EUROPEAN HEMATOLOGY ASSOCIATION

Targeting specific mutations in myeloproliferative neoplasms

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Take-home messages

- Mutations in three genes (i.e. *JAK2, CALR* or *MPL*) drive the development of an MPN phenotype and all lead to activated JAK-STAT signaling.
- Although the development of JAK inhibitors has significantly advanced MPN treatment, these agents have a number of adverse side effects and are not curative.
- Several avenues of research are being explored with the goal of improving the clonal selectivity of current MPN therapies, including combination treatments with existing JAK inhibitors, developing mutant specific JAK2 inhibitors, and potentially immunological targeting of mutant CALR.

Introduction

The discovery of the JAK2V617F mutation in 2005[1,2] was a major advance in the MPN field and subsequently prompted a closer look into the mutations that drive JAK2V617F–negative MPNs. In the ensuing years, mutations in *JAK2* exon 12 were identified in the majority of V617F-negative polycythemia vera (PV) patients,[3] and mutations in the thrombopoietin receptor, *MPL* were found in a small percentage of essential thrombocythemia (ET) and primary myelofibrosis (PMF) patients.[4] More recently, two groups identified recurrent mutations in the gene calreticulin (*CALR*) in the majority of patients with non-mutated *JAK2* and *MPL*.[5,6] Recent work indicates that *CALR* mutations confer a neomorphic function on mutant CALR that results in activation of MPL signaling.[7] Despite their distinct molecular etiologies, the unifying hallmark of all MPN subtypes is the pathological activation of JAK-STAT signaling. This finding has provided a robust scientific rationale for therapeutic inhibition of the JAK-STAT pathway in MPN patients.

Current state of the art

First-generation JAK inhibitors for treatment of MPN

The discovery of activating *JAK2* mutations changed the landscape of MPN treatment dramatically, quickly prompting the development of small molecule inhibitors of JAK2. First-generation JAK inhibitors are ATP-competitive antagonists of the JAK kinase domain and each inhibits the activity of one or more JAK isoforms to different degrees. Ruxolitinib, a dual JAK1/2 inhibitor, was the first of its class to be approved for treatment of PMF and post-PV/ET MF. Treatment with ruxolitinib has been shown to reduce spleen size and alleviate systemic symptoms of MF,[8] although the myelosuppressive effects of ruxolitinib, particularly anemia, are problematic for MF patients. Subsequent JAK2 inhibitor development efforts sought to overcome the myelosuppressive effects of ruxolitinib, and have had varying levels of success in clinical development. Pacritinib, a JAK2/FLT3 inhibitor, was designed for PMF patients presenting with low platelet counts, and was shown to reduce splenomegaly without worsening thrombocytopenia.[9] Momelotinib, a JAK1/2 inhibitor currently in phase 3 trials for PMF and post PV/ET MF, demonstrated efficacy in reducing splenomegaly while improving anemia in phase II.[10]

Combination therapy

To improve upon JAK inhibitor monotherapy, several preclinical combination treatments are currently being explored. There are a number of potential partners for ruxolitinib, including those that target aberrantly activated pathways that are either downstream of or convergent upon the JAK-STAT pathway (Figure 1A). A number of studies have shown that combined inhibition of JAK2 and the phosphatidylinositol 3-kinase (PI3K) pathway synergistically inhibits MPN cell proliferation.[11] A phase 1b clinical trial is underway to evaluate ruxolitinib in combination with the PI3K inhibitor, buparlisib (NCT01730248). Additionally, given the interaction between the JAK-STAT pathway and the RAS-RAF-MEK pathway, the

effect of combined MEK and JAK2 inhibition has been tested and synergistic effects on MPN cell viability *in vitro* and enhanced survival in a murine model of MPN were demonstrated.[12]

In addition to targeting aberrant signaling pathways that work in concert with the JAK-STAT pathway, combination therapies targeting proteins that stabilize JAK2V617F are also being explored. JAK2 has been shown to be a chaperone client of heat shock protein 90 (HSP90), and treatment with the HSP90 inhibitor, PU-H71 leads to degradation of both JAK2V617F and HSP90.[13] A recent study demonstrated improved efficacy when PU-H71 was combined with JAK2 inhibition in pre-clinical mouse models.[14] This resulted in a phase 2 clinical trial of the HSP90 inhibitor, AUY922 in patients with MF (NCT01668173), which although terminated early due to excess gastrointestinal toxicity, found that all 6 patients treated experienced at least a partial remission (PR).[15]

Limitations of first-generation JAK inhibitors

Despite their clinical benefits, first-generation JAK inhibitors were not the 'home run' they were expected to be. Because none of these agents is selective for the mutant form of JAK2, JAK activity in normal cells is also inhibited, leading to its myelosuppressive effects. Additionally, none of these agents has been shown to eradicate the JAK2 mutant clone or to significantly reduce the mutant JAK2 allele burden, and thus none can cure the disease. Moreover, it has recently been shown that MPN cells can acquire resistance to JAK inhibitors

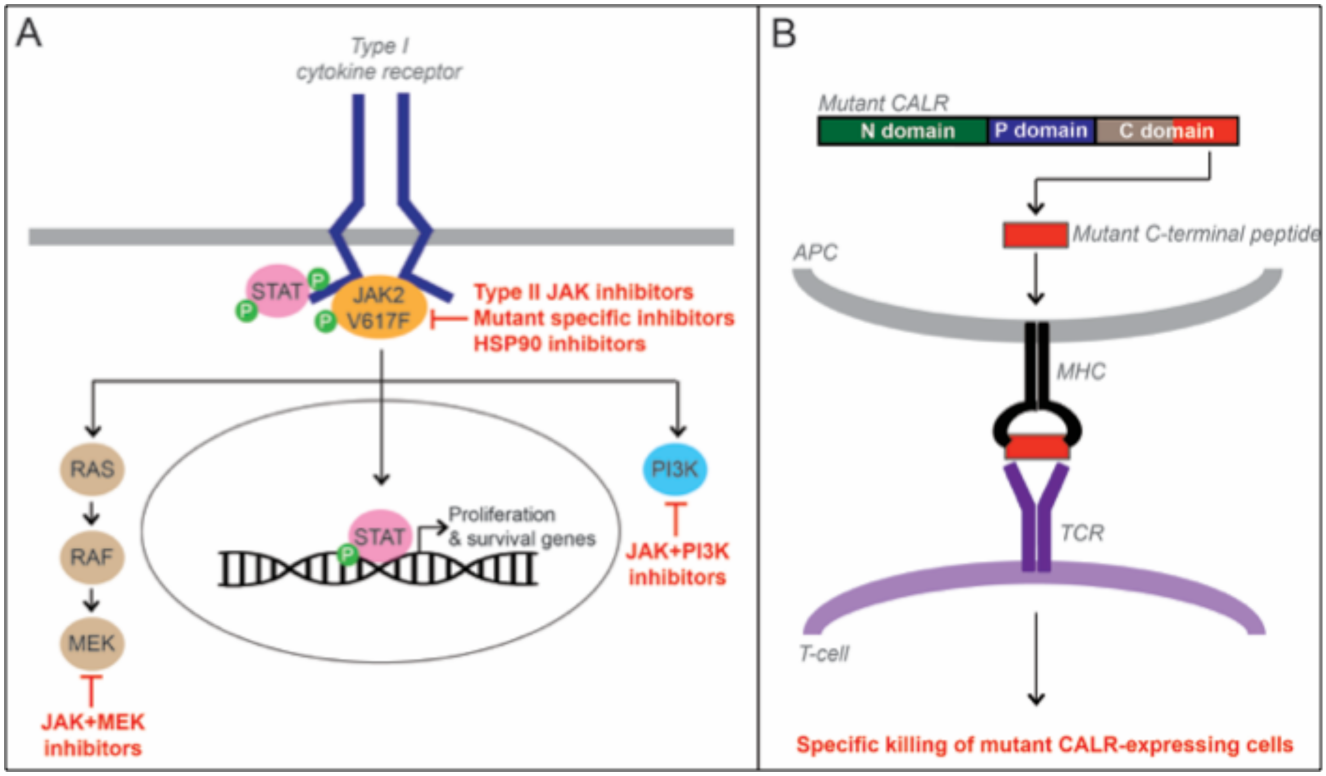

Figure 1. Enhancing the Clonal Selectivity of MPN therapy. (A) Schema showing potential points of intervention for newly developed JAK2 inhibitor monotherapies or combination therapies. Type II JAK inhibitors inhibit JAK2 in its inactive form, and preferentially inhibit JAK2V617F over wild type JAK2. Mutant specific JAK2 inhibitors are being developed to specifically target the JAK2V617F mutant protein. HSP90 inhibitors lead to the degradation of mutant JAK2. Combination therapies currently being explored include JAK inhibitors with PI3K inhibitors, and JAK inhibitors with MEK inhibitors. Both combinations have demonstrated synergistic inhibition of *JAK2*-mutant MPN cells in pre-clinical models. (B) Schema demonstrating one potential mechanism for immune responses to be generated against mutant CALR. Derivatives of the mutant C-terminal peptide of mutant CALR have been shown to elicit T-cell responses in *CALR*-mutant MPN patients following *ex vivo* peptide stimulation, suggesting that the mutant CALR C-terminus contains tumor-specific neo-epitopes that are targeted by T-cells.

through reactivation of JAK-STAT signaling via heterodimeric activation of JAK2 by JAK1/TYK2.[16] Together these limitations highlight the enduring need to identify improved therapeutic avenues for *JAK2*-, *MPL*-, and *CALR*- mutant MPNs.

Future perspectives

Type II JAK inhibitors

Type II JAK inhibitors, which stabilize the inactive unphosphorylated conformation of JAK2 resulting in more potent JAK2 inhibition, were recently developed. In addition to binding the ATP pocket, type II inhibitors bind supplemental adjacent sites, thus enhancing their specificity. A recent study demonstrated that CHZ868, a type II JAK inhibitor, overcame resistance to first-generation JAK inhibitors by binding the inactive conformation of JAK2.[17] CHZ868 was shown to be active in both *in vitro* and *in vivo* models of MPN and although type II inhibitors are not specific for mutant JAK2, CHZ868 was found to preferentially target Jak2V617F cells over Jak2 wild type cells in Jak2V617F mice.[17] If developed for clinical use, type II inhibitors may be more clonally selective for JAK2V617F-mutant cells than current JAK inhibitors.

Mutant-specific JAK2 inhibitors

The identification of the crystal structure of the pseudokinase domain of JAK2, which is the domain in which the V617F mutation occurs, is an important advance towards the development of JAK2V617F mutant-specific inhibitors.[18] Recent biochemical studies have advanced the understanding of the specific requirements for JAK2V617F driven activation and identified specific residues that could potentially be targeted with allosteric small molecule inhibitors.[19] Mutant-specific JAK2 inhibitors should overcome the myelosuppressive effects resulting from inhibition of wild type JAK2 in normal cells.

Mutant CALR immunotherapy

CALR mutations occur as insertions and/or deletions in exon 9, all of which cause a +1 base-pair frameshift. Although more than 50 mutations have been identified to date, all mutant CALR proteins possess the same tumor-specific 36 amino acid C-terminal peptide, making it an attractive target for anti-MPN immunotherapy. A recent study demonstrated that mutant CALR-derived peptides are capable of eliciting T-cell responses in mutant *CALR*-positive MPN patients following *ex vivo* peptide stimulation.[20] This result suggests that the mutant CALR C-terminus is immunogenic, and may represent a promising immunotherapeutic target in *CALR*-mutant MPN patients (Figure 1B).

Additional mutations and clinical implications

In addition to the aforementioned MPN phenotypic driver mutations (i.e. *JAK2, CALR, MPL*), co-occurring mutations in epigenetic and splicing genes are found in MPN patients (in particular in MF) and are associated with poor clinical outcome. These include mutations in genes that impact DNA methylation (e.g. *TET2, IDH1/2*), polycomb complex proteins (e.g. *ASXL1, EZH2*) and mutations in splicing factors (e.g. *SRSF2*).[21,22] Studies focused on developing treatment strategies that target these mutations or their molecular consequences are therefore warranted.

Acknowledgements

The authors thank Gabriela Soriano Hobbs for critically reviewing the manuscript.

The authors apologize that due to space limitations not all relevant references could be included.

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