Testing six cGMP-BM-hMSC samples for osteogenesis by ex vivo cellular matrix mineralization stainings, only four were positive for alizarin Red S and only three for Von Kossa. Four of these donors generated ≥15% new bone area in histological sections from bone formation assays in immunodeficient mice after 6 weeks. Notably, the ex vivo phenotype of matrix mineralization staining at two weeks only correlated well with in vivo bone formation in 50% of the samples. When testing a panel of twelve osteogenic biomarker genes after one week of osteogenic induction, a subset of genes showed statistically significant changes in expression level that correlated with ex vivo mineralization. No direct correlation between expression and bone formation was found for any individual gene. Nonetheless, cluster analyses among five consistently expressed genes distinguished the two donors with least bone forming potential, suggesting this approach may prove helpful for clinical trials.

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AUTOLOGOUS BONE MARROW MESENCHYMAL STROMAL CELLS CAN TREAT ARTHRITIC JOINTS OF RHEUMATOID ARTHRITIC PATIENTS: REPORT OF TWO PATIENTS

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Background: Adult human bone marrow (BM) contains a population of mesenchymal stromal cells (MSC) that contribute to tissue regeneration. Further interest in the clinical application of MSC has been generated by the observation that MSC can exert profound immunosuppression by inhibiting T-cell activities *in vitro*. Rheumatoid arthritis (RA) is a T-cell-mediated systemic autoimmune disease characterized by cartilage and bone destruction associated with local production of inflammatory mediators. Joint destruction renders RA a candidate disease for cartilage repair using MSC. However, the issue of whether MSC from patients with RA are functionally altered must be addressed before proceeding to clinical application. The aim of this study was to investigate the properties of BMMSC isolated from patients with active RA *in vitro* and the efficacy of treating arthritic joints of RA patients with autologous BMMSC by intra-articular injection.

Methods: Two patients were recruited. BMMSC were isolated and characterized (including tri-differentiation immunosuppression assay) before implanting the cells back into patients.

Results: BMMSC were successfully isolated from both patients. These BMMSC can differentiate into adipocytes, osteocytes and chondrocytes. Isolated MSC also showed immunosuppressive ability when co-cultured with activated autologous peripheral blood T-cells. Subsequently, successfully isolated and characterized MSC were injected back into patient A's knee joints and patient B's hips. At 1 month after autologous BMMSC implantation, significant reduction of rheumatoid factor was observed in both patients.

Conclusion: This preliminary study showed the properties of BMMSC from RA patients were comparable to healthy MSC, and can be used for autologous transplantation.

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DEVELOPING CELL-BASED THERAPY FOR MACULAR DEGENERATION USING IPS CELL DERIVED RPE TISSUE ON BIODEGRADABLE SCAFFOLDS

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Age-related Macular Degeneration (AMD) is one the leading causes of blindness in the world. It affects more than 10 million people just in the US. The disease is thought to initiate in the back of the eye in the photoreceptor-retinal pigment epithelium (RPE)-choroid complex. In the advanced stage of the disease, RPE cells atrophy leading to photoreceptor cell death and vision loss. Currently there is no effective therapy that reverses or prevents vision loss in AMD patients. The recent suggestion that stem cell-derived RPE can be used to replace atrophied cells has provided hope for a therapeutic intervention. However, the existing stem cells to RPE differentiation protocols are not robust enough to be directly compatible with current Good Manufacturing Protocols (cGMP). We are using a reporter iPS cell line that expresses RPE-specific green fluorescent protein (GFP) to further improve the existing RPE differentiation protocols. The GFP expression marks cells that attain an epithelial morphology, express RPE-specific genes, and become pigmented. Using the knowledge of developmental biology of the RPE, we have modified existing protocols to improve the differentiation efficiency up to 90%. This new step-wise differentiation protocol generates cells that express high levels of RPE-specific genes and show all the morphological features of RPE cells. These cells have been used to generate a functional RPE tissue on plastic semi-permeable membranes. These RPE tissues have been authenticated for their ability to perform several physiological functions performed by RPE cells. Currently, we are developing polarized RPE tissue on artificial scaffolds for pre-clinical testing in animal models. In conclusion, the use of the reporter iPS cell line has led a RPE-differentiation protocol that is scalable, cGMP-amenable, and represents a critical first step in the creation of transplantable tissue with fully-differentiated, functional monolayers of polarized RPE cells.

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CHALLENGE FOR COMMERCIAL CELL THERAPY PRODUCTS: EASY RECONSTITUTION AT THE BEDSIDE

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Promethera® HepaStem is the company's cell therapy product to treat serious metabolic liver disorders. Currently, an European Phase I/II clinical trial is ongoing for the treatment of children suffering from Crigler-Najjar syndrome and Urea cycle disorders. The challenge is to provide a drug product easy to reconstitute at the clinical site.

For this multi-country and multi-center clinical study, Promethera has developed a GMP-compliant fully closed formulation system based on Biosafe's Sepax 2 device. This technology allows an aseptic preparation of the final product in a non-sterile environment. The underlying idea was to prepare the product in a mobile unit brought near the clinical site, to timely and consistently deliver the cells within its shelf-life. A successful infusion of HepaStem, formulated in the mobile unit, has been accomplished in the Cliniques Universitaires Saint-Luc (Bruxelles, Belgium). Future patients enrolled in this clinical trial will receive HepaStem cells formulated in the mobile unit.

In order to guarantee a flexible, highly qualitative, and economically sustainable supply chain during commercialization, the phase III formulation step will be performed by reconstitution at the hospitals. This implies further fine-tuning of the current technology towards a fully automatic process with minimal manipulations, yet ensuring consistency in terms of safety, identity, purity, and therapeutic potency. This simple process will allow reconstitution to be handled by the medical team or the hospital pharmacist. The approach offers new opportunities in the field of stem cell therapy and cell-based products with a limited shelf-life. Promethera Biosciences® future development programs will implement this innovative supply delivery system.

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GENE-MODIFIED MESENCHYMAL STROMAL CELLS FOR STROKE: UPDATE ON CLINICAL DEVELOPMENT

CC Case
SanBio, Inc., Mountain View, CA

The cell therapy product SB623 comprises allogeneic bone marrow-derived mesenchymal stromal cells transiently transfected with a plasmid vector expressing the human Notch-1 Intracellular Doamin (NICD). The NICD transfection alters the secretory profile of the cells and improves their effectiveness in models of neurodegenerative diseases. SB623 is currently in a Phase I clinical trial in the US for chronic stroke. This presentation will focus on mechanism of action, the path to the clinic and additional indications.

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ESTROGEN ADMINISTRATION WITH SUBSEQUENT HUMAN UMBILICAL CORD DERIVED MESENCHYMAL STEM CELLS TRANSPLANTATION MAY IMPROVE HINDLIMB LOCOMOTOR DYSFUNCTION AFTER EXPERIMENTAL SPINAL CORD INJURY

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