stimulation paralleled that of endogenous CD40L in unedited T-cells. Additionally, activated T-cells expressing edited CD40L were able to bind CD40-Ig similar to unedited cells. When T-cells from X-HIGM patients were edited in this fashion, CD40L expression and CD40-Ig binding was restored, and repaired activated X-HIGM T-cells induced the expression of early activation markers and class switching of naïve B-cells in vitro. Finally, we found no differences in TCR repertoire or engraftment and stability in NSG mice between T cells that underwent gene editing vs. unedited cells. These results demonstrate the feasibility of site-directed gene repair to restore normally regulated CD40L protein expression and functional T-cell help.

483. Epigenetic Silencing of Hepatitis B cccDNA In Vitro and In Vivo Using AAV-Delivered Engineered Repressor Transcription Activator-Like Effector

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Hepatitis B virus (HBV) infection is hyper-endemic (>8 % chronic carriers) to parts of Asia and sub-Saharan Africa. Persistent HBV infection predisposes to liver diseases such as cirrhosis and hepatocellular carcinoma. Current anti-HBV therapies face several limitations. RNA interference-based therapies lack the robustness and specificity required for therapeutic effect while interferon-α and nucleotide/side analogs function post-transcriptionally and thus allow for the persistence of the covalently closed circular (cccDNA). cccDNA may persist indefinitely and enables re-initiation of HBV replication after withdrawal of treatment. Disabling the cccDNA is essential for the successful treatment of chronic HBV. Our group previously described effective anti-HBV transcription activator-like effector (TALE) nucleases (TALENs). Although potentially useful to counter HBV replication, one drawback is that viral sequences integrated into the host genome may be susceptible to digestion by the TALENs. Transcriptional silencing, rather than cleavage, of cccDNA may therefore be preferable to avoid causing undesirable mutations in the host. To this end, TALE binding domains designed to target the viral preS2 promoter and the basic core promoter/enhancer II regions were fused to a Krüppel-associated box repressor domain to generate repressor-TALEs (rTALEs). These rTALEs were shown to inhibit viral replication *in vitro* and *in vivo* without inducing significant toxicity. In an *in vivo* murine model using hydrodynamic transfection with an HBV replication-competent plasmid, a reduction in secreted HBV surface antigen (HBsAg) of 97% and 98% was seen at day 3 and 93% and 96% at day 5 for P1L and P1R respectively. To develop this approach as a feasible therapy, rTALE-encoding sequences were incorporated into recombinant adeno-associated viruses (rAAVs) and assessed in the HepG2.hNTCP-C4 cell line. These cells overexpress the HBV receptor, human sodium taurocholate co-transporting peptide (hNTCP). The HepG2.NTCP-C4 cell line is infectable with HBV and viral gene expression is dependent on formation of cccDNA. Measurement of viral markers of replication may thus be used as an indicator of inhibitory effects on cccDNA. The anti-HBV efficacy of the rTALEs and epigenetic modification of the targeted HBV DNA was characterized. Chromatin immunoprecipitation assays determined the binding of the rTALEs to the cccDNA and induction of epigenetic markers of viral gene suppression. Mobility shift assays confirmed specificity of rTALE binding. *In vivo* efficacy of rAAV-delivered rTALEs was evaluated in transgenic HBV mice. Our study provides valuable information on the potential therapeutic utility of rTALEs and demonstrates the feasibility of the approach for treatment of HBV.

484. Preclinical Proof of Concept of Transcriptional Silencing and Replacement Strategy for Treatment of Retinitis Pigmentosa Due To RHODOPSIN Mutations

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Silencing and replacement strategy is a promising approach to overcome mutational heterogeneity of genetic defects. In autosomal dominant retinitis pigmentosa (adRP) due to rhodopsin gene (RHO) approximately 200 different mutations have been described, posing a challenge for the design of effective therapeutics.

We designed a silencing and replacement strategy based on transcriptional silencing through an artificial zinc finger DNA-binding protein lacking effector domains (ZF6DBD), and tested both efficacy and safety in two animal models.

In a murine model of adRP, we show that AAV-mediate retinal delivery (AAV2/8-CMV-ZF6-DBD) is associated with selective transcriptional silencing of the human mutated allele resulting in morphological and functional (Electroretinography, ERG a-wave and b-wave responses) rescue. We then tested the effect of transcriptional silencing in the porcine large pre-clinical model. Delivery of a low dose (AAV2/8-CMV-ZF6-DBD, 1x10e10 vector genomes, vg) of the ZF6 transcriptional silencer to the porcine retina resulted in robust transcriptional silencing of the endogenous porcine RHO transcript. Cell sorting of transduced photoreceptors showed an almost complete RHO transcriptional silencing effect (90% RHO transcriptional repression), underscoring the potency of the system. To determine the safety of the zinc-finger silencer we performed extensive RNA-seq analysis on treated and control retinae. The data sets generated demonstrate selective RHO gene transcriptional repression and a remarkably low number of differential expressed genes (DEGs), supporting specificity and thus, safety. The co-administration to the porcine retina of the AAV-ZF6 silencer (AAV2/8-CMV-ZF6-DBD) and the AAV-RHO replacement (5x10e11 vg, AAV2/8-GNAT1-HumanRHO) constructs resulted in a balanced silencing and replacement effect. This data support the use of zinc-finger based RHO transcriptional silencing for the development of a clinical trial for adRP patients.

485. Targeted Epigenome Editing by CRISPR/Cas9-Based Repressors for Silencing of Distal Regulatory Elements

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The CRISPR/Cas9 genome engineering platform has emerged as a powerful tool for gene regulation. Catalytically inactivated, "dead" Cas9 (dCas9) can be fused to a variety of transcriptional modulators to activate or silence gene expression. The KRAB