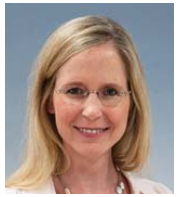
Myeloid Malignancies



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Mechanisms and targeting of oncogenic signaling in myeloproliferative neoplasms

Myeloproliferative neoplasms (MPN) are chronic leukemias with excessive proliferation of mature myeloid cells. They present as essential thrombocythemia (ET) with thrombocytosis, polycythemia vera (PV) with erythrocytosis, or myelofibrosis (MF) with expansion of megakaryocytes and bone marrow fibrosis. They lead to bone marrow failure or may transform to acute myeloid leukemia (AML). Hematopoietic stem cell transplantation is the sole curative therapy, but is limited to a subset of patients. It is the goal of our studies to contribute to novel therapeutic approaches for MPN patients by targeting the molecular signaling driving these diseases. MPN are characterized by hyperactive signaling of the JAK2 kinase due to acquired mutations in the JAK2 signaling pathway. JAK2 is an intracellular non-receptor tyrosine kinase essential for hematopoiesis representing the exclusive mediator of signaling from the thrombopoietin receptor MPL, the erythropoietin and GM-CSF receptors. JAK2 activates several signaling pathways including STAT transcription factors, the phosphoinositide-3 kinase (PI3K) pathway and the mitogen activated protein kinase (MAPK) pathway, which promote cell proliferation, differentiation and survival. In MPN, JAK2 signaling is constitutively activated by mutations in JAK2, MPL or the chaperone protein Calreticulin. The central role of JAK2 signaling in MPN has led to the development of JAK2 inhibitors which act as ATP mimetics and stabilize JAK2 in the active conformation (type I inhibition, e.g. ruxolitinib). However, type I JAK2 inhibitors have not met the expectations. They fail to reduce the mutant clone suggesting limited curative potential, and induce resistance. To improve therapeutic targeting of JAK2 signaling in MPN, we are pursuing several approaches:

More effective targeting of JAK2

A new mode of JAK2 inhibition has recently been reported which stabilizes the inactive form of JAK2 (type II inhibition). We found high potency of type II JAK inhibition in preclinical MPN models. We observed decreased mutant allele burden due to preferential inhibition of mutant JAK2 suggesting type II inhibition could lead to a class of agents with curative potential. Resistance to type I JAK inhibitors is also abrogated. We are continuing our studies on type II JAK2 inhibition, which appears to herald the development of mutant-selective inhibitors, as a basis for improved therapeutic options.

Resistance mechanisms to JAK2 inhibitors

Response to type I JAK inhibitors is often lost upon prolonged exposure. JAK2 resistance mutations occur *in vitro*, but have not been observed in patients. It has been shown that MPN cells functionally adapt and reactivate JAK2 signaling through formation of JAK2 heterodimers with other JAK family members such as JAK1 and TYK2. We found that this escape mechanism extends to type I JAK2 inhibitors in clinical development, and observed cross-resistance. These molecular studies of resistance mechanisms to JAK inhibitors may reveal new therapeutic targets, while studies on patient samples will provide insight into clinical JAK2 inhibitor resistance.

JAK2 signaling network

JAK2 induces activation of STAT-, PI3K- and MAPK signaling. Therapeutic targeting of these pathways in other malignancies was impeded by feedback or cross-talk signaling revealing intricate signaling networks. We could show that combined inhibition of several targets such as JAK2 and Bcl-2/Bcl-xL can provide superior benefit in JAK2-driven leukemias. We are investigating the signaling network

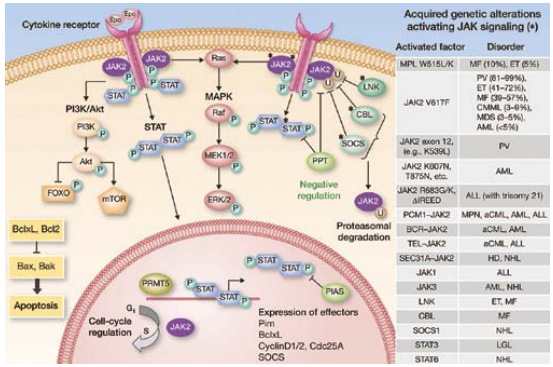

Fig. 1: Overview of JAK2 signaling. The JAK2 tyrosine kinase associates with hematopoietic cytokine receptors for EPO, TPO and GM-CSF. Upon ligand binding, JAK2 activates several signaling pathways including the STAT3 and STAT5 transcription factors, the PI3K/Akt pathway and the MAPK signaling pathway which includes RAS and the kinases RAF, MEK1/2 and ERK1/2. In MPN, JAK2 is constitutively activated by somatic mutations leading to excessive myeloid proliferation. The molecular interconnections between JAK2 and the downstream signaling pathways is not fully clarified. (Adapted from Meyer S. C. & Levine R. L., Clin. Cancer Res. 2014)

downstream of JAK2 to delineate the mechanisms limiting efficacy of JAK inhibitors and to inform novel therapeutic strategies. We aim to extend these studies to other myeloid malignancies with suboptimal clinical benefit of tyrosine kinase inhibitors.

JAK2 signaling in thrombopoiesis

Efficacy of therapeutic targeting with JAK2 inhibitors can be limited by on- and off-target toxicities. Thrombocytopenia is a significant side effect of JAK2 inhibition. We have shown that JAK2 regulates megakaryopoiesis including formation of megakaryocyte-biased stem cells, and are interested in differential effects of JAK2 inhibitors on thrombopoiesis.

Selected Publications

Meyer SC, Keller MD, Chiu S, Koppikar P, Guryanova O, Rapaport F, Ke X, Manova K, Pankov D, O'Reilly RJ, Kleppe M, McKenney AS, Shih AH, Shank K, Ahn J, Papalexi E, Spitzer B, Socci N, Viale A, Mandon E, Ebel N, Andraos R, Rubert J, Damassa E, Romanet V, Doelemeyer A, Zender M, Heinlein M, Rampal R, Singer R, Hoffman R, Sellers WR, Hofmann F, Murakami M, Baffert F, Gaul C, Radimerski T, Levine RL. CHZ868, a type II JAK2 inhibitor, reverses type I JAK inhibitor persistence and demonstrates efficacy in myeloproliferative neoplasms. (2015) Cancer Cell 28:15–28

Meyer SC, Keller MD, Woods BA, LaFave LM, Bastian L, Kleppe M, Bhagwat N, Marubayashi S, Levine RL. Genetic studies reveal an unexpected negative regulatory role for Jak2 in thrombopoiesis. (2014) Blood 124:2280–4

Meyer SC, Levine RL. Translational implications of somatic genomics in acute myeloid leukemia. (2014) The Lancet Oncology 15:e382–94

Meyer SC, Levine RL. Molecular pathways: molecular basis for sensitivity and resistance to JAK kinase inhibitors. (2014) Clinical Cancer Research 20:2051–9

Waibel M, Solomon VS, Knight DA, Ralli RA, Kim SK, Banks KM, Vidacs E, Virely C, Sia KC, Bracken LS, Collins-Underwood R, Drenberg C, Ramsey L B, Meyer SC, Takiguchi M, Dickins RA, Levine R, Ghysdael J, Dawson MA, Lock RB, Mullighan CG, Johnstone RW. Combined targeting of JAK2 and Bcl-2/Bcl-xL to cure mutant JAK2-driven malignancies and overcome acquired resistance to JAK2 inhibitors. (2013) Cell Reports 5:1047–59

Connection to Clinical Practice

Prof. Dr. J. R. Passweg, Prof. Dr. R. C. Skoda
Division of Hematology, University Hospital Basel

Characteristics of oncogenic signaling, therapeutic response and resistance in MPN patients

Our studies on oncogenic signaling and targeted therapeutic approaches in MPN are in close collaboration with Prof. R. Skoda who has established a long-term MPN patient cohort at University Hospital Basel, and Prof. J. Passweg, Head of the Division of Hematology at University Hospital Basel. We are studying clinical isolates of MPN patients with different mutational setup or at different stages of the disease for characteristics of oncogenic signaling and functional capacity of hematopoietic stem/progenitor cells. We are interested in the signaling dynamics in response to different therapies and upon development of resistance to conventional JAK2 inhibitors such as ruxolitinib. Collaborative studies with Prof. R. Levine based on the MPN cohort at Memorial Sloan Kettering Cancer Center New York, support these efforts. We aim to correlate the molecular findings with clinical characteristics of response or resistance to therapy in these MPN patients. These translational studies will facilitate potential clinical studies on improved targeting of JAK2 signaling in MPN in the longer term.

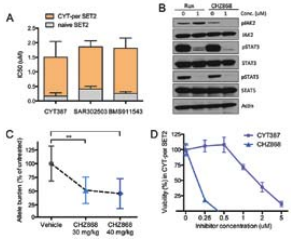

Fig. 2: Type II JAK2 inhibition provides a novel mode for improved targeting of JAK2 signaling in MPN. A. Conventional (type I) JAK2 inhibitors like CYT387 (CYT) induce cross-resistance in SET2 MPN cells. **B.** Type I JAK inhibitors like ruxolitinib (Rux) stabilize JAK2 in the phosphorylated form. The new type II JAK inhibitor CHZ868 stabilizes inactive, unphosphorylated JAK2. **C.** Type II JAK2 inhibition reduces mutant allele burden in MPN *in vivo* models. **D.** Type II JAK inhibition abrogates type I inhibitor resistance, shown for CYT387 resistant SET2 cells (Adapted from Meyer S. C. et al, Cancer Cell, 2015).