3966 Administration of FVIII-Expressing Human Placental Cells to Juvenile Sheep Yields Multi-Organ Engraftment, Therapeutic Plasma FVIII Levels and Alter Immune Signaling Pathways to Evade FVIII Inhibitor Induction

63rd ASH Annual Meeting and Exposition, December 11-14, 2021, Georgia World Congress Center, Atlanta, GA

Program: Oral and Poster Abstracts

Session: 801. Gene Therapies: Poster III

Hematology Disease Topics & Pathways:

Bleeding and Clotting, Biological, Translational Research, Hemophilia, Genetic Disorders, Immune Mechanism, Diseases, Gene Therapy, Therapies, Adverse Events, Biological Processes, Transplantation

Monday, December 13, 2021, 6:00 PM-8:00 PM

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We have previously reported that normal juvenile sheep that received weekly intravenous (IV) infusions of human (n=3) or an expression/secretion-optimized, bioengineered human/porcine hybrid (ET3) FVIII protein (n=3) for 5 weeks (20 IU/kg) developed anti-FVIII inhibitory antibodies (10-116 BU, and IgG titers of 1:20-1:245) by week 3 of infusion. By contrast, the IV infusion, or IP administration, of human placental mesenchymal cells (PLC) transduced with a lentiviral vector encoding a myeloid codon-optimized ET3 transgene (PLC-mcoET3) to produce high levels of ET3 protein (4.9-6IU/10^6 cells/24h) enabled the delivery of FVIII without eliciting antibodies, despite using PLC-mcoET3 doses that provided ~20-60 IU/kg ET3 each 24h to mirror the amount of FVIII protein infused. In addition, we showed that the route of PLC-mcoET3 administration (IP vs IV) did not impact the resultant plasma FVIII levels, with animals in these two groups exhibiting mean

increases in FVIII activity (quantified by aPTT) of 30.9% and 34.2%, respectively, at week 15 post-treatment. Here, we investigated whether the sites and levels of PLC-mcoET3 engraftment were dependent upon the route of administration and performed s sheep-specific multiplexed transcriptomic analysis (NanoString) to define the immune signaling pathways that thwarted FVIII/ET3 protein immune response when ET3 was delivered through PLC. Tissue samples were collected from various organs at euthanasia and RT-qPCR performed using primers specific to the mcoET3 transgene, to the human housekeeping transcript GAPDH, and to sheep GAPDH, to quantify PLC-mcoET3 tissue engraftment, and normalize the results. RT-qPCR demonstrated PLC-mcoET3 engrafted, in both IP and IV groups, in all the organs evaluated (liver, lung, lymph nodes, thymus, and spleen). Animals that received PLC-mcoET3 via the IP route displayed higher overall levels of engraftment than their IV counterparts. The spleen was the preferential organ of engraftment for both IP and IV groups (IP:2.41$\pm$1.97%; IV: 0.64$\pm$0.54%). The IP group exhibited significantly higher engraftment in the left lobe of the liver (IP: 1.36$\pm$0.35%; IV: 0.041$\pm$0.022%), which was confirmed by immunohisto-chemistry (IHC) with an antibody to the human nuclear antigen Ku80 and ImageJ analysis (IP:5.24$\pm$3.36%; IV: 0$\pm$0). Of note is that the IP route resulted in higher levels of engraftment in the thymus, while IV infusion yielded higher levels of PLC-mcoET3 in lymph nodes. Analysis of H&E-stained tissues demonstrated they were devoid of any abnormal histologic changes and exhibited no evidence of hyperplasia or neoplasia, supporting the safety of the cell platform, irrespective of the route of administration. To date, NanoString analysis of PBMC collected at day 0, week 1, and week 5 post-infusion demonstrated that animals who received FVIII protein had upregulation of UBA5 and BATF, genes involved in antigen processing and Th17 signaling pathways, respectively. Although both IV and IP recipients of PLC-mcoET3 also had an increase in BATF, the IV group exhibited upregulation of BTLA, a gene involved in immune-tolerance, and downregulation of NOTCH and DDL1, involved in T cell differentiation, as well as MAPK12 and PLCG1, genes involved in proinflammatory cytokine regulation and T signaling within the Th17 signature. In IP recipients, BTLA, NOTCH, and DLL1 were all downregulated. Since ET3-reactive Th_1 cells were not present in any of the treated animals, it is possible that the Th17 cells are responsible for the inhibitory antibodies seen in the juvenile sheep treated with FVIII/ET3 protein, while in animals receiving PLC-mcoET3, downregulation of genes involved in T cell differentiation and proinflammatory cytokine signaling keeps the immune system in check to avoid an immune response.

Disclosures: Doering: *Expression Therapeutics:* Divested equity in a private or publicly-traded company in the past 24 months. **Spencer:** *Expression Therapeutics:* Divested equity in a private or publicly-traded company in the past 24 months.