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POSTER ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Systematic Mapping of Gene-Editable Mutations in GATA2 and SAMD9/SAMD9L Syndromes

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Germline predisposition to bone marrow failure (BMF) and myelodysplastic syndromes (MDS) cause substantial economic and psychosocial burden and early mortality in affected individuals. Due to the limitations and risks of hematopoietic stem cell transplantation, there is an urgent need for new and innovative treatment approaches. Conventional lentiviral-based gene therapy is not feasible for most BMF/MDS predisposing genes because their uncontrolled overexpression is detrimental and lentiviral vectors might cause insertional mutagenesis. However, new technologies that precisely modify the endogenous allele can overcome these limitations. Base editing (BE) using cytosine base editors (CBE) and adenine base editors (ABE) as well as prime editing (PE) are advanced gene editing techniques with great therapeutic promise.

The goal of this project is to develop a comprehensive map of gene-editable mutations in the *GATA2*, *SAMD9*, and *SAMD9L* genes as a preclinical effort to facilitate gene editing trials. Germline heterozygous mutations in these key predisposing genes lead to highly penetrant disorders characterized by progressive immunodeficiency and BMF/MDS risk, with particularly high prevalence in children, as recently reported by us (Sahoo, Nature Medicine 2021) and others. For in depth analysis, we focused on the 5 most recurrent mutations in respective genes and devise strategies to correct mutations in cellular models.

We first created a comprehensive database encompassing reported and unpublished pathogenic mutations (single nucleotide variants [SNVs] and <20bp insertion/deletions[indels]). In total, we annotated 506 cases with *GATA2*, 195 cases with *SAMD9*, and 187 cases with *SAMD9L* mutations. SNVs were detected in 83.2% (421/506), 94.4% (184/195), and 93.1% (174/187) patients with *GATA2*, *SAMD9*, *SAMD9L* mutations, respectively, while remaining cases had indels. Among all *GATA2* cases, 59.9% (303/506) mutations are editable by ABE, 4.5% (23/506) by CBE, while remaining 35.6% (180/506) can be "rescued" by PE (**Fig. 1A**). For *SAMD9*, 34.4% (67/195) cases were found to be amenable to editing by ABE, 30.3% (59/195) by CBE, and 35.4% (69/195) using PE. For *SAMD9L*, 47.1% (88/187) are editable by ABE, 20.3% (38/187) by CBE, and 32.6% (61/187) using PE. Having a well-annotated mutation list, we proceeded to identify the top 5 most common mutations in each gene (**Fig. 1B**). Remarkably, all 5 mutations in both *GATA2* and *SAMD9* and 4 out of 5 top mutations in *SAMD9L* and amenable to BE. Next, we explored in depth the target editing window, protospacer adjacent motif (PAM) sites, and bystander editing effect (utilizing BE that recognize the canonical [NGG], and non-canonical PAM sequences [NG and near PAMless SpRY-NRN/NYN]. This stepwise approach resulted in successful identification of at least one potential guide RNA for each mutation with predicted low bystander editing. Ongoing work includes guide RNA validation, off-target analysis, and assessment of gene-editing efficiency using model cell lines.

In conclusion, we have undertaken a systematic analysis of the gene editing landscape for frequent germline driver mutations in BMF/MDS. Our findings reveal promising editing strategies for the majority of prevalent mutations in key predisposing genes *GATA2*, *SAMD9* and *SAMD9L* using CBE, ABE, or PE editors, and lay a groundwork to enable future clinical translation.

Disclosures Tsai: *Ensoma:* Membership on an entity's Board of Directors or advisory committees; *Kromatid:* Membership on an entity's Board of Directors or advisory committees; *Prime Medicine:* Membership on an entity's Board of Directors or advisory committees; *St. Jude Children's Research Hospital and MGH:* Patents & Royalties: S.Q.T. is a co-inventor on genome editing patents including GUIDE-seq and CHANGE-seq.. **Yen:** *Beam therapeutics:* Current equity holder in publicly-traded company; *IMAGO/Merck:* Consultancy. **Wlodarski:** *Novartis:* Honoraria.

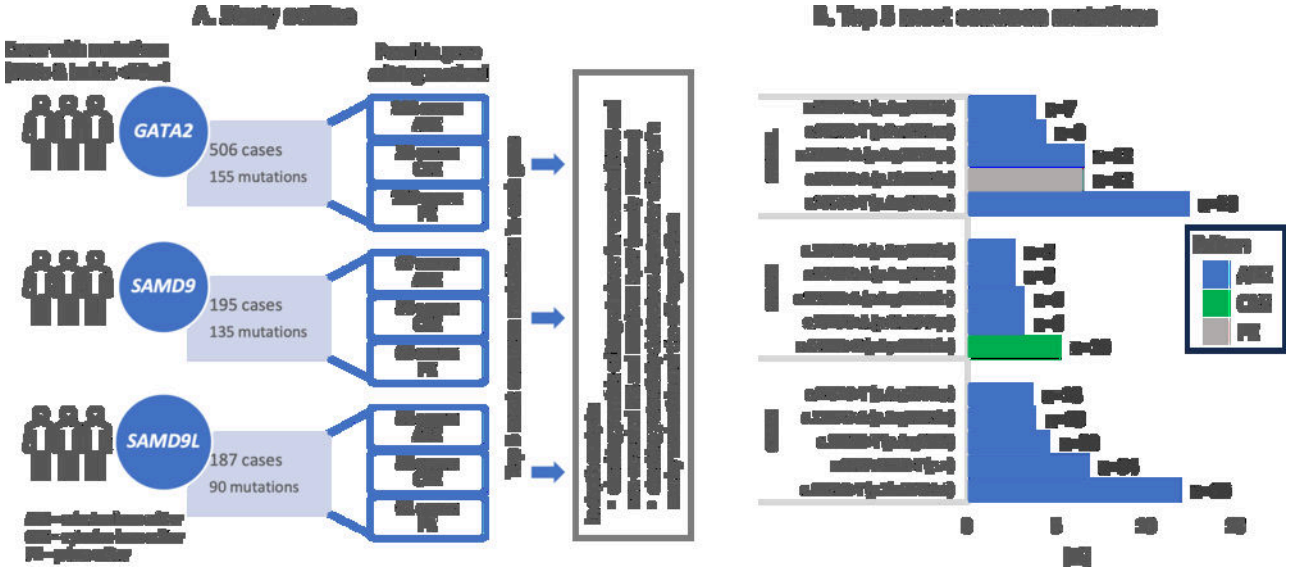

Figure 1

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