Abstract # 2197

Induced Pluripotent Stem Cell Gene-Editing Therapy In An Infertile Mouse Model To Restore In Vivo Spermatogenesis. Amanda Colvin Zielen, University of Pittsburgh, USA

Approximately 1% of men in the general population have azoospermia where sperm is absent from their ejaculate. Non-obstructive azoospermia (NOA) is more common (~85%) than obstructive azoospermia; 15% of NOA patients also have a maturation arrest phenotype (NOA-MA) where germ cells are present in the testes but fail to complete spermatogenesis, likely due to a genetic defect. Sperm recovery rates from men with NOA-MA are relatively low, severely limiting their options for having biological children. We hypothesized that if a single gene mutation is identified to cause NOA-MA in a patient, then ex vivo gene-editing of germ cells will restore gene function and in vivo transplantation of gene-corrected germ cells will regenerate spermatogenesis in infertile males. To test our hypothesis, we used CRISPR/Cas9 gene-editing to produce mice with an 11 base-pair deletion in the minichromsome maintenance 8 (Mcm8) gene at a location analogous to one of our Pittsburgh patients. Mutations in MCM8 are associated with male and female infertility as well as DNA damage/repair defects and cancer in clinical and murine studies. Mcm8-11/-11 mice exhibited an NOA-MA infertility phenotype. Homozygous Mcm8(-11) male mice were unable to sire offspring when paired with wildtype females, while heterozygous Mcm8+/-11 mice sired 3.6±0.13 litters in five months with 7±0.8 pups per litter. Compared with normal littermate controls, Mcm8-11/-11 testes were significantly reduced in size (Mcm8+/+ 115±4.9mg per testis, n=10; Mcm8+/-11 were 108±4.3 mg, n=20; Mcm8-11/-11 were 20.2±1mg, n=7). No sperm were recovered from the tail of the epididymis of Mcm8-11/-11 mice, and there was not a significant difference (p=0.25, t-test) in sperm counts (Mcm8+/+ 8.9±1.4 x106 sperm; Mcm8+/-11 6.6±1.0 x106 sperm) of wildtype and heterozygous mice. Hematoxylin and eosin staining of eight-week old Mcm8-11/-11 testis sections showed tubules varying between 0-3 germ cell layers, while heterozygous and wildtype sections had tubules with complete spermatogenesis. Preliminary immunohistochemistry staining identifies undifferentiated spermatogonia (SALL4), differentiating spermatogonia (STRA8), and spermatocytes (SYCP3) in Mcm8-11/-11 testicular sections; we are currently quantifying our results. Attempts to establish spermatogonial stem cell (SSC) lines from these animals for ex vivo gene-editing were not successful. Therefore, we isolated fibroblasts from Mcm8-11/-11 mice and programmed them to become induced pluripotent stem cells (iPSCs). Three novel Mcm8-11/-11 mouse iPSC lines were generated, cloned, and characterized (alkaline phosphatase staining, immunocytochemistry staining with pluripotency markers: OCT-4, SOX-2, NANOG, and SSEA-1; along with teratoma formation and karyotype analyses). A validated Mcm8-11/-11 iPSC clone was gene-edited using CRISPR/Cas9 and an oligonucleotide template containing the wildtype Mcm8 sequence. We identified four clones with one Mcm8 allele corrected back to the wild type (Mcm8+/-11). Gene-edited (Mcm8+/-11) clone 14 was differentiated to primordial germ-cell-like cells (PGCLCs) that exhibited a SSEA1+/CD61+ phenotype. FACS sorted, gene-edited PGCLCs were transplanted into four 5-week old W/Wv mice. Breeding trials are ongoing. This work was supported by NIH grants P50 HD096723 to KEO, T32 HD087194 to ACZ, and the Magee-Womens Foundation.