

Among diverse approaches in translational medicine, there is growing interest in use of mesenchymal stem cells (MSC) to enhance bone regeneration. We aimed to evaluate the osteogenic potency of human bone marrow (BM)-derived MSC isolated and expanded according to good manufacturing practice (GMP). Recently, numerous genes have been associated with the osteogenic differentiation, however their expression in differentiated hMSC ex vivo rarely predict in vivo bone formation. We wished to explore whether such biomarkers changes were applicable using primary hMSC grown in medium supplemented by platelet lysate at 1 week instead of 2 weeks time points. Testing 6 donor BM-hMSCs for osteogenesis using ex vivo matrix mineralization stainings revealed 4 were positive for Alizarin Red (ALZ) and 3 for Von Kossa (VK). In vivo bone formation assays, implanting hMSC with osteoconductive scaffold in NOD/SCID mice for 6 weeks, revealed that hMSC from 4 donors generated new bone ($\geq 15\%$ total area). Notably, the ex vivo phenotype of matrix mineralization staining at 2 weeks did not necessarily correlate with in vivo bone formation. To address the problem of donor-specific heterogeneity we selected hMSC from osteogenically functional donors when analyzing 1 week gene expression. Using hMSC from donors that were positive for ex vivo mineralization, 7 genes showed statistically significant changes using ALZ+ donors and 6 genes using VK+ donors. Using hMSC from bone-forming donors, 7 genes showed statistically significant changes and 5 of these were also relevant to ex vivo mineralization. Using these 5 genes for cluster analysis, a strong correlation with bone-formation was identified; distinctly clustering hMSC from bone-forming versus non-bone forming donors. We provide proof of principle that genetic analysis of hBM-MSC early responses to osteogenic factors ex vivo can indicate bone forming potential, suggesting that this approach may prove helpful for clinical trials.

342 QUANTITATIVE ANALYSIS OF BONE MARROW CONCENTRATE (BMC) PRODUCED USING SYNGENX™-2000 SYSTEM- A POINT OF CARE MEDICAL DEVICE

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Introduction: There is growing interest in the use of bone marrow-derived stem cells to treat a variety of acute and chronic conditions, including cardiovascular and orthopedic indications. The SynGenX™-2000 system is a novel point of care device for efficient and reproducible preparation of bone marrow concentrate (BMC) from bone marrow aspirate (BMA). The objective of this study was to characterize the cell counts of BMA from normal healthy donors and of BMC following processing with the SynGenX™-2000 using complete blood counts (CBC) and flow cytometer-based analysis, and to determine the viability of the CD34+ cells in the BMC.

Methods: Bone marrow aspirate (108 +/- 9 mL) was concentrated into a final volume of 20 +/- 0.2 mL in a 12 minute process using the SynGenX™-2000 system. All 10 bone marrow aspirates used in this study were processed and sample aliquots were analyzed within 8 hours of collection. For colony forming unit (CFU-II) assays, cells were plated at a density of 1 and 2x10⁴/plate. Flow cytometric analysis was performed using the Becton Dickinson FACS Calibur machine.

Results: The cell counts, cell enrichment factor and cell recoveries of the 10 samples are reported in the table. The SynGenX™-2000 system removed greater than 95% of the RBCs, resulting in a mean Hct of 6.5 ± 2.1% in the final 20 mL BMC product. The CD 34+ cell viability of the BMC was

98.8 ± 1.1% and microscopic evaluation of the distribution of red, white and mixed colonies demonstrated no change in the BMC as compared to the BMA.

Conclusion: The results demonstrated an average recovery of $\geq 90\%$ and 5 times enrichment for mononucleated cells (MNC), CD34+ and CD45+ cells in the final BMC product. The SynGenX™-2000 system demonstrated that it is an efficient, rapid and easy method for preparing bone marrow concentrate from human bone marrow aspirate at the point of care.

343 AUTOMATION OF UPSTREAM AND DOWNSTREAM MANUFACTURING PROCESS OF CELL THERAPEUTIC PRODUCT: GAIN IN QUALITY, YIELD AND CONSISTENCY WITH REDUCED MANPOWER

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Promethera Biosciences® produces the cell-therapy product, HepaStem, to treat serious metabolic liver disorders. Today, 20 patients have been treated during a European phase I/II clinical trial. Promethera is currently preparing its next clinical phases in US and Europe. To upscale the process, minimize manual operations & related-risks, and reduce costs, Promethera has developed a fully-closed semi-automated system from expansion to final filling. This next-generation process is based on ATMI's Xpansion bioreactor technology followed by in-line centrifugation and filling in Aseptic Technologies closed vials. HepaStem cells were successively expanded in mid-scale XP100 (61200cm²) and large-scale bioreactors XP200 (122400cm²) without change in growth-rate, population doubling-time, in-process impurities and quality. A 400-fold concentration of the harvested product (150000 cells/ml upon harvest to 60.10E6 cells/ml after centrifugation) with satisfactory preservation of viability and quantity was accomplished via ATMI's in-line centrifuge. Sterility was preserved at all-time. In-line filling in closed vials substantially reduced batch-to-batch variation in terms of content uniformity with less than 15% variation in terms of cell quantity/vial and less than 2% in terms of volume. To date, this combined closed process has passed all qualification steps with increased sterility risk mitigation. The first full-scale GMP-batches are currently being manufactured. In conclusion, the stream-lined expansion-centrifugation-filling process via combination of ATMI's closed Xpansion™ bioreactor/centrifugation with in-line filling in Aseptic Technologies closed vials offers a valuable technology for large-scale commercial production. This technology results in reduced costs, increased batch-to-batch consistency and is able to increase the yield of Promethera's production process by 10-fold while reducing manpower and global operational time by 50-to-60%.

344 MESENCHYMAL STROMAL CELLS FOR OSTEONECROSIS OF THE FEMORAL HEAD (ONFH). DATA FROM ONGOING CLINICAL TRIAL

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Background: ONFH is an ischemic event that eventually progresses to collapse, total articular degeneration and arthroplasty. It affects mainly young adults and it's associated to pain, functional limitation and a great socio-economic impact. Current treatments lack efficacy limiting its progression. The working hypothesis proposes that XCEL-MT-OSTEO-ALPHA (ex-vivo expanded autologous bone marrow mesenchymal stromal cells fixed in allogenic bone tissue, produced at Xcelia- Advanced Therapy Division of the Blood and Tissue Bank of Catalonia, under GMP conditions) is a useful product to achieve bone regeneration and avoid progression to collapse. This study has been funded by the Spanish government (MSSSI and MICINN) and the EU (ERDF).

(n=10)	BMA (x10 ⁶ /mL)	BMC (x10 ⁶ /mL)	Enrichment ("X")	Recovery (%)
TNC	25.2 ± 8.8	113.7 ± 38.4	4.6 ± 0.6	83.8 ± 7.6
MNC	5.8 ± 2.3	28.4 ± 6.9	4.9 ± 1.4	90.3 ± 22.2
Platelets	57.7 ± 18.7	278.5 ± 385.7	5.1 ± 1.8	93.1 ± 27.4
CD34+	0.28 ± 0.22	1.56 ± 1.00	5.7 ± 1.3	105.9 ± 24.3
CD45+	31190.3 ± 12473.4	150198.4 ± 54140.2	4.9 ± 0.7	90.3 ± 10.7
CFU-H	75434 ± 60755	383641 ± 291420	6.4 ± 3.5	118.3 ± 58.2