# The 2013 Stem Cells Discussion Forum: Working Towards Clinical Application

Thursday, 06 June 2013

The Royal College of Pathologists, Carlton House Terrace, London, SW1Y 5AF, United Kingdom

This event is discussion forum, focused on clinical applications of stem cell therapy. The aim is to offer participants a chance to explore aspects with the experts during round table and one-to one discussions.

Meeting Chair: Dr Glyn Stacey, UK Stem Cell Bank, Division of Cell Biology and Imaging.
This event has CPD accreditation and is part of the 2013 Euroscicon Stem Cell Trilogy.
To find out more see www.stemcells2013.com

9:00 – 9:45 Registration

9:45 – 10:00 **Introduction by the Chair**: *Dr Glyn Stacey,* UK Stem Cell Bank, Div of Cell Biology and Imaging, NIBSC, MHPRA.

10:00 – 10:30 **First-in-Man trials of Stem Cells as Medicines – from single cells to 3-D constructs**
*Dr Mark Lowdell,* University College London, UK

10:30 – 11:00 **Biologic Augmentation of Rotator Cuff Repairs**
*Dr. James Hoffman*, Coordinated Health, USA
Recurrent rotator cuff tears are a well documented problem. Following surgical correction, healing of the rotator cuff is impeded by muscle atrophy, fat infiltration, devascularization, and scar tissue formation. The structural integrity of the tendon is weakened, increasing the susceptibility to retear. Consequently, biologic adjuvants and mechanical reinforcements have been suggested as a means of recreating a biomechanically equivalent layer of connective tissue. Within, we present our initial clinical and histological findings evaluating the efficacy of a rotator cuff repair performed with platelet-rich plasma, a dermal allograft, and mesenchymal stem cells.

11:00 – 11:30 **Generating hESCs for Clinical Application: The Challenges for Academics.**
*Dr Zoe Hewitt,* Centre for Stem Cell Biology, The University of Sheffield
As momentum in the field of human embryonic stem cell (hESC) based therapies grows, researchers face the challenge of developing raw materials from which these therapies can be developed which are suitable for clinical application. Consequently, there has been significant emphasis placed on deriving new hESC lines using good manufacturing practices (GMPs) to provide standard mechanisms for assessing reliability and reproducibility of these raw materials. If GMP standards are to be met, the derivation process, which involves a range of procedures and mechanisms some of which are not yet fully understood, must be validated and implemented in a framework of strict quality management.

11:30 – 12:00 **Speakers' photo then mid-morning break, poster sessions and trade show**
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12:00 – 12:30 **A risk based, unit operation approach to manufacturing process science for cell based therapies**
*Dr Robert Thomas,* Loughborough University, UK
The EPSRC Centre for Innovative Manufacturing in Regenerative Medicine is developing manufacturing science for industrial production of advanced therapies. Key goals are reduction of risk and cost in translating development therapies to commercially viable products. Important steps include early process analysis, consideration of constituent units of operation, and risk assessment in the context of industrial operating restrictions and logistics. Key challenges such as identifying units of operation, required measurement system performance and frequency for process control, and application of engineering design for optimization, risk reduction, and decision making, will be discussed with examples from both academic and commercial development programs.

12:30 – 13:00 **Stem cell treatment for multiple sclerosis**
*Professor Neil Scolding,* Burden Professor of Clinical Neurosciences, University of Bristol, UK
Multiple sclerosis (MS) is a major cause of disability, particularly affecting young adults, in which patches of tissue damage occur throughout the brain and spinal cord. Recent advances in our understanding of how MS progresses have radically altered the way we think about cell therapy for this disease. The many and varied reparative properties of bone marrow derived stem cells may offer new and attractive possibilities for developing cell-based treatments for MS, with significant and positive implications also for other common neurodegenerative conditions.

13:00 – 14:00 **Lunch, poster sessions and trade show** *Please try to visit all the exhibition stands during your day at this event. Not only do our sponsors enable Euroscicon to keep the registration fees competitive, but they are also here specifically to talk to you*

**Oral Presentations**

14:00 – 14:15 **HUMAN FETAL DOPAMINERGIC PRECURSOR CELL TRANSPLANTATION: LIF-NANOTHERAPY TO PROMOTE (i) IMMUNE TOLERANCE, AND (ii) GRAFT SURVIVAL.**
Metcalfe Su M. *Brain Repair Centre, Addenbrooke's Hospital, Cambridge, CB2 0PQ, UK.*

14:15 – 14:30 **IMMUNOLOGICAL EFFECTS OF MESENCHYMAL STROMAL CELLS ON T CELL LINES FROM CROHN'S DISEASE PATIENTS**
R. Ciccocioppo, *Department of Internal Medicine, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Piazzale Golgi, 19; 27100 Pavia; Italy*

14:30 – 16:00 **Round Table Discussions**
Table 1: Mark Lowdell
Table 2: Professor Neil Scolding
Table 3: Robert Thomas
Table 4: Zoe Hewitt
Table 5: James Hoffman

16: 00 - 16:30 **Question and Answer Session**
Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day

16:30 – 17:00 Chairman's summing up

About the Chair

***Glyn Stacey,*** Head of the Division of Cell Biology and Imaging at NIBSC and Director for the UK Stem Cell Bank.
His scientific background has been in microbiology and cancer research. From 1989-1998 he worked at Porton Down, UK, where he developed cell banking procedures and the development of cells for manufacture of medical products and cell-based diagnostic assays. At NIBSC he has developed a broad remit relating to the quality and safety of new biological medicines and therapies based on the use of human and animal cells. He has also acted as an advisor to the UK Department of Health and the World Health Organization. He coordinates the International Stem Cell Banking Initiative funded by a consortium of funding agencies from 20 countries. He has recently overseen the establishment of a new and expanded GMP facility for banking stem cell lines. He has published numerous scientific papers and books on cell banking and quality control.

About the Speakers

**Neil Scolding** trained in neurology in Cardiff, Cambridge and London (National Hospital for Neurological Diseases), before being appointed foundation Burden Professor of Clinical Neurosciences at the University of Bristol and Frenchay Hospital. His main clinical research interests lie in multiple sclerosis and other inflammatory neurological diseases, and the biology and clinical development of stem cell therapies for MS and other neurodegenerative conditions. He has published four textbooks and some 150 research papers; is currently a Guarantor of the journal Brain; and is a member of the editorial board of various other journals, of the national bioethics committee ESBAC, and of the Association of British Neurologists Council.

Following **Zoe Hewitt** 's PhD at the Roslin Insitute in Edinburgh, where she studied the "elimination of undifferentiated human embryonic stem cells in vitro" as a method to address the associated risk of tumorgenicity from the accidental transplantation of undifferentiated hESCs within a therapeutic graft, Zoe moved to the Stem Cell Derivation Facility (SCDF) at the Centre for Stem Cell Biology, University of Sheffield to take up a role as Quality Manager. Since 2006 she has been responsible for commissioning and managing a Clean Room Facility for the production of clincal grade human embryonic stem cells with a goal of making these available for use in future therapeutics.

**Rob Thomas** is a Senior Lecturer and EPSRC Bio-manufacturing Fellowship holder at Loughborough University. He is a leading member of the bio-manufacturing research team working to develop the process science and the manufacturing capability for cell based regenerative medicine therapies, with a particular focus on haematopoietic lineage based processes. He is currently associate director of the EPSRC Centre for Innovative Manufacturing in Regenerative Medicine led from the Centre for Biological Engineering at Loughborough University.

Keywords: Clinical Trial Partnerships, stem cells, banking,manufacturing,scale-up,GMP, regenerative,IPS, ATMPs, somatic, stem cells, constructs, human embryonic stem cells, clinical grade, cGMP, macular degeneration, retinal pigment epithelium, RPE, AMD, multiple sclerosis, neurodegeneration,cell therapy, clinician-scientist, stem cell research, translation, career path, Stem Cells, Embryonic, clinical application, Manufacture, Cell Therapies, Process

**POSTER PRESENTATIONS**

## STUDY ON CLINICAL EFFICACY OF AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANAPLANTATION WITH ADVANCED MALIGNANT SOLID TUMOR IN CHILDREN.

Zhang Yi, Zhang Wei Ling, Huang Dong Sheng, Wang Yi Zhuo, Hong Liang, Zhu Xia,
Liu Ai Ping, Zhi Tian, Hu Hui Min
Department of pediatrics, Beijing Tongren Hospital, Capital Medical University.

**Objection:** Autologous Peripheral Blood Stem Cell Transplantation (APBSCT) is a treatment method of solid tumor in children. Our study objection is to assessment the value of high-dose Chemotherapy with APBSCT for malignant solid tumors in children. We analysised the clinical effect of the cases with advanced malignant solid tumors in children of Department of Pediatrics in our hospital on 2005.9-2011.11. **Method**: 38 cases of malignant advanced solid tumor, including 25 cases of neuroblastma (NB), 2 case of Primitive neuroectodermal tumor (PNET), 4 cases of Rhabdomyosarcoma (RMS), 1 case of Hepatoblastoma（HP）, 5 cases of retinoblastoma (RB), 1 case of Wilms tumor and 1 case of Malignant schwannoma. 14 cases were complete remission before APBSCT, and 14 cases were partly remission before APBSCT, and 10 cases were advanced stage before APBSCT. 27 Males and 11 Females. Average age of 38 cases is 6.3±3.4 a (1a 1m-14 a). The Mobilization programs in all cases were Conventional chemotherapy. Average MNC counts is 5.7±2.4*108/kg, and average CD34 counts is 3.8±3.3) *106/kg. The Pretreatment chemotherapy of 33 cases were CEM, In addition to the five cases of RB .The Pretreatment chemotherapy of 5 cases of RB were Cyclophosphamide ( CTX) and Carboplatin (CBP) and Etoposide (E). 2 case in advanced stage were treated by radiotherapy before high-dose chemotherapy.
**Result:** Medium Bone marrow reconstitution time （MBMRT） is 16.8±7.53 days( 11-37days). Medium Bone marrow reconstitution time of NB is 13.5 days, and MBMRT of PNET is 13.5 days, and MBMRT of RB is 18 days, and MBMRT of RMS is 12 days, and MBMRT of HP is 26 days, and MBMRT of Wilms tumor is 14 days, and MBMRT of malignant schwannoma is 11 days. Follow-up to the November 2012, 35 cases were followed. Medium follow-up time of 35 cases is 57 months (11-96m), 3 case with progressive were dead within +30 days after APBSCT, because of Multiple organ failure. Survival rate of 1 year is 65.7%, survival rate of 2 years is 40%. The total survival rate is 28.5%(10/35).
**Conclusion**: Clinical efficacy with high-dose chemotherapy and APBSCT in progressive cases was poor. However, high-dose chemotherapy and APBSCT with progressive cases of malignant solid tumor in chidren may prolong the survival time to some extent. Such, the clinical value of high-dose chemotherapy and APBSCT with malignant advanced solid tumor in children is confirmed and application value of it is sure.
Corresponding author: Huang Dong Sheng. Hds5180@sina.com
First author: Zhang Yi, Zhang Wei Ling.

## CLINICAL STUDIES OF TREATMENT OF CHILDREN HEAD AND NECK RHABDOMYSARCOMA

Zhang Weiling[1]; Zhang Yi[1]; Huang Dongsheng[1]*; Guo Fang[1]; Han Tao[1]; Hong Liang[1]; Hu Huimin[1]; Zhi Tian[1]
Department of Pediatrics, Tong Ren Hospital, Capital Medical University, Beijing 100176, China.
***Corresponding author**: Huang Dongsheng, M.D.

**Objective:** Rhabdomysarcoma (RMS), which frequently occurred in head and neck, is the most common soft tissue carcinoma in children and is highly malignant. The study aims to summarize the clinical data and evaluate the outcome of pediatric Rhabdomysarcoma in head and neck.
**Method:** Forty one (24 males, 17 female) children with newly diagnosed Rhabdomysarcoma in Bei Jing Tongren Hospital were enrolled between Nov. 2004 and May. 2011. The clinical data of patients with RMS was retrospectively reviewed and analyzed with Students' t and chis ($\chi^2$) test . The survival analysis was calculated based on the Kaplan Meier method by using SPSS 17.0 sofeware.**Results:** Of the 41 cases, 3 cases with stage III RMS received auto peripheral blood stem cell transplantation, in which 2 achieved complete remission (CR); 1 recurred after 6 months and died 1 year later. Eight cases were treated with $^{125}$I seeds Interstitial Implantation, in which 5 cases achieved CR, 2 cases achieved partial remission and 1 case died. The median follow-up time of 37 cases reviewed was 41 months, In total, 7 cases were partly remission and survival, 5 cases died for cerebral metastasis, the total survival rate was 86.48% (32/37), CR rate was 67.56% (25/37), PR rate was 18.91% (7/35).
**Conclusion:** Multidiscipline treatment including chemotherapy, radiotherapy, surgery and Auto-PBSCT for RMS is highly recommended.

## ANALYSIS ON MULTIMODALITY TREATMENT EFFECT OF ADVANCED PAEDIATRIC HEPATOBLASTOMA

Yi Zhang[1], Weiling Zhang[1], Dongsheng Huang[1*], Liang Hong[1], Yizhuo Wang[1], Xia Zhu[1], Huimin Hu[1], Pinwei Zhang[1], You Yi, Tao Han[1]
[1]Department of pediatrics , Beijing Tongren Hospital, Capital Medical University, Beijing, 100176, P.R.China.
*Corresponding author: Dongsheng Huang, professor, Department of pediatrics of Beijing Tongren Hospital, Capital Medical UniversityBeijing, 100176, P.R.China.

**Objective:** To investigate the effect of multimodality treatment of advanced paediatric hepatoblastoma and the factors affecting the prognosis. **Methods:** 35 childhood patients were treated with multimodality treatments consisting of chemotherapy, surgery, interventional therapy, and autologous peripheral blood stem cell transplantation and followed up every month. **Results:** 33 patients completed the follow-up, of which 17 were in complete remission, 5 were in partial remission, 1 got worse, and 10 died. The remission rate was 66.7% (22/33), and the overall survival rate was 69.7% (23/33). Patients with the mixed phenotype of hepatoblastoma had a worse prognosis than with the epithelial phenotype (P<0.001), and patients in stage IV had a lower survival rate than in stage III (P<0.001). **Conclusion:** Multimodality treatment can effectively improve remission rate and prolong the survival of children with the advanced hepatoblastoma. In addition, AFP, hepatoblastoma pathological classification and staging are of great use in prediction of prognosis.

## HUMAN FETAL DOPAMINERGIC PRECURSOR CELL TRANSPLANTATION: LIF-NANOTHERAPY TO PROMOTE (i) IMMUNE TOLERANCE, AND (ii) GRAFT SURVIVAL.

Metcalfe Su M.[1], Fahmy TM[2], Barker RA[1], Tyers P, He X, Strom TB[3], Zhao JW[1]
*[1]University of Cambridge, Brain Repair Centre, Addenbrooke's Hospital, Cambridge, CB2 0PQ, UK.*
*corresponding author email: smm1001@cam.ac.uk, [2]Yale University, Biomedical Engineering, New Haven, CT 06511 US., [3]Harvard University, BIDMC, Transplant Institute, 330 Brookline Avenue, Boston, MA 02215, USA*

Within the immune system there is an exquisite ability to discriminate between "self " and "non-self" that is orchestrated by antigen-specific T lymphocytes. Genomic plasticity enables differentiation of naïve CD4+ T lymphocytes into either regulatory cells (Treg) that express the transcription factor Foxp3 and actively prevent auto-immune self destruction, or effector cells (Teff) that coordinate attack upon their cognate target.

An example of such plasticity is our recent discovery that a major stem cell growth factor "LIF" (leukemia inhibitory factor) supports Treg maturation. In marked contrast, the closely related cytokine interleukin-6 (IL-6) drives development of the inflammatory, and potentially pathogenic, Th17 effector phenotype [1, 2]. This has revealed a LIF/IL-6 axis in T cell development and – *in vivo* - we found this axis to be sensitive to altered levels of LIF, or IL-6, using antibody or cytokine-trap to neutralize the respective cytokine. We went on to ask, can the LIF/IL-6 axis be exploited for therapy?

Since *in vivo* use of soluble recombinant LIF is hindered by its rapid degradation and excretion, we prepared a nano-particulate formulation of LIF intermixed with PGLA. This biodegradable, biocompatible LIF-nano device showed paracrine-type release of bioactive LIF into the local microenvironment over a period of several days. LIF-nano were decorated with avidin allowing attachment of biotinylated anti-CD4 for targeting of CD4+ T cells where LIF-nano treatment biased CD4+ cell responses towards Treg maturation both *in vitro* and *in vivo*. In contrast, IL-6 loaded nanoparticles directed CD4+ T cells towards Th17 development [3].

Given LIF's role in supporting stem cells, we next asked, do LIF-nano promote stem and precursor cells of the neural lineage? If so, then LIF-nano may provide dual therapeutic benefits for cell transplantation in Parkinson's Disease (PD). The TransEuro Project (http://www.transeuro.org.uk) is currently evaluating grafts of human fetal ventral mesencephalon (hfVM, rich in precursor dopaminergic cells) in treatment of PD. Working in parallel, we have shown that LIF-nano-treated hfVM yield some 3-fold more dopaminergic cells cp empty-nano controls. This has major implications for clinical transplantation, including reduced numbers of donors required to treat an individual patient. *In vivo* intra-striatal grafts in nude rats show successful integration of hfVM grafts plus dopaminergic cell maturation. Current experiments extend this finding to LIF-nano-treated hfVM grafts.

In summary, the value of LIF as a clinical therapeutic can be harnessed and specifically targeted using the nano-particulate PGLA formulation. Anticipated benefits include promotion of the donor-specific Treg lineage within the micro-environment of a graft, in addition to promotion and integration of the graft itself within the host tissue.

REFERENCES

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## IMMUNOLOGICAL EFFECTS OF MESENCHYMAL STROMAL CELLS ON T CELL LINES FROM CROHN'S DISEASE PATIENTS

R. CICCOCIOPPO, G.C. CANGEMI, E. BETTI, A. GALLIA, G.R. CORAZZA
*Department of Internal Medicine, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Piazzale Golgi, 19; 27100 Pavia; Italy*

**BACKGROUND.** Mesenchymal stromal cells (MSC) are multipotent non-haematopoietic stem cells capable of differentiating into several cell types and promoting tissue repair. Recently, they have also been found to exert potent immunological effects by acting non-selectively on almost all cells involved in the immune response. Among them, the effect on T lymphocytes is the most studied. It has been established that MSC can inhibit T-cell proliferative response induced by dendritic cells, alloantigens and mitogens, while inducing a modification of proliferative rate and immunophenotype. MSC represent, therefore, an interesting novel source for cellular therapy in those conditions caused by a dysregulated T-cell response, including Crohn's disease (CD), a chronic inflammatory bowel disease, due to the loss of immune tolerance towards components of the intestinal microbiota which occurs in genetically susceptible individuals. Specifically, an abnormal T-cell response to muramyl dipeptide (MDP), a peptidoglycan constituent of the bacterial wall, seems to play a crucial role in orchestrating the inflammatory cascade that leads to intestinal injury.
**AIM.** In a previous phase I-II study by our group, we demonstrated the feasibility, safety and efficacy of local injections of autologous bone marrow-derived MSC in the treatment of refractory fistulising CD.[1] Here, we aimed to investigate the *in vitro* effects of MSC on mucosal T cells in terms of mortality, proliferation, immunophenotype and cytokine production upon specific antigen stimulation.
**PATIENTS AND METHODS.** Mononuclear cells were isolated from bone marrow blood samples of 3 adult CD patients, plated and expanded *ex vivo* until passage 3, when the adherent population underwent morphological and immunophenotypical characterization which confirmed an almost pure population of MSC. For the generation of T cell lines, mononuclear cells were isolated by enzyme digestion of perendoscopic colonic biopsies from both inflamed and non-inflamed areas of 6 patients suffering from CD (F/M 4/2, mean age: 32 years, range 8-56) and healthy mucosa of 4 control subjects (F/M 1/3, mean age: 43 years, range 27-52) who underwent endoscopy for polyp screening. The cells were then expanded *in vitro* through weekly cycles of antigen stimulation by MDP (10 μg/ml; Sigma-Aldrich) and cytokine stimulation with interleukin (IL)-2 (40 U/ml; Chiron). For each experiment, parallel cultures were settled in the absence or presence of allogeneic MSC at two different MSC:T ratios (1:20, 1:200), as well as in the absence or presence of MDP. The effects of MSC on antigen-reactive T-cell lines were evaluated in terms of mortality rate and immunophenotype by flow cytometry (FACSScan, Becton Dickinson), and proliferation rate by incorporation of [3H]-thymidine ($^3$HTdR; 0,5μCi/well; Amersham, UK) after MDP re-stimulation. Moreover, the following cytokines were detected on cell-free supernatants by ELISA assay (SearchLight): transforming growth factor (TGF)-β1, interferon (IFN)-γ, interleukin (IL)-6, IL-8, IL-10, IL-21. Student *t* test was applied for statistical analysis, and a value of $p<0.05$ was considered statistically significant.
**RESULTS.** In the inflamed mucosa, T-cell lines showed a lower rate of mortality when generated with MDP in comparison with those obtained without MDP (18% *versus* 43%, $p<0.05$). In the presence of MSC, the mortality rates increased in both cases (to 55% and 65%, respectively; $p<0.01$ for both) at MSC:T ratio of 1:20, with no difference observed when using the lower ratio. MSC had no effect on the mortality rate of T-cell lines isolated from normal mucosa of both CD and healthy controls, regardless of the presence or absence of MDP. As regards the immunophenotype, we found a reduction in the activation marker CD25 (17% to 10%) and an increase in the regulatory marker CD69 (13% to 23%) when T cells were cultured with MSC at 1:20 ratio ($p<0.001$ for both). When analyzing the cytokine profile, a reduction in the pro-inflammatory IL-21 and IFN-γ, and an increase in the regulatory TGF-β1 ($p<0.01$) were evident. Finally, MSC inhibited T cell proliferative response to antigen stimulation, both when using cells isolated from inflamed and non-inflamed mucosa of CD patients ($p<0.005$ and $p<0.05$, respectively) at both MSC:T cell ratios applied.
**CONCLUSIONS.** Bone marrow-derived MSC display a robust immunomodulant and immunosuppressant effect *in vitro* on antigen-reactive pathogenic T cells isolated from CD mucosa. These results pave the way for a possible use of MSC as new therapeutic tool in CD.

[1]Ciccocioppo R. et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. Gut 2011;60:788-798.

## CELL THERAPEUTIC APPROACHES FOR THE TREATMENT OF EXPERIMENTALLY INDUCED LIVER FIBROSIS

Askarova S., Umbayev B., Tsoy A, Bulanin D., Ongarbayeva N., Shalakhmetova T.
*Center for Life Sciences, Nazarbayev University, 53 Kabanbay Batyr Ave, Astana, Republic of Kazakhstan, 010000*

**Introduction**
Acute and chronic liver diseases are common in Kazakhstan and other countries. These diseases are known to cause significant disability and death. In many cases, liver transplantation is the last resort for patients with end stage liver disease, but it is extremely expensive procedure associated with many risks. Cell transplantation is a potential therapeutic approach for treatment of liver diseases which could become a viable alternative to organ transplantation. However, morphological and functional changes in the liver of patients suffering from chronic liver fibrosis and cirrhosis restrict the effectiveness of direct cell transplantation. Therefore, extra hepatic sites for cell transplantation could be a good therapeutic approach for compensation of the liver functions. Thus, the aim of our study was to evaluate the engraftment of i.p. injected allogeneic hepatocytes into extra hepatic sites in albino rats with chemically induced liver fibrosis (LF).
**Materials and methods**

Albino rats were randomly divided into 4 groups as following: (1) intact group (n = 18); (2) rats with induced LF (n = 18); (3) rats with induced LF and transplanted with hepatocytes (n=18); to prevent immune response, groups 2 and 3 were subjected to immunosuppression by cyclosporine A (25 mg/kg, per day); (4) as a control, rats were treated with cyclosporine A only (n = 18). LF was induced with N-nitrosodimethylamine (NDMA), i.p., 10 mg/kg, three times a week for 4 weeks and confirmed by histological analysis of the liver samples. Hepatocytes transplantation (HT) has been performed two days after NDMA exposure cessation by i.p. injection of $5\times10^8$ freshly isolated allogeneic hepatocytes. Liver functions were assessed by quantifying blood biochemical parameters (ALT, AST, GGT, total protein, bilirubin and albumin) at 1 week, 1 month and 2 months after hepatocytes transplantation (HT). To confirm a hepatocytes' engraftment, immunohistochemistry of spleen, intestines, stomach and lungs has been conducted.

**Results**

We observed 30% mortality rate among rats with LF within 1 week after NDMA exposure cessation, while 100% of animals with HT survived. ALT, AST, GGT activities and bilirubin levels were markedly elevated in blood samples of LF rats compared to the control animals. However HT significantly improved ALT, AST, GGT activity as well as bilirubin levels. We also observed decreased levels of total protein and albumin in blood serum of rats with LF, while HT normalized these parameters. At the same time, we have not detected any statistical differences of studied parameters in the control group (4) treated with Cyclosporine A only, compared with the intact animals. HepPar1immunohistochemical staining of the different tissue sections demonstrated the presence of engrafted hepatocytes mainly within enlarged Peyer's patches (aggregated lymphoid nodules in the lowest portion of the small intestine).

**Conclusion**

The results of our study provide evidence that HT improved animal survival and liver functions by generating an ectopic hepatic mass inside the Peyer's patches. These observations point to the conclusion that hepatocyte transplantation into lymph nodes would improve efficacy of cell –based therapy of patients with liver fibrosis and cirrhosis.

## HUMAN SUSPENSION PERIPHERAL BLOOD STEM CELLS AS ALTERNATIVE SOURCE FOR CELULLAR THERAPY

Z.A. Shahrul Hisham[1*], Z.A. Intan Zarina[1] & M.A.W. Rohaya[2]

*[1]School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.[2]Department of Orthodontic, Faculty of Dentistry, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.*

*[*]Corresponding Author's Email: hisham@ukm.my / shahroy8@gmail.com*

Stem cells are well known to have potential in the development of innovative therapeutic strategies. To date stem cell in adherent form is widely used in many research areas especially in therapeutics studies. Nevertheless, non-adherent (suspension) cells from bone marrow demonstrate the same capabilities as other adherent cells. Therefore, this study was carried out to identify the capabilities of human suspension peripheral blood to differentiate into specialized cells, i.e., osteoblast and osteoclast. Peripheral blood mononucleated cells were isolated using the Ficoll-Paque™ density gradient separation method and *in vitro* cultured for 15 days. Stem cells in the isolated mononucleated cells were characterised using a multidisciplinary approach which was based on the expression of stem cell markers, morphology and the capacity to self-renew or proliferate and differentiate into specialized cells. The stemness analysis revealed that human peripheral blood mononucleated cells in suspension form were positive for hematopoietic stem cell (HSC) markers. The suspension mononucleated cells were subjected for osteoblast and osteoclast cells differentiations. Differentiation analyses then were done which comprise of viability, biochemistry, morphology and molecular biology (reverse transcription polymerase chain reaction; RT-PCR) analyses. The isolated human suspension mononucleated cells were able to maintain their stem cell properties during *in vitro* culture by retaining their capacity to proliferate and differentiate when exposed to the appropriate induction medium. Differentiation of human suspension mononucleated cells into osteoblasts was associated with increasing of Alkaline Phosphatase (ALP) enzyme activity and expression of the *OPN* and *SPARC* genes, while differentiation into osteoclasts was associated with increasing of Tartrate Resistant Acid Phosphatase (TRAP) enzyme activity and expression of the *TRAP* and *CATK* genes. In addition, the differentiated human suspension mononucleated cells also showed morphologies like osteoblast and osteoclast cells after stained by von Kossa and May-Grunwald-Giemsa, respectively. These indicate that human suspension mononucleated cells able to differentiate into osteoblast and osteoclast which originated from two different lineages, i.e., mesenchymal stem cells and hematopoietic stem cells, respectively. In conclusion, human suspension peripheral blood cell is potential in multilineage differentiation and can be categorized as multipotent stem cells. The potentiality in multilineage differentiation is an indication for this cell to be used in cellular therapy.

## DETERMINING THE IMMUNE PROPERTIES OF EQUINE STEM CELLS

Y. Paterson, N. Rash, R. Paillot, D.J. Guest.

*Centre for Preventative Medicine, Animal Health Trust, Newmarket, UK.*

Objectives:

At present much of the therapeutic work carried out using equine stem cells is centred around their use in over-strain and traumatic tendon injuries. Current clinical practices use autologous stem cells, however this poses a number of limitations which make the immediate treatment of acute injuries unfeasible. The use of allogeneic stem cells has therefore been investigated, with both embryo-derived stem cells (ESCs) and mesenchymal stromal cells (MSCs) having shown no detectable cell-mediated immune response following injection into injured allogeneic tendons. This work showed that ESCs survived in high numbers following injection whereas MSC had a much lower survival. It can therefore be suggested that the ESCs play a direct role in regeneration and repair through differentiation which would support their role in cell replacement therapies, whereas the low survival rate of MSCs may imply that they

have a more indirect trophic effect, with work carried out in other species suggesting that they may have immunomodulatory properties. The aim of this study was therefore to determine the immune properties of equine ESCs and MSCs using *in vitro* methods.
Methods:
To determine if equine ESCs and MSCs are immune privileged, mixed lymphocyte reactions (MLRs) were set up using ESCs and MSCs in co-culture with peripheral blood mononuclear cells (PBMCs) and the proliferative response of the PBMCs measured. The immunomodulatory activity of the MSCs was then measured by co-culturing MSCs with stimulated PBMCs and the effects on PBMCs proliferation measured. Cell-contact dependence of the MSCs was also investigated by co-culture with transwell inserts to separate the PBMCs from the MSC as well as by using conditioned media.
Results:
MLR studies have confirmed that both equine ESCs and MSCs do not induce proliferation of allogeneic PBMCs. Furthermore when MSC were co-cultured with stimulated PBMCs a strong inhibitory effect was observed on PBMC proliferation. This was further confirmed upon experimentation using transwell systems and conditioned media which again resulted in an inhibition of PBMC proliferation.
Conclusion
The result of this study therefore suggests that ESCs and MSCs do not elicit a cell-mediated immune response which supports the possibility of using allogeneic equine ESCs and MSCs in aiding the repair of other tissues in addition to the tendon. Findings following co-culture of MSCs with stimulated PBMCs also intimate their potential role in immune modulation, a property which would support their role in future treatment of inflammatory conditions. Confirmation of the MSCs lack of reliance on direct cell to cell contact also suggests that secreted trophic factors may be involved in bringing about their inhibitory effect. Together these results have provided key information which will help in the development of allogeneic treatments targeted for acute injuries which is unfeasible when using autologous cells. Furthermore, the results have provided insight into how to optimise the use of the stem cells, with ESCs potentially being most beneficial in injuries where cell replacement is required, such as fractures, and MSCs possibly being more suitable for treatment of inflammatory conditions such as osteoarthritis.