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REVIEW

Targeting the PI3K pathway in myeloproliferative neoplasms

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ABSTRACT

Introduction: Decreasing efficacy over time and initial suboptimal response to Janus kinase (JAK) inhibitors such as ruxolitinib in a subset of patients are critical clinical challenges associated with myeloproliferative neoplasms (MPNs), primarily myelofibrosis.

Areas covered: The role of phosphatidylinositol-3 kinase (PI3K) in MPN disease progression and treatment resistance and as a potential therapeutic target in patients who experience loss of response to JAK inhibition is discussed. Understanding the complex signaling networks involved in the pathogenesis of MPNs has identified potentially novel therapeutic targets and treatment strategies, such as inhibiting other signaling pathways in addition to the JAK/signal transducer and activator of transcription (STAT) pathway. PI3K plays a crucial role downstream of JAK signaling in rescuing tumor cell proliferation, with PI3Kδ being particularly important in hematologic malignancies. Concurrent targeting of both PI3K and JAK/STAT pathways may offer an innovative therapeutic strategy to maximize efficacy.

Expert opinion: Based on our understanding of the underlying mechanisms and the role of PI3K pathway signaling in the loss of response or resistance to JAK inhibitor treatment and initial results from clinical studies, the combination of parsaclisib (PI3Kδ inhibitor) and ruxolitinib holds great clinical potential. If confirmed in larger clinical trials, parsaclisib may provide more treatment options and improve clinical outcomes for patients with MPNs.

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1. Introduction

Myelofibrosis (MF) is a Philadelphia chromosome-negative myeloproliferative neoplasm (MPN). Patients may present with primary MF (PMF) or develop secondary MF from other MPNs, namely, polycythemia vera (PV) and essential thrombocythemia (ET) [1,2]. MF is marked by uncontrolled kinase signaling, clonal myeloproliferation, and altered levels of inflammatory cytokines [3,4]. Median survival of patients with MF is short, ranging from <2.5 years (high-risk patients) to ~11 years (low-risk patients) [5].

Three therapies are currently approved by the US Food and Drug Administration (FDA) for patients with intermediate- or high-risk MF: ruxolitinib, a dual Janus kinase (JAK)1/JAK2 inhibitor, fedratinib, a JAK2 inhibitor, and pacritinib, a JAK2 and FMS-like tyrosine kinase 3 inhibitor [6–8]. Treatment with JAK inhibitors can be associated with drug-related hematologic adverse events (AEs), including anemia and thrombocytopenia, which may lead to dose reduction and/or treatment discontinuation [9–11]. Furthermore, a subset of patients with MF with a good initial response subsequently experience loss or lack of response and thus do not achieve optimal prolonged benefit with JAK inhibitor therapy [12,13]. Allogeneic hematopoietic cell transplantation is the only curative therapy for MF; however, it is associated with serious treatment-related complications, and only select patients are eligible [14].

JAK signaling is dysregulated in all subtypes of MPNs, and in most patients, it is a result of mutations in *JAK2, CALR,* or *MPL* [1,3,15,16]. These mutations can lead to constitutive activation of signaling through cytokine receptors and increased phosphorylation of the downstream signal transducer and activator of transcription (STAT) proteins [1,15,16]. Phosphatidylinositol-3 kinase (PI3K) transmits important signals regulating a variety of physiological processes in nearly all tissue types studied to date. PI3K activity is stimulated by different oncogenes and growth factor receptors, and elevated PI3K signaling contributes to a wide range of human diseases and plays an important role in cancer [17]. In addition to JAK/STAT signaling, the PI3K pathway is reported to be activated in MPNs [3,16,18]. The PI3K pathway regulates survival and growth of cells in response to extracellular signals including cytokines and growth factors [19]. The objectives of this review are to discuss the role of the PI3K pathway in MPNs and the importance of PI3K signaling in the context of JAK inhibition and cellular resistance and highlight the preclinical rationale supporting investigating the combination of parsaclisib (PI3Kδ inhibitor) and ruxolitinib (JAK1/2 inhibitor) in patients with MPNs.

2. Role of PI3K in MPN pathogenesis

Constitutive activation of JAK/STAT signaling in MPNs activates other molecular pathways such as the PI3K pathway (Figure 1) [3,16,18]. The PI3K pathway is a critical intersection of cellular signaling where other pathways converge to

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Article highlights

- Decreased efficacy over time or initial suboptimal efficacy in a subset of patients, are an important unmet medical need for patients with myeloproliferative neoplasms (MPNs)
- Phosphatidylinositol-3 kinase (PI3K) pathway plays a fundamental role in MPN disease progression, treatment resistance, and is a potential therapeutic target in the subgroup of patients experiencing loss of response to Janus kinase (JAK) inhibition
- Simultaneous targeting of the PI3K and JAK/signal transducer and activator of transcription (STAT) pathways may offer a new therapeutic strategy to maximize the efficacy of JAK inhibition
- Promising clinical results have been observed in patients with MPNs treated with a combination of parsaclisib (PI3Kδ inhibitor) and ruxolitinib (JAK1/2 inhibitor); if confirmed in larger clinical trials, this may provide more treatment options and improved clinical outcomes for hard-to-treat patients with MPNs

regulate cell survival, growth, and proliferation [19]. There are three classes of PI3Ks, among which class I is commonly linked with cancer development. Class I PI3Ks consist of a p85 regulatory subunit and one of the four catalytic subunits: p110α, p110β, and p110δ (class 1A) and p110γ (class 1B) [19,20]. Although α and β isoforms are ubiquitously expressed in cells, the δ and γ isoforms are expressed only in hematopoietic cells, primarily leukocytes [19,20].

Class I PI3Ks phosphorylate the lipid substrate, phosphatidylinositol 4,5-bisphosphate (PIP2) to generate the active second messenger, phosphatidylinositol 3,4,5-triphosphate (PIP3) [19,20]. In quiescent cells, the regulatory and catalytic subunits are bound together, thus inhibiting the catalytic activity [19,20]. Upon stimulation, the regulatory subunit is mobilized to phosphotyrosine proteins at the cell membrane, and the catalytic subunit catalyzes the formation of PIP3 that in turn recruits protein kinase B (AKT) [19,20]. Subsequently, AKT is phosphorylated at the membrane and activates mammalian target of rapamycin (mTOR), a serine threonine kinase, and other proteins involved in cellular survival, proliferation, migration, and metabolism [20].

Activation of the PI3K pathway has been reported in *JAK2*V617F-mutated cells, V617F transgenic or knock-in mice, and cells from patients with MPNs [21,22]. Hyperphosphorylation of AKT indicating constitutive activation of PI3K pathway has been demonstrated in bone marrow biopsy samples from patients with MPN with *JAK2*V617F mutation and in *JAK2*V617F-mutated cells from mouse models [22]. Using an AKT inhibitor, Khan *et al*. reported strong inhibition of proliferation in MPN cell lines, reduced colony formation by primary hematopoietic progenitors from patients with MF, and reduced hepatosplenomegaly and megakaryocyte proliferation in a mouse model of MPN [18]. Activation of PI3K and

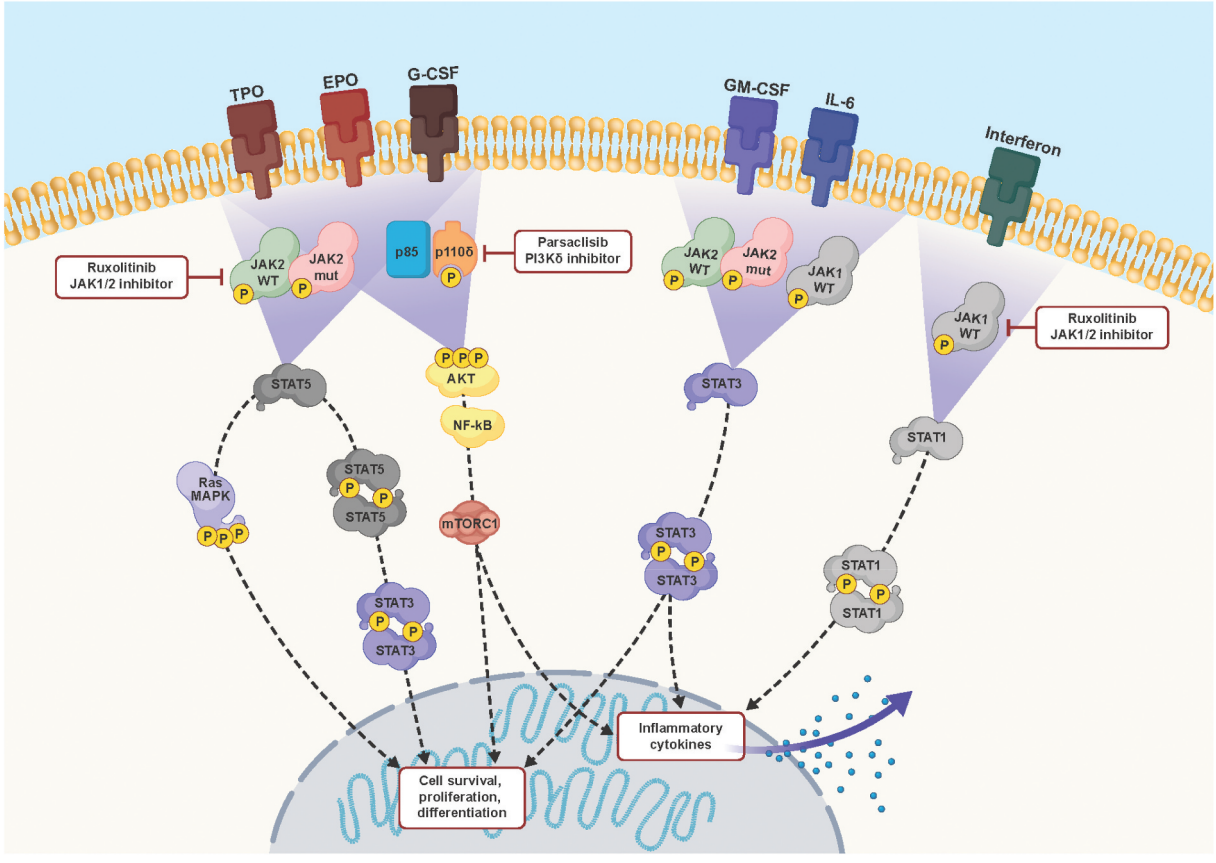

Figure 1. Combined inhibition of Janus kinase (JAK)2 and phosphatidylinositol-3 kinase (PI3K) in myeloproliferative neoplasm cells with multiple molecular pathways activated because of constitutive JAK/signal transducer and activator of transcription (STAT) signaling. AKT: protein kinase B; EPO: erythropoietin; G-CSF: granulocyte-colony stimulating factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL-6: interleukin-6; MAPK: mitogen-activated protein kinase; mTORC1: mammalian target of rapamycin complex 1; mut: mutation; NF-kB: nuclear factor-kappa B; TPO: thrombopoietin. Figure adapted from Hermouet S, *et al*. (2015) Pathogenesis of Myeloproliferative Neoplasms: Role and Mechanisms of Chronic Inflammation. *Mediators of Inflammation*. Volume 2015: Article ID 145293. doi.org/10.1155/2015/145,293 (CC BY; https://creativecommons.org/licenses/by/3.0/).

STAT5 also contributes to erythropoietin independence in *JAK2*V617F-mutated cells [22]. Interestingly, STAT5 is phosphorylated on both tyrosine and serine residues by JAK2- and PI3K/mTOR-dependent mechanisms, respectively, in *JAK2*-mutated cells [23].

Recently, Palam *et al*. [24] reported that activation of the PI3K pathway regulates proliferation in MPN cells with activating *KITD814V* mutation and/or loss of the epigenetic regulator TET2 (Tet methylcytosine dioxygenase-2). Using genetic and pharmacologic approaches, the authors demonstrated that proliferation of MPN cells is dependent on the α and δ isoforms of PI3K [24]. The combination of ruxolitinib and PI3K blockade synergistically inhibits survival of MPN cell lines, primary cells from patients, and reduces splenomegaly in an MPN mouse model.

Inflammatory cytokines such as interleukin (IL)-6 and IL-8 and growth factors including granulocyte-monocyte colony-stimulating factor (GM-CSF) and transforming growth factor-β are present at significantly higher levels in MPNs, and contribute to the expansion of *JAK2*-V617F-mutated cells [3]. Increased levels of IL-8, IL-10, IL-12, and IL-15, and the IL-2 receptor are reported to be independent predictors of poor survival in patients with PMF [25]. In MPNs, signaling through granulocyte-colony stimulating factor, thrombopoietin, and erythropoietin can activate wild-type or mutated JAK2, resulting in constitutive JAK/STAT pathway activation [3]. However, even in the absence of inflammatory cytokines or growth factors, normal signaling can be activated in *JAK2*V617F-mutated cells through PI3K and mitogen-activated protein kinase (MAPK) pathways [3].

PI3Kδ is reported to be the main isoform contributing to uncontrolled cell survival and proliferation in acute myeloid leukemia and acute promyelocytic leukemia through constitutive AKT phosphorylation [20]. PI3Kδ is also the key isoform involved in constitutive PI3K signaling leading to AKT activation and GM-CSF hypersensitivity in juvenile myelomonocytic leukemia [26] and is indispensable for the maximal response of hematopoietic stem and progenitor cells to inflammatory cytokines IL-1β and tumor necrosis factor-α [27]. Thus, the PI3Kδ isoform, through constitutive AKT phosphorylation, may contribute to unrestricted proliferation in MPNs even after inhibition of the JAK pathway.

3. PI3K overactive signaling in ruxolitinib resistance

Signaling via PI3K has been reported to be involved in escape mechanisms after inhibition of receptor tyrosine kinase pathways. In *KRAS* (Kirsten rat sarcoma viral oncogene homolog) mutated colorectal cancer cell lines, activation of PI3K leads to acquired resistance with the combination of MEK (MAPK/extracellular signal-regulated) inhibitor and anti-epidermal growth factor receptor treatment [28]. In *in vitro* cell cultures and primary cells from patients with chronic myeloid leukemia (CML), chronic eosinophilic leukemia, or Philadelphia-positive acute lymphoblastic leukemia, constitutive activation of the PI3K pathway as a consequence of epigenetic silencing of the *PTEN* (phosphatase and tensin homolog deleted on chromosome 10) gene has been shown to play a key role in the development of resistance to BCR-ABL tyrosine kinase inhibitors [29]. Similarly, mutations in NRAS (neuroblastoma RAS), KRAS, and TET2, which have been associated with MF, may also contribute to constitutive PI3K pathway activity [30,31].

The combination of ruxolitinib with either mTOR or AKT blockade has demonstrated synergistic survival inhibition of MPN cell lines and primary cells from patients with MPN [18]. However, incomplete inhibition of STAT5, a key molecule downstream of JAK2, has been reported after ruxolitinib treatment *in vitro* in both cell lines and primary cells from patients with MPN [23]. Combination of ruxolitinib with PI3K or mTOR inhibitors demonstrated the highest reduction in STAT5 activation in these cells [23]. The combination of ruxolitinib with a dual PI3K/mTOR inhibitor was shown to have synergistic effects, resulting in decreased proliferation in mouse models of MPN, as well as in clonogenic assays using cells from patients with PMF, and improved splenomegaly and survival in a *JAK2*V617F knock-in mouse model of MF [22]. Notably, the strong synergistic activity in these models was reported at drug doses lower than those shown to have activity when used as single agents [22]. MPN cells that survive JAK2 inhibition were found to be sensitive to the combination of ruxolitinib and a dual PI3K/mTOR inhibitor, suggesting that inhibition of the PI3K pathway may help overcome or prevent resistance to therapies targeting JAK2 [22]. These results suggest that concurrent blockade of the PI3K pathway may confer benefits in patients with MPNs who experience loss or lack of response with chronic ruxolitinib therapy (Figure 1).

Pan-PI3K inhibitors have broad and dose-dependent toxicity profiles; thus, efforts have been directed to develop isoform-specific alternatives [19,32]. PI3Kδ is the primary isoform expressed in CD34+ cells from chronic ruxolitinib-treated or ruxolitinib-naive patients with MF [21]. In chronic lymphocytic leukemia and other B-cell malignancies, constitutive PI3K pathway activation is dependent on the p110δ isoform [20]. Currently, four inhibitors targeting PI3K have received approval by the FDA for use in hematologic malignancies: idelalisib (targeting the PI3Kδ isoform), copanlisib (predominantly targeting the α and δ isoforms), and duvelisib (predominantly targeting the δ and γ isoforms) are approved for treatment of relapsed or refractory follicular lymphoma (FL); umbralisib, a multi-kinase inhibitor targeting PI3Kδ, casein kinase 1 epsilon (CK1ε), and mutated ABL-1 were recently approved by the FDA for treatment of relapsed or refractory FL and marginal zone lymphoma (MZL) [33–36]. However, clinical use of first-generation PI3K inhibitors has been limited primarily because of associated toxicities including diarrhea and/or colitis, hepatotoxicity, pneumonitis, and infections [32].

4. Parsaclisib as a unique PI3Kδ inhibitor in clinical studies

First-generation PI3Kδ inhibitors have an increased association with off-target toxicities. This effect may be structure-based [37]; thus, newer PI3Kδ inhibitors with different molecular structures have been designed. Parsaclisib (INCB050465) is a next-generation PI3Kδ inhibitor with a fundamentally different molecular structure from the first-generation PI3Kδ inhibitors currently approved to help limit toxicities [37]. Parsaclisib

is composed of a monocyclic scaffold with a pyrazolopyrimidine substituent compared with a bicyclic scaffold with a purine substituent for first-generation PI3Kδ inhibitors. It is highly selective for the PI3Kδ isoform with ~20,000-fold selectivity over α, β, and γ isoforms. In diffuse large B-cell lymphoma (DLBCL) xenograft models, parsaclisib alone inhibited tumor growth with enhanced effect when combined with other therapies [37].

In the first-in-human phase I/II CITADEL 101 study, parsaclisib was evaluated in patients with relapsed or refractory B-cell malignancies, alone or in combination with itacitinib, a JAK1 inhibitor, or chemotherapy [38]. A range of doses were evaluated starting from 5 to 45 mg once daily (QD) in the escalation phase followed by 20 and 30 mg QD in the expansion phase. An intermittent dosing at 20 mg QD for 9 weeks followed by once weekly (QW) was also explored [38]. Parsaclisib monotherapy produced rapid and durable objective responses with 93% of responses occurring at the first assessment ~9 weeks after treatment. Objective response rates across all tumor subtypes ranged from 30% in patients with DLBCL to 78% in patients with MZL. Notably, pharmacokinetics (PK) analysis showed near-maximal inhibition of PI3Kδ at all doses evaluated in this study [38].

The safety profile of parsaclisib was notably different compared with first-generation PI3Kδ inhibitors (e.g. idelalisib and duvelisib) with near absence of grade ≥2 transaminitis and no Hy's Law cases (alanine aminotransferase [ALT] or aspartate aminotransferase [AST] ≥3 × upper limit of normal [ULN] or total bilirubin ≥2 × ULN) [38]. No patients discontinued treatment owing to hepatotoxicity and any-grade diarrhea/colitis or rash led to treatment discontinuation in 13% (9/72) of patients [38]. Most diarrhea events were of grade 1 or 2, with the only reports of grade 3/4 diarrhea, colitis, and rash occurring after 9 weeks of treatment. Grade 4 neutropenia (6%) was observed only during QD dosing with the earliest occurrence reported after 21 days of treatment [38]. Further analysis showed that the safety profile associated with the 20-mg QD dose was consistent with that observed for all doses combined and for each individual dose level [38]. As late-onset AEs at the 20-mg QD dose level led to treatment discontinuation, a modified dosing regimen of 20 mg QD for 9 weeks followed by 20 mg QW was implemented to maintain a high response rate with prolonged tolerability [38]. Encouragingly, none of the 26 patients in the QW dosing regimen discontinued study treatment because of toxicities, and no grade 4 nonhematologic or hematologic events were reported [38]. Thus, 20 mg QW schedule seemed better tolerated than continuous 20 mg QD dosing and has been evaluated in subsequent clinical trials.

In the phase II CITADEL 202 study, parsaclisib was evaluated in patients with relapsed or refractory DLBCL who were either Bruton tyrosine kinase (BTK) inhibitor-treatment naive or had received prior therapy with the BTK inhibitor ibrutinib [39]. Parsaclisib monotherapy at 20 mg QD for 8 weeks followed by 20 mg QW demonstrated an objective response rate of 25.5% in BTK inhibitor-naive and 20.0% in patients who had received prior ibrutinib. The reported safety results were consistent with the phase I CITADEL-101 study with no new safety signals [39]. Most of the diarrhea/colitis and rash events were grade 1/2, and grade 3 or 4 ALT and AST values were reported in three (5.0%) and two (3.3%) patients, respectively. Grade 3/4 hematologic abnormalities (anemia, neutropenia, and thrombocytopenia) were infrequent and observed in three (5.0%), four (6.7%), and two (3.3%) patients, respectively [39].

In other phase II studies, two dosing schedules of parsaclisib at 20 mg QD for 8 weeks followed by either 20 mg QW (weekly dosing group) or 2.5 mg QD (daily dosing group) have been evaluated in patients with relapsed or refractory FL (CITADEL-203), MZL (CITADEL-204), and mantle cell lymphoma (CITADEL-205) [40–43]. In all these studies, parsaclisib demonstrated rapid and durable responses with an acceptable safety profile; grade ≥3 hepatoxicity, diarrhea/colitis, and rash were generally infrequent [40–43]. Thus, early clinical studies with parsaclisib have established promising clinical efficacy and safety profile, with low observed rates of common nonhematologic and hematologic toxicities associated with PI3K inhibitors.

5. Combination studies with ruxolitinib and parsaclisib or other targeted therapies in MF

Preclinical evidence on the role of the PI3K pathway in the pathogenesis of MPN, and promising results from phase I and II studies of parsaclisib in patients with lymphoma, supported the rationale to investigate parsaclisib treatment in patients with MF. In the ongoing phase II, 50465-201 study (NCT02718300), addition of parsaclisib to ruxolitinib is being evaluated in patients with MF and a suboptimal response to ruxolitinib [44]. Suboptimal response to ruxolitinib for the study has been defined as treatment with ruxolitinib for ≥6 months with a stable dose (5–25 mg twice daily [BID]) for ≥8 weeks immediately before enrollment *and* a palpable spleen length of >10 cm below the left subcostal margin upon physical examination *or* palpable spleen length of 5–10 cm below left subcostal margin upon physical examination with active symptoms of MF at the screening visit defined as one symptom score ≥5 or two symptom scores ≥3 each. Patients had to have platelet count ≥50 × 10^9/L in the 4 weeks before screening, and the study has no exclusion for anemia. The study design included four parts with the first part being safety run-in equivalent to a phase I study. Patients remained on their stable dose of ruxolitinib that they had been taking for the past 8 weeks and were randomized to receive add-on parsaclisib at 10 or 20 mg QD for 8 weeks followed by the same dose QW thereafter (daily-weekly group) or parsaclisib at 5 or 20 mg QD for 8 weeks followed by 5 mg QD thereafter (all-daily group) [44].

Based on results from an interim analysis, all-daily dosing seemed more efficacious than the daily-weekly dosing schedule with higher spleen volume responses observed at both week 12 (−13.0% vs −1.9%) and week 24 (−21.8% vs −3.5%) [44]. A similar trend was observed for improvement in symptom scores with better responses demonstrated in the all-daily group. New-onset grade 3 thrombocytopenia was reported in 19% (6/32) and 26% (9/35) of the patients in the daily-weekly and all-daily groups, respectively; grade 4 thrombocytopenia was reported in 21% (7/33) of patients in the daily-weekly group but not in the all-daily group. Notably, hemoglobin

levels remained steady in both parsaclisib dosing groups during the study. Additionally, no colitis or grade ≥2 diarrhea or rash were reported in the all-daily group [44]. Thus, the addition of parsaclisib to ruxolitinib was well tolerated with limited grade 3/4 AEs. In a subgroup analysis based on low (50–<100 × 10^9/L) or higher (≥100 × 10^9/L) baseline platelet counts, the addition of parsaclisib (administered daily-weekly or all-daily) to ruxolitinib was efficacious with an acceptable safety profile in patients from both the low and higher baseline platelet count groups [45]. This suggests a unique safety profile of parsaclisib in patients with MF, with low incidences of high-grade hematologic and infrequent high-grade nonhematologic events. Furthermore, addition of parsaclisib to ruxolitinib shows encouraging efficacy in terms of regaining spleen and symptom responses in patients with MF who demonstrate a suboptimal response to ruxolitinib alone, including commonly more difficult to treat patients who may have thrombocytopenia associated with JAK inhibitors. Based on the encouraging results from the 50465-201 phase II study, parsaclisib all-daily dosing was selected for further efficacy and safety evaluation.

Different combination strategies with JAK2 inhibition are currently being explored in clinical studies in patients with MF. CPI-0610 is a novel bromodomain and extraterminal domain protein inhibitor that is being investigated in an ongoing multicohort, phase II study (NCT02158858), including as an 'add-on' to ruxolitinib for patients with MF who have experienced suboptimal disease control with ruxolitinib [46]. However, the criteria defining suboptimal response in that study are not clearly defined. Additionally, patients with transfusion-dependent or transfusion-independent anemia as well as those with a platelet count of ≥75 × 10^9 were included in the 'add-on' group. The most recent results show week 24 median spleen volume change of −21.9% in the transfusion-dependent cohort and −15.8% in the transfusion-independent cohort. Grade ≥3 hematologic toxicities were thrombocytopenia (24.2%) and anemia (8.5%). Most common grade 3 nonhematologic toxicities were fatigue (5.7%), diarrhea (4.3%), and respiratory tract infections (4.3%) [46].

Navitoclax, a BCL-2/BCL-xL inhibitor, has also been evaluated in combination with ruxolitinib in a phase II study (NCT03222609) in patients with MF pretreated with ruxolitinib [47]. The study enrolled patients with palpable splenomegaly (≥5 cm below the costal margin) or spleen volume of ≥450 cm^3 pretreated with a stable dose of ≥10 mg ruxolitinib for ≥8 weeks before the first dose of navitoclax and with a platelet count of ≥100 × 10^9/L. Spleen volume reduction of ≥35% at week 24 (primary study endpoint) was achieved by 26.5% of patients; observed week 24 median spleen volume change was −36.9%. The most common reported grade ≥3 AEs were thrombocytopenia (56%), anemia (32%), and pneumonia (12%); the most common serious AEs were pneumonia (12%) and splenic infarction (6%) [47].

6. Future plans for parsaclisib in comparison with other phase III combination studies

The encouraging efficacy with additional spleen responses observed in the phase II study evaluating add-on parsaclisib to ruxolitinib informed the development of two phase III studies in patients with MPN (Table 1). LIMBER-304 (INCB 50465-304; NCT04551053) is designed to further evaluate parsaclisib in the 'add-on' setting [48]. The efficacy and safety of parsaclisib plus ruxolitinib will be assessed in patients with suboptimal response, defined as receiving treatment with ruxolitinib for ≥3 months with a stable dose ranging from 5 to 25 mg BID for at least 8 weeks immediately before enrollment and with palpable spleen length of ≥5 cm below left subcostal margin and active MF symptoms (Total Symptom Score [TSS] ≥10 using the Screening Symptom Form) at the screening visit. Importantly, the study design includes stratification by platelet count (≥100 × 10^9/L vs 50 to <100 × 10^9/L) and Dynamic International Prognostic Scoring System (DIPSS) risk category (intermediate-1 vs. intermediate-2 vs. high). In LIMBER-313 (INCB 50465-313; NCT04551066), the combination of parsaclisib and ruxolitinib will be assessed in the frontline setting [49]. The study will enroll patients with MF who demonstrate the need for MF-directed therapy (i.e. palpable spleen ≥5 cm below left subcostal margin and active MF symptoms [TSS ≥10 using the Screening Symptom Form] at screening) who have not received prior JAK or PI3K inhibitor therapy. Like the add-on setting, the design for this study also includes stratification by platelet count and DIPSS risk category. In addition, baseline platelet count will be used to determine the starting ruxolitinib dose.

The PI3K pathway plays an important role in inflammatory cytokine signaling downstream of mutated *JAK2* and possibly contributes to cellular resistance to JAK inhibition. The results from the add-on setting will provide evidence whether targeting PI3Kδ can reverse suboptimal response to JAK1/2 inhibition seen in a subset of patients and help regain responses to ruxolitinib. Furthermore, results from LIMBER-313 in the frontline setting will help to understand if targeting both PI3Kδ and JAK1/2 can help to ameliorate the inflammatory component of MF in patients naive to JAK inhibition. Moreover, findings from both studies will be crucial to confirming synergistic effects of the combined inhibition of PI3K and JAK1/2 in patients with MPNs.

Other phase III combination trials with ruxolitinib in MPNs are summarized in Table 1. Notably, in combination trials of ruxolitinib with navitoclax and CPI-0610, although the key outcomes are similar to the phase III parsaclisib plus ruxolitinib studies, none of the trials appear to have well-defined criteria for defining suboptimal response to ruxolitinib. With a better understanding of MF disease, the definition of suboptimal response to ruxolitinib is evolving, and it is becoming more important to distinguish between patients who relapse or are refractory to JAK inhibitors from those who do not achieve an adequate response [13]. Moreover, the combination trials with either navitoclax or CPI-0610 do not stratify patients enrolled by baseline platelet count. Since thrombocytopenia is considered a negative prognostic factor for patients with MF, stratification by platelet count in the combination trials with parsaclisib may help determine the effect of targeting JAK1/2 and PI3K together in patients with low platelet counts who generally have poor prognosis and reduced survival rates.

Table 1. Summary of phase III studies with ruxolitinib in combination with other targeted therapies in MPNs.

Study name/NCT number[a]	Study arms	Key inclusion criteria				Primary outcomes
		Prior or current ruxolitinib	Platelet count	Splenomegaly	Symptom burden	
LIMBER-304/ NCT04551053 (recruiting)	Parsaclisib plus ruxolitinib vs. placebo plus ruxolitinib	None	Two groups: ≥100 × 10^9/L vs. 50 to <100 × 10^9/L	Palpable spleen of ≥5 cm below the left costal margin on physical examination	Active MF symptoms at screening with a TSS of ≥10 using the Screening Symptom Form	Proportion of patients achieving targeted reduction in spleen volume from baseline to week 24
LIMBER-313/ NCT04551066 (recruiting)	Parsaclisib plus ruxolitinib vs. placebo plus ruxolitinib	≥3 months with a stable dose for at least the last 8 weeks before day 1 of the study	Two groups: ≥100 × 10^9/L vs. 50 to <100 × 10^9/L	Palpable spleen of ≥5 cm below the left costal margin on physical examination	Active MF symptoms at screening with a TSS of ≥10 using the Screening Symptom Form	Proportion of patients achieving targeted reduction in spleen volume from baseline to week 24
TRANSFORM-1/ NCT04472598 (recruiting)	Navitoclax plus ruxolitinib vs. placebo plus ruxolitinib	None		Length ≥5 cm below costal margin or volume ≥450 cm^3	At least two symptoms with a score ≥3 or a total score of ≥12, as measured by the MFSAF v4.0	Percentage of patients who achieve spleen volume reduction of ≥35% at week 24
TRANSFORM-2/ NCT04468984 (recruiting)	Navitoclax plus ruxolitinib vs. best available therapy	≥24 weeks but stopped or continued despite relapsed/ refractory status OR <24 weeks with documented disease progression defined by appearance of new splenomegaly ≥28 days with intolerance defined as new RBC transfusion requirement	≥100 × 10^9/L	Length ≥5 cm below costal margin or volume ≥450 cm^3	At least two symptoms with a score ≥3 or a total score of ≥12, as measured by the MFSAF v4.0	Percentage of patients who achieve spleen volume reduction of ≥35% at week 24
MANIFEST-2/ NCT04603495 (recruiting)	CPI-0610 plus ruxolitinib vs. placebo plus ruxolitinib	None	None defined	Volume ≥450 cm^3	At least two symptoms with an average score ≥3 or an average total score of ≥10 over the 7-day period before randomization using the MFSAF v4.0	Spleen volume reduction of ≥35% from baseline to week 24

[a]Study details from ClinicalTrials.gov as of the time of writing this review (6 April 2022).
MFSAF v4.0: Myelofibrosis Symptom Assessment Form version 4.0; MPN: myeloproliferative neoplasm; RBC: red blood count; TSS: Total Symptom Score.

7. Conclusions

Based on our understanding of underlying mechanisms and role of signaling via the PI3K pathway in loss of response or resistance to ruxolitinib treatment and initial results from clinical studies, the combination of parsaclisib and ruxolitinib holds great clinical potential and, if confirmed in larger clinical trials, may provide more treatment options and improved clinical outcomes for patients with MPNs.

8. Expert opinion

Targeting the JAK/STAT pathway with ruxolitinib has substantially improved the management of patients with MPNs. Long-term follow-up of patients treated with ruxolitinib demonstrated durable responses and significant survival benefit versus the best available therapy up to 5 years of follow-up [2,50,51,52]. Unfortunately, despite meaningful clinical benefits, many patients lose response over time.

In clinical practice, treatment-related anemia and thrombocytopenia prevent dose optimization and often lead to early discontinuation of ruxolitinib in a subset of patients with MF [11], and ruxolitinib is not an option for patients with severe thrombocytopenia (platelet count $<50 \times 10^9$/L) cannot be treated with ruxolitinib. Additionally, some patients with a promising initial response develop primary or secondary resistance to ruxolitinib [11]. Thus, in a subset of patients, an adequate and optimal response to ruxolitinib is not achieved, presenting a significant unmet clinical need. Therefore, novel combination treatment strategies are needed that can provide deeper and more durable responses to ruxolitinib in these patients.

Preclinical evidence strongly suggests that the PI3K pathway could be a compensatory survival pathway, contributing to inadequate responses or development of resistance to ruxolitinib, observed in a subset of patients with MF. As the PI3K pathway has emerged as a potential therapeutic target, both pan-PI3K and isoform-specific inhibitors have been tested in clinical trials with ruxolitinib in MF. Unfortunately, the combination of ruxolitinib and the pan-PI3K inhibitor buparlisib did not show promising results, and further investigation was terminated. Similarly, a study evaluating the combination of ruxolitinib and idelalisib (PI3K α and δ inhibitor) was prematurely terminated because of safety concerns [53]. In another study assessing the combination of ruxolitinib and umbralisib (PI3Kδ and CK1ε inhibitor) in patients with MF, anemia, decreased neutrophil count, and diarrhea were the most common grade 3 AEs [54]. Currently, there are no advanced phase II or III studies reported with this combination as per ClinicalTrials.gov (accessed 6 April 2022).

Parsaclisib is a next-generation and highly selective inhibitor of PI3Kδ, the predominant isoform in hematopoietic cells [19,38]. Promising results from the ongoing phase II study of add-on parsaclisib to ruxolitinib in patients with MF, who experienced suboptimal response with ruxolitinib, have shown potential to improve responses to ruxolitinib and a differentiated safety profile with limited grade 3/4 AEs [44]. Future phase III studies will further evaluate the combination of parsaclisib with ruxolitinib in the add-on setting in patients already receiving ruxolitinib, and in the frontline setting in PI3K- and JAK-naïve patients with MF. With anticipated confirmatory larger clinical trials, in the next 5 years there may be an improved alternative treatment option combining PI3K and JAK inhibition for patients with MPNs who currently experience a suboptimal response with ruxolitinib.

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