correlated with the formation of higher-titer anti-FVIII antibodies. On the other hand, for the HemA mice treated with G-LVs, the blood loss increased with increasing dosages of G-LVs except the mice treated with high-titer G-LVs, in which FVIII proteins produced in megakaryocytes leaked into circulation and induced anti-FVIII antibodies. Most interestingly, partial phenotypic correction of the mice treated with low to medium-titer of G-LVs were achieved and maintained at day 160. In order to induce long-term immune tolerance to the transgene hFVIII in the HemA mice treated with intra-bone marrow injection of E-LVs or G-LVs, different immunosuppressive agents are also investigated. These data indicate direct transduction of bone marrow cells by LVs has the potential to correct the HemA phenotype in unconditioned mice.

829. Evaluation of Liver Gene Therapy by Lentiviral Vectors in a Dog Model of Hemophilia B
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Gene therapy holds promise as a cure for hemophilic patients. We previously demonstrated phenotypic correction of hemophilia B after a single administration of a microRNA-regulated lentiviral vector (LV) encoding FIX in a mouse model. We have now tested our gene therapy strategy in hemophilia B dogs, a spontaneous large animal model of the disease. We administered intraportalily 1.5x10^{10} LV integration units to an 8-month old, 20 Kg hemophilia B dog. The infusion was well tolerated and uneventful with only minor self-limiting hepatocellular toxicity. At the current follow up (19 months post infusion) the dog is alive and well and has experienced no spontaneous bleeds for more than 1 year. Whole blood clotting time has been stably shortened and anti-canal FIX inhibitors tested negative. Quantitative measurement of canine FIX activity by aPTT showed 0.2% of normal levels. While these results are encouraging, FIX levels are still below the therapeutic threshold of 1% of normal. Since increasing the dose represents a challenge for the current manufacturing capacity of purified LV, it becomes crucial to maximize the LV potency (efficacy per dose). Moreover, while liver gene therapy by microRNA-regulated LV has proved safe in hemophilic mice, concerns remain regarding the risk of insertional mutagenesis and a careful assessment of the safest vector design still needs to be performed before clinical translation. In order to increase the potency of our LV for liver-directed gene therapy, we compared the performance of four different hepatocyte-specific promoters in terms of efficiency and safety. First, we assessed promoters’ activity in hemophilia B mice. We then assessed the potential for gene transactivation at and around the integration site of the different promoters, taking advantage of site-specific integration into two genomic loci in hepatic cell lines. Our results show that whereas all the promoters have the potential of deregulating neighboring genes, the choice of a strong synthetic promoter such as the ET does not result in an increased risk of perturbation of endogenous gene expression. To assess the impact of this finding in vivo, we are monitoring the distribution of vector integrations over time in normal and tumor-prone mice. We then evaluated a codon-optimized FIX transgene to maximize protein output from a single expression unit. By this approach we achieved 4-fold higher clotting activity in hemophilia B mice treated with a matched dose of re-coded as compared to the original vector. Based on these results we are now treating a dog with the codon-optimized LV and we will report short-term follow up of this experiment. The preclinical evaluation of our strategy, which includes feasibility, safety and efficacy evaluation in large animals and optimization of efficacy and safety in mice, will allow us defining the risk-benefit ratio of our strategy in view of clinical translation.

830. Targeting B Cells To Express a Tolerogenic Fusion Protein Using Pseudotyped Lentiviral Vectors Displaying a Single Chain Antibody Against Human CD20
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Antigen-specific tolerance induction is an important goal that has great potential in preventing or reversing undesirable immune responses. Previously, we utilized B-cell delivered gene therapy to achieve this goal in multiple mouse models for human autoimmune diseases and inhibitor formation to clotting factor VIII (FVIII) in hemophilia. In our model, syngeneic B cells are retrovirally transduced ex vivo to express an antigen-immunoglobulin G (IgG) fusion protein. Such B cells are highly tolerogenic upon adoptive transfer to naive or even primed recipients. In the current study, we focus on creating a gene therapy vector that can directly target B cells in vivo, and aim to apply this to treat inhibitor formation to FVIII. We have taken advantage of the recently developed lentiviral vector re-targeting technology that utilizes a virus pseudotyped to display a human CD20 single chain antibody. Because the C2 domain of FVIII is an important target for hemophilia inhibitors, we PCR amplified the C2 as a fusion protein with a murine IgG1 heavy chain. This was cloned upstream of the GFP in the pS2E1 transduction plasmid. The αCD20-C2-Ig-GFP lentiviral vector was generated by co-transfection of 293T cells with the transfer plasmid together with two other packaging plasmids. The generated HIV-1 lentiviral vector was pseudotyped with measles virus glycoproteins displaying a single chain anti-CD20 antibody. As a control, a chicken ovalbumin (OVA) expressing vector, αCD20-OVA-Ig-GFP, was also generated. As expected, the αCD20-C2-Ig-GFP supernatant efficiently transduced up to 70% of the CD20 expressing human B cell line, Raji cells, in terms of GFP expression analyzed by flow cytometry. In addition, the αCD20-C2-Ig-GFP vector selectively transduced B cells from human PBMC in vitro. Our next step is to infect mouse B cells expressing human CD20 transgene, which can be used to test the tolerance effect using this vector in hemophilia A (FVIII-/-) mice that express human CD20 transgene. Our results could facilitate the clinical translation of the B-cell gene therapy for tolerance induction purpose (Supported by NIH grant AI035622 and HL061883 to DWS).

831. BAFF Inhibition by Exon Skipping in Sjögren’s Syndrome
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In patients with Sjögren’s syndrome (SjS), a disease mainly affecting the excretory organs, B-cell activating factor of the TNF family (BAFF) is increased. Inhibition of BAFF using antibodies or soluble receptors improves the course of animal models of