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Blood 142 (2023) 996–997

The 65th ASH Annual Meeting Abstracts

# ORAL ABSTRACTS

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

### Divergent Functions of ERK2 Substrate Binding Modalities in Myeloproliferative Neoplasm

*Billy Truong*[1], *Monika Toma, PhD*[2], *Yong Zhang, MDPhD*[3], *Susan Shinton*[4], *Muheng Li*[4], *Xiang Hua, MS*[5], *Dietmar Kappes, PhD*[5], *Paul Shapiro, PhD*[6], *Tomasz Skorski, MD PhD*[2], *David L. Wiest, PhD*[7]

[1] Fox Chase Cancer Center, Fox Chase Cancer Center, Philadelphia, PA
[2] Fels Cancer Institute for Personalized Medicine, Temple University School of Medicine, Philadelphia, PA
[3] Penn State Health Milton S. Hershey Medical Center, Hershey
[4] Nuclear Dynamics and Cancer Program, Fox Chase Cancer Center, Philadelphia, PA
[5] Transgenic Animal Facility, Fox Chase Cancer Center, Philadelphia, PA
[6] School of Pharmacy, University of Maryland, Baltimore, MD
[7] Fox Chase Cancer Center, Temple Health, Philadelphia, PA

**Introduction:** JAK2-V617F driven myeloproliferative neoplasms (MPNs) are intractable blood disorders marked by the elevated production of erythrocytes and myeloid cells in the bone marrow. While current JAK2 inhibitors offer some respite, they fail to eradicate the mutated cell population due to acquired treatment resistance. Treatment resistance arises from compensatory activation of the RAS-RAF-MEK-ERK mitogen-activated protein kinase (MAPK) pathway; however, even combination therapy targeting both JAK2 and the MAPK signaling has led to drug resistance. Instead of attempting to inhibit kinase activity, we propose to attenuate ERK2 substrate interactions that drive cancer. We have discovered that the two substrate binding domains of ERK2, D and DBP, play opposing roles in MPN pathogenesis. The DBP domain attenuates MPN pathogenesis and promotes oncogene-induced-senescence (OIS) through physical association with the DEF motif of Egr1, while the D-domain promotes progression, through Polq induction.
**Methods:** To study the divergent effects of ERK2 DBP and D domain on MPN progression, we introduced single point mutations in the substrate binding domains of ERK2 using CRISPR-Cas9. The expression of mutant alleles of ERK2 is conditionally activated by excision of the floxed allele using Vav-Cre. Similarly, we constructed a CRISPR knock-in mouse model, Egr1-Y253A, to examine the role of the Egr1 DEF motif in regulating OIS. To assess the response of individual malignant clones to Polq inhibitor (Polqi), we employed single-cell NGS and the Tapestri platform to detect clinically relevant mutations in 54 genes associated with myeloid neoplasms. For the inhibitor studies, we used human MPN cell lines and independent MPN patient cohorts.
**Results:** Ectopic expression of JAK2 $^{V617F}$ in DBP-inactivated progenitors enhanced their clonogenic potential and abrogated OIS in vitro. This occurs because mutating the ERK2-DBP domain prevents interaction with the senescence-inducing transcription factor, Egr1. Egr1-Y253A mutant progenitors display a similar phenotype in colony formation and OIS function supporting our previous findings that physical association with the DBP domain is required for senescence induction.
Because disabling the DBP domain accelerated MPN progression (Zhang et al, Blood, 2022), it is suggested that the D domain promotes progression. Induction of Polq protein is observed with ectopic expression of oncogenic MPN drivers in a myeloid cell line, which is inhibited by blocking ERK activity. The increased expression of Polq is crucial for MPN growth and survival, as the growth of HSPCs transduced with MPN oncogenic drivers is attenuated in the absence of Polq. Notably, Polq expression is upregulated in HSPCs from ERK2-DBP mutant mice, both before and after transduction with oncogenic JAK2 and CALR, thereby suggesting that the remaining substrate interaction domain, ERK2-D, is responsible for its induction. Interestingly, the ERK2-D domain not only interacts with Polq but may also regulate its expression through ubiquitin-dependent degradation involving the E3-ubiquitin ligase, c-CBL.
D-domain inhibitor (76) substantially reduced disease burden in a xenotransplantation model of SET2 MPN line. We found that targeting the ERK2-D domain substrate, Polq, was similarly effective. Lin-CD34+ MPN HSPC were more sensitive to Polq inhibitor, ART558, compared to normal implicating Polq as a therapeutic target in MPNs. Results from single cell sequencing analysis of a triple-negative MPN which transitioned to AML, indicated a high degree of clonal variation in sensitivity to Polqi, with WT1 clones being sensitive and NRAS mutant clones exhibiting resistance. Thus, a combination of Polqi + ERK2-Di might

exhibit a synergistic anti-MPN effect by attenuating the resistance mechanism of malignant subclones. As an initial test of this possibility, we treated SET2 cells with Polqi + ERK2-Di combination therapy, which revealed a synergistic response.
**Conclusion:** The findings suggest that a combination therapy, such as Polqi + ERK2-Di, may be a promising strategy to overcome treatment resistance arising from clonal variations in sensitivity to single agents. By targeting both the D domain and inhibiting the downstream substrate Polq, a synergistic anti-MPN effect may be achieved, potentially offering a more effective therapeutic approach for patients with MPNs.

**Disclosures** No relevant conflicts of interest to declare.

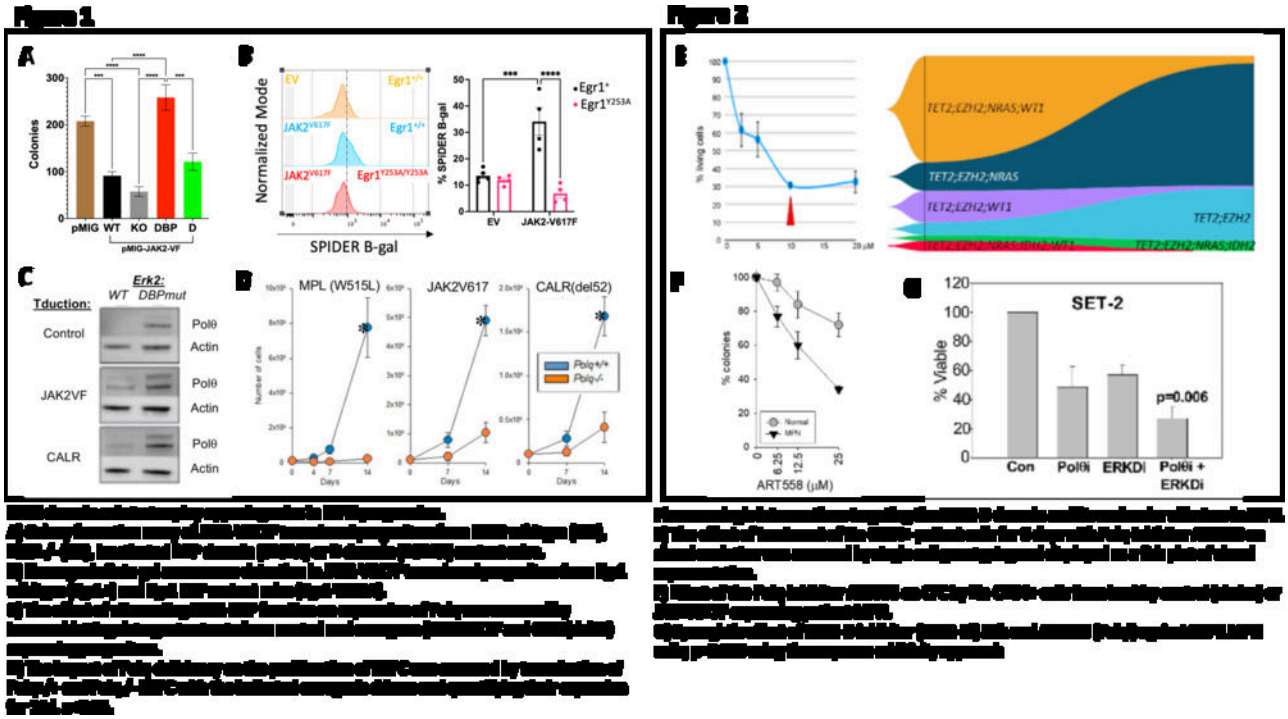


**Figure 1**

https://doi.org/10.1182/blood-2023-191180