(PF1956) DISCOVERY OF A FIRST-IN-CLASS ALLELE-SELECTIVE XNAZYME THAT KNOCKS DOWN MUTANT JAK2 V617F MRNA IN A MOUSE MODEL OF POLYCYTHEMIA VERA

Topic: 15. Myeloproliferative neoplasms - Biology & translational research

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Background

Polycythemia vera (PV) is a clonal myeloproliferative neoplasm driven by the somatic JAK2 V617F mutation in 95% of patients. This results in dysregulated erythroid progenitor proliferation and subsequent erythrocytosis with increased blood viscosity and thrombotic risk. Current JAK2 inhibitors lack selectivity for the V617F mutant allele, thereby interfering with WT JAK2-dependent hematopoiesis and leading to cytopenias. In contrast, allele specific knockdown of JAK2 V617F has the potential to be disease-modifying by preferentially suppressing the mutant clone and reducing variant allele burden while sparing WT JAK2 dependent hematopoiesis.
Therapeutic oligonucleotide modalities such as siRNAs and antisense oligonucleotides are powerful tools for targeting the fundamental genetic drivers of disease on the RNA level, providing opportunity to modify disease at its root, however achieving single nucleotide precision with existing technologies remains extremely challenging.
Here, we present 'X-Zymes', chemically evolved catalytic xeno-nucleotide enzymes (XNAzymes) engineered to enable potent, durable and single base allele-selective RNA silencing in vivo.

Aims

To evaluate the allele selectivity and target engagement of X-Zyme targeting mutant JAK2 V617F in preclinical models of PV.

Methods

Fully modified X-Zymes were designed to selectively cleave JAK2 V617F transcripts while sparing wild-type JAK2. Potency and selectivity were assessed using kinetic cleavage assays and allele-specific knockdown in cellular reporter systems. For in vivo proof of concept, the candidate X-Zymes were formulated with NOV340 liposomes and evaluated in a bone marrow transplantation model of PV (SclCre;JAK2-V617F;GFP). Mice were treated with a single IV dose and target engagement was assessed by RT-qPCR quantification of mutant-selective JAK2 V617F knockdown in sorted erythroid progenitors (BFU-E and CFU-E).

Results

X-Zymes demonstrated potent, allele-selective catalytic cleavage of JAK2 V617F with discrimination against wild-type JAK2 and achieved mutant-selective knockdown in cellular assays. NOV340 encapsulation enabled efficient, dose-dependent delivery of the X-Zyme payload to erythroid populations in both bone marrow and spleen. In the PV mouse model, X-Zyme administration resulted in selective knockdown of the V617F mutant allele, and reduction in the frequency of erythroid progenitor cells (CFU-E/BFU-E).

Summary/Conclusion

X-Zymes enable allele-selective degradation of the mutant JAK2 V617F transcript, a mechanistically distinct approach from JAK inhibitors. X-Zymes were delivered to erythroid progenitors and demonstrated median knockdown of mutant JAK2 V617F mRNA by 70%. These data support a potentially disease-modifying strategy for V617F-driven myeloproliferative neoplasms.