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POSTER ABSTRACTS

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

ALK2 and JAK2 Inhibition for Improved Treatment of Anemia in Myelofibrosis Patients: Preclinical Profile of an ALK2 Inhibitor Zilurgisertib in Combination with Ruxolitinib

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Introduction: Anemia is a common occurrence in patients with myelofibrosis (MF) and is associated with the need for red blood cell transfusion and poor clinical prognosis. JAK inhibitors such as ruxolitinib are used extensively to treat symptoms of MF and improve quality of life and overall survival. However, JAK inhibitors may also contribute to myelosuppression. Therapeutic interventions that allow for optimization of JAK inhibition with no concern of anemia would benefit patients with MF and potentially lower the rate of treatment discontinuation or suboptimal dosing. Recent studies have demonstrated that inhibition of ACVR1/ALK2, a bone morphogenetic protein receptor that is upstream of the transcriptional regulation of hepcidin, could reduce serum hepcidin levels in patients with MF and improve anemia (Verstovsek S et al. Lancet 2023; Oh S et al. Clin Lymphoma Myeloma Leuk 2022). Reducing levels of hepcidin, a key regulator of plasma iron levels, and restoring erythropoiesis would broadly benefit patients with MF. Based on these recent data, we developed zilurgisertib, a potent and selective ALK2 inhibitor that could be administered in combination with ruxolitinib at doses titrated to the needs of patients with MF.

Methods and Results: In biochemical and cellular assays, zilurgisertib inhibited ALK2 kinase activity and SMAD1/5 phosphorylation with IC_{50} values of 15 nM and 63 nM, respectively. In Huh-7 cells stimulated with BMP-6, zilurgisertib inhibited hepcidin production with an IC_{50} of 20 nM, demonstrating the compound is a potent ALK2 inhibitor capable of regulating iron homeostasis via hepcidin. To assess possible off-target effects of zilurgisertib, kinome profiling at 10 μM ATP was performed at Reaction Biology (Malvern, PA) to determine the overall specificity across 356 kinases (see Figure). At 200 nM, zilurgisertib only inhibited ALK2, ALK1 (to 50%), and ALK6 (to 48%). In addition, zilurgisertib at 20 μM did not affect viability of HEK293 cells, a human cell line commonly used to assess general cell health and compound toxicity. Similarly, zilurgisertib did not affect viability of human fibroblasts or endothelial cells at concentrations up to 5 μM.

To test whether the combination of ruxolitinib and zilurgisertib could suppress hepcidin and restore erythropoiesis in an in vivo mouse model of cancer-induced anemia, B16F10 cells were injected intraperitoneally, mimicking a metastatic tumor that leads to anemia 1 week after injection. In a dose-dependent manner, zilurgisertib improved hemoglobin by 2 to 3 g/dL and red blood cell counts, while reducing both liver pSMAD and circulating hepcidin levels by 50% or more compared with vehicle control. The combination of zilurgisertib with ruxolitinib had no effect on this activity, suggesting that inhibition of JAK2 does not inhibit erythropoiesis restored following ALK2 inhibition.

Conclusion: Taken together, the potent and selective on-target activity of zilurgisertib suggests that ALK2 inhibition could reduce hepcidin and improve anemia, and that the combination of zilurgisertib with ruxolitinib is a rational and attractive approach to mitigate anemia in patients with MF. The combination of ruxolitinib and zilurgisertib is currently being evaluated in a phase 1 clinical trial in patients with anemia due to myeloproliferative disorders (NCT04455841).

Disclosures Stubbs: *Incyte Corporation:* Current Employment, Current equity holder in publicly-traded company. **Pusey:** *Incyte Corporation:* Current Employment, Current equity holder in publicly-traded company. **Wen:** *Incyte Corporation:* Current Employment, Current equity holder in publicly-traded company. **Drake:** *Incyte Corporation:* Current Employment, Current equity holder in publicly-traded company. **Zolotarjova:** *Incyte Corporation:* Current Employment, Current equity holder in publicly-traded company. **Smith:** *Incite Corporation:* Current Employment, Current equity holder in publicly-traded company. **Covington:** *Incyte Corporation:* Current Employment, Current equity holder in publicly-traded company. **Zhang:** *Incyte Corporation:* Current Employment, Current equity holder in publicly-traded company. **Macarrón:** *Incyte Corporation:* Current

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Figure. Kinome activity of zilurgisertib (IC_{50}s determined at 100 μM ATP)

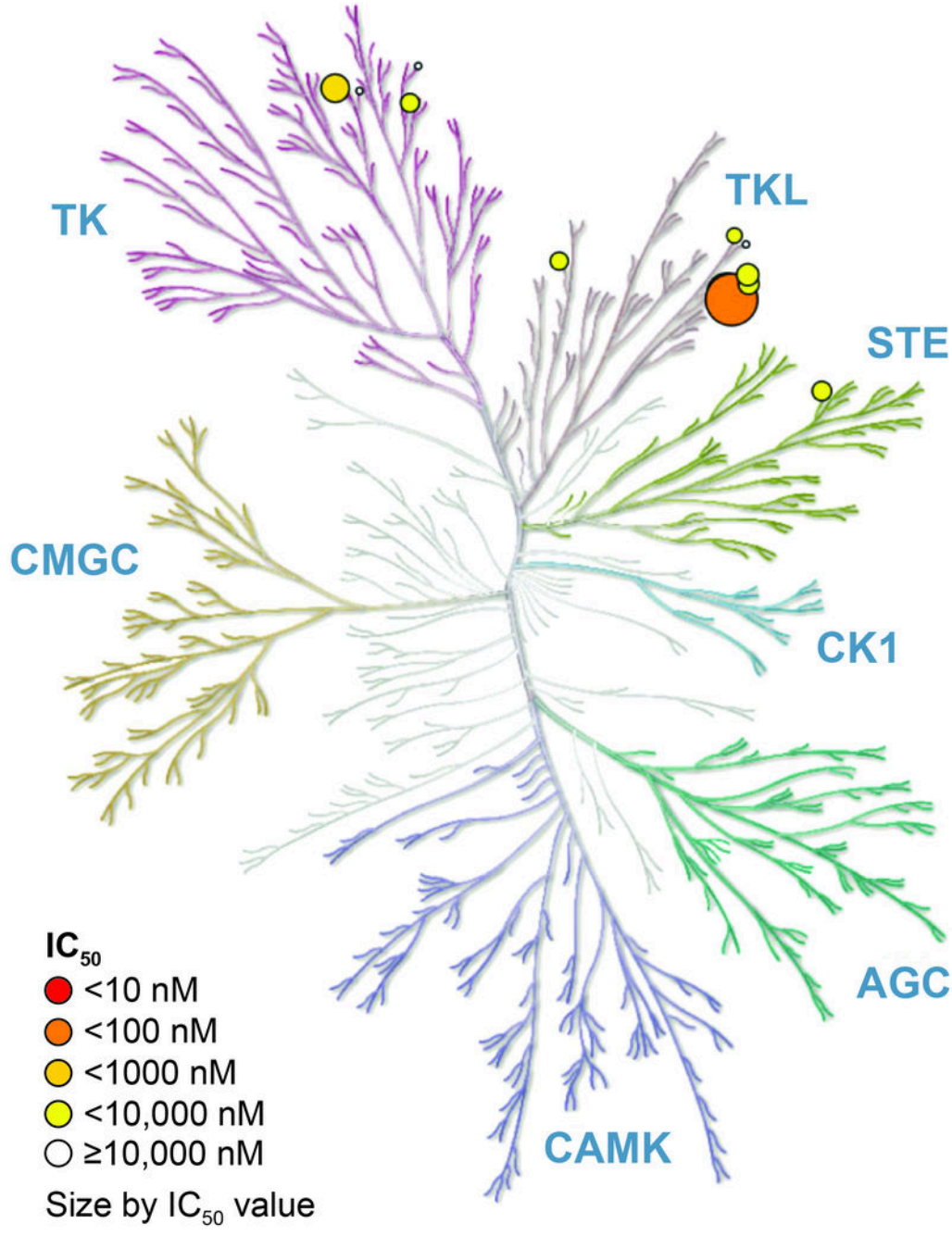

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Figure 1

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