Targets in MPNs and potential therapeutics

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Abstract

Philadelphia-negative classical Myeloproliferative Neoplasms (MPNs), including Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF), are clonal hemopathies that emerge in the hematopoietic stem cell (HSC) compartment. MPN driver mutations are restricted to specific exons (14 and 12) of Janus kinase 2 (*JAK2*), thrombopoietin receptor (*MPL/TPOR*) and calreticulin (*CALR*) genes, are involved directly in clonal myeloproliferation and generate the MPN phenotype. As a result, an increased number of fully functional erythrocytes, platelets and leukocytes is observed in the peripheral blood. Nevertheless, the complexity and heterogeneity of MPN clinical phenotypes cannot be solely explained by the type of driver mutation. Other factors, such as additional somatic mutations affecting epigenetic regulators or spliceosomes components, mutant allele burdens and modifiers of signaling by driver mutants, clonal architecture and the order of mutation acquisition, signaling events that occur downstream of a driver mutation, the presence of specific germ-line variants, the interaction of the neoplastic clone with bone marrow microenvironment and chronic inflammation, all can modulate the disease phenotype, influence the MPN clinical course and therefore, might be useful therapeutic targets.

1. Introduction

The constitutively activated Janus kinase (JAK)/Signal Transducers and Activators of Transcription (STAT) signaling pathway is fundamental for Myeloproliferative Neoplasms (MPN) pathogenesis (Fig. 1), irrespective of driver mutation, being encountered even in "triple-negative" MPN cases (without JAK2, thrombopoietin receptor (MPL/TPOR) and calreticulin (CALR) mutations) (Rampal et al., 2014). Importantly, several benign conditions characterized by polyclonal hematopoiesis, namely Hereditary Erythrocytosis and Hereditary Thrombocytosis, can mimic "triple-negative" MPNs, leading to diagnostic and therapeutic challenges (Rumi and Cazzola, 2017).

Allogeneic hematopoietic stem cell (HSC) transplantation is the only curative treatment option for MPNs (Zhang et al., 2020). However, due to its considerable risk of mortality and morbidity, this procedure is usually applied to younger Primary Myelofibrosis (PMF) patients exhibiting unfavorable prognostic scores (Kröger et al., 2015). The other therapeutic strategies are mainly palliative, focusing on cytoreduction, prevention of thrombotic events, alleviation of constitutional symptoms, anemia and splenomegaly (Barbui et al., 2018). The increasing knowledge of the complex molecular mechanisms that contribute to MPN development and progression (Grinfeld et al., 2017; Vainchenker and Kralovics, 2017) creates the

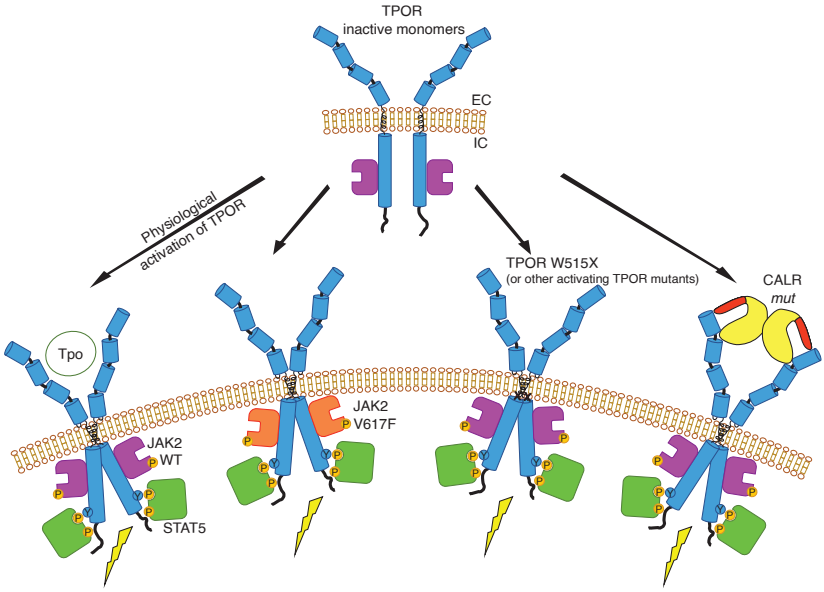

Fig. 1 The "MPN driver mutations" restricted to specific exons of JAK2, MPL/TPOR and CALR genes are involved directly in clonal myeloproliferation and generate the MPN phenotype. *JAK2,* Janus kinase 2; *TPOR*, thrombopoietin receptor (MPL/TPOR); *CALR mut*, calreticulin mutants; *Tpo*, thrombopoietin; *STAT5*, signal transducers and activators of transcription 5; *Y*, tyrosine; *P*, phosphorylation; *EC*, extracellular; *IC*, intracellular.

premises for more personalized therapies. In this chapter, we will address the therapeutic targets and related inhibitors that showed promising results in recent years, some of them being already approved in clinical practice, while most are tested in clinical trials or at least on cellular systems and preclinical animal models.

2. JAK2 mutations

2.1 Behind JAK2V617F: The molecular background

Among all driver mutations of MPNs, *JAK2*V617F is the most prevalent. It accounts for up to 96% of Polycythemia Vera (PVs), 50% of Essential Thrombocythemia (ETs) and 50–60% of Primary Myelofibrosis (PMFs) cases (Baxter et al., 2005; James et al., 2005; Kralovics et al., 2005; Levine et al., 2005; Passamonti et al., 2006). Although predominantly homozygous or carrying homozygous subclones early in the disease in PVs, *JAK2*V617F is found to be mainly heterozygous in ETs (Dupont et al., 2007; Li et al., 2014; Scott et al., 2007). It has been shown in transgenic mice that *JAK2*V617F gene

dosage induces a graded continuous phenotype, starting with ET at low gene dosage, erythrocytosis (PV phenotype) at higher gene dosage and myelofibrosis at very high gene dosage (Tiedt et al., 2008). These results have still to be translated to the human disease setting where either heterozygous or homozygous status can be detected; thus possibly gene dosage data could be interpreted as different levels of JAK2V617F biochemical activity, with low levels inducing ET, medium levels PV and very high levels myelofibrosis. Interestingly, since JAK2V617F expression eventually down-modulated cell-surface TPOR (Moliterno et al., 2006; Pecquet et al., 2012), the PV phenotype can be isolated to medium doses of JAK2V617F biochemical activity due to down-modulation of active TPOR complexes, while the erythropoietin receptor (EPOR) is not down-modulated (Pecquet et al., 2012).

The activation of JAK2 by the V617F mutation remains incompletely understood but seems to rely on a conformational change in the Janus kinase homology 2 (JH2) domain, leading to activation of the adjacent kinase domain (JH1) (Bandaranayake et al., 2012; Dusa et al., 2010; Leroy et al., 2016a, 2019). Furthermore, recently it has been demonstrated that JAK2V617F induces inside-out dimerization of dimeric cytokine receptors (Leroy et al., 2019; Wilmes et al., 2020).

2.2 JAK2V617F inhibition strategies

Since the discovery of the *JAK2*V617F mutation in 2004 (reported in 2005) (Baxter et al., 2005; James et al., 2005; Kralovics et al., 2005; Levine et al., 2005), several drugs have been developed to specifically target the JAK2V617F mutant (Fig. 2). However, no molecule with the ability to only target the mutant has been found to date. Among several JAK2 kinase domain inhibitors tested, only ruxolitinib and fedratinib have been approved by the FDA and EMA (Harrison et al., 2012; Pardanani et al., 2015a; Vannucchi et al., 2015; Verstovsek et al., 2012) because they combine adequate JAK inhibition and are not hampered by (too many) deleterious side effects.

Ruxolitinib is a non-specific JAK1 and JAK2 inhibitor, which inactivates both the wild type (WT) and JAK2V617F. It targets the ATP-binding site of JAKs in their active conformation (Type I inhibitor). Quintás-Cardama et al. (2010) first showed that ruxolitinib inhibited interleukin-6 signaling and proliferation of JAK2V617F-carrying Ba/F3 cells and preferentially suppressed erythroid progenitor colony formation from *JAK2*V617F PV patients compared with healthy donors in primary cultures. In a mouse model of *JAK2*V617F-driven MPN, oral ruxolitinib markedly reduced

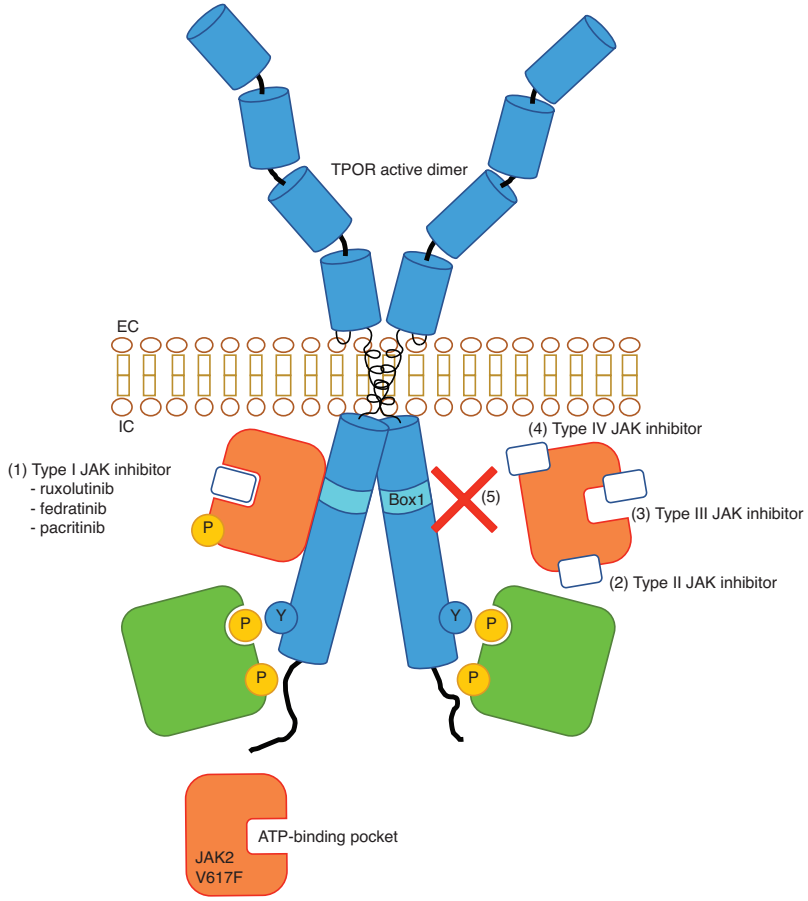

Fig. 2 JAK2 inhibition strategies. Type I inhibitors target the ATP-binding site of JAKs in their active conformation (1). Type II inhibitors interact with JAK2 kinase domain in the inactive conformation (2). Type III and IV inhibitors are allosteric inhibitors targeting sites close (3) or distant (4) of the ATP-binding pocket. Other strategies could target interaction of JAK2V617F and TPOR or of JAK2 and TPOR mutants (5). *JAK*, Janus kinase; *TPOR*, thrombopoietin receptor; *Y*, tyrosine; *P*, phosphorylation; *EC*, extracellular; *IC*, intracellular.

splenomegaly and circulating levels of inflammatory cytokines, and preferentially eliminated neoplastic cells, resulting in significantly prolonged survival without myelosuppressive or immunosuppressive effects.

In the same year 2010, Verstovsek et al. (2010) published the results of a phase 1–2 dose-escalation study (Study 251) for ruxolitinib in MF patients (Table 1). The results of the Study 251 were unprecedented at the time

Table 1 Ongoing studies for targeted therapies in MPNs.

Study ID number	Brief title of the study	Status (first posted)	Condition	Number of patients	Study type	Study phase	Intervention/drugs	Activity	Primary outcome measures
NCT00509899	Open label ruxolitinib (INCB018424) in patients with myelofibrosis and post polycythemia vera/essential thrombocythemia myelofibrosis (expansion of Study 251)	Completed (August 1, 2007)	- MF - PV - Thrombocytosis	154	Interventional	Phase 1 Phase 2	Ruxolitinib (INCB018424)	JAK1 and JAK2 inhibitor	Safety and tolerability of INCB018424
NCT00952289	Controlled myelofibrosis study with oral JAK inhibitor treatment: the COMFORT-I trial	Completed (August 6, 2009)	MF (primary, post-ET or post-PV)	309	Interventional	Phase 3	Ruxolitinib vs placebo	JAK1 and JAK2 inhibitor	Spleen reduction $\geq$35% (at week 24)
NCT00934544	Controlled myelofibrosis study with oral Janus-associated kinase (JAK) inhibitor treatment-II: the COMFORT-II trial	Completed (July 8, 2009)	MF (primary, post-ET or post-PV)	219	Interventional	Phase 3	Ruxolitinib vs best available therapy	JAK1 and JAK2 inhibitor	Spleen reduction $\geq$35% (at week 48)
NCT01437787	Phase III study of SAR302503 in intermediate-2 and high risk patients with myelofibrosis (JAKARTA)	Completed (September 21, 2011)	MF (primary, post-ET or post-PV)	289	Interventional	Phase 3	Fedratinib (SAR302503) vs placebo	JAK2 (and FLT3) inhibitor	Spleen reduction $\geq$35% (at cycle 6)
NCT01523171	Phase II, open label, single arm study of SAR302503 in myelofibrosis patients previously treated with ruxolitinib (JAKARTA2)	Completed (February 1, 2012)	MF (primary, post-ET or post-PV)	97	Interventional	Phase 2	Fedratinib (SAR302503) after ruxolitinib	(JAK1 and) JAK2 inhibitors	Spleen reduction $\geq$35% (at cycle 6)

NCT01773187	Acritinib versus best available therapy to treat myelofibrosis (PERSIST-1)	Terminated (January 23, 2013)	MF (primary, post-ET or post-PV)	327	Interventional	Phase 3	Pacritinib vs best available therapy	JAK2 (and IRAK1) inhibitor	Spleen reduction ≥35% (at week 24)
NCT02055781	Oral pacritinib versus best available therapy to treat myelofibrosis with thrombocytopenia (PERSIST-2, PAC326)	Terminated (February 5, 2014)	MF (primary, post-ET or post-PV)	311	Interventional	Phase 3	Pacritinib vs best available therapy	JAK2 (and IRAK1) inhibitor	Spleen reduction ≥35% (at week 24)
NCT03165734	A phase 3 study of pacritinib in patients with primary myelofibrosis, post polycythemia vera myelofibrosis, or post-essential thrombocythemia myelofibrosis (PACIFICA)	Recruiting	MF (primary, post-ET or post-PV)	348 (estimated)	Interventional	Phase 3	Pacritinib vs physician's choice	JAK2 (and IRAK1) inhibitor	Spleen reduction ≥35%
NCT04173494	A study of momelotinib versus Danazol in symptomatic and anemic myelofibrosis patients (MOMENTUM)	Recruiting	MF (primary, post-ET or post-PV)	180 (estimated)	Interventional	Phase 3	Momelotinib vs Danazol	JAK1 and JAK2 (and ACVR1) inhibitor	Total symptom score (TSS) response rate at week 24 measured using the myelofibrosis symptom assessment form v4.0
NCT03566446	CALR exon 9 mutant peptide vaccine to patients with CALR-mutant myeloproliferative neoplasms	Active, not recruiting	- MPN, unclassifiable - ET - MF	10 (estimated)	Interventional	Phase 1	CALR long 36 peptide (vaccines)	Anti CALR mutants vaccine	Adverse events evaluated by CTCAE 4.03 (time frame: 1 year)

Continued

Table 1 Ongoing studies for targeted therapies in MPNs.—cont'd

Study ID number	Brief title of the study	Status (first posted)	Condition	Number of patients	Study type	Study phase	Intervention/drugs	Activity	Primary outcome measures
NCT02158858	A phase 2 study of CPI-0610 with and without ruxolitinib in patients with myelofibrosis (MANIFEST)	Recruiting	MF	271 (estimated, phase 2) 10 (phase 1)	Interventional	Phase 1 Phase 2	CPI-0610 +/− ruxolitinib	BET inhibitors	- Phase 1: frequency of dose-limiting toxicities (DLTs) associated with CPI-0610 administration during the first cycle (first 21 days) of treatment - Phase 2: spleen response and red blood cell transfusion independency
NCT01712308	Sotatercept in treating patients with myeloproliferative neoplasm-associated myelofibrosis or anemia	Recruiting	- MF - Myelodysplastic/ myeloproliferative neoplasm, and - Anemia	60 (estimated)	Interventional	Phase 2	Sotatercept	TGF-β superfamily inhibitor	- Incidence of foxicities - Anemia response
NCT03194542	A safety and efficacy study to evaluate luspatercept in subjects with myeloproliferative neoplasm-associated myelofibrosis who have anemia with and without red blood cell-transfusion dependence	Recruiting	- Primary MF and - Anemia	103 (estimated)	Interventional	Phase 2	Luspatercept	TGF-β superfamily inhibitor	Anemia response

NCT04103645	Intra-patient dose escalation study to investigate safety and feasibility of vactosertib in treating anemic MPN patients	Recruiting	- MPN and - Anemia	37 (estimated)	Interventional	Phase 2	Vactosertib (TEW-7197)	TGF-β superfamily inhibitor	- Number of adverse events - Change in symptoms of the disease - Change in spleen size - Change in transfusion dependency - Change in hemoglobin values - Change in EPO levels
NCT04218071	Actuate 1901: 9-ING-41 in myelofibrosis	Recruiting	MF	58 (estimated)	Interventional	Phase 2	9-ING-41 +/− ruxolitinib	GSK-3β inhibitor	Response rate (time frame: 3–24 months)
NCT01981850	A phase 2 study of PRM-151 in participants with myelofibrosis	Completed (November 13, 2013)	MF (primary, post-ET or post-PV)	125	Interventional	Phase 2	PRM-151 +/− ruxolitinib	Recombinant serum amyloid P (SAP)	Overall response rate according to International Working Group consensus criteria for treatment response in myelofibrosis with myeloid metaplasia (Time frame: 6 months)

Continued

Table 1 Ongoing studies for targeted therapies in MPNs.—cont'd

Study ID number	Brief title of the study	Status (first posted)	Condition	Number of patients	Study type	Study phase	Intervention/drugs	Activity	Primary outcome measures
NCT03065400	PD-1 inhibition in advanced myeloproliferative neoplasms	Completed (February 7, 2017)	- MF (primary, post-ET or post-PV) - MPN in accelerated/blast phase	10	Interventional	Phase 2	Pembrolizumab	PD-1	At least one clinical improvement (CI, PR, CR) by combined European Leukemia Net -International Working Group (ELN-IWG) criteria after six cycles of pembrolizumab therapy
NCT04097821	Platform study of novel ruxolitinib combinations in myelofibrosis patients (ADORE)	Recruiting	MF (primary, post-ET or post-PV)	243 (estimated)	Interventional	Phase 1 Phase 2	- Siremadlin +/− ruxolitinib - Crizanlizumab +/− ruxolitinib - MBG453 (sabatolimab) +/− ruxolitinib - LTT462 +/− ruxolitinib - NIS793 +/− ruxolitinib	- p53-MDM2 interaction inhibitor (siremadlin) - P-selectin blocker (crizanlizumab) - TIM-3 inhibitor (MBG453) - ERK1/2 inhibitor (LTT462) - TGF-β inhibitor (NIS793)	- Incidence and severity of dose limiting toxicities within the first 2 cycles - Response rate at the end of cycle 6 or cycle 8
NCT03662126	KRT-232 versus best available therapy for the treatment of subjects with myelofibrosis who are relapsed or refractory to JAK inhibitor treatment	Recruiting	MF (primary, post-ET or post-PV)	385 (estimated)	Interventional	Phase 2 Phase 3	KRT-232 vs best available therapy	MDM2 inhibitor	Spleen reduction ≥35% (at week 24)

NCT03669965	KRT-232 compared to ruxolitinib in patients with phlebotomy-dependent polycythemia vera	Active, not recruiting	PV	295 (estimated)	Interventional	Phase 2	KRT-232	MDM2 inhibitor	Response at week 32 defined as having achieved both: absence of phlebotomy eligibility beginning at the week 8 visit and continuing through week 32, reduction in spleen volume ≥35% at week 32
NCT04113616	An open-label, multicenter, phase 1b/2 study of the safety and efficacy of KRT-232 combined with low-dose cytarabine or decitabine in patients with acute myeloid leukemia	Recruiting	- AML - AML secondary to MPNs	160 (estimated)	Interventional	Phase 1 Phase 2	KRT-232 with cytarabine or decitabine	MDM2 inhibitor	- Number of dose-limiting toxicities of KRT-232 in combination with cytarabine or decitabine (Time frame: 28 days), - Proportion of patients achieving complete remission (CR) or complete remission with partial hematological improvement (CRh)
NCT01693601	Panobinostat and ruxolitinib in myelofibrosis (PRIME trial)	Completed (September 26, 2012)	MF (primary, post-ET or post-PV)	20	Interventional	Phase 1 Phase 2	Panobinostat +ruxolitinib	HDAC inhibitor	- Number of patients - With adverse events - That achieve at least stable disease

Continued

Table 1 Ongoing studies for targeted therapies in MPNs.—cont'd

Study ID number	Brief title of the study	Status (first posted)	Condition	Number of patients	Study type	Study phase	Intervention/drugs	Activity	Primary outcome measures
NCT01761968	Long-term study evaluating the effect of givinostat in patients with chronic myeloproliferative neoplasms	Active, not recruiting	MPNs	90 (estimated)	Interventional	Phase 2	Givinostat	HDAC inhibitor	Long-term safety and efficacy (Time frame: 3 months)
NCT03935555	Assess the safety, tolerability oral PU-H71 in subjects taking ruxolitinib	Recruiting	MF (primary, post-ET or post-PV)	24 (estimated)	Interventional	Phase 1	PU-H71	HSP90 inhibitor	- Safety and tolerability - Treatment response (Time frame: week 24)
NCT03373877	Evaluation of ruxolitinib in combination with PU-H71 for treatment of myelofibrosis	Terminated (December 14, 2017)	MF (primary, post-ET or post-PV)	4	Interventional	Phase 1	PU-H71 +ruxolitinib	HSP90 inhibitor	- Incidence of adverse events (Time frame: 12 months) - Maximum tolerated dose of PU-H71 (Time frame: 7 months) - Recommended phase 2 dose of PU-H71 (Time frame: 12 months) - Pharmacokinetic profile of PU-H71 (Time frame: 12 months)

NCT01393509	The first-in-human phase I trial of PU-H71 in patients with advanced malignancies	Active, not recruiting	MPNs	47	Interventional	Phase 1	PU-H71	HSP90 inhibitor	- Safety (Time frame: 2 years) - Tolerability (Time frame: 2 years) - Pharmacokinetic - Maximum tolerated dose (Time frame: 2 years)
NCT03136185	IMG-7289 in patients with myelofibrosis	Recruiting	MF (primary, post-ET or post-PV)	75 (estimated)	Interventional	Phase 2	IMG-7289	LSD1 inhibitor	- Safety (Time frame: 28 days) - Tolerability (Time frame: 28 days)
NCT04262141	IMG-7289 in patients with essential thrombocythemia or polycythemia vera	Recruiting	- ET - PV	24 (estimated)	Interventional	Phase 2	IMG-7289	LSD1 inhibitor	Hematologic response rates (Time frame: 24 weeks)
NCT03222609	A study evaluating tolerability and efficacy of navitoclax alone or in combination with ruxolitinib in participants with myelofibrosis	Recruiting	MF (primary, post-ET or post-PV)	164 (estimated)	Interventional	Phase 2	Navitoclax (ABT-263) +/− ruxolitinib	Bcl-2 inhibitor	Spleen reduction ≥35% (at week 24)
NCT04354727	A study of APG-1252 in patients with myelofibrosis who progressed after initial therapy	Recruiting	MF	60 (estimated)	Interventional	Phase 1 Phase 2	Palcitoclax (APG-1252) +/− ruxolitinib	Bcl-2/xL inhibitor	- Dose-limiting toxicity (Time frame: 28 days) - Spleen volume measurement reduction

Continued

Table 1 Ongoing studies for targeted therapies in MPNs.—cont'd

Study ID number	Brief title of the study	Status (first posted)	Condition	Number of patients	Study type	Study phase	Intervention/drugs	Activity	Primary outcome measures
NCT02098161	LCL161 in treating patients with primary myelofibrosis, post-polycythemia vera myelofibrosis, or post-essential thrombocytosis myelofibrosis	Active, not recruiting	MF (primary, post-ET or post-PV)	50 (estimated)	Interventional	Phase 2	LCL161	SMAC mimetic	Objective response rate (after 84 days (three courses) of treatment)
NCT02593760	A study to evaluate the efficacy and safety of vismodegib in combination with ruxolitinib for the treatment of intermediate- or high-risk myelofibrosis	Completed (November 1, 2015)	MF (primary, post-ET or post-PV)	10	Interventional	Phase 1	Vismodegib or placebo + ruxolitinib	Hedgehog pathway inhibitor	- Spleen reduction ≥35% (at week 24) - Complete and partial remisions rate (at week 24)
NCT01243073	Open label study to evaluate the activity of imetelstat in patients with essential thrombocythemia or polycythemia vera	Completed (November 18, 2010)	- ET - PV	20	Interventional	Phase 2	Imetelstat (GRN163L)	Telomerase inhibitor	Hematologic response
NCT02426086	Study to evaluate activity of two dose levels of imetelstat in participants with intermediate-2 or high-risk myelofibrosis previously treated with Janus kinase inhibitor	Completed (April 24, 2015)	Primary MF	107	Interventional	Phase 2	Imetelstat (GRN163L)	Telomerase inhibitor	- Spleen reduction ≥35% (at week 24) - Reduction in total symptom score (TSS) ≥50% (at week 24)

PV, polycythemia vera; *ET*, essential thrombocythemia; *MF*, myelofibrosis; *MPN*, myeloproliferative neoplasm; *AML*, acute myeloid leukemia; *BET*, bromodomain and extra-terminal motif; *HDAC*, histone deacetylase; *SMAC*, second mitochondria-derived activators of caspases.

(Vannucchi, 2010) with both prolonged reduction in blood parameters and spleen volume and massive improvements in symptoms and quality of life. These latter results were biologically accompanied by a reduction in signal transduction and proinflammatory cytokine levels. In the Study 251, ruxolitinib achieved improvements irrespectively of JAK2 mutational status or subtype of MF (i.e., primary, post-PV or post-ET MF). As seen in rodent models, only a modest decrease in mutant *JAK2*V617F allele burden was observed. After the COMFORT-I and -II phase 3 controlled trials (Harrison et al., 2012; Verstovsek et al., 2012) ruxolitinib was approved in 2012 by the FDA and the EMA for the treatment of MF. While bone marrow transplantation remains the only curative treatment for MF (Kröger et al., 2015; Zhang et al., 2020), other studies have since confirmed the efficacy of ruxolitinib and it is considered as a first-line therapy for patients with intermediate or high-risk MF, including primary, post-PV or post-ET MF.

For PV, ruxolitinib was approved by the FDA in 2014 (Vannucchi et al., 2015) (and by the EMA in 2015) and was the first FDA-approved treatment for patients with PV, who have an inadequate response or are intolerant to hydroxyurea.

With respect to ET treatment, ruxolitinib has not been shown to be more efficient or better tolerated than best available therapies (Finazzi, 2017; Harrison et al., 2017) and is therefore not currently recommended. Indeed, while ruxolitinib has shown similar efficacy and, for a subset of patients with particularly troublesome symptoms, has even been shown to be appeasing and could therefore be considered when possible, its relatively high costs combined with potential toxicities, particularly infectious, relating to the long-term exposure required in patients with ET, are probably the main barriers to its routine implementation.

2.3 Other JAK inhibitors in MF

Since the approval of ruxolitinib, many other JAK inhibitors have been assessed for the treatment of MPNs (Table 1), mainly with the aim of being more targeted to JAK2 and lessening the side effects of ruxolitinib, especially cytopenias.

After it was granted priority review and orphan drug designation, fedratinib has been the second JAK inhibitor to be approved by the FDA for the treatment of MF, both in first and second line after ruxolitinib. It was granted marketing authorization with the same indications by the EMA in December 2020. Fedratinib is an oral, selective inhibitor with

activity against WT and mutant JAK2, rationally designed while developing a series of pyrimidine-based inhibitors to target JAK2. Fedratinib was assessed as first-line MF treatment in the placebo-controlled, phase 3 JAKARTA study and as post-ruxolitinib treatment in the single-arm, phase 3 JAKARTA2 study (Harrison et al., 2013, 2019a). Especially, fedratinib was well tolerated in patients with platelets counts between 50 and 100.000/mm (Zhang et al., 2020) (compared with ruxolitinib). However, FDA approved fedratinib in August 2019 with a warning about the risk of severe and fatal encephalopathy, including Wernicke encephalopathy. Therefore, patients should have normal thiamine levels before starting and periodic monitoring of thiamine levels during treatment is also recommended (Gangat et al., 2019; Pardanani et al., 2015b; Talpaz and Kiladjian, 2021).

Recently, among other molecules, pacritinib, a JAK2/FLT3/IRAK1/CSF1R inhibitor, has revealed a very potent JAK2 inhibitory activity without myelosuppressive effects. It has also been shown to reduce spleen size and improve clinical outcome in patients with MF in two phase 3 studies (PERSIST-1, PERSIST-2) (Mascarenhas et al., 2018; Mesa et al., 2017) as well as in a Phase 2 dose-finding study (PAC203) (O'Sullivan et al., 2019) including patients with severe thrombocytopenia. Pacritinib is currently being evaluated in the PACIFICA trial, which was designed to evaluate its efficacy vs physician's choice in patients with MF and severe thrombocytopenia (NCT03165734) (Harrison et al., 2019b).

Globally, many clinical trials in MPNs, and especially MF, are being conducted lately with a focus on the potential benefits of alternative JAK inhibitors (momelotinib in the MOMENTUM clinical trial NCT04173494) or agents for patients with cytopenias with or without transfusion dependence. The challenge with JAK2 inhibitors in the context of ET and PV is that the disease burden is quite low compared to the potential side effects of aggressive therapies.

Although difficult to assess due to the lack of uniform criteria, failure of ruxolitinib is reported in many reports and is estimated to lie around 50% between 6 months to 1.5 years after initiation of the treatment (Fonseca et al., 2013; Palandri et al., 2020; Pemmaraju et al., 2020). Ruxolitinib failure is linked to a variety of causes, including suboptimal response, treatment-related toxicities and progression to secondary AML. Moreover, most type I inhibitors can induce therapy resistance due to several mechanisms:

- enhancement of JAK2 activation loop phosphorylation despite kinase inhibition (Andraos et al., 2012), most likely by exposing the activation loop tyrosines to other kinases,

- heterodimerization between tumor overexpressed JAK2 and JAK1 or TYK2 that results in reactivation of JAK2 signaling (Koppikar et al., 2012),
- theoretically certain JAK2 non-canonical mutations may exhibit longer half-life and hence less sensitivity to JAK2 inhibitors; this is the case for JAK2 R867Q or JAK2, S755R/R938Q described in hereditary thrombocytosis (Marty et al., 2014),

Type II JAK inhibitors, that interact with a JAK2-inactive conformation, might be efficient in Type I JAK inhibitor resistance and might exhibit increased specificity and efficacy due to their interaction with a less conserved kinase site (the DFG-pocket) (Andraos et al., 2012). NVP-BBT594 and NVP-CHZ868 have been developed and showed to be effective in preclinical models but were not further developed (Andraos et al., 2012; Meyer et al., 2015). Due to their binding efficiency, type II JAK2 inhibitors might induce profound cytopenia, limiting their potential use in PV or ET (Vainchenker et al., 2018).

Allosteric inhibitors would theoretically be more specific than Type I and Type II inhibitors targeting sites closer (type III) or distant (type IV) to the ATP-binding pocket, such as the pseudokinase domain of JAK2V617F. Selective JAK2V617F inhibitors would be desirable, since JAK2 WT is essential for normal hematopoiesis and its efficient inhibition will induce cytopenia (Leroy et al., 2016a, 2019; Leroy and Constantinescu, 2017).

However, two recent studies have shown that secondary mutations in JAK2V617F that restore wild-type JAK2 function for most cytokine receptors are inhibiting signaling of IFN gamma through its receptor complex as JAK2V617F conformation is close to the active conformation of wild-type JAK2 when associated with the ligand-stimulated IFN gamma receptor (Hammarén et al., 2018; Leroy et al., 2019). The IFN gamma receptor complex is presumed to adopt a tetramer structure (Marsters et al., 1995) and JAK2 activation within this tetramer would seemingly require the same pseudokinase residues as JAK2V617F (Hammarén et al., 2018; Leroy et al., 2019). Thus, the conformation that stabilizes JAK2V617F might be the same as the conformation that allows activation of the IFN gamma receptor complex where the IFNγR1 is coupled with JAK1 and IFNγR2 is coupled with JAK2 (Marsters et al., 1995), suggesting that specific JAK2V617F inhibitors would potentially inhibit IFN gamma signaling. Hence, other approaches, such as antifibrinolytic agents, telomerase inhibitors, bromodomain and extra-terminal motif (BET) inhibitors, and numerous combinatorial strategies are also being explored. We will focus on those

strategies which might be called "targeted" in the following subchapter dedicated to "other targeted therapies."

One other approach in relation to JAK2 in MPNs could target the differences between interaction of JAK2V617F or WT with TPOR, EPOR, G-CSFR or other receptors. While Box 1 is conserved, recent X-ray crystal structure data suggested unique interactions for each receptor (Ferrao et al., 2018; Moraga et al., 2015). Detachment of JAK2V617F from TPOR or of JAK2 from TPOR mutants would interrupt the activating events leading to MPNs.

2.4 Concerns emerging from JAK2 inhibition in myelofibrosis

Extensive experience with ruxolitinib showed that in rare patients latent viral infections can be re-activated and as a consequence it has become routine practice to test for viral infections before the start of treatment (Harrison et al., 2012; Verstovsek et al., 2012).

Following sporadic observations of co-occurrence of B-cell non-Hodgkin's lymphoma during treatment of MF patients with ruxolitinib, a recent study reported a significantly higher incidence of lymphoma (5.8% of 69 patients with MF and ruxolitinib) vs only 0.36% of 557 MF patients treated with conventional therapy (Porpaczy et al., 2018). This 16-fold increased risk was confirmed in another cohort of 929 patients with MPNs (15-fold increase). Lymphomas were of aggressive B-cell type, extranodal, or leukemic with high MYC expression and did not carry the MPN driver mutations; the median time from initiation of ruxolitinib therapy to diagnosis was 25 months. Preclinical studies using mice devoid of STAT1 revealed that JAK2 inhibition *in vivo* led to appearance of aberrant B-cells in such a context, suggesting that inhibition of STAT1 in the presence of a pre-existent B-cell clone may increase risk of lymphoma development (Porpaczy et al., 2018). It remains to be confirmed whether long term treatment with ruxolitinib requires extensive and sensitive searches for preexisting aberrant B-cell clones.

Another concern is represented by reports that during ruxolitinib treatment, subclones carrying the driver mutation (most frequently JAK2V617F) and loss of function mutations in ASXL1 appear to be selected (Newberry et al., 2017). In an unpublished case from VH and SNC we were able to detect after 1 year of ruxolitinib treatment an allele frequency of 35% of a pathogenic ASXL1 mutation, whereas before treatment this mutation was <3%. The reasons behind such a selection are unknown but suggest that loss of ASXL1 may help the fitness of the *JAK2*V617F clone under ruxolitinib.

3. TPOR/MPL inhibition strategies

The thrombopoietin receptor is less frequently mutated in MPNs (2% of ET and 5% of PMF) (Vainchenker and Kralovics, 2017) and TPOR mutations do not occur in PV. The most prevalent mutations are W515K/L/R/S/A (Pardanani et al., 2006; Pikman et al., 2006) and S505N (Ding et al., 2004, 2009), which lead to dimerization of the receptor in an active conformation in absence of ligand and activation of downstream signaling. Both mutations occur in *TPOR* exon 10, which roughly corresponds to the transmembrane and amphipathic juxtamembrane region of the receptor. S505N leads to dimerization of TM domains in an active interface due to the strong amino-acid polarity between the two Asn and to unraveling of the helicity of the TM domain above H499 (Ding et al., 2009; Leroy et al., 2016b). On the other hand, mutations of W515 of the RWQFP motif lead to suppression of an inactivating anchor in the lipid bilayer, enabling dimerization of the upstream TM helices in an active configuration (Defour et al., 2013; Leroy et al., 2016b).

The TPOR TM dimer can adopt several activating interfaces (Staerk et al., 2011) which induce various levels of activation of the receptor and might lead to different phenotypes of the disease. While in the human, only one interface is activated by Ala-substitutions, novel mutations have been described lately in the TM domain, that also activate the receptor (Levy et al., 2019).

Other much rarer mutations outside the TM or JM domains activate TPOR, such as S204P/F in the extracellular domain and Y591F/N in the cytosolic domain (Cabagnols et al., 2016; Feenstra et al., 2016). Tyrosine 591 is involved in the negative regulation of TPOR signaling (Drachman and Kaushansky, 1997; Pecquet et al., 2010). When phosphorylated it might serve as a docking site for SH2 domain-containing proteins that negatively regulate TPOR. When mutated to Phe or Asn, phosphorylation at this site is no longer possible and this negative regulation is lost (Sangkhae et al., 2014).

To our knowledge, there is no treatment targeting TPOR in MPNs to date (Fig. 3). Tryptophan 491 located at the outer part of the lipid bilayer has recently been shown to have a role in activation of the receptor by either S505N or W515K. When mutated to Ala (W491A), it suppressed ligand-independent activation of TPOR S505N or W515K. Since this region is on the extracellular side of the juxtamembrane-transmembrane domains, one would imagine that it would be an easy target for TPOR

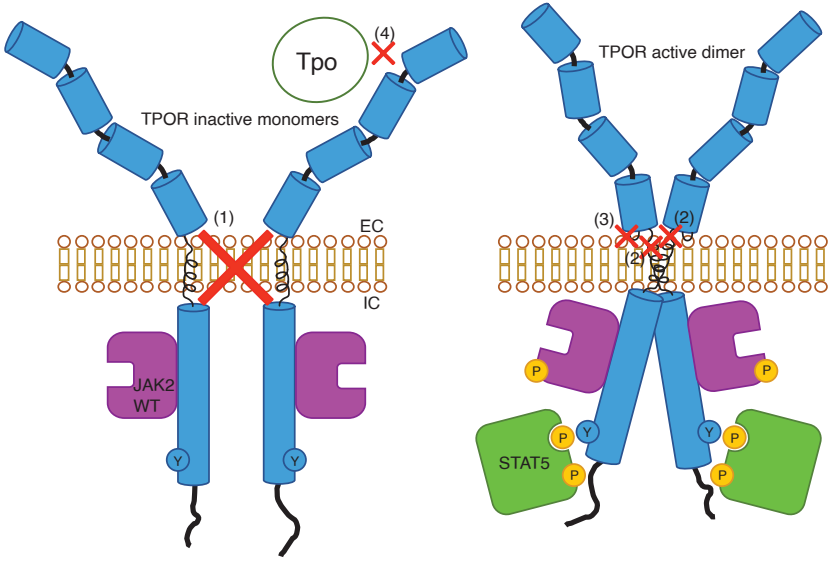

Fig. 3 TPOR inactivating strategies could target: (1) dimerization of the receptor in an active conformation by ligand or by oncogenic mutations, (2) the extracellular juxtamembrane domain at the vicinity or at W491 and H499, where stabilization of active TPOR dimers occurs. Other molecules could aim at preventing the change in the tilt-angle of the extracellular domain necessary for activation of the receptor (3) or prevent Tpo binding (4). *TPOR*, thrombopoietin receptor; *Tpo*, thrombopoietin; *Y*, tyrosine; *P*, phosphorylation; *EC*, extracellular; *IC*, intracellular.

antagonist (antibodies, small molecules, nanobodies, diabodies and others), which might even have specificity for the mutants encountered in PV or MF and not disrupt TPOR wild-type signaling. Other compounds targeting TPOR, but which might lack specificity, could also target the region around H499, as it changes conformation from a random coil in the inactive state to an alpha-helix in the active state. Because TPOR activation also occurs through a change in the tilt angle of the TM domain with the cell surface (Defour et al., 2013), small molecules binding to the outer part of the JM or TM regions could also prevent activation by maintaining the high natural tilt of the ligand-free TPOR. Similarly, increasing the distance between TPOR monomers with diabodies might prevent activation, as suggested for EPOR by Moraga et al. (2015). Targeting TPOR with more or less specificity for the mutants might finally trigger internalization and degradation or antibody-dependent cytotoxicity.

Although potentially beneficial for a small number of patients, it may also be of interest to identify small molecules that bind to the intracellular region

adjacent to the membrane close to and without overlapping with W515, that may adopt a common structural change upon W515L/K/R/S/A mutation.

Finally, antagonist antibodies that prevent Tpo binding have been described (Wang et al., 2016). They prevent Tpo effects and could be useful in the rare cases of Tpo-overexpression. Nevertheless, such antibodies do not inhibit TPOR activation by MPN driver mutations but may decrease the myeloproliferative phenotype as was shown in a Tpo-knockout mouse model of retrovirally-induced MPN by CALR mutants (Marty et al., 2016).

4. CALR mutations

4.1 CALR inhibition strategies

Mutations in the last exon of calreticulin (*CALR*), exon 9, are responsible for approximatively 25% of ET and MF, and occur at a higher frequency than TPOR mutations (Klampfl et al., 2013; Nangalia and Green, 2017). While more than 50 mutations are described, all lead to the same +1 frame-shift in exon 9 that is responsible for the synthesis of a CALR molecule with a novel C-terminus devoid of the KDEL motif, but rich in positively-charged amino acids. Two mutations are predominant, a 52 bp deletion (p.L367fs*46), also called type 1, and a 5 bp insertion (p.K385fs*47), also called type 2. The remaining mutations are classified as type 1-like and type 2-like following the size of the remaining WT sequence. Type 1 *CALR* mutations are predominant in MF, whereas the frequency of both types is more similar in ET. While *CALR* mutations are usually heterozygous, homozygous mutations have been observed, more particularly for type 2 mutations (Klampfl et al., 2013; Nangalia and Green, 2017).

CALR is not a signaling molecule, but a chaperone of the endoplasmic reticulum (ER) involved in quality control of N-glycosylated protein and calcium storage (Michalak et al., 2009). The WT exon 9-coded sequence and the calcium-binding sites are nearly entirely lost in the del52 mutant while ins5 retains around 50% of negative charges (Klampfl et al., 2013). The new C-terminus changes the function of the molecule and allows CALR mutants to tightly bind to TPOR through an interaction between their lectin domain and N-glycosylated residues of the TPOR extracellular domain. They stabilize a dimeric state and transport TPOR to the cell surface where such dimers are able to signal through JAK2 (Araki et al., 2016; Chachoua et al., 2016; Elf et al., 2016; Marty et al., 2010; Pecquet et al., 2019). As a rogue chaperone CALR is nevertheless not sufficient for oncogenic transformation and TPOR remains absolutely required for

the induction of cytokine-independent growth of factor dependent cell lines by CALR mutants (Chachoua et al., 2016). At ASH 2018, Pecquet et al. furthermore reported that CALR mutants are also secreted and found in the serum of MPNs patients (Pecquet et al., 2018). Secreted CALR mutants remain capable of inducing TPOR-induced cell proliferation, a rogue cytokine propriety possibly playing an auto- and paracrine role for mutated HSCs as well as influencing the bone marrow and spleen microenvironment in MPN CALR-mutated patients.

4.2 Potential therapies targeting CALR mutants

The necessary interaction of CALR mutant and TPOR to induce oncogenic transformation has opened a new field for the research of targeted therapies (Figs. 1 and 4). Both the soluble extracellular domain of TPOR (Chachoua et al., 2016) and a peptide electrostatically complementary to

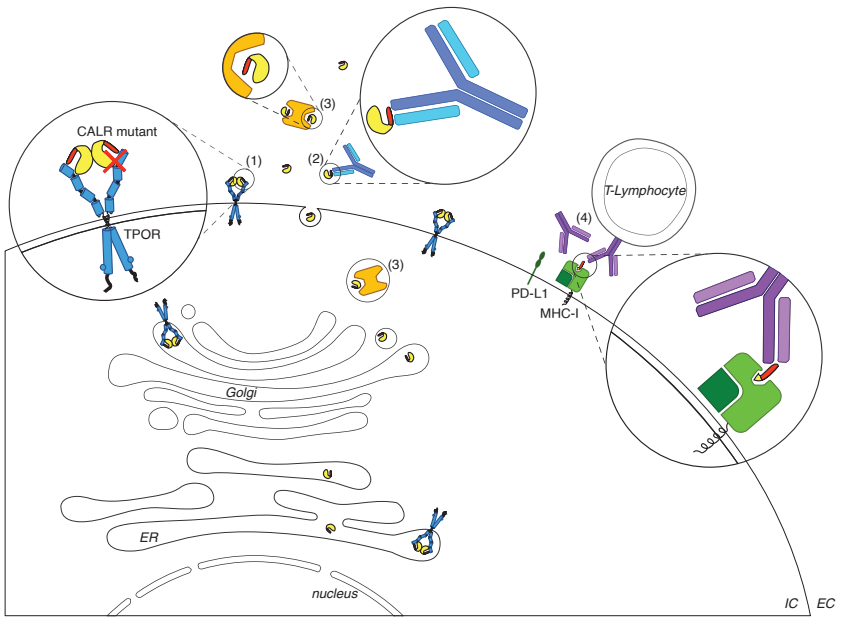

Fig. 4 CALR mutants inactivating strategies could target (1) interaction of CALR with TPOR. Antibodies (2) or small molecules (3) directed at CALR mutants could be used to target and remove CALR mutated proteins. Used as a novel antigen, the C-terminal of the mutated CALR could as well be used to develop vaccines or T-cell-mediated immunotherapy (4). *TPOR*, thrombopoietin receptor; *CALR*, calreticulin; *MHC-I*, major histocompatibility complex I; *PD-L1*, programmed death-ligand 1; *ER*, endoplasmic reticulum; *EC*, extracellular; *IC*, intracellular.

the positively charged sequence of CALR mutants prevent TPOR activation in CALR-mutated target cells (Nivarthi et al., 2016; Pronier et al., 2018), thus small molecules destabilizing TPOR-CALR interaction could be synthetized. These molecules would nevertheless need to be able to inhibit both cell surface and intracellular complexes, as part of the signaling through TPOR-CALR complexes might occur at the intracellular level (Pecquet et al., 2019). This would indeed be a hinge for the development of antibodies preventing such interactions, while antibodies targeting mutated CALR could be used to target and remove circulating and paracrine CALR mutated proteins as well as to eliminate CALR expressing cells, lowering the burden of pathogenic clones. Used as a novel antigen, the C-terminal of the mutated CALR could also be used to develop vaccines or T-cell-mediated immunotherapy. If such therapies would however depend on HLA presentation of the novel peptide, which might be hampered in MPN patients (Arshad and Cresswell, 2018), a trial for a CALR Exon 9 Mutant Peptide Vaccine to Patients With CALR-mutant Myeloproliferative Neoplasms has already been announced by a single center in Denmark, although it is not yet open (NCT03566446) (Table 1). Other development of such targeted antibodies is also ongoing but remains in preclinical stages. On the other hand, immunization with peptides derived from the novel C-tail of mutant CALR did not lead to antibody response in mice transplanted with a mixture of bone marrow from wild-type vs del52 KI mice, while mice transplanted with wild type bone marrow responded (Balligand et al., 2019). These data suggest that the presence of the antigenic sequence in the HSCs may lead to tolerance.

5. Other targeted therapies

The development of targeted therapies aims to actually inhibit causative alterations specifically in the tumor. While the three driver mutations are found as single mutations in MPNs, they can be associated with numerous additional acquired mutations (Grinfeld et al., 2018; Lundberg et al., 2014), which can modulate the phenotype or the evolution of the disease. Furthermore, understanding the biology of MPN has revealed how certain physiological pathways are disrupted in the diseases (Fig. 5). Alongside the rapid development of targeted therapies, this has led to an increasing number of trials in MPNs, in combination or not with JAK inhibitors. While there is indeed no standard of care for patients with intermediate or high-risk MF who

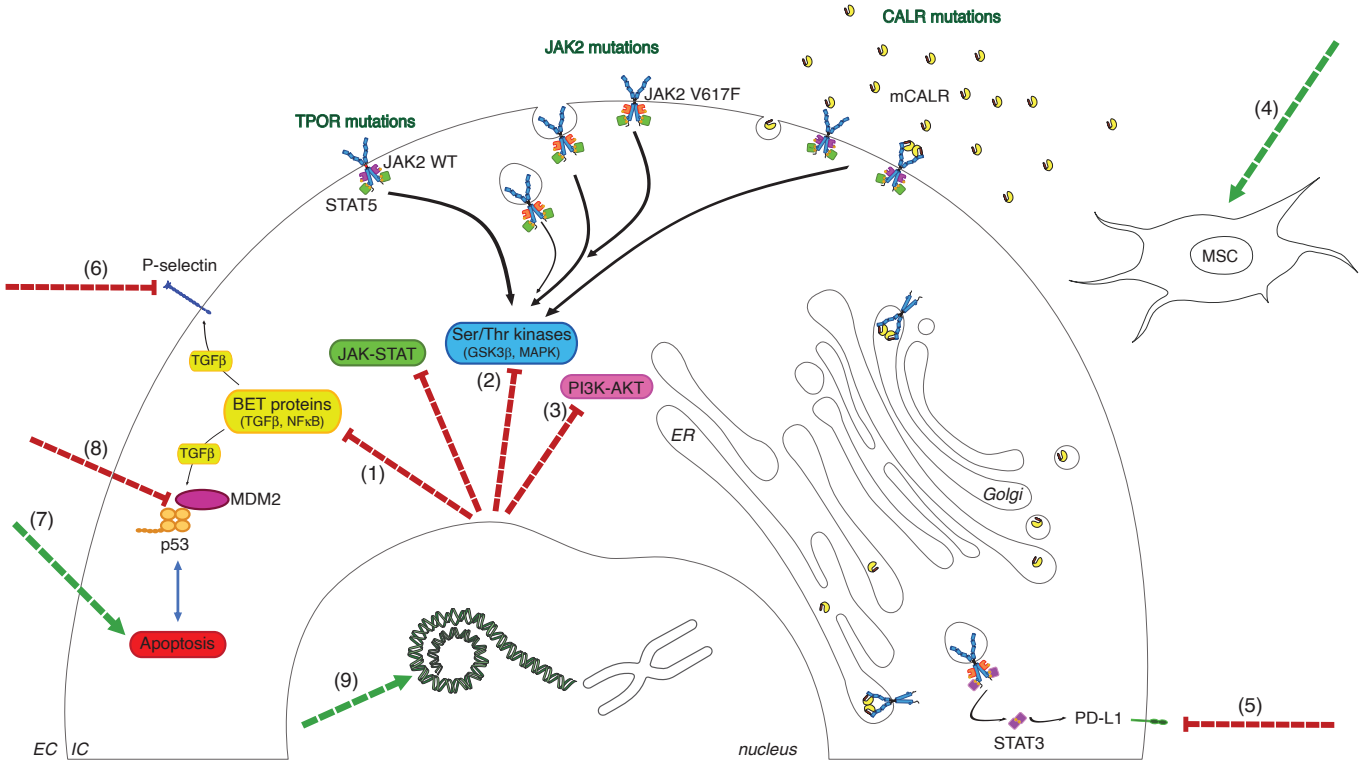

Fig. 5 See figure legend on opposite page.

have failed, become intolerant or resistant to JAK inhibitors, combination of JAK inhibitors with various drugs may lead to clinical benefits in patients.

We will describe here (Table 1) some of the ongoing trials for targeted therapies and also mention potential therapeutic targets that were addressed in preclinical studies.

5.1 Bromodomain and extra-terminal family of proteins

Bromodomain-containing proteins are chromatin-reading proteins with a wide variety of functions, from chromatin remodeling to transcriptional mediation and co-activation (Belkina and Denis, 2012). They contain two tandem bromodomains and an extra-terminal domains. Their main activity is to bind to acetylated histones via the bromodomains. Over the past 15 years, BET proteins have been shown to regulate key oncogenic pathways, especially in myeloid malignancies (Zuber et al., 2011). Such targeted pathways include those of NFκB and TGFβ signaling, which are important drivers of pro-inflammatory cytokine expression and bone marrow fibrosis, respectively, and are involved in myelofibrosis pathogenesis (Le Bousse-Kerdilès, 2012). BET proteins may also play a role in post-MPN secondary AML cells acting as enhancers for the transcription of pro-oncogenes such as c-MYC, BCL2 and CDK4/6 (Fiskus et al., 2014a).

BET inhibitors have been shown to inhibit growth *in vitro* and *in vivo* and induce apoptosis in cultured and primary AML cells (Dawson et al., 2014; Fiskus et al., 2014b; Saenz et al., 2017). Preclinical studies in MPNs suggest

Fig. 5 Other targeted therapies in MPNs could rely on inhibition of downstream pathologically-activated signaling pathways in MPNs (JAK/STAT, PI3K-Akt and Serine/Threonine kinases such as GSK3β) by specific pharmacological inhibitors alone or in combination. BET proteins regulate several pathogenic pathways in MPNs, including NFκB and TGFβ. BET inhibitors and other inhibitors of the TGFβ pathway (1) could be used in MF patients. Other trials aim at targeting Ser/Thr kinases as GSK-3β (2) or the PI3K/AKT/mTOR (3) pathway, as their inhibitors have anti-neoplastic and/or anti-fibrotic properties. The niche plays a role in MPN progression and treatment acting on MSCs or other of its components could be used to decrease MF (4). New strategies have also emerged with the development of immune checkpoint inhibitors (5) or P-selectin inhibitors (6). Targeting apoptosis (7) or increasing p53 levels in MPN cells is another strategy that might be induced by type I interferon treatment, but which is also addressed today by disrupting the interaction between MDM2 and p53 (8). Finally, certain trials have considered targeting histones through histone deacetylases (HDAC) or histone demethylases inhibitors (9). *BET*, bromodomain and extra-terminal motif; *Ser/Thr*, Serine/threonine; *MF*, myelofibrosis; *MSC*, mesenchymal stromal cell; *ER*, endoplasmic reticulum; *EC*, extracellular; *IC*, intracellular.

that a combination of BET inhibitors and JAK inhibitors may result in synergistic reduction of splenomegaly, bone marrow (BM) fibrosis and mutant cell burden (Kleppe et al., 2018).

CPI-0610, a selective and potent small molecule BET inhibitor, has shown clinical activity and a wide therapeutic window in a Phase 1 lymphoma study (Abramson et al., 2015; Blum et al., 2018). Preliminary data of the MANIFEST trial (Mascarenhas et al., 2019a) indicated that CPI-0610 alone or "add-on" to ruxolitinib is generally well-tolerated and provides clinical benefits in MF patients with inadequate responses or who were refractory to ruxolitinib. Alongside, improvement in bone marrow fibrosis and anemia revealed the potential for meaningful disease modification and the trial is currently running for gaining more significant power.

5.2 Inactivation of the TGF-β pathway

Members of the TGF-β superfamily, which include activin and the growth differentiation factor 11 (GDF11), are secreted by bone marrow stromal cells and play a role in proliferation and differentiation of the erythroid precursors. Sotatercept, a TGF-β superfamily inhibitor, which acts as a ligand trap to inhibit activin and GDF11 (soluble activin receptor 2A-IgG Fc fusion), improved anemia in preclinical models of β-thalassemia and Diamond-Blackfan anemia and is being evaluated for treatment of patients with anemia in MPN (NCT01712308). Luspatercept, another ligand trap inhibitor of TGF-β (fusion between activin receptor type 2B extracellular domain and an IgG fragment), leading to inhibition of the SMAD signaling pathway, also demonstrated clinical efficacy for the treatment of anemia (Suragani et al., 2014) in β-thalassemia (Cappellini et al., 2020) and myelodysplastic syndromes (Platzbecker et al., 2017). It has been approved in 2019 by the FDA and in 2020 by the EMA for the treatment of anemia in blood transfusion-dependent patients with β-thalassemia (Markham, 2020) and is currently being evaluated in association with ruxolitinib in a phase 2 multicentric trial for the treatment of anemia in patients with PMF (NCT03194542). The differences between luspatercept and sotatercept have been summarized recently and appear to involve the fact that luspatercept does not alter GDF11 signaling like sotatercept (Guerra et al., 2019).

Based on inhibition of the same signaling pathway, a phase 2, intra-patient dose escalation study is currently open to assess the potential of using the TGFβ receptor inhibitor vactosertib (TEW-7197) in patients with MPNs and anemia (NCT04103645).

5.3 Targeting fibrosis

Following the hypothesis that reduction in bone marrow fibrosis might also help to restore hematopoiesis and improve cytopenias, studies have aimed at identifying fibrosis-driving cells and inhibit their activity.

9-ING-41 is a potent selective GSK-3β inhibitor with clinical anticancer activity, as well as significant pre-clinical ability to reverse pathologic fibrosis in multiple models of pulmonary and pleural fibrosis. The Actuate 1901 Study (NCT04218071) is expected to start soon in patients with advanced and poor prognosis MF. This phase 2 study tackles fibrosis and will investigate 9-ING-41 as a single agent or in combination with ruxolitinib. As 9-ING-41 could potentially act as an anti-neoplastic agent and anti-fibrotic agent in patients with MF, it may reduce the dose of ruxolitinib needed for optimal therapeutic response and/or reverse myelosuppression.

As chronic inflammation driven by an abnormal production of cytokines and other soluble proteins by both malignant and non-malignant cells was suggested to play an important role in MPN progression, leading to a progressive impairment of bone marrow niche (Kleppe et al., 2015; Koschmieder et al., 2016), alternative therapies that address bone marrow components and their products were proposed and investigated mainly in preclinical studies.

Serum amyloid P (SAP, pentraxin-2), also known as fibrocyte inhibitor, is a constitutive, anti-inflammatory, innate immune plasma protein, that has been shown to successively reduce fibrotic disease of the kidney. SAP levels were found to be lower in patients with MF. In xenograft mice models, increasing SAP levels prolonged survival and delayed bone marrow fibrosis. The recently completed NCT01981850 study investigated PRM-151, a recombinant human SAP, either as single agent or in combination with ruxolitinib, in patients with PMF and post ET/PV MF. Decreased bone marrow fibrosis was one of the outcomes measured in this phase 2 study and results are pending.

It has been demonstrated in mouse models of bone marrow fibrosis that $Gli1^{+}$ mesenchymal stromal cells (MSCs) represent the progenitors of myofibroblasts, being involved in myelofibrotic transformation. Treatment with Gli antagonist 61 (GANT61) of MSCs isolated from mouse models as well as from patients with myelofibrosis inhibited myofibroblast differentiation and, consequently, alleviated bone marrow fibrosis, indicating that Gli proteins may serve as promising therapeutic targets (Schneider et al., 2017). Also, the MPN local neuropathy induced by alteration of sympathetic nerve fibers

that innervate bone marrow nestin positive MSCs having a role in normal HSC regulation can be targeted therapeutically by β3-adrenergic agonists. In this respect, treatment with the human β_3-adrenergic agonist mirabegron increased the number of nestin positive MSCs by restoring their sympathetic regulation and leading to a reduction of bone marrow fibrosis (Arranz et al., 2014). Recently, it was suggested that the Wnt inhibitor Dickkopf-related protein 1 (Dkk-1), measured in plasma of MPN patients, could differentiate between ET and prefibrotic PMF (Mambet et al., 2018). As Dkk-1 might be associated with bone marrow niche alterations and especially neoangiogenesis, it might represent a future therapeutic target (Mambet et al., 2018).

Other avenues for the treatment of MPNs have also emerged with the development of the immune checkpoint inhibitors. Of interest, JAK2V617F activates persistent TPOR-dependent STAT3 signaling that induces transcriptional activation at the promoter of Programmed Death Ligand 1 (PD-L1) leading to enhanced PD-L1 expression at the surface of monocytes, megacaryocytes and platelets (Prestipino et al., 2018) and thus reducing T-cell responses against the mutated clone. Data in a xenotransplantation model suggest that inhibition of PD1 may be a promising strategy in MPN treatment (Prestipino et al., 2018; Veletic et al., 2018; Wang et al., 2019) and Mascarenhas et al. completed in May 2020 the NCT03065400 trial (May 28, 2020 being the final data collection date for primary outcome measure, clinicaltrials.gov/ct2/show/NCT03065400) studying the anti-PD-1 pembrolizumab in patients with accelerated or blast phase disease (MPN-AP/BP) refractory to or intolerant to conventional therapies such as decitabine, and for whom hematopoietic stem cell transplant was no therapeutic option (exploratory cohort).

In another international trial comparing different approaches in different arms (NCT04097821) and now open to enrolment of MF patients, ruxolitinib is being tested in one arm in combination with another immune checkpoint drug, MBG453, an inhibitor of the inhibitory T-cell receptor T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3; hepatitis A virus cellular receptor 2; HAVCR2), with potential immune checkpoint inhibitory and antineoplastic activities. The second arm is investigating the adjunction of crizanlizumab, a humanized monoclonal antibody that binds P-selectin and blocks interaction with its ligands (including leukocyte PSGL-1). Indeed, it is now accepted that reduced GATA1 content in megakaryocytes as a consequence of ribosomal deficiency is a hallmark of primary MF and a driving event in the disease. Among others, GATA1 deficiency leads to abnormal activation of the TGF-β/P-selectin axis in

megakaryocytes of primary MF patients, which in turn contributes to the development of MF (Zetterberg et al., 2013). Consequently, deletion of the P-selectin gene prevented development of myelofibrosis in a GATA1-low mouse model of MF (Spangrude et al., 2015) and inhibition of P-selectin is thought to constitute a new target in MF treatment (Ling et al., 2018; Zingariello et al., 2019).

5.4 The p53 pathway

Finally, the third drug tested in the trial in combination with ruxolitinib is HDM201 (siremadlin), a potent and selective orally bioavailable inhibitor of p53-MDM2 (HDM2 in human) interaction, that activates p53 and induces robust p53-dependent cell cycle arrest and apoptosis in human p53 wild-type tumor cells (Jeay et al., 2018). Of interest, in patients with chronic-phase MPN, *p53* is usually wild-type, and malignant hematopoietic stem cells (HSCs) express low levels of the p53 protein (Nakatake et al., 2011). It was hypothesized that since interferon is an effective therapy in patients with MPNs (Gowin et al., 2012), this may be due, at least in part, to interferon-linked increase in p53 expression in malignant HSCs. Furthermore, TGF-β1 induces expression of HDM2 which might drive high levels of MDM2. p53 is indeed reduced in MPN cells secondarily to the overexpression of its major regulators MDM2 and MDM4 (HDM4/HDMX), which are expressed at levels higher than normal in MF $CD34^+$ cells (Lu et al., 2012; Nakatake et al., 2011). Hence, targeting the interaction of MDM2 and p53 might be of interest in MF, as it has already been suggested with idasanutlin (Lu et al., 2012). Idasanutlin is an MDM2-p53 binding cell cycle inhibitor, the first long-acting MDM2 antagonist investigated in clinical trials. *In vitro*, it binds selectively and with high affinity to the p53 site on the surface of the MDM2 molecule and can effectively displace p53 from MDM2, leading to stabilization, accumulation of p53 protein and activation of the p53 pathway. The phase 1 study of idasanutlin in PV was presented by Mascarenhas et al. in 2017 at ASH (Mascarenhas et al., 2017) and published in 2019 (Mascarenhas et al., 2019b). It showed promising hematologic, symptomatic, pathologic and molecular responses, indicating idasanutlin is a promising novel agent for PV. Moreover, *in vitro* combination of low doses of pegylated interferon alfa-2a with nutlin-3 resulted in selective and significant inhibition of JAK2V617F-positive CD34+ PV colony formation compared to normal colony formation. The global phase 2 trial that was evaluating idasanutlin for hydroxyurea-resistant

or intolerant PV patients was however discontinued by its sponsor in April 2020 as the sponsor decided to stop the development of idasanutlin in the PV indication. If idasanutlin presents indeed a much increased activity compared to its precursor nutlins, some authors fear that it might display similar weaknesses, which are limited elimination of cancer cells and the generation of p53-mutated drug-resistant subpopulations (Skalniak et al., 2018). It is important to emphasize that secondary AML on MPNs frequently carry TP53 mutations or overexpression of MDM4, and that leukemic transformation is associated with acquired homozygosity or compound heterozygosity for mainly missense TP53 mutations (Goyal et al., 2020; Lundberg et al., 2014).

Nevertheless, other trials with nutlins keep ongoing. First results of the Phase 2 trial on the use of KRT-232, a novel oral small molecule inhibitor of MDM2 (NCT03662126), were presented at EHA 2020 annual congress and demonstrated a dose-dependent relationship for safety and efficacy as well as promising clinical activity and tolerability in patients with poor prognosis MF. In the meantime, KRT-232 is also studied in a global, open-label Phase 2a/2b study to determine its efficacy and safety in the treatment of patients with phlebotomy-dependent PV (NCT03669965). As nutlins have also showed durable response in the treatment of selected patients with AML (Yee et al., 2014), another multicenter phase 1b/2 trial (NCT04113616) is recruiting for the treatment with KRT-232 of patients with AML secondary to MPN.

Finally, other drugs have been proposed in combination with ruxolitinib and are under evaluation. Because JAK2V617F-expressing cells that co-express TPOR are synergically inhibited by the combination of JAK2 and PI-3 kinase or mTOR inhibitors (Bartalucci et al., 2013; Choong et al., 2013), one trial investigated the combination of ruxolitinib and BKM120 (Buparlisib, a PI3K inhibitor) in MF. However, it was terminated prematurely by its sponsor in 2019. Yacoub et al. (Abstract Book, 2020) presented interesting results at the EHA 2020 annual congress for the addition to ruxolitinib in MF patients with a suboptimal response of parsaclisib, a potent, highly selective, next-generation PI3Kδ inhibitor. The addition of parsaclisib appeared to be well tolerated and resulted in further reduction in spleen volume and improvement in symptom burden.

Other trials are considering or have considered different histone deacetylase (HDAC) inhibitors such as panobinostat (NCT01693601), vorinostat (Andersen et al., 2013) or givinostat (NCT01761968) (Rambaldi et al., 2010). HDAC inhibitors have multiple potential mechanisms of action

in MPN cells, especially through down-regulation of JAK2 via inhibition of the chaperone protein function of heat shock protein 90 (HSP90) (Akada et al., 2012; Fiskus et al., 2011; Guerini et al., 2007; Wang et al., 2009).

Givinostat and vorinostat are active in patients with PV and ET, alleviating efficiently symptoms as pruritus and inducing spleen and hematologic responses in a substantial proportion of patients, apparently regardless of *JAK2* mutational status (Andersen et al., 2013; Rambaldi et al., 2010) and although the reduction of the allele burden of mutated *JAK* was modest. Secondary effects as asthenia and gastrointestinal complaints were however present. Nevertheless, givinostat continues to be developed for PV (NCT01761968).

HSP90 can also be directly targeted and inhibitors have been developed as antineoplastic drugs. PU-H71 is one of them and started to be studied for various solid tumors and non-Hodgkin lymphoma (Speranza et al., 2018). Although evidence of its efficiency *in vivo* still seems scarce, some trials are studying its efficiency in MPNs in combination with ruxolitinib (NCT03935555, NCT03373877, NCT01393509).

5.5 LSD1: Inhibition of lysine specific demethylase 1A

Another proposed epigenetic therapy consists of targeting histone demethylases. Lysine-specific demethylase 1A (LSD1) is a flavin-dependent monoamine oxidase, which demethylates mono- and di-methylated lysines, specifically histone 3 lysines 4 and 9. LSD1 has been shown to play an important role in cell proliferation and in various tumors. Elevated levels of LSD1 have been found in diverse cancers and were related with cellular effects such as epithelial-mesenchymal transition (EMT), cell proliferation and differentiation, stem cell biology and malignant transformation (Fang et al., 2019). LSD1 overexpression was also found in hematologic malignancies, such as AML or MPNs, with a critical role for self-renewal of malignant myeloid cells and differentiation of myeloid progenitors (Magliulo et al., 2018; Niebel et al., 2014). IMG-7289 (bomedemstat) is an orally available LSD1 inhibitor. After IMG-7289 reduced peripheral cell counts, spleen size, inflammatory cytokines, mutant allele frequencies and marrow fibrosis in mouse models of MPN (Jutzi et al., 2018), trials were started in the human. Preliminary results from the open-label Phase 2b study NCT03136185 of IMG-7289 in myelofibrosis showed a trend toward improvement in symptom burden in most patients and modest reduction in spleen volumes in a subset of patients, despite under-dosing and slow dose escalation (Pettit et al., 2019). Hence this study

keeps on recruiting with more aggressive dosing and IMG-7289 is now also being evaluated in patients with ET and PV (NCT04262141).

In addition, another target being considered for MF treatment is the apoptosis pathways through the B-cell lymphoma 2 (Bcl-2) protein, as preclinical studies have demonstrated cytotoxic activity of navitoclax, a novel small-molecule that targets and binds with high affinity to multiple anti-apoptotic Bcl-2 family proteins (including Bcl-xL, Bcl-2, and Bcl-W) against myeloproliferative neoplasm-derived cell lines (Lu et al., 2010). In a phase 2 single-arm, multicenter, open-label study (NCT03222609) assessing the efficacy and safety of navitoclax combined with ruxolitinib in patients with MF, navitoclax was well tolerated with clinically meaningful spleen responses, allelic burden reductions, improvement in total symptom score and encouraging improvements in bone marrow fibrosis (in comparison to treatment with ruxolitinib alone in the patients who had been on therapy before) (Harrison et al., 2019c). Because the development of navitoclax was initially hampered by dose-limiting thrombocytopenia (Mason et al., 2007), an on-target effect resulting from inhibition of Bc-xL, other more specific Bcl-2 inhibitors have been developed. Among them, palcitoclax (APG-1252) has shown reduced toxicity on platelets (Lakhani et al., 2020) and is currently being tested in the treatment of MF (NCT04354727).

Evasion from programmed cell death is a hallmark of cancer and can be achieved in cancer cells by overexpression of inhibitor of apoptosis proteins (IAPs). Second mitochondria-derived activators of caspases (SMAC) directly bind to IAPs and promote apoptosis. SMAC mimetics have been investigated in various types of cancer and are hypothesized to be particularly effective in diseases with high inflammation and NFκB activation. Given that elevated TNFα levels with strong NFκB activation are a feature of patients with MF (Fleischman et al., 2011; Tefferi and Vainchenker, 2011), the SMAC mimetic LCL-161 was investigated in MF patients in the phase 2 NCT02098161 trial and showed interesting activity, especially in patients, who had failed on JAK inhibitors (Pemmaraju et al., 2019). Moreover, further arguments indicate that JAK2 signaling might even be required for the differential sensitivity of mutant cells to SMAC mimetics, suggesting that the addition of JAK2 inhibitors may reduce the ability of SMAC mimetics to selectively target JAK2V617F mutant cells (Craver et al., 2020).

Finally, other targets have also been considered, as the sonic hedgehog pathway, that could be targeted by vismodegib (NCT02593760), therapy directed at CD123 (tagraxofusp) or telomerase inhibitor such as imetelstat.

If telomerase inhibition by imetelstat was able to induce hematologic response in all 18 ET patients in the NCT01243073 trial (Baerlocher et al., 2015), caution was advised because the treatment was accompanied by significant myelosuppression (Tefferi et al., 2015). In patients with relapsed or refractory MF, imetelstat did not appear to significantly increase overall survival in all patients of the NCT02426086 trial (preliminary results) but might have been more efficient in the subgroup of patients with shorter telomeres at diagnosis (Mascarenhas et al., Oral and Poster Abstracts n°347 at 62nd ASH Annual Meeting 2020).

6. Conclusion

In conclusion, while the number of treatments directed at MPNs approved by government agencies remains very low, efforts are under way to identify new treatment strategies for MPNs, with a particular focus on MPN-specific altered pathways. It should however be noted, that none of above-mentioned treatments have so far proven effective in curing MPNs. In this race to treat rare diseases, the number of patients included in each trial is also relatively small, requiring international efforts. Finally, it should be noted that indications for enrolment in clinical trials may particularly not be an easy choice in MPNs, neither for the patients nor for the clinician, as evolution of the diseases remains slow for many patients and should be compared with the possible burden of treatment-associated toxicities.

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