28. Intravenous Administration of Lentiviral Vectors Expressing Hyperactive Factor IX Converts Severe Into Mild Hemophilia B in a Canine Model

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Lentiviral vectors (LVs) are attractive vehicles for liver-directed gene therapy by virtue of their ability to stably integrate in the genome of target cells and the lack of pre-existing immunity against vector components in most humans. Over the past years, we have developed a LV platform that can achieve stable transgene expression in the liver, induce transgene-specific immune tolerance and establish correction of hemophilia in mouse models upon systemic administration. This LV is designed to stringently target transgene expression to hepatocytes through transcriptional and microRNA-mediated regulation. We then investigated the efficacy and safety profile of portal vein administration of LVs expressing wild-type, codon-optimized (c.o.) or c.o. and hyperactive factor IX (FIX) in a canine model of hemophilia B. We produced large-scale batches of LVs qualified for in vivo administration and treated adult hemophilia B dog by portal vein administration. We observed long-term stable reconstitution of canine FIX activity up to 1% of normal and significant amelioration of the clinical phenotype in 3 treated dogs (>9 years cumulative follow up). LV infusion was associated with transient signs of inflammation and mild hepatotoxicity, which could be abrogated by pretreatment with anti-inflammatory drugs. There was no detectable long-term toxicity or development of FIX inhibitors. In the perspective of clinical translation and to increase therapeutic efficacy, we next treated an 11-kg, hemophilia B dog by peripheral vein administration of LVs expressing the c.o. and hyperactive canine FIX at a 5-fold higher dose than those previously administered. At the current follow-up (3 months after gene therapy) FIX activity is 6-9% of normal. Intravenous LV administration, coupled with a 1-day anti-inflammatory and anti-histamine pre-treatment, induced mild and self-limiting leukopenia and elevation of aminotransferases. Treatment of more hemophilia B dogs is underway to confirm and extend these results. Overall, our studies, which suggest comparable efficacy of LV by both portal and peripheral vein administration, position LV-mediated liver gene therapy for further pre-clinical development and clinical translation. LVs may thus complement other available vectors to address some of the outstanding challenges posed by liver gene therapy of hemophilia and conceivably other diseases.

29. Clinical Trial Showing EPO-Independence for 7 Months by Prolonged Secretion of Autologous EPO by TARGT™

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Recombinant human EPO (rHuEPO) along with iron supplementation corrects anemia in most patients with End Stage Renal Disease (ESRD) but is associated with supra-physiological peak serum concentration (C_{max}) of EPO and may cause thromboembolic complications.

The Transduced Autologous Restorative Gene Therapy system (TARGT™) is an *ex-vivo* gene therapy that provides autologous, continuous proteins or peptide therapy in the physiological range. The system consists of several 2 x 30 mm pieces of dermal tissue (Micro-Organ, MO), extracted under local anesthetic in which its fibroblasts cells are transduced with a Helper-Dependent Adenoviral Vector (HDAd) containing the EPO gene expression cassette. After culture, and measurement of EPO production, one or more transduced MOs (TARGTs) are re-implanted as required for delivering the target dose. Patients are treated with local steroid injection to stabilize secretion. The system allows dose flexibility and the TARGTs may be removed or added according to the *in-vivo* secretion levels.

We present here initial results from an-ongoing open label ascending dose clinical study of $TARGT_{EPO}$ in patients with anemia due to ESRD. We have completed the enrollment of patients in the first out of 3 cohorts (the low dose) with 6 EPO-dependent patients treated with a total of up to 3 $TARGT_{EPO}$ units each, secreting a total of 25 IU/Kg/day of autologous EPO. All patients continued their previous regimen of intravenous supplemental iron.

Patients follow-up post implantation is still ongoing with one patient being followed with stable EPO secretion and resulting stable Hb for over 7 months. Results obtained suggest stabilization of serum EPO levels at the physiological range of £20 mIU/ml and the resulting Hb levels between 9-12 g/dL without rHuEPO or transfusion while TARGTs are still functioning. Comparative analysis of serum EPO levels revealed significantly lower C_{max} with $TARGT_{EPO}$ compared to rHuEPO. Also, comparison of extrapolated Area Under the Curve (AUC) of rhEPO *vs.* actual $TARGT_{EPO}$ AUC, revealed that $TARGT_{EPO}$ maintained Hb within the desired range in patients with an order of magnitude smaller exposure to EPO compared to rHuEPO. This observation may have significant clinical benefits. No treatment related serious adverse events have been reported. $TARGT_{EPO}$ is a promising novel therapy for anemia and potentially for other protein deficient diseases.

30. Phase II Clinical Trial of Gene Therapy for Adenosine Deaminase-Deficient Severe Combined Immune Deficiency (ADA-SCID) Using a γ-Retroviral Vector

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We report follow-up of subjects treated in a Phase II study of gene therapy for ADA-SCID. Between 2009 and 2012, ten ADA-deficient SCID patients were treated by γ-retroviral-mediated gene transfer (MND-ADA) to their bone marrow CD34+ cells. The subjects were given non-myeloablative chemotherapy (busulfan @ 90 mg/m2) and were withdrawn from PEG-ADA enzyme replacement therapy (ERT) prior to infusion of autologous gene-modified cells. Subject age at the time of treatment ranged from 3 months to 15 years (median = 11.5 months). Follow-up times range from 2 to 5 years. All but one subject, who was 15-years old at the time of treatment, remain off PEG-ADA ERT with immune reconstitution that reached maximal level between