Abstract: P1028

Title: PRECLINICAL EVALUATION OF INCB160058 - A NOVEL AND POTENTIALLY DISEASE-MODIFYING THERAPY FOR JAK2V617F MUTANT MYELOPROLIFERATIVE NEOPLASMS

Abstract Type: Poster Presentation

Topic: Myeloproliferative neoplasms - Biology & translational research

Background:

The *JAK2*V617F mutation is the most common oncogenic driver in myeloproliferative neoplasms (MPNs), with nearly all patients with polycythemia vera and over half of patients with primary myelofibrosis (MF) and essential thrombocythemia positive for the somatic mutation. Although approved therapies for MPNs, such as ruxolitinib, directly inhibit the kinase domain (JH1) activity of JAK2 and exhibit high clinical efficacy, they do not address *JAK2*V617F allelic burden or achieve molecular remission of disease.

Aims:

Preclinical characterization of INCB160058, a pseudokinase (JH2)-targeting, first-in-class, orally bioavailable small molecule with the ability to selectively target JAK2V617F+ cell populations from patients with *JAK*-mutant MPNs.

Methods:

INCB160058 was designed using structure- and function-guided molecular techniques and characterized by biochemical and cell-based assays. In vitro assays were conducted with engineered cell lines and patient-derived cells, and in vivo analyses were performed in MPN PDX mouse models

Results:

INCB160058 was rationally designed to bind with picomolar affinity to the JH2 domain of JAK2V617F at the canonical ATP-binding site with high specificity (>2500-fold over JH1 domain of JAK2). Live cell single-molecule fluorescence microscopy showed that INCB160058 binding to JAK2V617F blocked ligand-independent thrombopoietin receptor dimerization, and consequently led to loss of JH1 kinase activity while preserving cytokine-dependent thrombopoietin receptor dimerization. Selectivity for JAK2V617F over wildtype (WT) JAK2 was explored using CD34+ human multipotent hematopoietic stem cells derived from patients with *JAK2*-mutant MF, engineered *JAK2*-mutant human hematopoietic cancer cell lines, and murine BaF3 cell lines. INCB160058 treatment selectively reduced pathogenic phospho-STAT5 levels and suppressed colony formation in JAK2V617F+ CD34+ cells but not in CD34+ cells from healthy volunteers. Importantly, continuous exposure of mutant and WT JAK2 cells to INCB160058 in cocultures at concentrations below IC50 resulted in progressive elimination of JAK2V617F+ cells without affecting WT cells. At the end of the testing period, the *JAK2*V617F-harboring population was no longer detectable in the coculture assay. In NSG mice subcutaneously inoculated with JAK2V617F-expressing SET2 cells, INCB160058 was tolerated and exhibited significant antitumor activity. In addition, following INCB160058 treatment, a significant reduction in the engraftment of total human cells, including human stem and progenitor cells (hCD45+ mCD45− Lin− CD34+ CD38−) and human erythroid progenitors (hCD45− mCD45− Ter119− hCD71+ hCD235a+), was observed in NSGS mice xenotransplanted with JAK2V617F+ CD34+ cells. Moreover, INCB160058 treatment also led to normalization of various pathogenic cytokines, such as interleukin-6 (IL-6) and IL-8. Importantly, these observations were absent in NSGS mice engrafted with CD34+ cells from healthy volunteers following INCB160058 treatment, further demonstrating the selectivity of INCB160058 for JAK2V617F.

Summary/Conclusion:

Our study demonstrates INCB160058 as a high-affinity JH2-binding inhibitor of JAK2V617F that blocks

cytokine-independent activity of JAK2V617F while preserving cytokine-dependent signaling. Extended treatment with INCB160058 results in the specific elimination of mutant *JAK2*V617F-harboring cells in mouse models and human cancer cells, with minimal impact on WT counterparts. Clinical testing of INCB160058 may allow patients with MPNs to achieve molecular remission and afford them an opportunity to overcome the disease.

Keywords: Myeloid malignancies, Targeted therapy, Myelofibrosis, Animal model