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Mesenchymal stem/stromal cells genetically engineered to produce vascular endothelial growth factor for revascularization in wound healing and ischemic conditions

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Mesenchymal stem/stromal cells (MSCs) may be able to improve ischemic conditions as they can actively seek out areas of low oxygen and secrete proangiogenic factors. In more severe trauma and chronic cases, however, cells alone may not be enough. Therefore, we have combined the stem cell and angiogenic factor approaches to make a more potent therapy. We developed an engineered stem cell therapy product designed to treat critical limb ischemia that could also be used in trauma-induced scarring and fibrosis where additional collateral blood flow is needed following damage to and blockage of the primary vessels. We used MSCs from normal human donor marrow and engineered them to produce high levels of the angiogenic factor vascular endothelial growth factor (VEGF). The MSC/VEGF product has been successfully developed and characterized using good manufacturing practice (GMP)-compliant methods, and we have completed experiments showing that MSC/VEGF significantly increased blood flow in the ischemic limb of immune deficient mice, compared to the saline controls in each study. We also performed safety studies demonstrating that the injected product does not cause harm and that the cells remain around the injection site for more than 1 month after hypoxic preconditioning. An on-demand formulation system for delivery of the product to clinical sites that lack cell processing facilities is in development.

CLINICAL TRIALS OF GENE AND CELL THERAPY FOR CRITICAL LIMB ISCHEMIA

Prior clinical trials have investigated injection of vascular endothelial growth factor (VEGF) gene therapy agents to promote angiogenesis and restore blood flow in chronic conditions such as critical limb ischemia (CLI). These therapeutic agents seemed promising in early-stage clinical trials but were not significantly better than controls in Phase III trials. Other clinical trials using the patient's own stem cells from the marrow and injected into the ischemic leg had shown some benefit, although the final assessments are not yet completed, and patients fall into two categories, "responders"

ABBREVIATIONS: CLI = = critical limb ischemia; GMP = good manufacturing practice; IM = intramuscular; MSC(s) = mesenchymal stem/stromal cell(s); VEGF = vascular endothelial growth factor.

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and "nonresponders," making it difficult to meet overall endpoints.

One approach to treatment of CLI is inducing formation of collateral blood vessels that bypass the primary blockage and restore tissue perfusion to initiate healing. Proteins in the VEGF family have long been considered prime candidates for promoting such therapeutic angiogenesis, and several clinical trials have been carried out to test these molecules as CLI therapeutics. Proteins, however, have a short half-life and are usually extremely expensive. Gene therapy for therapeutic angiogenesis presents an alternative to recombinant protein administration. Clinical studies that delivered proangiogenic cytokines as genes began later than many of the studies involving recombinant protein formulations of these cytokines, in part due to issues regarding the appropriate vectors and promoter sequences to be employed, as well as additional safety concerns surrounding the field of human gene therapy.

Food and Drug Administration (FDA)-approved Phase I and Phase II clinical trials involving intramuscular (IM) injection of naked DNA or viral vectors have tested the delivery of angiogenic factors, including VEGF, indicated a potential benefit of VEGF administration for CLI.[1-3] Patients with advanced myocardial ischemia have been treated with a VEGF-A_{165} plasmid delivered through the endocardium via a percutaneous approach for refractory coronary artery disease and peripheral artery disease.[4-7] Another open-label study treated 30 patients with "no-option" advanced coronary artery disease and refractory angina, with a VEGF-A_{165}–coding plasmid, found a significant improvement in symptoms, and a modest improvement in exercise treadmill time.[8] The RAVE study[9] was a large (107 patients), randomized Phase II, double-blind, placebo-controlled clinical study using an adenovirus coding for VEGF-A_{121} in patients with unilateral advanced intermittent claudication. Unfortunately, no differences in outcome could be identified between Ad/VEGF-A_{121} and placebo-treated patients. However, no major safety issues associated with Ad/VEGF-A_{121} were identified.

Although Phase I and II trials of VEGF delivered via plasmid and as purified protein injections showed potential clinical benefit in treatment of CLI, Phase III studies in this indication did not achieve significant efficacy. There was, however, benefit observed for administration of the pCMV-vegf165 plasmid in IM gene transfer for treatment of moderate to severe claudication due to chronic lower limb ischemia.[10,11] The failure of these agents to significantly affect therapeutic angiogenesis in the most severe forms of CLI (Rutherford Category IV/V) has been attributed to the mechanisms of VEGF delivery, because expression from plasmids is suboptimal and transient and the VEGF protein has a short half-life. We hope to circumvent this issue by delivering VEGF from mesenchymal stem/stromal cells (MSCs), which could work in synergy with the other paracrine angiogenic factors naturally produced by MSCs.

Numerous clinical trials have demonstrated the biosafety of systemic infusion of allogeneic MSCs into patients with various diseases.[12,13] These cells are ideal for this application due to their ease of isolation and expansion, low immunogenicity in allogeneic settings,[14] and ability to secrete paracrine factors that stimulate regeneration.[12] Furthermore, MSCs show tropism toward sites of hypoxia[15] and are stimulated to express angiogenic factors in hypoxic environments,[16] which make them particularly advantageous for application in ischemic disease.

Clinical studies have provided extensive safety data for nonmatched allogeneic marrow-derived MSCs administration in patients through FDA-approved clinical trials. Liew and O'Brien[17] reviewed the progress of using MSCs to treat CLI. Early-phase trials for heart and limb revascularization have shown safety and early indication of efficacy (REVASCOR-Mesoblast, RESTORE-CLI-Aastrom, Multistem-Athersys, and others). The lack of infusion-related serious adverse events in these trials demonstrates the safety of MSCs and other expanded adherent marrow-derived cell infusion, when performed carefully and according to specific clinical regimens. Cell therapy for CLI was recently reviewed, with an excellent integration of the preclinical and clinical studies.[18]

POTENCY ASSAYS FOR MSCs IN ANGIOGENESIS

In contrast to features such as their osteogenic potential or their ability to suppress the immune system, the proangiogenic activity of MSCs remains difficult to predict before experimental use. The common and simple methods to isolate and expand MSCs from the marrow lead to a rather heterogeneous population.[19,20] Nonetheless, many characteristics of the cells are consistent and only minimally vary between donors or culture conditions. For example, their surface phenotype is homogenous regarding the absence of hematopoietic markers (CD45, CD34, CD14) and the presence of mesenchymal markers CD73, CD90, and CD105.

Marrow-derived MSCs may be better at promoting blood flow restoration than MSCs derived from adipose tissue.[21] Studies have demonstrated that the therapeutic potency of MSCs can vary greatly depending on the donor.[12] Marrow-derived MSCs have a long safety history and have shown efficacy in some clinical trials, while other late-stage trials have failed. Expansion and conditioning procedures are important for optimizing their survival in vivo after delivery, as are identifying the best cell batches before clinical use. It is thus imperative to define surrogate potency assays, such as rate of expansion, viability and identity, sterility, cytokine release, in vitro vascularization tube-forming assay, and in vitro induction of target cell mobility, as assessed by videomicroscopy.[22]

DEVELOPMENT OF THE MSC/VEGF PRODUCT

The rationale for delivering VEGF from MSCs in our novel product is to extend the duration of local expression of VEGF and thereby promote angiogenesis. Through interaction with the FDA and pioneering scientists in the field of gene therapy, we developed a protocol to generate the MSC/VEGF product to be as safe and efficient as possible, using lentiviral vector technology. The keys to our approach include the use of a third-generation lentiviral vector,[23] no other transgenes to minimize immunogenicity, and a limit of one to two proviral insertions per cell, to minimize the risk of insertional mutagenesis. A strong promoter (MNDU3; previously used in human gene therapy[24]) and an enhancer element acting in cis (woodchuck hepatitis posttranscriptional regulatory element [WPRE][25]) maximize transgene expression, while minimizing viral load. The MSC/VEGF was produced in a good manufacturing practice (GMP)-compliant manner in the laboratory using the same standard operating procedures, reagents, and formulations to be used in future GMP batches. Cells were then cryopreserved as several "tox batches" for future in vivo testing.

The preclinical studies published by our group[26,27] demonstrate that MSC/VEGF may be useful in promoting angiogenesis, because the cells show tropism toward hypoxic sites and deliver high levels of VEGF from the introduced transgene at the sites of ischemia, promoting reperfusion of the ischemic tissue. MSC/VEGF are more effective than unmodified MSC (without the VEGF transgene) in restoring blood flow in animal models.[26,27] MSC/VEGF cells do not persist indefinitely at the site of ischemia. MSC/VEGF can initiate angiogenesis that should be carried on by endogenous mechanisms once tissue perfusion is restored.

We published proof-of-concept data showing that engineering MSCs overexpressing VEGF-A_{165} (hereafter VEGF) show a much greater potential to restore blood flow in a murine model of hind limb ischemia, compared to control MSCs.[26] We then manufactured and tested the efficacy of clinically compliant MSC/VEGF, made under current GMP conditions.[27] We are currently testing MSC/VEGF in a large animal model (rabbits), to further determine the optimal implementation of the cell/gene therapy.

MSC/VEGF ON-DEMAND PRODUCT FORMULATION

Gene-modified allogeneic MSCs are highly relevant products for clinical cell and gene therapies. To transport these cellular products for clinical administration, cryopreservation, formulation, and transportation methods must be developed. There is evidence that MSCs need a refractory phase after cryopreservation, before injection, to allow best in vivo survival and function.[28,29] It is possible that some MSC trials may not be meeting endpoints because the cells are cleared too rapidly due to effects from cryopreservation.[28-30]

If, rather than the "thaw and infuse" method, culture-recovered and hypoxic conditioned cells could be used, the therapy could have higher likelihood to be successful. We have shown retention of MSCs prepared in this manner at higher levels than unconditioned cells.[31] Ten percent of the human MSCs are retained at the 1-month time point using our methods, after IM injection, compared to only 1% without the preconditioning.[31] Another key postthaw conditioning protocol could be lowering the sugars in the preconditioning media, since MSCs are slow to switch to glycolysis in times of glucose deprivation, such as in ischemic tissues.[32]

The final certified lot of MSC/VEGF will be banked in liquid nitrogen in the GMP facility. When the patient's clinic date is identified, the GMP facility will receive notice that the cells should be thawed for recovery and formulation 48 hours before injection. Final release criteria are done, and the FDA does not require a 14-day sterility test to be repeated if culture is under 4 days. In the last steps of manufacturing of MSC/VEGF, the cell product is thawed on demand, cultured, exposed to hypoxic conditions, and then formulated to be delivered and released for administration without refreezing (Fig. 1). We have demonstrated that such procedures increase the survival and retention of MSCs in vivo.[31] Consequently, it is anticipated that MSC/VEGF will be more active in promoting therapeutic angiogenesis in CLI than unmodified MSCs or VEGF (gene or protein).

Preformulated product will be received at the administering center in twenty-eight 1-mL syringes, proper chain-of-custody documentation will be in place, and our stability studies indicate that the product is stable for 24 hours during shipping and administration.[33] The product will be administered as 28 IM injections to the index limb isolated to the injection grid within the lower and upper anterior, medial, and posterior aspect of the index leg.

By using the on-demand product thaw, condition, and formulation method, in theory the syringes can be sent out to centers that do not have a marrow transplant unit, which traditionally processes and formulates cells for cell therapy applications or a formal cell processing facility. The clinician at the receiving end would have the syringes ready to administer upon receipt through the investigational pharmacy. As we look to the future of cell therapies, the formulation and packaging and the area of the hospital or pharmacy where cell processing for administration is done at each clinical site will be increasingly important variables for successful and consistent usage of the cellular product.

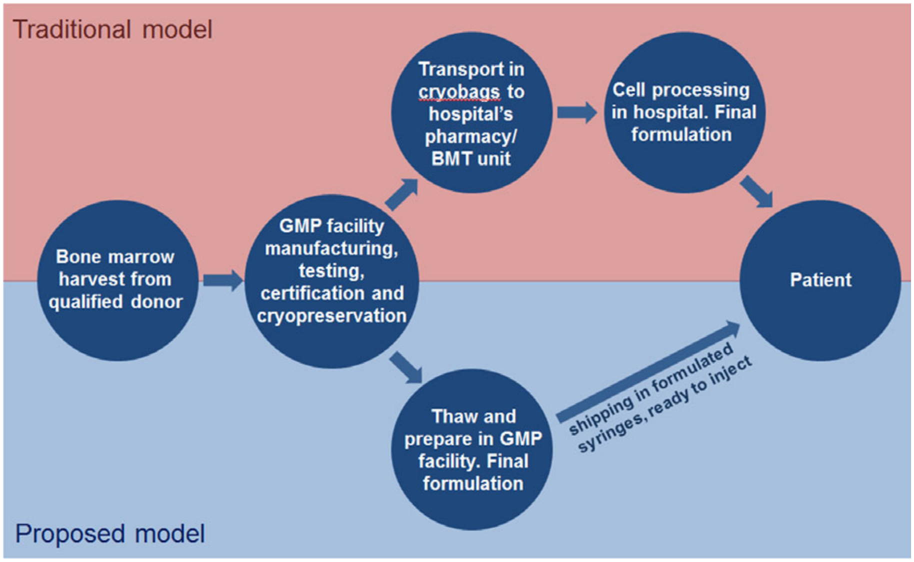

Fig. 1. On-demand formulation strategy. In the traditional model, cells are manufactured in a GMP facility and shipped to the clinical site as cryopreserved units. At the clinical site, the units are checked in through the pharmacy and transferred in the cryoshipper to the hospital cell processing laboratory. The cells are held until the patient is in clinic and then thawed, washed, checked for viability, and formulated into the batch of syringes needed for IM injection in the patient's limb, following a grid. There is much room for inconsistency and variability in cell handling, due to human factors and site-specific conditions. In the proposed model, all formulation is done in a consistent manner in the GMP facility, closely following established standard operating procedures. Orders are placed, and vials of cryopreserved VEGF/MSCs to fulfill the order are thawed, preconditioned, and formulated into batches of 28 syringes in the GMP facility. Syringes are shipped overnight to the clinic for receipt, transfer to the vascular clinic, and injection into the patient. [Color figure can be viewed at wileyonlinelibrary.com]

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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