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different prime-editing guideRNAs (pegRNAs) and nicking guideRNAs (ngRNAs), produced mRNA of these and the prime editor via in vitro transcription and formulated LNPs through microfluidics. We tested gene-editing in different cell-models, using flow cytometry for our fluorescent reporter system (fluoPEER), as well as ddPCR and DTECT, to quantify genomic editing efficiency.

We were able to generate pegRNAs that allowed editing of the *MMUT*-mutation in a mouse cell line with 30% efficiency. We optimized mRNA production and formulation of LNPs for mRNA delivery in rat hepatocytes, human hepatocytes, HEK293T cells, HepG2 cells, and mouse fibroblasts, resulting in an average of 30% editing efficiency *in vitro*.

In this proof-of-concept study, we demonstrate that we can generate mRNA of the prime-editing tools encapsulated in LNPs to gene-edit different (liver) cell lines with an efficiency that we anticipate to be clinically relevant. Upon validation in animal models, we expect that this strategy may be used to develop truly transformative therapies for patients with (liver dominant) metabolic diseases.

P0638

Leveraging precise genome editing through homology-independent targeted integration (HITI)

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Homology-independent targeted integration (HITI) is a form of genome editing that allows precise insertion of arbitrary sequences via the NHEJ pathway and can be used to disrupt and replace dominant gain-of-function mutations. The HITI approach was investigated as a potential therapeutic strategy for addressing pathogenic mutations in the human *SERPINA1* gene which encodes alpha-1 antitrypsin (AAT). Wild type AAT is produced in the liver and is secreted via the circulation to the lungs, where it functions as a serine protease inhibitor. AAT primarily inhibits neutrophil elastase in the lungs to regulate the inflammatory immune response. Misfolded aggregates of AAT, caused by pathogenic mutations, lead to liver damage through the (gain-of-function) generation of inclusion bodies and also lung damage through loss of wild-type antiprotease function. Here, we modelled therapeutic interventions at the *SERPINA1* locus by integrating a fluorescent reporter transgene (*mNeonGreen)* into the *SERPINA1* exon 1 at a position ideally suited to correct a range of pathogenic mutations. Using droplet digital PCR, a successful integration of the transgene was achieved with an efficiency of 6.3% in cells (HEK293T HITI reporter cell line). Upon investigation, a subset of edited cells showed insertions and or deletions (indels) at the Cas9/gRNA mediated double-strand break sites. However, Amplicon-EZ next generation sequencing (NGS) of 80,000 samples confirmed that these indels were most often limited to only 5–12 bp in length and did not interfere with the desired transgene editing outcome, because the indels exclusively fell in untranslated intronic sequences. Interestingly, a subset of sequences, also identified by NGS, were initially thought to harbour larger indels within part of the inserted *mNeonGreen* expression cassette. In contrast, high-resolution melting (HRM) analysis of such sequences found there was no significant difference in melting temperature (Tm) between such samples and Sanger sequence verified TOPO cloned sequences ($p>0.9999$, $n=9$). The precision of HRM analysis was demonstrated by comparing TOPO clones harbouring sequence-verified indels, with significant differences in Tm observed with as little as 5 bp difference in 331 bb of analysed sequence ($p<0.0001$, $n=9$). Thus, the HITI approach was able to alter the *SERPINA1* locus by inserting a 1.6 kb reporter transgene via NHEJ without indels affecting the function of the inserted sequence. Adapting this editing approach to use a therapeutic *SERPINA1* cDNA has the potential to address AAT deficiency. This approach potentially can be used to address other dominant gain-of-function disorders where disruption of pathological sequences and replacement with fully functional native sequences are simultaneous therapeutic goals.

P0639

Zip Editing: an easy-to-use tool to increase CRISPR-Cas9 HDR-editing efficiency

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Genome editing using CRISPR-Cas9 holds great promise in the treatment of genetic diseases as a safer alternative to additive gene therapy. Cas9 nuclease is very efficient to introduce double-strand breaks. Those are repaired by end-joining pathways resulting in insertions and deletions (InDels) or by homology-directed repair (HDR) using an exogenous template to introduce a desired modification. The latter repair mechanism is less frequently used by cells because it occurs only in S-G2 cell cycle phases and depends in part on the availability of the exogenous template at the site of editing at the moment of the repair. To increase the presence of the exogenous template delivered as single-strand DNA oligonucleotides (ssODN), several solutions has been developed to import it with the RNP complex (Cas9 + gRNA) but mostly rely on modifications of the Cas9 nuclease to link the ssODN. We propose a new editing tool, called Zip-Editing (ZE) to import the ssODN template with the RNP complex that doesn't rely on Cas9 modification. It is also not based on the modification of the cell cycle, or of DNA repair mechanisms, which can have deleterious genotoxic effects and are a major safety concern in the development of gene therapies. Thus, this system can be used with commercially available Cas9s, gRNAs and ssODNs and can also be very easily adaptable to a new target to introduce any type of modification. We tested ZE to edit precisely different targets (eGFP, UROS, CFTR) in several cell lines (HEK-293T, K562) and in primary cells (human foreskin fibroblasts, human pulmonary basal and nasal cells, human hematopoietic stem and progenitor cells) for some relevant for gene therapy of genetic diseases. We reached an increase in HDR-editing efficiency up to 12-fold as compared to a condition where ssODN template is not imported with the RNP complex. These results are very encouraging to impose ZE as a new tool to precisely edit the genome and to be part of the gene editing toolbox to treat or model diseases.