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Blood 142 (2023) 313–314

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Mitotic Dysregulation Sensitizes Malignant Stem Cells to CHK1 Inhibition in *SF3B1*-Mutant Myeloid Neoplasms

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Myelodysplastic syndromes (MDS) arise from the acquisition of driver mutations in hematopoietic stem cells (HSCs). Splicing factor SF3B1 mutations are recurrent initiating lesions, found in up to 30% of patients with MDS. *SF3B1* mutations are associated with aberrant splicing marked by recognition of alternative 3' splice sites (a3'ss). However, the causal role of mis-spliced genes in disease pathogenesis remains largely unexplored. Although *SF3B1* mutations and downstream mis-spliced genes provide attractive therapeutic targets, targeted therapies remain unavailable.

Here, we developed a novel method of precise gene editing of CD34 $^{+}$ hematopoietic stem and progenitor cells (HSPCs) to introduce an *SF3B1* K700E mutation at the endogenous locus, enabling stoichiometric expression of mutant protein. An intronic fluorescent marker was introduced upstream of the mutation for isolation of edited cells. Global splicing analysis in *SF3B1* K700E knock-in HSPCs recapitulated canonical targets identified in *SF3B1*-mutant (SF3B1m) MDS patients. Annotation of a3'ss mis-spliced genes showed profound enrichment of gene ontology (GO) terms related to cell cycle regulation, mitotic checkpoint signaling, and chromosome segregation. Consistently, cell cycle analysis of SF3B1m HSCs revealed accumulation of cells in G2/M phase, suggesting a previously unidentified stall in G2/M progression driven by *SF3B1* mutations.

To identify mis-spliced genes that cause this G2/M delay, we compared a3'ss mis-spliced genes in the cell cycle GO category across multiple model systems. BUBR1 and CDC27 were consistently mis-spliced in all datasets, resulting in reduced mRNA and protein levels. BUBR1 is a mitotic kinase involved in the spindle assembly checkpoint and chromosome alignment, and CDC27 is a component of the anaphase-promoting complex. Knockdown of BUBR1 or CDC27 in normal CD34 $^{+}$ HSPCs recapitulated G2/M delay. BUBR1 and CDC27 are thus attractive therapeutic targets to impair cell cycle progression of SF3B1m cells. While there are no direct inhibitors of BUBR1 kinase, BUBR1 is phosphorylated by CHK1 during mitosis, and its expression is highly correlated with response to a selective CHK1 inhibitor prexasertib in a large panel of cancer cell lines (Blosser *et al.*, *Oncotarget* 2020). Indeed, SF3B1m K562 cells and CD34 $^{+}$ HSPCs were highly sensitized to prexasertib. Knockdown of BUBR1 or CDC27 in wild-type cells induced CHK1 phosphorylation, especially upon prexasertib treatment, and sensitized normal cells to prexasertib. These data indicate that mis-splicing of BUBR1 or CDC27 delays G2/M progression leading to CHK1 activation, which sensitizes SF3B1m cells to prexasertib.

To determine if CHK1 inhibition can eradicate SF3B1m HSCs, we leveraged our gene editing system to test drug responses in CD34 $^{+}$CD133 $^{+}$CD38 $^{-}$ primary immunophenotypic HSCs. Prexasertib selectively eliminated SF3B1m HSCs, including high-risk *SF3B1/ RUNX1* and *SF3B1/ STAG2* genotypes, while control AAVS1-edited HSCs were maintained. Furthermore, prexasertib depleted CD34 $^{+}$ cells in primary SF3B1m MDS patient samples. To test prexasertib efficiency *in vivo*, we transplanted NSGS mice with iPSC-derived HSPCs reprogrammed from a patient with high-risk MDS and *SF3B1/ RUNX1* co-mutations, and treated with a standard preclinical regimen. While all the vehicle-treated mice showed consistent engraftment, none of the prexasertib-treated mice were engrafted. Together, these data indicate that prexasertib selectively targets SF3B1m HSCs *in vitro* and eradicates SF3B1m MDS *in vivo*.

In conclusion, we developed a precise gene editing strategy of human HSCs to identify prexasertib as a promising therapy for SF3B1m myeloid neoplasms, and implicate the mitotic function of CHK1 as a SF3B1m sensitivity. The safety and toxicity profiles displayed in early phase clinical trials make prexasertib a suitable agent for further clinical investigation in SF3B1m MDS and its advanced stages.

Disclosures Elena: *Blueprint Medicines Corporation, Cogent and Gilead:* Other: Advisory Board Fees.

https://doi.org/10.1182/blood-2023-188476