Abstract: P1002

Title: EFFICACY OF INCA033989 IN CHRONIC AND ADVANCED FORMS OF CALRDEL52 AND CALRINS5 MYELOPROLIFERATIVE NEOPLASMS (MPN) MODELS

Abstract Type: Poster Presentation

Topic: Myeloproliferative neoplasms - Biology & translational research

Background:

Mutations in the calreticulin gene (*CALRmut*) acquired by hematopoietic stem cells (HSCs) are one of the main drivers of myeloproliferative neoplasms (MPNs). CALRmut binds to the thrombopoietin receptor and activates oncogenic JAK2/STAT cell surface signaling. A novel anti-CALRmut monoclonal antibody (INCA033989 [INCA]) was recently shown to antagonize CALRmut-induced oncogenic signaling and improve disease outcomes in *CALRdel52* mice, a mouse model of MPN.

Aims:

To investigate the therapeutic potential of INCA using in vitro and in vivo MPN

Methods:

We performed competitive transplantation studies using a mix of bone marrow (BM) cells from either *CALRdel52* or *CALRins5* homozygous knock-in mice, and from C57BL/6 wildtype mice ubiquitously expressing GFP. These models produce an MPN phenotype associated with blood thrombocytosis and BM megakaryocyte hyperplasia. Hematologic and molecular responses were assessed after INCA treatment with histopathology and flow cytometry, and the mechanism of action was investigated in HSCs using cell-cycle and single-cell RNA sequencing (scRNA-seq) assays. Further, the therapeutic potential of INCA was explored in vitro using CD34+ cells from patients with *CALRmut* MPNs, and in vivo in patient-derived xenografts (PDX) of post-MPN acute myeloid leukemia (AML).

Results:

The *CALRdel52* competitive transplantation model showed a substantial increase in platelet counts and megakaryocyte hyperplasia in untreated mice, resembling the essential thrombocythemia phenotype observed in human disease. In contrast, 12-week INCA treatment not only resolved the thrombocytosis but significantly reduced the proportion of platelets, red blood cells, and granulocytes positive for *CALRdel52*. Notably, histopathologic analysis of the BM indicated the megakaryocyte hyperplasia observed in untreated mice was significantly reduced upon INCA treatment. Consistent with the aforementioned hematologic benefits, the proportion of *CALRdel52* HSCs, progenitors, and precursors in the BM was markedly reduced in mice treated with INCA vs untreated counterparts. No disease relapse or signs of thrombocytosis were observed after stopping treatment during a 10-week follow-up. Similarly, in the *CALRins5* competitive transplantation model, 22-week INCA treatment resulted in platelet count normalization, a significant decrease in megakaryocyte hyperplasia, and a decrease in the proportion of platelets, HSCs, progenitors, and precursor cells expressing *CALRins5*. The efficacy of INCA in inhibiting spontaneous growth was confirmed in vitro using megakaryocytes differentiated from CD34+ cells from patients with type 1 and type 2 *CALRmut* MPNs (type 1, n=9; type 2, n=5). Mechanistically, comparisons of scRNA-seq data from *CALRdel52*-treated vs untreated mice showed INCA deregulates DNA replication, apoptosis, and p53-dependent metabolic pathways in HSCs, as shown by gene set enrichment analysis. In vivo studies showed 2-month INCA treatment also decreased cell cycling of mouse *CALRdel52* HSCs and overall oncogenic signaling in human megakaryocytes, as assessed by Western blot. In addition, flow cytometry and histopathology analyses showed that INCA efficiently targeted blood, BM, and spleen blasts derived from patients with post-MPN AML carrying *CALRdel52/ins5* and *TP53* mutations in in vivo PDX models; the decrease in AML blast cells was associated with improved mouse survival.

Summary/Conclusion:

Together, our results show INCA efficiently targets *CALRdel52* and *CALRins5* cells in chronic and advanced forms of MPNs, demonstrating therapeutic potential and warranting further investigation.

Keywords: Platelet count, Megakaryocyte, Myeloid malignancies