discrete groups we call immune profiles. We identified specific immune profiles with improved survival ($p<0.01$). What is particularly noteworthy is that these immune profiles identified patients with similar immune status independent of the underlying diagnosis. This analysis also allowed determination of unique relationships between immune markers. For example, two critical myeloid phenotypes (the immunosuppressive $CD14^{+}HLA\text{-}DR^{lo/neg}$ monocytes and $Lin^{-}CD33^{+}HLA\text{-}DR^{-}$ myeloid derived suppressor cells) independently segregated among patients. We have now developed our third generation immune profiling panel. This panel consists of a 10 color, 8-tube protocol set capable of identifying more than 100 distinct phenotypes. We are using this panel to identify unique immune profiles in patients undergoing cell-based therapy including dendritic cell vaccines and mesenchymal stem cell therapies. To date, we have evaluated approximately 200 patients and healthy volunteers using this approach. We will present data on the power of this approach in cancer and demonstrate its potential to select and monitor patients undergoing cell based therapies.

29
PROGRESS TOWARDS THE CGMP PRODUCTION OF PLURIPOTENT STEM CELL DERIVED RED BLOOD CELLS

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Blood transfusion is a wide spread and important clinical intervention, however problems persist both nationally and internationally in maintaining adequacy of supply, managing the risk of transmission of infectious agents and immune incompatibility between donor and recipient. Human embryonic and induced pluripotent stem cells (hESCs & hiPSC) have unique properties in that they can be maintained indefinitely in culture in an undifferentiated state and yet retain the ability to form all the cells and tissues within the body. They therefore offer a potentially scalable source from which to generate red cells (RBCs) for use in clinical transfusion. We have evaluated hESC lines derived under Good Manufacturing Practice (GMP) conditions in compliance with UK regulatory requirements for clinical products and are preparing master cell bank stocks of the lead line for clinical use, RC9. We are able to differentiate these RC9-hESCs to form haematopoietic progenitor cells (HPC) which subsequently result in $\geq$95% conversion to erythroid cells (GlyA+, CD45-, haemoglobinised) with up to 350,000 fold expansion in cell numbers, in a stroma-free, animal product-free suspension based culture system. The culture process starts with a short embryoid body stage followed by sequential changes in inductive cytokines and growth factors taking up to 30 days. We have also demonstrated that this methodology is similarly effective for hiPSC. The erythroid cells express foetal (alpha/gamma) rather than embryonic (epsilon/zeta) haemoglobin and achieve reasonable enucleation. Many challenges exist in taking this product through to clinical trial including optimisation of the differentiation an maturation protocol, scale-up and optimisation of process control in manufacturing, cGMP-translation and cost-control, and the regulatory and commercial challenges of moving through to first in man clinical studies.

30
EXPANSION AND DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO NEURAL PROGENITORS: A SIMPLIFIED BIOREACTOR PROCESS REPLACING NOGGIN WITH 2 SMALL MOLECULES

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Scaling up the production of human pluripotent stem cells (hPSCs) holds the key to the future realization of cell therapy. Conventionally, the cultivation and expansion of hPSCs is still based on the static tissue culture plate with limited surface area and requires repetitive passaging for expansion. We have developed a microcarrier based process for the expansion and differentiation of hPSCs to neural lineage. Using this platform, hPSCs in serum free medium can be expanded to 3×10^6 cells/ml with 15 fold expansion in a 100ml spinner flask. These expanded hPSCs were differentiated to neural progenitors (NPCs) using two small molecules (Dorsomorphin and SB 431542). The process was simple as medium exchange without manual manipulation required for embryoid body formation. By replacing recombinant protein Noggin with these two small molecules, the neural differentiation was shortened by 4 days to generate above 90% $PSA\text{-}NCAM^{+}$ NPCs, achieving a higher cell concentration of 15×10^6 NPCs/ml (398 NPCs/hPSCs seeded) as compared to 6.1×10^6 NPCs/ml (163 NPCs/hPSCs seeded) achieved using Noggin based protocol. These NPCs can be further differentiated to dopaminergic neurons with positive expression of tyrosine hydroxylase. In conclusion, the microcarrier platform is robust and scalable. hPSCs and NPCs generated are significant higher than the conventional 2D platform. It is our long term goal to further develop this microcarrier based hPSC expansion and differentiation to specific cell types for cell therapy, disease studies, drug screening, and tissue engineering.

31
GMP PROTOCOL TO GENE-ENGINEER AND PROCESS HUMAN T-CELLS REVISITED: MEDIUM CRITICALLY DETERMINES YIELD, FUNCTION AND DIFFERENTIATION STATE OF CD8+ T-CELLS

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We have previously designed and validated a GMP protocol for retroviral gene transduction and expansion of human T-cells. Here, we have revisited the choice for optimal medium (composition) and tested different and newly available media for their effects on yield as well as phenotypical and functional properties of receptor-engineered T-cells. GMP-defined (serum-free) media that were tested included AIMV and Optimize (Invitrogen), Xvivo15 (Lonza), CellGro (CellGenix) and TexMACS(Miltenyi). We tested these media with or without supplementation of 2% plasma. In addition, we tested 3 blended media (i) AIMV (20%)+RPMI(80%)+2% plasma (EMC standard); (ii) AIMV(50%)+RPMI(50%)+5% human serum; and (iii) Xvivo15(20%)+RPMI(80%)+2% plasma. First experiments used CAR-engineered T-cells.The non-supplemented media performed the least with respect to T-cell yield, transduction efficiency and function of CAR T-cells. The best performing media with respect to these parameters included 3 plasma-supplemented media (AIMV, Xvivo15 and TexMACS) and the 3 blended media, including the EMC standard. Five of these media, supplemented with IL15+IL21, were re-tested in parallel to generate MC2/A2 TCR T-cells. In terms of T-cell yield, transduction and function, AIMV+2% plasma performed the best, whereas Xvivo+2% plasma performed the least. Notably, with regard to the preservation of early T-cell differentiation this sequence was reverse. Taken together, this study shows the impact of culture media on yield and phenotypical and functional properties of human T-cells. Importantly, the medium resulting in highest T-cell yield led to more differentiated T-cells, and vice versa. This study provides important information with respect to medium selection to gene-engineer and process T-cells for clinical studies.

32
PRECLINICAL CHARACTERIZATION OF DUOC-01, A CANDIDATE CELL THERAPY PRODUCT DERIVED FROM HUMAN BANKED UMBILICAL CORD BLOOD INTENDED FOR USE IN TREATMENT OF DEMYELINATING DISEASES

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Allogeneic umbilical cord blood [CB] transplantation can slow or reverse progression of central nervous system demyelination in inherited metabolic diseases. Clinical observations suggest that several months are required after transplant for donor derived cells in the brain to provide benefit. We are

developing DUOC-01 as a bridging cell product administered intrathecally to patients early post-transplant to provide therapeutic effects prior to CNS engraftment by cells from the CB transplant. DUOC-01 is manufactured under cGMP conditions from the 20% compartment of the same CB unit used for systemic transplantation by a modification of a previously described method. We performed preclinical characterization of DUOC-01. Time lapse imaging showed that the cultures evolve into attached, motile, highly active cell populations resembling macrophages. The cells express characteristic myeloid macrophage markers including CD45, CD11b, and Iba1. Cells had activities of 11 disease-relevant lysosomal enzymes similar to wild type blood leucocytes. All DUOC-01 batches secreted IL-6 and IL-10. Some secreted TGF-β, IL-1β, INF-γ, TNF-α or very low amounts of IL-12 or IL-2. IL-4, IL-5 and IL-13 were not detected. Peripheral blood mononuclear cells [MNC] proliferated and released cytokines in response to DUOC-01. CB MNC did not respond to DUOC-01 made from the same unit, and DUOC-01 did not proliferate in response to mismatched MNC. Following intrathecal injection DUOC-01 cells were targeted to and persisted in brains and spinal cords of newborn NOD/SCID-IL2Rγ^{null} mice for up to 56 days. Brains of NOD-SCID mice injected intrathecally or intracerebrally with DUOC-01 showed no tumors, ectopic tissue growth, or gross clinical abnormalities during 56 days of observation. DUOC-01 accelerated remyelination in NOD/SCID-IL2Rγ^{null} mouse brains following curpizone feeding. Thus, preclinical studies suggest that DUOC-01 has promise as a candidate cell therapy for demyelinating diseases.

33

TREATMENT OF CEREBELLAR ATAXIA WITH MESENCHYMAL STEM CELLS: A PHASE I/II TRIAL

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Spinocerebellar ataxias (SCA), are determined rare diseases by the Office of Rare Diseases Research at the National Institutes of Health. SCA causes progressive difficulty with coordination and gait which interferes in performing normal daily functions. SCA patients die from respiratory failure, aspiration pneumonia, or severe infection within 20 years of onset. There are no approved therapeutics for treating SCA (spinocerebellar ataxia). PolyQ SCAs are caused by an extensive CAG sequence repeat which encodes for expanded polyQ residues within the mutated protein. All polyQ SCA patients clinically present limb and gait ataxia because the same ataxia interactome is shared among subgroups. Extensive polyQ in cells, including Purkinje neurons, leads to cell dysfunction and triggers cell apoptosis. Loss of Purkinje cells leads to the symptoms and disease outcomes of SCA. Our pre-clinical research has achieved pre-clinical evidence suggesting adipose tissue-derived mesenchymal stem cell (ADMSC) transplantation ameliorates motor function deterioration of SCA in SCA2 transgenic mice by rescuing cerebellar Purkinje cells (Journal of Biomedical Science 2011, 18:54; Chang, et al). The infusion of ADMSC-derived Stemchymal MSCs into SCA patients may be safe and may demonstrate evidence of ameliorating motor function deterioration by arresting continued loss of Purkinje cells to premature apoptosis caused by oxidative stress from excessive PolyQ expression. Our trial design includes a single 7 x107 Stemchymal cells infusion into seven patients with 12 months follow up. Primary outcome measures for safety include vital signs, clinical lab tests and adverse events. Secondary outcome measures for early evidence of efficacy include changes in the scale for the assessment and rating of ataxia (SARA) score, changes in sensory organization test (SOT) score, changes in adaptation test (ADT) scores and changes in electronystagmogram (ENG). At 10 months, Phase I / II safety and early efficacy data supports the feasibility of using allogeneic Stemchymal(TM) Cell Therapy in the treatment of SCA patients. Longer term follow-up and larger, well-controlled clinical trials will be required to get to reach a definitive conclusion for Stemchymal treatment of SCA.

34

FUCOSYLATION OF EX VIVO EXPANDED CORD BLOOD (CB) CELLS IMPROVES ENGRAFTMENT IN NOD/SCID MICE

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CB is as an alternate stem cell source for patients receiving high dose chemotherapy, however the time to neutrophil and platelet engraftment is delayed compared to BM or PBPC products. We have demonstrated faster engraftment with CB products expanded in MSC co-culture leading to an ongoing phase 3 trial. Also studies have demonstrated that fucoyslation of unmanipulated CB also results in faster engraftment in patients. Therefore we hypothesized that fucosylation of expanded CB may provide faster engraftment. CBs were expanded using co culture on MSCs and expanded cells were fucoyslated with fucosyltransferase (FT) VI. CD34+ cells were isolated from the same CB as a control cell population. Immunodeficient (NSG) mice were injected with CD34+ cells, expanded CB cells, or fucosylated expanded cells. Expansion resulted in an increase of 9 fold of TNC and 116 fold increase in CD34+ cells. The expanded cells resulted in faster engraftment of human (hu) CD45+ cells when injected into NSG mice compared to animlas injected with CD34+ cells. The levels of huCD45 in recipient mice was dose dependent with higher cell doses (140 M) resulting in earlier engraftment of huCD45+ cells with a maximum level of 25% huCD45+ cells in the PB, compared to a lower dose (50 M) which engrafted at less than 1% of huCD45+ cells. Treatment with FTVI resulted in an increase of fucosylation from 72% to 98% CLA+ cells. Animals injected with fucosyalted expanded cells achieved up to 25% huCD45+ cells in the PB compared to non fucosylated expanded cells and the engraftment of human cells was more rapid. These data suggest that fucosylation of expanded CB cells may provide enhanced engraftment in the setting of low cell doses such as CB transplantation. Further studies are being performed to support evaluation of fucosylated expanded CB cells in clinical trials in the near future.

35

EX-VIVO EXPANSION OF CORD BLOOD HEMATOPOIETIC STEM AND PROGENITOR CELLS FOR TRANSPLANTATION USING AN ANTIOXYDANT-SUPPLEMENTED MEDIUM AND A CYTOKINE COCKTAIL INDUCING HYPOXIC-LIKE CELLULAR RESPONSE

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We recently developed a clinical grade ex vivo cord blood (CB) expansion procedure enabling a massive amplification of hematopoietic progenitors without any loss of stem cell potential as revealed on the basis of serial engraftment of Nod/SCID mice (Ivanovic et al, Cell Transplant. 2011). This procedure, in line with our concept "Oxygen Stem Cell Paradigm" (Ivanovic, J Cell Physiol 2009), is based on Day 14 liquid cultures of CB CD34+ cells, in medium Macopharma HP01 (containing several antioxidant molecules and nicotinamide) and in presence of Stem Cell Factor - SCF (100 ng/ml), fms-Related Tyrosine Kinase 3 – Ligand - FLT3-ligand (100ng/ml), Megakaryocyte Growth and Developmental Factor - MGDF (100 Ng/ml) (these two cytokines acting in favor of HIF-1alpha transcript stabilization) and Granulocyte – Colony Stimulating Factor -G-CSF (10 Ng/ml). This cocktail had to be modified due to the commercially unavailability of clinical grade MGDF molecule. So, MGDF was replaced by Thrombopoietin - TPO in five-fold lower dose (20 ng/ml) and culture time was reduced to 12 days (Duchez et al Cell Transplant 2012). That way, a mean expansion fold of 400, 80, and 150 was obtained for total cells, CD34+ cells and Colony Forming Cells – CFC, respectively. This amplification was associated with a slight enhancing effect on stem cells (Scid Repopulating Cells - SRC). These are the ultimate pre-clinical modifications of a clinical grade expansion protocol which is already employed in an ongoing clinical trial (adult allogeneic context) started in 2010). The preliminary results are encouraging – rapid and durable hematopoietic reconstitution after injection of one ex vivo expanded CB unit only.