Induced Pluripotent Stem Cell-Derived Mesenchymal Stromal Cells Promote Muscle Regeneration in a Diabetic Mouse Model of Critical Limb Threatening Ischemia

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Critical limb threatening ischemia (CLTI), the end stage of peripheral arterial disease (PAD), is diagnosed in 500,000 patients each year, often results in amputation, and has a ~50% 5-year mortality rate. Diabetic CLTI patients experience especially high morbidity and mortality, and no effective non-surgical therapy exists for this population. Our Phase II MOBILE trial demonstrated that autologous bone marrow nucleated cells were unable to prevent amputations in diabetic patients; however, data from a Phase I trial shows that allogeneic bone marrow-derived mesenchymal stromal cells (BMD-MSC) stimulated angiogenesis in ischemic muscle, including diabetics. While allogeneic MSC may be an effective cell preparation to treat diabetic CLTI, passaging-related cell senescence prevents generation of sufficient cell numbers for therapeutic use. The development of induced pluripotent stem cell (iPSC)-derived MSC overcomes cell senescence issues and offers the possibility of genetic modifications to enhance cell function. The current study was designed to determine potential mechanisms by which iPSC-MSC stimulate muscle regeneration in a rodent CLTI model.

The CLTI mouse model was created by ligation/excision of the femoral artery in male polygenic diabetic TallyHo mice. Mice with intramuscular administration of iPSC-MSC displayed positive indicators of muscle regeneration compared to vehicle control mice. Real-time PCR performed with gastrocnemius muscle obtained 7- or 30-days post iPSC-MSC injection showed an increase in mRNA expression for MyH3, MyoD1, Mrc1, FoxP3, and VEGF-A vs. vehicle treated muscle, indicating promotion of muscle regeneration, M2-biased macrophage expression, T regulatory cell (Treg) expansion, and vascular proliferation. Downregulation of the NADPH oxidase subunit $p47^{phox}$ indicated a decrease in oxidative stress in the treated mice. The results are consistent with iPSC-MSC promotion of muscle regeneration via a Treg mediated stimulation of the M1-M2 macrophage phenotypic shift. Thus, human iPSC-MSC could be an effective treatment to stimulate muscle regeneration and ameliorate CLTI in diabetic patients.