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STUDY THE MECHANISM OF ACTION OF ELIXCYTE®, AN ALLOGENIC STEM CELL PRODUCT, ON OSTEOARTRITIS

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Keywords: Osteoarthritis, Mesenchymal stem cells.

Background & Aim: Osteoarthritis (OA) is characterized by progressive and irreversible cartilage degeneration, which causes pain, stiffness and decreased function. Mesenchymal stem cells have been shown to possess broad immunoregulatory and anti-inflammatory abilities, and to promote cartilage regeneration and chondrocyte protection. To evaluate the safety and efficacy of intra-articular (IA) injection of allogeneic adipose-derived stem cells ELIXCYTE® for knee osteoarthritis, a phase I/II, randomized, active-control, single-blind, multiple-center clinical trial was conducted (NCT02784964). The results showed that ELIXCYTE® had earlier onset and significant better therapeutic efficacy than HA on WOMAC, VAS, and KSCRS functional activities scores. In order to study the mechanism of action of ELIXCYTE® and identify potency markers with correlation to the clinical outcomes, in vitro studies revealing chondrocyte proliferation, chondrocyte protection, immunomodulation and anti-inflammation abilities of ELIXCYTE® are conducted.
Methods, Results & Conclusion: ELIXCYTE® was shown to promote chondrocyte proliferation, which was positively correlated to FGF1, FGF2, TGFβ1, and PDGF-BB. In IL-1β induced chondrocyte damage, ELIXCYTE® protected chondrocyte by promoting type II collagen and aggrecan and reducing MMP13 expression. In addition, ELIXCYTE® inhibited T cell proliferation through indoleamine 2,3-dioxygenase. Also, ELIXCYTE® was capable to reduce pro-inflammatory cytokine secretion from activated M1 macrophage. In order to translate preclinical research to clinical application, we evaluate the functional abilities of ELIXCYTE® in disease mimic environment. First of all, synovial fluids from 12 osteoarthritis patients with stage 2 or 3 undergoing hydrarthrosis were collected. Chondrocytes are cocultured with ELIXCYTE® in synovial fluids for three days. Gene expression of ELIXCYTE® are analyzed to investigate its response under synovial fluids. Type II collagen, aggrecan and MMP13 of chondrocytes are analyzed to reveal the protective effects by ELIXCYTE®. In our preliminary data, gene expression of ELIXCYTE® of anti-inflammation, immunomodulation and chondrocyte proliferation were triggered by synovial fluids. Cytokine profiling of synovial fluids will be established. In conclusion, ELIXCYTE® can modulate the inflammation environment of OA to relief the pain and promote cartilage repair via chondrocyte regeneration and extracellular matrix replenishment to improve the functional activities of OA knee.

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DEVELOPMENT OF AN OPTIMIZED LENTIVIRAL TRANSDUCTION MEDIUM AND PROCESS TO MANUFACTURE GENETICALLY MODIFIED MSC WORKING CELL BANKS

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Keywords: Lentivirus, Mesenchymal Stem Cells, Medium.

Background & Aim: Background. Human Mesenchymal stem/stromal cells (hMSCs) are widely used in clinical development and have an excellent safety profile demonstrated in over 1,000 clinical trials, making them ideal candidates for cell-based gene therapies. hMSCs may be genetically engineered to improve their inherent therapeutic properties or to secrete proteins for new applications. A critical challenge, however, is efficiently modifying hMSCs within the context of cGMP manufacturing, especially given the historically low transduction efficiency of primary cells. Here we describe the development of a manufacturing process using a novel medium called RoosterGEM™, optimized for maximum lentiviral transduction to generate working cell bank (WCB) vials of genetically modified hMSCs that could be expanded to a final cell product.
Methods, Results & Conclusion: Methods. Various lentiviral promoters, MOI, and transduction processes were investigated for the efficient transduction of human umbilical cord and bone marrow MSCs (hUC- and hBM-MSCs). Briefly, both hUC- and hBM-MSCs were thawed and transduced with lentiviral vectors expressing Green Fluorescent Protein (GFP) or Zs Green at MOIs ranging from 2 to 50. Transduction efficiencies were assessed by fluorescent microscopy and quantified by flow cytometry. WCBs of transduced cells were created and further expanded to a final product PDL to characterize hMSC Critical Quality Attributes (CQAs), including phenotypic markers (CD90 and CD166), multilineage differentiation and cytokine secretion (HGF, IL-8, TIMP-1, TIMP-2 and VEGF). Results & Conclusions. Two hUC-MSC lots and two hBM-MSC lots were efficiently transduced with lentiviral vectors expressing GFP or Zs Green using a novel genetic engineering medium, RoosterGEM™. The use of this medium resulted in a 2-5 fold increase in transduction efficiencies above growth medium alone. The technique of Spinoculation in which the virus and cells are centrifuged together did not markedly increase transduction efficiencies. Significant differences were observed in the transduction efficiency of lentivirus from different vendors ranging from 20% to 90%. Overall, the use of RoosterGEM™ resulted in WCBs with over 90% transduction efficiency, while maintaining MSC CQAs. This novel medium optimizes a critical unit operation of gene modification and provides a useful tool for the generation and expansion of engineered hMSCs for use in clinical therapies.

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NEEDLE TO NEEDLE BIOPROCESSING OF CELL THERAPIES

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Keywords: Bioprocessing, Donor source, Serum free media.

Background & Aim: As cell therapies move higher up the value chain, the complexity of the process from cell sourcing to manufacturing and delivery becomes evident and the integration of each step is more critical. We have built an allogeneic stem cell manufacturing (ASTEM) platform which covers several critical steps such as: donor screening, novel isolation technology, development of robust serum free media, heparan sulphate additives for improved performance, reporter cell line for process monitoring, degradable microcarriers, a spiral vane impeller for cell suspension, DMSO free cryopreservation solution and in vitro functional assays of cartilage repair.
Methods, Results & Conclusion: We will present results of these advances with several examples (See Fig. 1): 1) Donor screening for GSTT1 null phenotype showing higher proliferation rates than heterozygous and homozygous donors; 2) Isolation with microcarriers for enhanced yields of MSC from mononuclear cell fractions of the BM harvests; 3) Development of 3 variants of serum free media for higher proliferation rates, potency and functions benchmarked against commercial media; 4) Heparan sulphate additives that stabilize bFGF that reduce the dose needed for media; 5) A reporter cell line that can be used in process monitoring; 6) A new Bach impeller with lower Power numbers for mixing compared to conventional axial flow impellers; 7) Biodegradable microcarriers fabricated with high throughput microfluidics; 8) A DMSO free cryopreservation solution that allowed longer handling of