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501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Celmod Induced Disruption of Granulopoiesis Continuum Identified Using Humanized Cereblon Mice

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Introduction: CRBN mediated degradation of Ikaros by Pomalidomide (Pom) and Mezigdomide (Mezi) results in robust antiproliferative activity against myeloma cells, with concomitant activation of immune cells such as T and NK cells. However, degradation of Ikaros has been shown to mediate maturation arrest of neutrophils leading to neutropenia. Unlike chemotherapy drugs, CELMoD driven neutropenia is an on-target adverse effect. Although the next generation cereblon E3 ligase modulators are potent and highly selective, neutrophil biased adverse effects remain persistent. Here, we sought to define the impact of Ikaros degradation through a longitudinal assessment of granulopoiesis in the bone marrow of humanized CRBN mice treated with Pom and Mezi.

Methods: We have performed multi-parametric studies leveraging a humanized CRBN (hCRBN) C57BL/6 mouse model. Driven by the endogenous mouse CRBN promoter, human CRBN was inserted into the mouse CRBN gene, preventing expression of mCRBN. These hCRBN mice are capable of specific degradation of CELMoD mediated substrates, thus enabling an *in vivo* model to understand pathophysiological mechanisms. Homozygous hCRBN mice were treated daily with Pom and Mezi via oral gavage until days 7, 14 and 21 when mice were euthanized. Peripheral blood and bone marrow were collected for flow cytometric and molecular analyses. Critical population classes such as multipotent progenitors, lineage committed progenitors, pre-myelocytes, immature neutrophils and mature neutrophils were defined by immunophenotypes, and pharmacodynamic parameters such as Aiolos/Ikaros degradation were evaluated. All data is represented as fold change over median of vehicle control.

Results: The hCRBN mouse model was able to recapitulate Pom and Mezi driven clinical neutropenia. Peripheral absolute neutrophil count gradually decreased with increased dosing (QD7, QD14 and QD21) in mice treated with Pom (26%, 45% and 74%) and Mezi (35%, 57% and 70%). Treatment with Pom and Mezi resulted in disruption of early hematopoietic multipotent progenitor populations leading to a widespread effect at multiple stages of haematopoiesis. Intriguingly, in bone marrow these effects were both temporally differential for multi-potent progenitors, and across various stages of neutrophil maturation. Myeloid biased, MPP3 population increased to ~55% and ~103% at QD7, followed by a steady decline, renormalizing to baseline at QD14 and QD21 for Pom and Mezi respectively. The first myeloid lineage committed progenitors, CMP population, reduced to 21% (Pom) and 42% (Mezi) at QD7 stabilizing at ~17-21% below baseline by QD21. In contrast GMP population, descendants from CMP, increased to ~50% (Pom) and ~117% (Mezi) at QD7. For Pom treatment (but not with Mezi), GMP proportions renormalized to baseline at QD21. Pre-myelocytes, one of the earliest neutrophil maturation stages, shrunk to ~24-30% at QD21 without any differences between Pom and Mezi. On the other hand, immature neutrophils steadily diminished to 51% (Pom) and 70% (Mezi) at QD21. This effect was more pronounced in mice treated with Mezi. Mature neutrophils in bone marrow, part of a highly dynamic reservoir that extravasates into blood, increased to 10-18% at all the time points for both Pom and Mezi. Overall, impairment of granulopoiesis is more profound in hCRBN mice treated with Mezi than Pom, owing to the more potent Ikaros degradation. Mechanistically, degradation of neo-substrates such as Ikaros affect the myelopoiesis and neutrophil maturation but are likely not the sole drivers. Addition of corticosteroids such as dexamethasone have been shown to mitigate IMiD/CELMoD mediated neutropenia in the clinical setting. Mezi treated animals who were co-administered dexamethasone demonstrated partially enhanced early progenitor populations compared to single agent Mezi. To investigate patterns of Ikaros transcriptional control, specific populations were flow sorted in parallel, for focused molecular analyses such as RNAseq using the SMARTseq platform and will be included in the presentation.

Conclusion: These studies delineate previously unreported effects of CELMoDs on a multifaceted continuum of myelopoiesis. These insights will help rationally design dose and schedule considerations for CELMoD treatment regimens alone, or in combination with other therapeutic agents to alleviate CELMoD induced neutropenia.

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