan¹, P. Hill¹, F. Allaf¹, J. Anderson², P. Guillot¹, G. Bou-Gharios¹; ¹Imperial College, London, UNITED KINGDOM, ²Hammersmith Hospitals Trust, London, UNITED KINGDOM.

Fetal cells trafficked during pregnancy persist for decades in maternal bone and bone marrow, but their biological role remains unclear. We investigated whether fetal microchimeric cells (FMC) participate in tissue repair after pregnancy. In humans, we collected tumour specimens, bone marrow and detailed reproductive histories from women undergoing thoracotomy. Y-FISH and immunostaining was performed on paraffin-embedded sections and cytopins. Male cells were found in lung and thymus (median 1 [1-43] male cells/section, 1:645 to 1:2000 cells) from all women with known male pregnancies, but not controls without sons. The majority of male cells were clustered in tumour tissue, compared to surrounding healthy tissue ($p < 0.0001$) and at sevenfold greater frequency than in bone marrow ($p < 0.0001$). To track functional FMC recruitment in mice, we monitored wild-type mothers with fetuses transgenic for luciferase/LacZ driven by collagen-type-I promoter FMC using bioluminescence after full-thickness skin biopsy, unilateral nephrectomy or unilateral ischaemia-reperfusion renal injury. After confirming bioluminescence in mice embryos, we detected luciferase activity *in vivo* in pregnancy in maternal excisional wounds one week after injury, which then diminished with healing to be undetectable postpartum. Fresh injury two weeks postpartum similarly resulted in fetal col1A2 activation compared to controls mated with non-transgenic males. Bioluminescence was detected in response to unilateral ischaemia-reperfusion or unilateral nephrectomy, but in the contralateral kidney as well as the injured side. FMC were preferentially located in tubular epithelium, and more frequent after serial than single pregnancies. In conclusion, fetal cells persist after pregnancy and are increased at sites of tissue-injury. Activation of the collagen type I promoter in response to maternal tissue injury both identifies the functional microchimeric cells as definitively fetal, and suggests a non-haemopoietic origin. We speculate that microchimeric fetal cells may be mesenchymal stem cells, either recruited from marrow or proliferating locally.

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HUMAN MULTIPOTENT MESENCHYMAL STROMAL CELLS INHIBIT PROLIFERATION OF PBMC IN THE ABSENCE OF FUNCTIONAL IFN R1 AND IDO EXPRESSION

F. Gieseke, S. Viebahn, R. Handgretinger, I. Müller; University Children's Hospital Tübingen, Tuebingen, GERMANY.

Human multipotent mesenchymal stromal cells (MSC) are known to inhibit proliferation and effector functions of T cells, B cells and NK cells. Here, the tryptophan degrading enzyme indoleamine 2,3-dioxygenase (IDO) has been shown to be involved. Although IDO is not expressed in MSC constitutively, it can strongly be induced by interferon- γ (IFN γ). Contradictory results have been published regarding the role of IFN γ -induced IDO expression in immunomodulation by MSC and it is still a matter of debate whether IDO is essential in this setting. Therefore, we carried out proliferation assays with PBMC stimulated by IL-2 and OKT-3 either in the presence or absence of HLA-mismatched MSC. The effect of MSC was similar in cultures with or without exogenous IFN γ . Addition of the IDO inhibitor 1-methyl-tryptophan to co-cultures of PBMC and MSC gave variable results and did not restore proliferation completely in each experiment. These results suggested that expression of IDO in MSC was not solely responsible for the inhibition of PBMC. Subsequently, we isolated MSC from bone marrow of a child with a mutation in the subunit I of the IFN γ receptor (IFN γ R1) leading to a non-functional IFN γ receptor. MSC^{IFN γ R1^{-/-}} behaved normal in terms of proliferation and plasticity. Interestingly, MSC^{IFN γ R1^{-/-}} were also able to suppress the proliferation of PBMC. This showed that MSC inhibit PBMC independent of functional IFN γ R1. In MSC^{IFN γ R1^{-/-}}, IDO was neither expressed constitutively nor was it induced by incubation with IFN γ as assessed by RT-PCR. cDNA array analysis of MSC revealed distinct expression patterns affected by IFN γ . Based on these transcriptional data, functional assays were performed for HLA-G and other candidate molecules, which were found to contribute to MSC-mediated inhibition of PBMC proliferation. Taken together, these findings demonstrate that neither IFN γ R1-mediated effects of IFN γ nor IDO are essential for MSC to inhibit PBMC proliferation.

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REGULATION OF MHC CLASS II EXPRESSION AND ANTIGEN PROCESSING IN MURINE AND HUMAN MESENCHYMAL STROMAL CELLS BY IFN- γ , TGF- β AND CELL DENSITY.

J. Galipeau, R. Romieu-Mourez, M. François, M. Boivin, J. Stagg; McGill University, Montreal, PQ, CANADA.

Mesenchymal stromal cells (MSC) possess immunosuppressive properties, yet when treated with IFN- γ they acquire antigen presenting cell (APC) functions. To gain insight on MSC immune plasticity, we explored signalling pathways induced by IFN- γ required for MHC class II (MHC II)-dependent antigen presentation. IFN- γ -induced MHC II expression in mouse MSC was enhanced by high cell density or serum deprivation and suppressed by TGF- β . This process was regulated by the activity of the type IV class II transactivator (CIITA) promoter independently of STAT1 activation and the induction of the IRF-1-dependent *B7H1/CD137* encoding gene. The absence of direct correlation with the cell cycle suggested that cellular connectivity modulates IFN- γ responsiveness for MHC II expression in mouse MSC. Accordingly, an inhibitor of gap junction-mediated intercellular communications, significantly blocked IFN- γ -induced MHC II but not B7-H1 expression in mouse MSC. TGF- β signalling in mouse MSC involved ALK5 and ALK1 TGF- β RI, leading to the phosphorylation of Smad2/Smad3 and Smad1/Smad5/Smad8. An opposite effect was observed in human MSC where IFN- γ -induced MHC II expression occurred at highest levels in low density cultures; however, TGF- β reduced IFN- γ -induced MHC II expression and its signalling was similar as in mouse MSC. This suggests that the IFN- γ -induced APC features of MSC can be modulated by TGF- β , serum factors and cell density *in vitro*, although not in the same way in mouse and human MSC, via their convergent effects on CIITA expression.

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OSTEOGENESIS IMPERFECTA PHENOTYPE RESCUED BY INTRAUTERINE TRANSPLANTATION OF HUMAN FIRST-TRIMESTER FETAL MESENCHYMAL STEM CELLS.

P. V. Guillot, O. Abass, C. Chapon, D. Bassett, S. Shefelbine, G. Bou-Gharios, J. Chan, H. Kurata, K. Bakhoo, G. Williams, J. Polak, N. Fisk; Imperial College London, London, UNITED KINGDOM.

Genetic diseases with prenatal onset could be treated by intrauterine stem cell therapy. Osteogenesis imperfecta mice (oim) have a mutation in the *pro α 2(I)* gene, resulting in non-functional α 2(I) chains with accumulation of abnormal α 1(I)3 collagen, leading to fractures and abnormal bone. We treated oim mice by intrauterine transplantation (IUT) of first trimester human fetal mesenchymal stem cells (MSC) to investigate donor cell ability to engraft and repair/prevent bone damage. IUT of fetal MSC resulted in a two-thirds reduction in fractured humeri, ulna, tibia and femur (i.e. 28.6% by 4 weeks, 34.2% 8 weeks, 30.7% 12 weeks (all $P < 0.01$)), along with improved bone strength in 3-point bending tests (at 8 weeks, increased tibial structural yield force (62.5%), ultimate force (71.5%), ultimate stress (46.7%) and stiffness (54.3%, all $P < 0.01$)) and reduced tibial bowing ($P < 0.01$). Bone structural properties were improved, i.e. femoral cortical thickness increased 26-98% in 4-12 week-old oim ($P < 0.05$); and tibial bowing decreased 22% ($P < 0.05$). The length of ulna, tibia, and femur bones was similarly increased. Tibial growth plate height was decreased in 4 (10.4%), 8 (24.9%) and 12 (24.2%, all $P < 0.001$) week transplanted mice in the direction of wild type. Engrafted donor cells were identified over 12 weeks in haemopoietic (liver, spleen, blood, bone marrow) and non-haemopoietic organs (lung, heart, brain, kidneys, bones, thymus), with chimerism higher in bone compared to other organs ($P < 0.001$). In bones donor cells were clustered in areas of bone formation and at sites of healing. In kidney, homotrimer accumulation was reduced by a third in 4-12 week old oim mice ($P < 0.01$). Finally, hydroxyproline content was lowered ($P < 0.001$), and the missing *col1a2* protein detected. IUT of fetal MSC contributes to bone formation and reduces bone pathology in OI, providing a scientific basis for MSC treatment in affected humans.

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DERIVATION AND IMMUNOLOGICAL CHARACTERIZATION OF MSCS FROM HUMAN ESCSP. Trivedi, P. Hematti; *University of Wisconsin-Madison, Madison, WI.*

Human ESCs could potentially provide an unlimited number of different types of clinically applicable cells if we can develop methodologies to efficiently derive safe and functional cells from undifferentiated human ESCs, and strategies to overcome the immunological rejection of the ESC-derived cells by the host. We have devised a novel and reproducible methodology to derive pure populations of mesenchymal stromal cells (MSCs) from human ESC lines H1, H7 and H9. We start with culturing undifferentiated human ESCs on matrigel plates and through a multi-step process of about a 4-weeks period we are able to derive a pure population of cells that have uniformly fibroblast/mesenchymal looking morphology. These cells express a pattern of cell surface marker antigens similar to what has been reported for adult human MSCs: they are positive for CD29, CD44, CD54, CD71, CD90, CD105, and are negative for hematopoietic markers such as CD34 and CD45. Using established differentiation protocols we could differentiate these human ESC-derived MSCs into adipocytes, osteocytes, and chondrocytes as verified by immunohistochemistry and RT-PCR assays. Most importantly, these cells do not express markers of undifferentiated human ESCs such as SSEA-4. We also investigated the immunological properties of these ESC-derived MSCs and compared them to bone marrow (BM) derived MSCs. These cells express HLA class-I antigens but not class-II antigens; however, the level of expression of HLA class-II antigens increased after treatment with interferon-gamma. Furthermore, when we used ESC-derived MSCs in one way mixed lymphocyte culture reactions as stimulator cells they did not induce proliferation of responder peripheral blood lymphocytes that were labeled with CFSE. MSCs derived from BM have shown encouraging results in various clinical trials. We propose that ESC-derived MSCs could provide a universal source of MSCs for regenerative medicine and potentially enhancing the engraftment of other cells/tissues derived from the same ESC lines.

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MESENCHYMAL STEM CELLS ADMINISTERED TO THE CNS OF RHESUS MACAQUES EXHIBIT ENHANCED ENGRAFTMENT BUT A SIMILAR ANATOMICAL DISTRIBUTION IN NEONATAL VS. ADULT RECIPIENTS.D. G. Phinney¹, I. A. Isakova¹, K. C. Baker², J. Dufour²; ¹*Tulane University Health Sciences Center, New Orleans, LA*, ²*Tulane National Primate Research Center, Covington, LA.*

To evaluate their potential as vectors to treat neurological disorders, we injected mesenchymal stem cells (MSCs) from a male rhesus macaque into the caudate nucleus of eight female, infant macaques and correlated engraftment levels in brain with effects on animal health, development, and behavior. Real-time PCR was used to estimate engraftment levels of MSCs and map their anatomical location in brain. This analysis revealed that a significantly greater percentage of injected MSCs survived within the brains of low dose ($5.05 \pm 3.0\%$) vs. high dose ($0.17 \pm 0.12\%$) recipients at 6 months post-transplant. Fine mapping studies further demonstrated that transplanted cells preferentially engrafted within specific anatomical structures including the somatosensory cortex, primary motor cortex, caudate nucleus, putamen, thalamus, hippocampus and cerebellum. Moreover, when these data were compared to that obtained previously for young adult macaques, MSC engraftment levels were found to be on average 17.8-fold higher ($p < 0.05$) with a maximal observed difference of 180-fold in neonatal vs. adult recipients. Nevertheless, no long-term adverse effects of MSC engraftment on animal health, development or behavior were detected after extensive monitoring. Specifically, no evidence of an immune response against the allogeneic donor MSCs was detected in mixed lymphocyte reactions. Furthermore, behavioral tests revealed no effect of MSC engraftment on the social and behavioral state ($p > 0.01$) of infants at either time point post-surgery and neuro-behavioral assessments confirmed that the cognitive and motor abilities of all transplant recipients were comparable to an age-matched sample of normal infants ($N=20$). Collectively, these data indicate that MSCs may be effective cellular vectors to treat neurological disorders due to their low immunogenicity, enhanced engraftment and dynamic distribution in the neonatal brain. Moreover, our data demonstrate that MSC administration is safe and produces no adverse effects on neurological development and animal behavior.

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CONTROLLING THE GRAFT VERSUS HOST RESPONSE - ARE MESENCHYMAL STEM CELLS WHAT WE ARE LOOKING FOR?C. R. Kleiveland, G. Kvalheim, M. Wang, M. S. Dyrhaug, T. Lea; *Rikshospitalet-Radiumhospitalet HF, Oslo, Oslo, NORWAY.*

To delineate the mechanism behind the immunosuppressive principle of MSC we initially investigated the impact of titrated amounts of bone marrow-derived human MSCs on co-culture systems with T-cells activated with polyclonal activators like PHA, Con A, solid phase-coupled anti-CD3/CD28 antibodies as well as mixed leukocyte cultures (MLCs). To this end, the inhibitory effect of hMSCs on T-cell proliferation was variable, ranging from negligible to a moderate inhibition. Furthermore, the inhibitory effect was still detectable when replacing hMSCs with culture supernatants from hMSCs, suggesting that the inhibitory principle was mainly soluble. We further studied the effect of hMSC culture supernatants on cytokine production from T-cells activated by solid phase-coupled anti-CD3/CD28 antibodies. A quantitative 25-plex fluorimunoassay revealed that the T-cells, upon co-culture with hMSC supernatants, produced less IL-2 and IFN- γ but increased the production of IL-4, IL-5, IL-13, IL-15 and IL-17. Thus, there was a shift in cytokine production profile, from a TH1 to a TH2 type of response. The change in cytokine profile became more prominent when employing supernatants from hMSCs that had been stimulated with TNF- α , an effect that was accompanied by an increase in prostaglandin E2 (PGE2) by the hMSCs. Thus, our data point to PGE2 as an important immunoregulatory component in these systems. The same studies also indicated a minor increase in TGF- β production, and flow cytometric analysis of T-cells expanded in hMSC-derived culture supernatants revealed a slight increase in FoxP3-positive cells. In conclusion, we find that molecular interplay between hMSCs and activated T-cells *in vitro* creates an environment that enhances PGE2-production, inhibits IL-2 production and shifts the T-cell cytokine profile in an anti-inflammatory direction. Additionally, the enhancement in TGF- β production and the increased frequency of FoxP3-positive regulatory T-cells suggest that several factors together could be responsible for hMSC-mediated immunosuppression. Separately, each of the observed effects was moderate

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MULTISTEM® (MULTIPOTENT ADULT PROGENITOR CELLS) USED IN TREATMENT OF PIGS WITH ACUTE MYOCARDIAL INFARCTIONA. Ting¹, L. Zheng², S. Medicetty¹, N. Lehman¹, X. Wang², M. Jameel², A. Mansoor², Q. Li², C. Panetta², C. Swingen², J. Zhang², R. Deans¹; ¹*Athersys, Inc., Cleveland, OH*, ²*University of Minnesota, Minneapolis, MN.*

MultiStem® are adherent adult bone marrow derived stem cells capable of differentiating into cells from multiple germ lineages. Previously, MultiStem® demonstrated benefit in a rodent model of acute myocardial infarction (AMI). Here, we tested the ability of MultiStem® to treat AMI in porcine models. Bone marrow derived adherent cells were obtained from Yorkshire pigs and transfected with beta-galactosidase (b-Gal). Myocardial infarction was induced in Yorkshire pigs by direct LAD ligation or occlusion. In the permanent LAD ligation model, the pigs (with or without cyclosporine) received a direct injection of saline or 50 million allogeneic MultiStem® in the peri-infarct zone. After 4 weeks, ejection fraction was significantly improved in animals that received stem cells, with or without cyclosporine, compared to those that received saline (41% and 42% vs 30%, $p < 0.05$). Furthermore, there was significant improvement in the bioenergetics of the peri-scar area and evidence of neovascularization in the cell-treated animals compared to the control animals. In the transient (60 minute) LAD occlusion model, the pigs received allogeneic MultiStem® (50 million/ml) or saline through a transarterial catheter. After 4 weeks post-MI and cell infusion, significant improvement in ejection fraction was observed in animals that received MultiStem® (46 vs 35 %, $p < 0.05$). In a separate experiment, the AMI model pigs that were injected with MultiStem® through a transarterial catheter showed extensive b-Gal positive cell distribution in the scar and periscar areas. The total cell retention at 2 and 8 weeks post-MI in all the pig studies (direct injection or transarterial) was 0.3-1%. Administration of cells using either catheter platform was safe, with no evidence of arrhythmia or interference with reperfusion. These data demonstrate that allogeneic MultiStem® improve the loss of cardiac function that occurs post-MI.