

Predictive Model of Human Mesenchymal Stromal Cell Therapeutic Efficacy in PTOA Treatment

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INTRODUCTION: Injury initiates a slow chronic cycle of joint degeneration resulting in post-traumatic osteoarthritis (PTOA). Worldwide millions of people are affected by PTOA, and it is one of the leading causes of disability in the United States. In addition, there is no known cure or disease modifying therapy to prevent progression of PTOA. Previous pre-clinical studies have made great strides in understanding the pathophysiology of this disease and in identifying potential therapies. Cell therapies, including use of human mesenchymal stromal cells (hMSCs), have shown potential as a therapeutic option for PTOA. However, clinical translation has been limited by factors such as heterogeneity and variability in potency of MSCs isolated from different donors. Thus, there is a critical need to understand the therapeutic potential of hMSCs to optimize its regenerative treatment efficacy in PTOA disease progression. The objective of this study was two-fold: 1) first generate a predictive model of MSC therapeutic efficacy based on hMSC secretome data and therapeutic efficacy data from a pre-clinical PTOA model, and 2) prospectively validate the predictive capacity of the model using hMSCs from four additional donors and testing the cells through the predictive pipeline (Fig 1). We hypothesized that the model would predict hMSC donors to be therapeutic based on secretome signatures and that predicted hMSCs treatment efficacy would be validated in our PTOA preclinical model by showing improved disease outcomes such as decreased pain behaviors, and structural degradation when treated with therapeutic donor hMSCs compared to control.

METHODS: *In vitro* studies: Bone marrow derived hMSCs were purchased from RoosterBio. The hMSCs from four donors for the training study and four donors for the validation study were cultured for 24 hours in control media (RoosterNourishTM-MS-C media, RoosterBio) or an OA simulated microenvironment where the media was stimulated with IL-1 β (20 ng/ml). Media was then collected and analyzed using a multiplexed immunoassay panel (Luminex) to evaluate hMSCs secretome. *In vivo* studies: All procedures were conducted following IACUC protocol approval. Weight matched male Lewis rats (Charles River Laboratory) were randomly assigned to medial meniscal transection (MMT) or sham (n=8) surgery groups. Prior to injury, baseline evoked pain sensitivity was evaluated using von Frey filament testing and spontaneous limb function was assessed by Dynamic Weight Bearing testing (DWB 2.2.6, BIOSEB; validation study only). Rats then underwent either MMT or sham surgery (MCL transection only) according to group. Von Frey and DWB analyses were performed again 1, 3, 4, and 6-weeks post-injury. Three weeks post-injury MMT rats received an intra-articular injection (50 μ L saline or saline with 5*10⁵ cells suspension) according to randomly assigned treatment group. In the training study: MMT/saline control and four MMT/hMSC (2.1, 2.2, 2.3, 2.4) treatment groups (n=8 per group). In the validation study: MMT-saline and two MMT-hMSC (D1, D2) treatment groups (n=8 per group). Three weeks post-injection, rats were euthanized and the hindlimbs collected for evaluation of PTOA structural pathology, which is ongoing. Structural changes were evaluated using contrast enhanced μ CT and histology. Pain data was normalized to baseline and evaluated by 2-way ANOVA with significant effects (p<0.05) further assessed by post-hoc analysis. *Predictive model:* PLSDA and PLSR models were generated in MATLAB 2021 (Mathworks) using a function written by Cleiton Nunes (Mathworks File Exchange). The model was trained on z-scored data from the training study using secreted cytokines level read outs as the independent variables and the different hMSC donors/treatments as the binary outcome variables. Validation data set was then run on the trained model, projected onto the multidimensional space, and correlated to the therapeutic latent variable (LV) axis to predict therapeutic efficacy.

RESULTS SECTION: Our initial training data *in vitro* study revealed donor hMSC heterogeneity in secreted cytokines in response to an OA simulated microenvironment (fig.1A). The initial training data preclinical study results showed variability in structural disease outcomes between the donor hMSC treated groups. Articular surface roughness in the medial tibial plateau was significantly (p<0.05) lower in the donor hMSC 2.3 and 2.4 MMT groups compared to the MMT/saline group (fig.1B). Similarly, cartilage lesion volume showed no difference between hMSC 2.3, 2.4 and the sham group (fig.1C). While hMSC 2.1, hMSC 2.2 and MMT/saline groups were significantly higher compared to sham. These data suggest hMSC 2.3 and 2.4 yielded more therapeutic outcomes compared to other donors. Data from these initial studies were used to train a PLSR predictive statistical model (fig.1D). Comparably, in the validation study there was variability in hMSCs response to IL-1 β stimulation (2A). The predictive model projections prospectively revealed hMSCs from four additional donors separated along LV axis 1 which was predicted to be associated with therapeutic efficacy (fig.2B) thereby predicting less (hMSC-D1) and more therapeutic (hMSC-D2) donors. In the preclinical model, spontaneous pain behavior results showed significantly higher injured to uninjured hindlimb weight ratio at all timepoints in the sham group compared to MMT groups (p<0.05) (fig. 3A). At 6 weeks post injury MMT-saline group weight ratio was significantly less compared to MMT-hMSC D1 group (p=0.0427). Representative images from μ CT qualitatively showed cartilage degradation and lesions in the MMT surgery groups compared to sham while the hMSC D2 treatment group displayed less cartilage degradation (Fig. 3B).

DISCUSSION: The results supported our hypothesis that the PLSR predictive model would predict therapeutic efficacy of the donors. The hMSC secretome was distinct between donor hMSCs which supports previous studies indicating the heterogeneity and variability of MSCs from different donors. Using our predictive model pipeline, we were able to classify hMSCs therapeutic potential based on their paracrine secretion of immunomodulatory cytokines. Consistently identifying therapeutic donors will optimize regenerative cell therapy treatment for improved clinical translation. In the *in vivo* validation study, injury triggered pain behaviors while the hMSC treatments exhibited protective effects in reducing pain behaviors. Treatment with the predicted therapeutic hMSC donor qualitatively showed protective effects against structural cartilage degradation and cartilage lesion formation. These findings suggest variable therapeutic benefits of hMSCs that can be prospectively predicted in the trained PLSR predictive model which further supports our hypothesis. Ongoing structural analysis will confirm the chondroprotective therapeutic benefits of hMSCs in inhibiting structural degradation and disease progression.

SIGNIFICANCE/CLINICAL RELEVANCE: The study showed that hMSCs therapeutic efficacy may be predicted, which will optimize clinical translation. Results from this study will advance PTOA therapies by reliably predicting therapeutic donors and limiting variability for regenerative treatment strategies.

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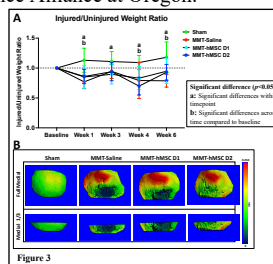
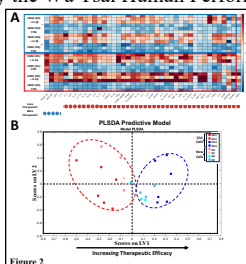
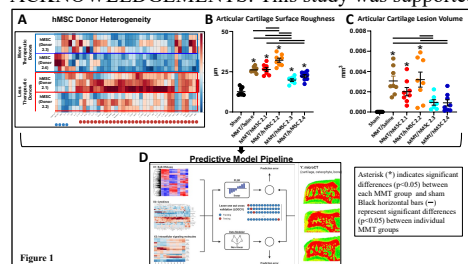


Figure 1. Initial Studies: A) Paracrine signaling response profile of hMSCs in an IL-1 β OA simulated microenvironment. B) Effects of donor hMSCs on articular cartilage surface roughness and C) articular cartilage lesion volume results outcomes in a preclinical study D) Predictive model pipeline used to identify hMSC therapeutic potential.

Figure 2. Validation studies: hMSCs cultured in an IL-1 β OA simulated microenvironment. A) Paracrine secretion of immunomodulatory cytokines B) PLSDA Predictive model pipeline used to identify therapeutic and less therapeutic donors.

Figure 3. Validation preclinical study evaluating predicted therapeutic hMSCs effects on pain behaviors and structural outcomes A) spontaneous pain behaviors (DWB) B) Representative cartilage thickness heatmap images from contrast enhanced microCT.