storage disorders). Overall, stem cell gene therapy provided stable TP expression and long-term biochemical correction in MNGIE mice without genotoxicity or apparent phenotoxicity, which will be further evaluated for somatic and neurological phenotype correction and optimized to develop a clinical protocol to treat MNGIE patients.

280. Combination of Low-Dose Gene Therapy and Monthly Enzyme Replacement Therapy Improves the Phenotype of a Mouse Model of Lysosomal Storage Disease

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Enzyme replacement therapy (ERT) is the current standard of care for Mucopolysaccharidosis type VI (MPS VI) that is caused by deficiency of arylsulfatase B (ARSB), which results in widespread accumulation and excretion of toxic glycosaminoglycans (GAGs). However, ERT is associated with inconvenient multiple and costly administrations and fails to ameliorate cardiac, visual and bone abnormalities.

To overcome ERT limitations, we developed a successful gene therapy approach based on a single administration of AAV2/8 that targets liver of MPS VI animal models. Importantly, we showed that a single systemic administration of AAV2/8 at high doses (2x10e12 gc/kg) is at least as effective as the current ERT therapeutic regimen based on weekly infusions of recombinant human ARSB (rhARSB). If this translates to humans, gene therapy could replace ERT for MPS VI. However, the administration of high doses of AAV2/8 requires a challenging and costly production process, and results in cell-mediated immune responses that eliminate transduced hepatocytes. Similarly, weekly ERT infusions are costly and require high patient compliance.

We therefore evaluated in a mouse model of MPS VI whether the combination of low doses of AAV2/8 at 6x10e11 or 2x10e11 gc/kg with a rarified ERT schedule (1mg/kg once a month) may be as effective as the single treatments at high doses or frequent regimen.

Significant increase of ARSB activity was found in liver of all treated mice. Detectable but low activity was variably observed in spleen and kidney and was associated with significant reduction of tissue GAGs, regardless of treatment and ARSB activity levels, similarly to what observed in mice treated with high doses of AAV2/8 or weekly ERT. This supports previous data indicating that low enzymatic levels improve MPS VI visceral phenotype. Evaluation of GAG storage in myocardium and heart valves is in progress.

Urinary GAG, which are a sensitive biomarker of systemic clearance of lysosomal storage and, thus of therapeutic efficacy, are slightly reduced in mice treated with either monthly ERT or 2x10e11 gc/kg of AAV2/8. The reduction is more consistent at 6x10e11 gc/kg. Importantly, urinary GAG decreased more in mice receiving the combined therapy than in those receiving single treatments. In particular, urinary GAG reduction in mice treated with both 6x10e11 gc/kg of AAV2/8 and monthly ERT was comparable to that obtained following administration of either high doses of AAV2/8, i.e 2x10e12 gc/kg, or weekly ERT.

The data collected so far show similar efficacy between low-dose gene therapy combined with rarified ERT and the corresponding single treatments at high doses or frequent regimen. This should increase the safety and reduce the risks and costs associated with both therapeutic approaches.

Cancer – Targeted Gene and Cell Therapy II

281. Engineering Hematopoiesis for Tumor-Targeted Interferon-alpha Delivery Inhibits Multuple Myeloma and B Cell Malignancies

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A protective and immunosuppressive tumor microenvironment is considered a key factor for the failure of anti-cancer treatments. We developed a strategy to turn a pro-tumoral into an anti-tumoral microenvironment by transplanting genetically engineered hematopoietic stem/progenitor cells (HSPC). We exploit a transcriptionally and post-transcriptionally regulated Interferon-alpha (IFNa) cassette to drive specific expression of this pleiotrophic anti-tumor cytokine in tumor-infiltrating monocytes/macrophages (IFNa gene therapy). When applied to spontaneous mouse or orthotopic human breast cancer models, IFNa gene therapy with mouse or human HSPC, respectively, inhibited tumor and metastases growth by activating innate and adaptive immune cells in the tumor (Escobar et al, Sci Transl Med 2014). To test this approach in clinics, we aim for a first-in-human trial of IFNa gene therapy in patients undergoing autologous transplantation, a procedure widely practiced for Multiple Myeloma (MM) and Lymphoma. To detect potential untoward effects of chronic IFNa exposure on human hematopoiesis, we transduced mobilized peripheral blood CD34+ cells with the human IFNa lentiviral vector (LV). A single round of transduction yielded vector copy numbers (VCN) of 0.4 to 4 according to the protocol used, with 30-90% transduction efficiency and 1-4 VCN per cell. This IFNa-engineered graft was mixed with mock-transduced CD34+ cells in various proportions and transplanted into NSG mice. All mice engrafted and showed long-term, multilineage hematopoietic output, with a dose-dependent decrease in the human graft at high, supratherapeutic VCN. To explore the efficacy of IFNa gene therapy on MM, we intravenously injected NSG mice reconstituted with IFNa LV-transduced CD34+ cells (VCN:1-3; chimerism 20-50%) with the human MM.1S cell line and followed MM growth by bioluminescence imaging and serial blood and BM exams. IFNa gene therapy strongly delayed myeloma bone disease and improved survival (9 wks vs. 13 wks median survival, p=0.03). Moreover, we tested the efficacy of IFNa gene therapy on human primary Ph+ B-ALL blasts as a model of high-grade B cell malignancies. Strikingly, IFNa gene therapy substantially reduced B-ALL growth in NSG and NSG3GS mice (p=0.002 and p=0.015), and Imatinib treatment, the standard of care for Ph+ALL, gave an additive effect when combined with IFNa gene therapy. These encouraging results pave the way for a phase I/II trial in patients with MM or B cell malignancies. To better understand the mechanism of IFNa gene therapy, we are setting up immuno-competent mouse models of MM and lymphoid malignancies. We developed a spontaneous model of high grade B cell leukemia/lymphoma based on ectopic expression of miR-126. IFNa gene therapy significantly reduced leukemic burden. Moreover, we derived more immunogenic leukemic subclones engineered with the ovalbumin or huCD20 antigen. These lines will help defining the immunomodulatory effect of IFNa gene therapy and allow testing combination with monoclonal antibodies or adoptive T cell transfer.