**ASGCT 2019 – Abstract Joost van Haasteren**

**Title:** Genome Editing Of The Liver For Treatment Of Alpha-1 Antitrypsin Deficiency Using Homology-Independent Targeted Integration (HITI)

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**Abstract (3500 characters excluding spaces):**

Homology directed repair (HDR) is often selected for correction of genomic mutations for therapeutic benefit due to its favourable repair fidelity, but activity of the main repair pathway, non-homologous end joining (NHEJ) is several-fold higher in most systems. Using NHEJ, Homology-Independent Targeted Integration (HITI) allows for precise integration of a chosen sequence by cutting both the genome and donor sequence. This approach is applicable for diseases such as Alpha-1 Antitrypsin (AAT) deficiency in which genome editing could allow for expression of functional AAT whilst simultaneously reducing expression of the highly prevalent, toxic PI*Z form of the gene. Here, we assess the feasibility of HITI genome editing for AAT deficiency in liver cell lines and human liver organoids.

A fluorescent reporter 293T cell line containing an mCherry cDNA flanked by two defined murine saCas9 gRNA target sites provided a platform to test four permutations of HITI dependent genomic integration. Each permutation could support a different therapeutic application, using mCherry as a proxy for the target mutant gene. One permutation proved optimal for the treatment of the PI*Z mutant version of AAT. In this permutation, the integration event is targeted to the 5` UTR region of AAT and the integrated cDNA (reporter or therapeutic) is followed by a transcription blocker to prevent expression of the downstream PI*Z allele. Transfection of Huh-7 liver carcinoma cells with a 1:2 molar ratio of SaCas9/HITI Donor plasmid was effective, both in expressing the integrated cDNA of mNeonGreen from the AAT promoter as well as in preventing endogenous AAT expression in ~12% of total cells despite only ~30% transfection efficiency (n=4). A ddPCR copy number variation assay at the 5`-integration junction confirmed this observation. Sequencing showed that HITI integration was almost seamless (~90% in 78 clones from 2 different loci) with any mutations limited to 1 or 2bp indels, implying that NHEJ is less error-prone than expected. Analysis of a pilot capture-seq experiment proved that the majority of the integration events occurred at the intended integration site.

This proof-of-concept in a cell line is currently being translated to more relevant models, including human and murine liver organoids. Human liver samples were obtained, with consent, from liver resection surgery and healthy tissue, as assessed by a pathologist, was processed into liver organoids. Organoids were transfected with plasmids or transduced with recombinant adeno-associated virus (rAAV) as a single cell suspension to allow access to the basolateral membrane of the hepatocytes. Transduction of mouse organoids with rAAV of various serotypes showed that rAAV capsid 6.2 was the most efficient with 45% and 39% of organoids EGFP-positive 2 days post-transduction (n=2 independent experiments). Human liver organoids cultured as immature, actively dividing cells were differentiated into mature liver organoids as evidenced by AAT expression, providing a human liver model for ongoing genome editing studies.

We conclude that HITI is capable of efficiently integrating a DNA sequence into a targeted location in the genome in a variety of therapeutically applicable permutations, one of which can provide potential correction of AAT deficiency regardless of the patients' mutation. Human liver organoids provide a physiologically and genetically relevant *in vitro* model of the human liver, acting as an appropriate stepping stone to *in vivo* studies in mouse disease models.