Abstract: P1019

Title: ALK2 AND JAK2 INHIBITION FOR IMPROVED TREATMENT OF ANEMIA IN MYELOFIBROSIS PATIENTS: PRECLINICAL PROFILE OF AN ALK2 INHIBITOR ZILURGISERTIB IN COMBINATION WITH RUXOLITINIB

Abstract Type: Poster Presentation

Topic: Myeloproliferative neoplasms - Biology & translational research

Background:

Anemia is associated with a need for red blood cell (RBC) transfusion and poor clinical prognosis in patients with myelofibrosis (MF). Janus kinase (JAK) inhibitors such as ruxolitinib are used to treat MF symptoms, and improve quality of life and overall survival, but also contribute to myelosuppression. Treatments that optimize JAK inhibition while avoiding anemia would benefit patients with MF and could lower adverse effects that lead to discontinuation and suboptimal dosing. Recent studies show inhibition of ACVR1/ALK2, a bone morphogenetic protein (BMP) receptor upstream of transcriptional regulation of hepcidin, could reduce serum hepcidin levels in patients with MF and improve anemia (Verstovsek et al. *Lancet* 2023; Oh et al. *Clin Lymphoma Myeloma Leuk* 2022). Reducing hepcidin, a key regulator of plasma iron levels, and restoring erythropoiesis would benefit patients with MF being treated with JAK inhibitors. We have developed zilurgisertib, a potent, selective, and dose-titratable ALK2 inhibitor that could be administered in combination with ruxolitinib for patients with MF.

Aims:

To assess potency and selectivity of zilurgisertib for ALK2 inhibition and evaluate preliminary efficacy for improving anemia in combination with ruxolitinib.

Methods:

Potency and selectivity of zilurgisertib were determined by monitoring inhibition of ALK1, 2, 3, and 5 kinase activity at 100 μM ATP. Inhibition of phosphorylated SMAD (pSMAD) 1/5 was also measured in HeLa cells and hepcidin inhibition was measured in Huh7 cells. Possible off-target effects of zilurgisertib were assessed by kinome profiling at 10 μM ATP to determine specificity across 356 kinases (Reaction Biology, Malvern, PA, USA) and by cell-based assays. An in vivo mouse model of cancer-induced anemia was used to test if the combination of zilurgisertib and ruxolitinib could suppress hepcidin and restore erythropoiesis; B16F10 cells were injected intraperitoneally in mice, mimicking a metastatic tumor that leads to anemia 1 week after injection.

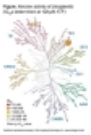
Results:

In biochemical and cellular assays, zilurgisertib potently inhibited ALK2 kinase activity and SMAD1/5 phosphorylation. In BMP-6-stimulated Huh7 cells, zilurgisertib inhibited hepcidin production, indicating it is a potent ALK2 inhibitor capable of regulating iron homeostasis via hepcidin. In kinome profiling assays, zilurgisertib at 200 nM only inhibited ALK2 and ALK1 at >50% (**Figure**). Cell-based assays showed 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, or human fibroblasts or endothelial cells at concentrations up to 5 μM. Finally, anemic mice treated with zilurgisertib showed dose-dependent improvement in hemoglobin (by 2-3 g/dL) and RBC counts, whereas liver pSMAD and circulating hepcidin levels were reduced by ≥50% compared with vehicle control. This activity was unaffected by the combination of zilurgisertib with ruxolitinib.

Summary/Conclusion:

Zilurgisertib is a potent and selective ALK2 inhibitor that can reduce hepcidin levels and improve anemia. Zilurgisertib directly inhibits ALK2 activity, reducing phosphorylation of the direct target SMAD1, and levels of

the SMAD target gene hepcidin. These data suggest JAK2 inhibition does not inhibit erythropoiesis restored following ALK2 inhibition. The combination of zilurgisertib with ruxolitinib is therefore a rational and attractive approach to mitigate anemia in patients with MF. This combination is currently being evaluated in a phase 1 clinical trial in patients with anemia due to myeloproliferative disorders (NCT04455841)



Keywords: Anemia, Ruxolitinib, Kinase inhibitor, Myelofibrosis