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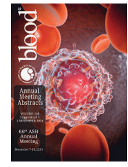

The 66th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

Targeting the *JAK2*-V617F Mutation in Polycythemia Vera Using CRISPR/AAV6 Genome Editing

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Polycythemia Vera (PV) is a chronic myeloproliferative neoplasm characterized by an overproduction of red blood cells (RBCs) and can progress to myelofibrosis and acute myeloid leukemia (AML). In ~95% of patients, PV is associated with the *JAK2*-V617F gain-of-function mutation. This mutation results in constitutive activation of the JAK/STAT signaling pathway, circumventing the normal erythropoietin-dependent regulation of erythropoiesis. Current therapies, such as hydroxyurea, interferon-alfa and ruxolitinib, primarily elicit hematologic and symptom responses, with some potential for reduction of *JAK2*-V617F allele burden, but are not curative. The challenge in developing definitive treatments for PV lies in the dominant nature of the *JAK2*-V617F mutation and the capacity of mutant cells to clonally expand. This necessitates the complete eradication or correction of all mutant cells to prevent disease recurrence. To address these limitations, we present a novel genome editing approach utilizing CRISPR-Cas9 RNP in conjunction with AAV6 delivery, aimed at precise correction of the *JAK2*-V617F mutation in hematopoietic stem cells (HSCs), potentially enabling autologous stem cell transplantation as a curative therapy.
Our genome editing strategy employs a dual-guide CRISPR-Cas9 system designed for high-efficiency correction of the *JAK2*-V617F mutation. This approach utilizes two guide RNAs: one specifically targeting the V617F mutation site, and another directed at an intronic region ~100 base pairs downstream. The mutation-specific guide alone shows 51±7.9% indel formation and consequent mutation deactivation. However, the synergistic effect of the dual-guide system significantly enhances editing efficiency, resulting in ~99% knockout or deactivation of the mutant allele in affected cells. Notably, this approach preserves wild-type alleles, with only intronic indels observed in non-mutant sequences.
To facilitate translational applications, we engineered two homology directed repair (HDR) AAV6 vector constructs with varying homology arm sequences: HA1-JAK2cDNA-PGK-tNGFR and HA2-JAK2cDNA-PGK-tNGFR. Both vectors integrate codon optimized cDNA of the downstream exons along with a PGK promoter-driven truncated nerve growth factor receptor (tNGFR) as a selection marker, allowing MACS purification of edited cells. When applied to HSCs, these vectors successfully edited 43.1±10.2% and 46.8±7.6% of cells, respectively, as determined by the presence of the tNGFR marker. MACS purification yielded enriched populations of edited cells, with 96.6±1.9% and 97.2±1.6% purity for the two vectors, respectively.
To assess the clinical relevance of our genome editing approach, we developed an in vitro model utilizing HSCs isolated from bone marrow aspirates of PV patients, obtained through a Stanford IRB-approved protocol. This model recapitulated key pathophysiological features of PV, including accelerated erythroid differentiation, enhanced erythrocyte proliferation, and an elevated mutant allele frequency. Our editing strategy, applied to patient-derived samples without MACS purification, eliminated 94.8±3.4% of mutant alleles, with 14.9±0.3% of these alleles reverting to the wild-type sequence. Notably, this genetic correction led to functional improvements. In a 14-day erythroid differentiation culture, our approach mitigated the aberrant cellular phenotype, reducing early erythrocyte proliferation and differentiation on day 4 by 7.4±6.6 and 33.4±8.5 fold, respectively. These reductions effectively restored cellular behavior to levels comparable with healthy control HSCs. Crucially, the overall erythrocyte differentiation capacity remained intact post-editing, with 94.8±6.7% of edited patient samples maturing into RBCs as evaluated by CD34-/CD45-/CD71+/CD235a+ immunophenotyping. These findings demonstrate our editing strategy's potential to correct the mutation, normalize cellular phenotypes, and preserve hematopoietic functions.

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These preliminary results demonstrate an early proof of concept for developing a one-time gene therapy for PV. While further long-term studies, including serial transplantation experiments, are needed to assess the impact on clonal expansion, the integration of this genome editing strategy with emerging stem cell engraftment approaches may serve as a starting point for advancing PV treatment.

Disclosures Gotlib: *Incyte:* Consultancy, Honoraria, Research Funding. **Porteus:** *Kamau Therapeutics:* Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees; *Allogene Therapeutics:* Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees; *Versant Ventures:* Other: Advisor; *CRISPR Tx:* Current equity holder in publicly-traded company.

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