Session Gene Targeting and Gene Correction I

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172 - Evaluation of On-Target and Off-Target Precision of AAVHSC-Mediated Genome Editing

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Keywords
Gene Correction/Modification/Targeting,AAV Vectors,Genetic Diseases

Authors
Jason B. Wright, Serena Dollive, Thia St.Martin, David Knowlton, Hillard Rubin, Meghan Scarpitti, Laura Smith, Saswati Chatterjee, Albert Seymour

Research and Development, Homology Medicines Inc., Bedford, MA

Disclosures
J.B. Wright: 1; Commercial Interest ***i.e. Company X***; Homology Medicines Inc..

Abstract
Variation within the human genome underlie a vast spectrum of diseases. The discovery of genome editing technologies now provide the potential to correct pathogenic mutations and bring the possibility to cure genetic diseases. However, the development of effective and safe therapeutic editing technologies will require highly sensitive assays to identify and quantitate the accuracy of genome editing. Thus it is critical to develop unbiased methodologies to identify i) on-target editing errors, including insertions/deletions (indels) that are commonly seen during non-homologous end joining and ii) unintended off-target mutations, including random viral integration. Adeno-associated virus (AAV) vectors are proven nonpathogenic gene therapeutic tools. AAVHSCs (AAV-Hematopoetic Stem Cell derived), a novel, naturally-occurring family of AAVs mediate highly efficient (HR) homolgous recombination-based genome editing (Smith et al ASGCT 2017). Here we measure AAVHSC HR mediated genome editing by targeted insertion of a promoterless fluorescent reporter gene into Intron 1 of the PPP1R12C gene on Chromosome 19 in CD34+ primary human hematopoetic stem cells. Editing was measured using fluorescence expression in parallel with quantitative genotyping by edit-specific droplet digital PCR and next generation sequencing (NGS) of the target site. Analysis of on-target NGS reveal high precision of AAVHSC editing, with an on-target indel rate of less than $2.63e^{-6}$ indels/cell, comparable to the error rate of Taq polymerase. Additionally, there was no sequence evidence of incorporation of viral DNA elements including inverted terminal repeats (ITR). To identify off-target genome alterations, we developed non-hypothesis driven approaches which use the vector genome as bait to capture all sequences in proximity to the vector thus identifying both on-target and off-target genomic integration events. Analysis of whole genome NGS mapping data revealed that 99.972% of captured sequences perfectly map to the genomic target, whereas off-target reads were observed at frequencies comparable or lower than the expected rate of AAV integration, (609 of 2,211,588 reads, $<1.25e^{-4}$ events / cell). Further characterization of off-target insertion revealed that repetitive elements, when included in the vector correlate with off-target insertion events into similar repetitive elements, thus providing valuable insights for improved vector design. Lastly, we have employed these genome editing analytical tools to the development of therapeutic AAVHSC genome editing vectors for the correction of genetic disease. In conclusion, we have developed and employed novel genome editing characterization tools and show that AAVHSC-mediated genome editing is highly precise for on-target editing and is accompanied by rare off target integration events. Thus, this evaluation of AAVHSC-mediated editing provides

a path toward novel genome editing therapeutics for the treatment of human genetic diseases.