Current Hematologic Malignancy Reports (2019) 14:310–327
https://doi.org/10.1007/s11899-019-00527-7

MYELOPROLIFERATIVE NEOPLASMS (B STEIN, SECTION EDITOR)

The Rationale for Immunotherapy in Myeloproliferative Neoplasms

Lucia Masarova[1] · Prithviraj Bose[1] · Srdan Verstovsek[1]

Published online: 21 June 2019

Abstract

Purpose of Review The classic, chronic Philadelphia chromosome negative myeloproliferative neoplasms (MPN)—essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF)—are clonal malignancies of hematopoietic stem cells and are associated with myeloproliferation, organomegaly, and constitutional symptoms. Expanding knowledge that chronic inflammation and a dysregulated immune system are central to the pathogenesis and progression of MPNs serves as a driving force for the development of agents affecting the immune system as therapy for MPN. This review describes the rationale and potential impact of anti-inflammatory, immunomodulatory, and targeted agents in MPNs.

Recent Findings The advances in molecular insights, especially the discovery of the Janus kinase 2 (JAK2) V617F mutation and its role in JAK-STAT pathway dysregulation, led to the development of the JAK inhibitor ruxolitinib, which currently represents the cornerstone of medical therapy in MF and hydroxyurea-resistant/intolerant PV. However, there remain significant unmet needs in the treatment of these patients, and many agents continue to be investigated. Novel, more selective JAK inhibitors might offer reduced myelosuppression or even improvement of blood counts. The recent approval of a novel, long-acting interferon for PV patients in Europe, might eventually lead to its broader clinical use in all MPNs. Targeted immunotherapy involving monoclonal antibodies, checkpoint inhibitors, or therapeutic vaccines against selected MPN epitopes could further enhance tumor-specific immune responses.

Summary Immunotherapeutic approaches are expanding and hopefully will extend the therapeutic armamentarium in patients with myeloproliferative neoplasms.

Keywords Interferon · JAK inhibitors · Immunotherapy · Myeloproliferative neoplasms

Introduction

The classic, chronic Philadelphia chromosome negative myeloproliferative neoplasms (MPNs), essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF), represent a heterogeneous group of stem cell disorders characterized by clonal myeloproliferation, ineffective hematopoiesis, varying degrees of bone marrow fibrosis, constitutional symptoms, and splenomegaly, with overall survival ranging from months to decades [1]. It has been suggested that these classic MPNs could be different phenotypic presentations of one biological continuum, from ET to PV to MF and ultimately leukemic transformation (acute leukemia, AML) [2].

Central to the pathology of MPNs is a constitutively activated Janus kinase and Signal Transducer and Activator of Transcription proteins (JAK-STAT) signaling pathway, caused by various, mostly mutually exclusive driver mutations, such as *JAK2V617F*, *MPLW515L/K*, *CALR* exon 9 indels, or other less known mutations within the JAK/STAT signaling pathway [3–6]. In addition, several non-driver mutations have been found in patients with MPN (e.g., *ASXL1*, *IDH1*, *IDH2*, *EZH2*, *SRSF2*) [7, 8]. Although the prognostic significance of molecular mutations has been extensively studied, their exact role in disease pathogenesis remains to be fully established.

This article is part of the Topical Collection on *Myeloproliferative Neoplasms*

✉ Lucia Masarova
lmasarova@mdanderson.org

Prithviraj Bose
pbose@mdanderson.org

Srdan Verstovsek
sverstov@mdanderson.org

[1] Department of Leukemia, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 0428, Houston, TX 77030, USA

There are various reasons to believe that driver mutations are not solely responsible for disease initiation or its phenotype. It has been shown that in a subset of patients, the *JAK2V617F* mutation might be a late event rather than a disease initiating mutation [9, 10]. Furthermore, as further elucidated in this review, there is solid evidence that chronic inflammation and a dysregulated immune system play indisputable roles in the biology of these diseases.

Optimal management of MPN patients includes complex decision-making, considering specific disease type, individualized prognosis, comorbidities, age, and the risks and benefits of available therapies. Reduction of thrombotic risk is the major goal of therapy in patients with PV and ET. Most patients should receive aspirin, and cytoreductive therapy is reserved for those with high-risk disease (e.g., age > 60 years, previous thrombosis, JAK2 mutation per revised IPSET score [11]). For years, hydroxyurea (HU) and anagrelide (in ET) represented the most frequently used cytoreductive agents. Interferon has been used "off-label" in both diseases (frontline or subsequent line) until recently, when a novel, monopegylated formulation received approval for frontline PV patients in certain countries [12]. In PV, second-line therapy is currently represented by the JAK2 inhibitor ruxolitinib. Interferon and ruxolitinib both represent unconventional agents with immune-modulatory activity and will be further discussed below. Therapies for MF range from observation and supportive care to medical therapy aimed to relieve symptoms or improve cytopenias to potentially curative allogeneic stem cell transplantation (SCT). SCT is only available for a small subset of fit patients as it is associated with considerable mortality and morbidity [13, 14]. The JAK1/2 inhibitor ruxolitinib, currently the backbone of medical therapy for MF, will be further discussed below.

Multiple efforts are currently ongoing to identify agents from various therapeutic classes, targeting cellular metabolic and apoptotic pathways, and different phases of the cell cycle, to improve the outcome of patients with MPNs. These approaches were extensively reviewed elsewhere [15•, 16•, 17•]. In this review, we will focus upon the agents targeting the immune system and cytokine milieu in MPN, immunomodulatory agents, and targeted immune therapy.

Rationale for Immune Therapy

Chronic inflammation and chronic oxidative stress are involved in the pathogenesis of a variety of cancer types and likely play significant roles in patients with MPNs [18••, 19••, 20••]. There is a hypothesis that patients with MPN have profound immune dysregulation and defective immune surveillance [21••]; because of which, they are sometimes referred to as "A Human Inflammation Model for Cancer Development" [22••].

In MPNs, cells from all myeloid lineages, including those involved in the immune and inflammatory response, may belong to the malignant clone [23]. This might cause a defective immune response and overexpression of inflammatory cytokines and receptors, further aggravating chronic inflammation.

Upregulation of inflammatory cytokines and growth factors has been reported in MPN, including tumor necrosis factor α, hepatocyte growth factor, platelet-derived growth factor, vascular endothelial growth factor, lipocalin-2, interleukin (IL)-6, IL-8, IL-2R, IL-12, and IL-15. Many of these independently predict inferior survival [24, 25]. Most inflammatory cytokines activate JAK/STAT3 or JAK2/STAT5 pathways, enhancing survival of many cell types, including fibroblasts, monocytes, macrophages, endothelial cells, and hematopoietic progenitors, and contributing to disease phenotype and pathogenesis including increased bone formation [26–28]. Activation of the nuclear factor-κ-B (NF-κ-B), JAK1/STAT, and HIF-α further enhance production of these cytokines via the same signaling pathways and create an endless vicious cycle [29–31]. Furthermore, inflammatory cytokines are also produced by non-malignant cells in the bone marrow and its microenvironment, such as the stem cell niche, fibroblasts, monocytes, neutrophils, and endothelial cells, thus further promoting the growth of MPN cells [32–36].

Concurrently, patients with MPNs also have dysregulated genes related to the immune system and inflammation, such as interferon-inducible gene [37], regulatory T cells (e.g., Tregs characterized as CD4 + CD25+ FOXP3+ [38]), natural killer cells, human leukocyte antigen (HLA) class I and II molecules, β2-microglobulin, molecules processing HLA I antigens (such as LMP2, LMP7, TAP1/2, and tapasin) [37, 39], and antioxidative stress genes (*ATM*, *TP53*, *CYBA*, *NRF2*, *PTGS1*, *SIRT2*) [40, 41]. In addition, an increased expression of immunosuppressive cells, such as myeloid-derived suppressor cells (MDSC), contributing to tumor escape from immune surveillance, has been reported [42].

Further, the most common driver mutation in MPN, *JAK2V617F*, was not only implicated in generating reactive oxygen species as an important inflammatory driver [40], but was also found to enhance programmed death receptor 1 (PD-L1) expression via STAT3/STAT5 phosphorylation on the surface of monocytes, MDSCs, megakaryocytes, and platelets [43•]. Overexpressed PD-1 contributes to inactivation and dysfunction of immune reactive T cells.

Demonstration of the immunogenicity of MPN driver mutations, especially *CALR* exon 9 and *JAK2V617F*, provides the rationale for the development of potential immunotherapeutic targets [44••, 45••, 46••, 47••, 48••]. For instance, Holmstrom et al. identified presence of spontaneous T cell responses against PD-L1–derived epitopes of *JAK2V617F* and *CALR* exon 9 mutant proteins in 71% of patients with MPN. Collaborators from Belgium

(laboratory of Stefan Constantinescu) recently showed that mutant *CALR* acts as a "rogue cytokine," is secreted from cells, and directly activates neighboring cells expressing MPL at the surface [49••].

Significant disease burden with debilitating symptoms, such as fatigue, pruritus, night sweats, weight loss, bone pain, and/or fever [50••, 51], caused by overexpression of inflammatory cytokines, negatively impacts quality of life of MPN patients. Many scoring tools were developed to better assess this symptom burden, e.g., the Myelofibrosis Symptom Assessment Form (MF-SAF), the Myeloproliferative Neoplasms-SAF (MPN-SAF), or the Myeloproliferative Neoplasms Symptom Assessment Form Total Symptom Score (MPN-SAF TSS), and they have now become essential parts of treatment response assessment [52, 53].

In the context of the above, immune-based therapy able to affect MPN pathology and disease presentation is intuitively attractive, based on sound rationale, and very much needed.

Immune Therapy in MPN

Interferon Alpha

The robust anti-proliferative, anti-angiogenic, and immunomodulatory effects of interferon, particularly interferon alpha (INF-α) have been known for decades. Additional findings suggesting that INF-α might alter MPN biology and maybe restore some components of impaired immune surveillance, making this agent particularly compelling [54].

One of the major mechanisms of action of INF-α on cells includes signaling via JAK-STAT pathway and activating Janus kinase 1, which ultimately leads to expression of anti-proliferative and pro-apoptotic genes (e.g., caspase 4, caspase 8, tumor necrosis–related apoptosis-inducing ligand, death-activating protein kinases, Fas/CD95) [55, 56]. INF-α also impairs thrombopoietin (TPO)-induced intracellular signaling via upregulation of suppression of cytokine signaling -1 (SOCS-1) secretion, thus inhibiting activation of the TPO and MPL receptors, as well as phosphorylation of JAK2, JAK3, and STAT5 [57].

Anti-proliferative and anti-angiogenic properties of INF-α could also be in part attributed to the effect on the bone marrow hematopoietic progenitors and cytokines, e.g., inhibition of pluripotent and lineage restricted circulating hematopoietic progenitor cells, granulocyte–macrophage colony-stimulating factor, fibroblast growth factor, vascular endothelial cell growth factor, IL-1b, IL-8; or stimulation of IL-1 receptor antagonist and transforming growth factor-β [58–62]. In addition, INF-α directly impairs endothelial cell proliferation and upregulates angiostatic chemokines, such as CXCL9, CXCL10, and CXCL11 [63].

INF-α might stimulate and mobilize previously dormant stem cells [64]. In patients with PV, treatment with INF-α increased the rate of apoptosis and the phosphorylation of p38 mitogen-activated protein kinase in PV CD34-positive cells, which might lead to depletion of *JAK2V617F*$^{+}$ MPN-propagating stem cells [65].

Recent studies have also shed light on the impact of INF-α on the immune system. Treatment with INF-α significantly increased the amount of regulatory Tregs and T-effector cells in the circulation, decreased the frequency of circulating myeloid and plasmacytoid dendritic cells [66], improved the proportion of immune-stimulating NK cells, i.e., increased CD56^{bright} NK cells and decreased CD56^{dim} ones [67], and led to rapid differentiation of monocytes into dendritic cells, which are capable of inducing T and B cell immune responses [68]. Treatment with INF-α was also associated with upregulation of the expression of tumor-associated antigens (e.g., MPD6 [69]) and correction of dysregulated HLA genes (e.g., upregulation of *BAT4*, *HCG26/8*, *HCP5*, *HLA-A/B/C*, *HLA-E/F/G*, *HLA-DOB*, etc.; downregulation of *CIITA*, *HLA-DOA*, and *HLA-DPB2* [70]). Furthermore, Skov et al. recently showed that therapy with INF-α might have a major impact upon antioxidative immune defense as it upregulated previously downregulated oxidative stress genes (e.g., *CXCR4*, *TP53*, *NRF2*, *ATOX1*, *SEPP1*, *CYBB*, and *MSRA*) [71].

The impact of INF-α upon the immune system in MPN has been extensively studied, and its benefit has been shown in ET, PV, and early phase MF patients (selected studies are summarized in Table 1 [72, 73–79, 81, 82•, 83••, 84–99]). Herein, we will only briefly discuss the important clinical data from recent phase 3 studies which compared INF-α against HU in the frontline setting.

The DALIAH trial compared recombinant INF-α-2a and r-INF-α-2b to HU. Patients ≤ 60 years were assigned to INF-α-2a vs 2b only (1:1), whereas patients > 60 years were randomized (1:1:1) to all three agents. After 36 months of therapy, overall response rate (ORR), complete hematologic response (CHR), and maintenance of CHR were higher with HU. Drug-related discontinuation was higher in the r-IFNα group (34–45% vs 13% for HU), but the rate of grade ≥ 3 adverse events (AEs) was similar in all groups. Among 70 *JAK2V617F*-mutated patients, the rate of partial molecular response (≥ 50% decrease in JAK2 allele burden) was between 20 and 30% in all groups; however, the median *JAK2V617F* reduction was greater with r-INF-α (~ 70% vs 38% with HU) [80].

The MPN-RC-112 trial (MPD-RC 112) compared frontline pegylated INF- α-2a (PEG-INF) to HU. After a median follow-up of 89.9 weeks (in patients treated > 24 months), PEG-INF provided non-significantly higher rate of ORR (60% vs 41% for HU, respectively, $p = 0.22$). Interestingly, treatment with HU was associated with a higher rate of bone

Table 1 Selected studies of interferon in MPN

	Regimen	No of pts	Median follow-up	Responses (type, %) [¶]	Ref.
MPNs	r-INF-α-2	63 [31 MF, 12 PV, 17 ET]	4 years	MF: PR 3%; spleen CI 26% ET: CR 6%, PR 82% PV: CR 8%, PR 34%	[72]
	PEG-INF-α-2a	75 [19 MF, 36 PV, 20 ET]	3.8 years	[IWG-MRT/ELN] MF: PR 10.5%, CI 21% ET: CR 25%, PR 20%, CR/PR 20% PV: CR 8.3%, PR 39%, CR/PR 39%	[73]
	PEG-INF-α-2a	118 [17 MF, 55 PV, 46 ET]	17 months	[IWG-MRT/ELN] MF: PR 12%, CI 18%, SD 41%; ET CR 63%, CI 18%, SD 41% PV CR 54%, PR 33%	[74]
	r-INF-α-2b/PEG-INF-α-2a/PEG-INF-α-2b	102 [4 MF, 74 PV, 19 ET]	42 months	CHR: ET 95%, PV 68%, 50% MF w/ normalization plt and WBC PMR 50%, CMR 11%	[75, 76]
	PEG-INF-α-2a	115/77 eval. [42 ET, 35 PV]		[IWG-MRT/ELN] ORR [24 mos]: ET 74%, PV 74%; Morph BM CR 11%	[77]
	PEG-INF-α-2a	83 [43 PV, 40 ET]	83 months	[IWG-MRT/ELN] CHR 75%, CMR 18%; morph BM CR 22%	[78, 79]
	r-INF-α-2 vs HU (DALIAH)	205 [41 MF, 90 PV, 72 ET]	3 years	[IWG-MRT/ELN] ORR ET and PV and preMF 41% INF-α vs 71% HU; in PMF ~ 25% INF-α vs 57% HU; ORR (all) 58% INF-α vs 66% HU PMR 28% INF-α vs 23% HU	[80]
	PEG-INF-α to HU	168 [81 ET; 87 PV]	90 weeks	[IWG-MRT/ELN] ORR 81.7%—non-inferiority 24 months; morph BM CR: ET 32%, PV 6%	[81]
	PEG-INF-α-2a/α-2b + RUX (COMBI)	50 [18 MF, 32 PV]	12 months	[IWG-MRT/ELN] MF: CR 9%, PR 22% CHR 92%, sust. 58%, PMR 20%; PV: PR 9%, CHR 88%, sust. 44%, PMR 19%	[82•]
PV	r-INF-α	55	13 years	CHR 100% by 2 years, 100% spleen RED 77%, > 50% spleen RED 90%	[83••]
	PEG-INF-α-2a	37	31.4 months	CHR 94.6%, MR 72,4%, CMR 24%	[84, 85]
	(PVN1)	40	77.4 months	CHR 82%, MR 83%, CMR 28%	
	Ropeg (PEGINVERA)	51	5.1 years 18.5 months	CHR 64.3%, MR 74%, CMR 28.6% CHR 47%, CMR 21%	[86, 87]
	PEG-INF vs HU (PROUD-PV - > CONT-PV)	254 (83 analyzed Cont-PV)	12 months 36 months	CHR 43%, median spleen RED by 21.3% -PEG-INF non-inferior to HU- CHR 53%, 70.5% durable; MR 66% -PEG-INF superior to HU-	[88••, 89]
ET	PEG-IFN-α-2a	31	142 months	CHR 67%, CMR 6%	[90]
MF	r-INF-α-2b/PEG-INF-α-2a	17	3.3 years	[IWG-MRT] CR 12%, PR 41%, spleen CI 76%; PMR 16%; Morph BM CR 27%	[91]
	r-INF-α-2b/PEG-INF-α-2a	30	80.3 months	[WG-MRT] CR 7%, PR 30%, CI 14%; spleen CI 50%; morph BM reticulin grading impr 25%	[92]
	PEG-INF-α-2a	62	26.3 months [mean]	[IWG-MRT/EUNMET] Hgb CI 64%; WBC CI 100%, PRBC-I 38.5%; CI plt 63% TSS CI 83%, spleen CI 46.5%	[93]
			58 months	[EUNMET] PMR 37%, CMR 7%	[94]
	Ropeg	25	24 months	[IWG-MRT] Hgb and plt CI 50% No disease progression	[95]

[¶] Refer to original studies for unspecified criteria (individual criteria per authors)

r-INF = recombinant interferon, dose range: 1–5.0 × 10^6 IU/D (most studies used 3.0 × 10^6 IU/D); PEG-INF dose: 0.5–3.0 μg/kg/week; *PR* partial remission, *CR* complete remission, *CI* clinical improvement, *SD* stable disease, *CHR* complete hematologic remission, molecular responses = decrease in JAK2 V617F allele burden in % (PR > 50% decrease in JAK2 allele burden, CMR undetectable JAK2V617F); *Hgb* hemoglobin, *plt* platelets, *WBC* white blood cells, *PRBC-I* red blood cells independence, *TSS* total symptom score,

PMR partial molecular remission, > 50% decrease in JAK2 allele burden, *CMR* complete molecular remission, undetectable JAK2 allele burden, *ORR* overall response rate, *Morph BM CR* morphologic bone marrow complete remission, *RED* reduction

marrow best response (33% vs 17% PEG-INF, $p = 0.05$). PEG-INF was associated with a higher incidence of grade ≥ 3 AE (46.3% vs 27.5% for HU) [81].

Ropeginterferon α-2b (Ropeg, *Besremi*®), novel, monopegylated isoform, was compared against HU in the phase 3, randomized, multicenter PROUD-PV study (continuing after 12 months as CONTINUATION-PV) in PV patients, who were either treatment naïve or who had already been treated with HU for <3 years and were neither intolerant nor complete responders. The study met its primary endpoint of non-inferiority for CHR rate at 12 months (43% vs 46% for HU, $p = 0.003$). The change in median spleen length was

similar for both arms (21% for Ropeg vs 28% for HY; $p = 0.22$). Treatment-related AEs were higher on HU (76% vs 60% on Ropeg); and 12-month discontinuation rates were similar (13% HU vs 16.5% Ropeg) [89]. Recently updated data on 83 (Ropeg) and 70 (HU) patients who completed 36 months on the CONTINUATION-PV trial showed that the rate of CHR with Ropeg (vs HU) increased over time (70.5% vs 51%; $p = 0.012$), as did the rates of CHR plus symptom improvement (53% vs 38%; $p = 0.04$), and molecular response (66% vs 27%, $p < 0.0001$). Furthermore, Ropeg was able to reduce non-JAK allele burden (e.g., *TET2*), while HU only suppressed *JAK2V617F*. The incidence of AEs (90% for Ropeg, 91% for HU) and treatment-related AEs (75% for Ropeg, 79% for HU) was similar [88••]. Following these results, in February 2019, Ropeg (*Besremi*®) received approval in Europe as a monotherapy in PV patients without symptomatic splenomegaly, independent of previous HU exposure [12].

The biggest limitation of INF-α, including PEG-INF, has been treatment-related toxicity which lead to treatment discontinuation in more than one third of all patients [78]. This shortcoming might be mitigated by the use of Ropeg, which showed similar toxicity rates and overall tolerability as observed with HU, and could finally allow us to exploit the full potential of interferon in clinical practice.

IMIDS

The group of immunomodulatory drugs collectively known as IMIDs has been used in MF patients with anemia, due to their known anti-inflammatory, anti-angiogenic, anti-proliferative effects, and cytokine modulatory properties [100]. Via inhibition of NF-κ-B pathway, IMIDs inhibit various pro-inflammatory and apoptotic cytokines (e.g., IL-2R, IL-6, IL-10, IL-15, TGF-β, TNF-α) and upregulate anti-inflammatory cytokines (e.g., IL-2, INF-γ). IMIDs also directly enhance cytotoxic T cells and NK-cell activity and strongly inhibit the suppressor functions of regulatory T cells [101, 102]. Heterogeneity of responses treated with different IMIDS: thalidomide (*Thalomid*®) and its more potent derivatives lenalidomide (Revlimid®) and pomalidomide (Pomalist®), may be attributed to various treatment schedules, doses, combinations with steroids, and different response criteria. Although some approaches resulted in anemia response as high as 70%, the average response rate with IMIDs was about 30% (selected trials are summarized in Table 2) [103–117]. The only IMID evaluated in a phase 3 trial was pomalidomide which, when randomized 2:1 to placebo, failed to meet the primary endpoint of transfusion (PRBC) independence (16% vs 16%, $p = 1.00$).

JAK Inhibitors

The discovery of the *JAK2V617F* mutation has led to the development of a number of JAK1/JAK2 inhibitors, and although ruxolitinib (*Jakafi*®, *Jakavi*®) still remains the only approved one, much effort is ongoing to bring more agents into clinic.

In this session, we will provide a brief overview of the most relevant JAK2 inhibitors evaluated in clinical trials and further discuss immune-related aspects of these agents. Table 3 summarizes clinical data from the largest studies of selected JAK inhibitors, and Table 4 outlines ongoing clinical trials with the agents discussed (monotherapy and combinations).

Ruxolitinib, a type 1 inhibitor of JAK1 and JAK2, stabilizes the active conformation of the tyrosine kinase and is approved for intermediate- and high-risk MF [2011] and PV refractory or intolerant to HU [2014]. In MF, approval followed the results from two randomized, phase 3 (COMFORT I and II) studies, which demonstrated ruxolitinib's ability to substantially alleviate symptoms, reduce splenomegaly, and improve OS (Table 3). These results were subsequently confirmed in another trials (ROBUST, JUMP) [118••, 119••, 120–123]. In PV refractory or intolerant to HU, ruxolitinib in two phase 3 (RESPONSE I and II) trials showed improved control of spleen and symptoms and also resulted in superior control of blood counts and decreased need for phlebotomies as also shown in another trials (Table 3; Extended Treatment Phase, RELIEF) [124••, 125••, 126–129]. In HU-resistant or intolerant ET, where ruxolitinib is not approved for clinical use, the agent showed similar control of counts and spleen size as best available therapy (BAT), but significantly better symptom reduction (Table 3, MAJIC-ET) [130, 131].

Pacritinib is a JAK2 and FLT3 (fms-like tyrosine kinase 3) inhibitor with limited myelosuppression [132] and was evaluated in two phase 3 randomized (PERSIST I and II) trials in comparison to best available therapy (PERSIST II allowed ruxolitinib as BAT) [133, 134]. In both PERSIST trials, pacritinib failed primary endpoint of ≥ 50% symptom reduction on intention to treat analysis (Table 3). Moreover, PERSIST-2 accrual was temporarily closed after the Food and Drug Administration (FDA) imposed a full clinical hold on the pacritinib development program because of concerns over excess mortality.

Momelotinib is a dual JAK1/JAK2 inhibitor with ability to improve anemia, possibly via direct inhibition of the type I activin A receptor and subsequent reduction of hepcidin production in the liver [135]. This agent failed to meet the predefined endpoints in the phase 3, randomized SIMPLIFY I and II trials (SIMPLIFY-II allowed ruxolitinib as BAT and was conducted in ruxolitinib-pretreated patients; Table 3) [136, 137].

Febratinib is a JAK2-selective inhibitor that showed encouraging results in the phase 3 JAKARTA trials in ruxolitinib-naïve and ruxolitinib-pretreated MF patients (Table 3) [138, 139]. Development of this agent was also

Table 2 Selected studies of IMIDs in MPN

Regimen, dose daily [mg]	No of Pts	Response (%)[¶]	Ref.
THAL 200	15	Hgb 25%, Plt 21%, spleen 25%	[103]
THAL 50–100	21, 63	[Dupriez]: Hgb 26%, PRBC-I 39%, Plt 22%, spleen 19%	[104, 105]
		[Modified Dupriez]: Hgb 29%, PRBC-I 30%, Plt 38%, spleen 41%	
THAL 50 − PRED	15, 15, 21	[Dupriez]: Hgb 62%, PRBC-I 40%, Plt 75%, spleen 19%	[106–108]
		[EUNMET]: Hgb 43%, PRBC-I 29%, spleen 31%	
		[IWG-MRT]: Hgb 37.5%, spleen 35%, PR 1, CI 10	
LEN 10	68	Hgb 22%, Plt 50%, spleen 33%	[109]
LEN 10 − PRED	40, 48	[IWG-MRT]: Hgb 19–32%, spleen 10–42%	[110, 111]
POM > 0.5–3	19, 58, 50	[IWG-MRT]: Hgb 10–37%, PRBC-I 7–16%, Plt 3–50%, spleen 1–29%	[112–114]
POM 0.5–3 +/− PRED	29, 84, 94	[IWG-MRT]: Hgb 16–39%, PRBC-I 20%, Ptl 2–8%, spleen 1–8%	[112, 114, 115]
POM 0.5 − PRED	58	[IWG-MRT]: Hgb 5%, PRBC-I 10%, spleen 3%, ORR 16%	[116]
POM vs placebo	252	Hgb 16%, PRB-I 16%, plt 22%	[117]

[¶] Refer to original studies for unspecified criteria (individual criteria per authors)

THAL thalidomide, *PRED* prednisone, *LEN* lenalidomide, *POM* pomalidomide, *Hgb* hemoglobin (refers to response in anemia), *plt* platelets (refers to response in thrombocytopenia), *PRBC-I* red blood cells independence, *PR* partial remission, *CI* clinical improvement, *ORR* overall response rate

temporarily halted by the FDA because of concerns for Wernicke's encephalopathy (potentially caused by inhibition by fedratinib of thiamine neuronal uptake [140]).

After the resolution and/or clarification of the aforementioned developmental issues affecting these newer JAK inhibitors, they have either already resumed (pacritinib, fedratinib) or plan to enter clinical testing in the near future (momelotinib) (Table 4).

The effect of JAK inhibitors is mostly explained by their strong anti-proliferative and anti-inflammatory properties. JAK inhibitors have robust immunomodulatory activity and significantly reduce circulating pro-inflammatory cytokines (e.g., C-reactive protein, IL-1RA, macrophage inflammatory protein 1β (CCL4), TNFα, IL-6) [33••, 141] via JAK-STAT pathway inhibition. In studies with ruxolitinib, the levels of inflammatory cytokines significantly decreased in patients on therapy and have been correlated with an improvement in constitutional symptoms and clinical response [118••]. Additionally, a clinical trial with fedratinib showed that decrease in inflammatory cytokines was also correlated with improvement in splenomegaly [138].

As mentioned previously, the significant symptom burden in patients with MPN negatively impacts their quality of life and life expectancy [50••, 51] and requires attention even in otherwise well-controlled disease. Despite different symptom assessment tools used in various JAK2 inhibitor studies [118••, 119••, 124••, 125••, 131], treatment with these agents significantly improved total symptom score (TSS; Table 3 for individual studies), as well as individual symptom scores, and overall quality of life measures. These improvements occurred regardless of baseline characteristics and disease severity [142–146].

The ability of JAK inhibitors to reduce symptom burden and improve performance status and disease-associated cachexia [147] has been postulated to be the major mechanism underlying the survival benefit of these drugs [148]. Although with longer follow-up, there is increasing evidence of improvement in bone marrow morphology (up to 50% of patients might achieve some regression in marrow fibrosis after 60 months) [149], complete molecular remissions are rare (3 and 6 patients in RESPONSE-I and COMFORT-I trials, respectively) [150, 151], and true disease modifying activity of these agents is debatable.

Another limitation of ruxolitinib includes the likelihood of losing response over time (median duration of spleen response 3 years), development of treatment resistance, on-target anemia and thrombocytopenia stemming from JAK2 inhibition frequently limiting optimal dosing [152], and potent immunosuppression with increased risk of infections. Ruxolitinib via inhibition of cytokine signaling leads to impaired immune response. Ruxolitinib markedly reduces the production of cytokines (IL-12, IL-23, INF-λ, IL-2, TNF-α) important for dendritic cell differentiation, migratory activity, and stimulatory functions, thus impairing their effective ability to activate T cells. Ruxolitinib also reduces antigen-specific T cell

Table 3 Selected studies of JAK inhibitors in MPN

Trial	Drugs	Diagnosis	Response	Toxicity/comments
RUXOLITIB (RUX)				
COMFORT I, phase 3	RUX (155) vs placebo (154)	MF, ≥ int-2 risk, plt ≥ 100, blasts ≤ 10%	At 24 wks: ≥35% SVR 41.9% vs 0.7%; ≥ 50% TSS ↓ 45.9% vs 5.3% At 5 years: 27.7% on RUX; DoR spleen: 168.3 wks OS NR vs 200 wks (HR 0.69; 95% CI, 0.50–0.96; $p = 0.025$), No new Gr ≥ 3 hem AE after 42 months. NMSC similar in both (2.7 vs 3.9 per 100 P-Y)	RUX AE Gr ≥ 3: HGB 45%, plt 13%, ANC 7% ANC; fatigue 5%
COMFORT II, phase 3	RUX 146 vs BAT 73	MF, ≥ int-2 risk, plt ≥ 100, blasts ≤10%	At 48 wks: ≥ 35% SVR 28% vs 0% At 5 year: 26.7% on RUX; DoR spleen: 3.2 years OS NR vs 4.1 yrs. (HR, 0.67; 95% CI, 0.44–1.02; $p = 0.06$/w/ crossover: OS NR vs 2.7 years (HR 0.44; 95% CI, 0.18–1.04; $p < 0.05$), Gr ≥ 3 HGB 8.8%, plt 5.9%. NMSC rate ↑ RUX (6.1 vs 3.0 per 100 P-Y)	RUX AE Gr ≥ 3: diarrhea 23%, edema 22%, HGB 34%, Plt 6%
RESPONSE, phase 3	RUX 110 vs BAT 112	PV requiring PHL, splenomegaly, R/I to HU	At 32 wks: Hct and ≥ 35% SVR 22.7% vs 0.9%; Hct 60.0% vs 19.6%; and ≥ 35% SVR 38.2% vs 0.9%; CHR 23.6% vs 8.9%, ≥ 50% TSS ↓ 49% vs 5% At 5 years: 66% on RUX; probability of maintaining Hct and ≥ 35% SVR 74% (for 224 wks), median duration NR. OS 91.9% vs 91.0% (HR = 0.95; 95% CI 0.38, 2.41). The most frequent AEs (< 10%) anemia, pruritus, diarrhea. RUX: TEE rate ↓ (1.2 vs 8.2 per 100 P-Y), NMSC rate ↑ RUX (7.0 vs 2.1 per 100 P-Y)	RUX: ↑ NMSC and HVZ. RUX AE Gr ≥ 3: dyspnea 2.7%, HGB 1.8%, Plt 5.4%
RESPONSE 2, phase 3	RUX 74, BAT 75 (HU 49%)	PV requiring PHL, no splenomegaly, R/I to HU	At 28 wks: Hct 62% vs 19%; CHR 23% vs 5%, ≥ 50% TSS ↓ 45% vs 23% At 156 wks: 88% on RUX; Hct, CHR and ≥ 50% TSS ↓ maintained in 42%, 24%, and 48%, resp. Median duration of Hct and CHR: NR and 35.9 wks, resp. No new Gr ≥ 3 AEs. RUX: TEE ↓ (2.6 vs 3.7 per 100 P-Y), NMSC ↑ RUX (3.4 vs 1.1 per 100 P-Y)	No Gr ≥ 3 HGB. Overall Gr ≥ 3 AE ↓ on RUX (25.6 vs 45.4 per 100 P-Y)
RESPONSE ETP, phase 2	161 RUX	RESPONSE 1 expansion (PV with splenomegaly, R/I to HU)	At 24 wks: Hct 45.3%; CHR 18%, ≥ 50% TSS ↓ 33.8%. At any time: ≥ 50% spleen length reduction 86.7%	The most frequent AE: HGB 32%, headache 24.5%, diarrhea 14.5%.
RELIEF, phase 2	RUX 54 vs HU 56	PV on HU with symptoms	At 16 wks: ≥ 50%↓ TSS-C (cytokine symptom cluster; tiredness, itching, muscle aches, night sweats, sweats while awake) 43.4% vs 29.6% ($p = 0.14$)/in post hoc: 47% vs 25% ($p = 0.035$)	No unexpected RUX safety signals.
RUX in ET, phase 2	RUX 39	ET R/I HU	At 12 wks: 55% plt response ≤ 600×10^9/L; ≥ 50%↓ TSS majority pts., 100% SVR > 50%	RUX Gr ≥ 3 AEs: infection (2 pts)
MAJIC-ET, phase 2	RUX 58 vs BAT 52 (71% HU)	ET R/I HU	At 1 year: CHR 47% vs 44%, ≥ 50% TSS 32% vs 0%	RUX Gr ≥ 3 HGB 19%; ~ rate of discontinuation, TEE
PACRITINIB				
PERSIST 1	Pacritinib (400 mg QD) 220 vs BAT 107	MF, ≥ int risk, spleen ≥5 cm, ANC > 0.5, blasts ≤ 10%, RUX naïve	At 24 wks: ≥ 35% SVR 19% vs 4.7%; ≥ 50% TSS ↓ 19% vs 10% per ITT ($p = 0.24$)/36% vs 14% ($p = 0.03$) in evaluable population; PRBC-I 25% vs 0%	plt < 100 were in 32% pts with similar benefit in SVR; Pacr: Gr ≥ 3 HGB 17%, plt 12%, diarrhea 5%.
PERSIST 2	Pacritinib 211 (400 mg QD in 104; 200 mg BID in 107) vs BAT 100 (45% RUX)	MF, ≥ int risk, spleen ≥ 5 cm, ANC > 0.5, blasts ≤ 10%, plt ≤ 100, incl RUX exposed (48%)	At 24 wks: ≥ 35% SVR 18% vs 3%; ≥ 50% TSS ↓ 25% vs 14% ($p = 0.08$)/Pacr BID: ≥ 35% SVR 22% vs 3% and ≥ 50% TSS ↓ 32% vs 14% (both $p < 0.05$)	Pacr: cardiac AEs 13% (QD) and 7% (BID) vs 9% BAT; Gr ≥ 3 AEs: Plt 31%, HGB 27%. D/c due to AEs 14% (QD), 9% (BID) vs 4% BAT. Enrolled stopped [FDA].
MOMELOTINIB (MMTB)				
SIMPLIFY-1	MMTB (200 mg QD) 215 vs RUX 217	MF ≥ int-2 risk, spleen ≥ 5 cm, ANC > 0.75, blasts ≤ 10%, plt ≥ 50, RUX naive	At 24 wks: ≥ 35% SVR 26.9% vs 29%; ≥ 50% TSS ↓ 28.4% vs 42.2% ($p > 0.05$), PRBC-I 66.5% vs 49.3%, PRBC-d 30.2% vs 40.1%. Post hoc: ≥ 50% TSS ↓ 26% vs 6% ($p = 0.0006$)	MMTB: peripheral neuropathy 10% (5% RUX), Gr ≥ 3 HGB 5.6% (23% RUX), Plt 7% (4.6% RUX). D/c due to AEs 13% (5.6% RUX)
SIMPLIFY-2	MMTB 104 vs BAT 52 (89% RUX)	MF ≥ int-2 risk, spleen ≥5 cm, ANC > 0.75, blasts ≤ 10%, plt ≥ 50, RUX failed	At 24 wks: ≥ 35% SVR 7% vs 6%, ≥ 50% TSS ↓ 26.2% vs 5.9%, PRBC-I 43% vs 21%, PRBC-d 60% vs 52%	MMTB: peripheral neuropathy 11% (0% BAT); Gr ≥ 3 HGB 14% (14% BAT), Plt 7% (6% BAT).

Table 3 (continued)

Trial	Drugs	Diagnosis	Response	Toxicity/comments
FEDRATINIB				
Ph 2	FEDR (400 mg QD) 83	MF ≥ int-2 risk, spleen ≥ 5 cm, RUX preRX	≥ 35% SVR 55% ≥ 50% reduction in TSS 26%	Gr ≥ 3 HGB 38%, plt 22%. Early termination [FDA].
JAKARTA				
Ph 3	FEDR (400 mg QD in 96, 500 mg QD in 97) vs placebo 96	MF ≥ int-2 risk, spleen ≥ 5 cm, blasts ≤ 10%, plt ≥ 50, RUX naïve	At 24 wks: ≥ 35% SVR 36%/40% (F 400 mg/500 mg QD) vs 1%; ≥ 50% ↓ TSS 36%/34% vs 7%	Gr ≥ 3 HGB 43–60% (400–500 QD); 8% d/c due to AEs. Early terminated [FDA].
JAKARTA				

BAT best available therapy, *plt* platelets, *PHL* phlebotomy, *wks* weeks, *SVR* spleen volume reduction, *TSS* total symptoms score, *HGB* hemoglobin (refers to anemia), *Hct* hematocrit, *PRBC-I* red cell independence, *PRBC-d* red blood cells dependence, *CI* clinical improvement, *AE* adverse event, *Gr* grade, *ANC* absolute neutrophil counts, *DoR* duration of response, *OS* overall survival, *NR* not reached, *R/I to HY* refractory/intolerant to hydroxyurea, *NMSC* non-melanoma skin cancers, *HVZ* herpes zoster, *P-Y*, person-years, *TEE* thrombo-embolic events, *QD* once daily, *BID* twice daily, *D/c* discontinuation, *BM* bone marrow, *P2RD* phase 2 recommended dose

proliferation and induction of response mediated by cytotoxic T cells [153, 154]. Patients treated with JAK inhibitors (ruxolitinib, fedratinib) were also found to have long-lasting decrease and "functional silence" of Tregs, T helper (Th)-17 cells [155–157] and decreased number as well as reduced killing activity of natural killer (NK) cells [158]. This observed immune suppression might be responsible for the increased incidence of certain infections reported in patients treated with ruxolitinib (e.g., reactivation of tuberculosis and hepatitis B, herpes zoster, cryptococcal pneumonia, toxoplasma retinitis, progressive multifocal leukoencephalopathy [159–163]). With regard to this important issue, however, the overall incidence of infectious AEs noted in the ruxolitinib pivotal studies was acceptably low and higher incidence of serious infections was not observed with longer follow-up. The most frequent new grade ≥ 3 AEs in MF patients were pneumonia (~15%), sepsis (~6.5%), and urinary tract infections (~6%). Incidence of herpes zoster occurred at higher rates in MF and PV patients treated with ruxolitinib (~11% in COMFORT studies, and 5.4% in RESPONSE) as compared to placebo or BAT, although most events were only grade ≤ 2.

In contrast to the above data, an interesting report was published on the role of ruxolitinib in a child with an inherited immunodeficiency disorder with a gain-of-function mutation in *STAT1* (characterized by severe T cell dysfunction and immunodeficiency). In this patient, therapy with ruxolitinib led to improvement of T cell differentiation (normalization of Th1 and T helper responses, and improved Th17 differentiation) and decreased the rate of infections [164].

Given the immunosuppressive effect of ruxolitinib, the most worrisome consequence of prolonged therapy is increased incidence of secondary malignancies. The incidence of non-melanoma skin cancers has been similar between ruxolitinib and placebo in COMFORT -I, but it has been higher with ruxolitinib in all subsequent studies (Table 3, [120, 123, 126]). However, there is no solid evidence of increased risk for other cancers ([165, 166]) or serious autoimmune disorders with ruxolitinib. Clinical consequences, if any, of long-term therapy with ruxolitinib require longer follow-up. Considering the immune dysfunction of patients with MPN, accurate screening, prophylaxis, and effective treatment of infections remain an indispensable part of clinical management of these patients regardless of the therapy used.

Combinations

Combination of immune-modulating drugs appears promising as it could result in an additive, if not synergistic effect and decreased toxicity. Here, we will review the available clinical data with the combination of ruxolitinib with INF-α and ruxolitinib with IMIDs.

The most appealing combination is the use of ruxolitinib and INF-α. Downregulation of cytokines by ruxolitinib might

Table 4 Ongoing studies in MPN (immune-based trials)

Therapeutic class	Drug [study]	Eligible diagnosis (MPNs) ¶	Phase	Clinicaltrial.gov identifier
PEG-INF	PEG-INF-α2a and α2b [DALIAH]	PV, ET, MF	3	NCT01387763**
	PEG-INF-α-2b (P1101)	MF	2	NCT02370329
	PEG-INF-α2a	ET, PV	2	NCT00452023**
PEG-INF + JAK inhibitor	PEG-INF-α-2a and 2b + Ruxolitinib [COMBI]	PV, MF	2	EudraCT2013-003295-12**
	PEG-INF-α-2a + Ruxolitinib [RUXOPEG]	MF	1/2	NCT02742324
Ropeg-INF vs BAT (all)	Pegylated-Proline-INF-α-2b (AOP2014) vs BAT [CONTINUATION-PV]	PV (cont of PROUD-PV)	3	NCT02218047**
JAK inhibitor (s)	Fedratinib [FREEDOM]	MF	3	NCT03755518
	Pacritinib	MF	2	NCT03165734
	Itacitinib (INCB039110) +/− Ruxolitinib	MF	2	NCT03144687
	INCB050465 + Ruxolitinib	MF	2	NCT02718300
JAK inhibitor vs anagrelide	Ruxolitinib vs. Anagrelide [RESET-272]	ET	2	NCT03123588
JAK inhibitor vs BAT	Ruxolitinib vs Anagrelide vs PEG-INF [RUXBETA]	ET	2/3	NCT02962388**
	Ruxolitinib vs Standard BAT [Ruxo-BEAT]	ET, PV	3	NCT02577926
MDM2 inhibitor vs JAK inhibitor	KRT-232 vs Ruxolitinib	PV	2	NCT03669965
Hypomethylating agent + JAK inhibitor	Azacitidine + Ruxolitinib	MF (MDS/MPN)	2	NCT01787487
BCL2 inhibitor + JAK inhibitor	Navitoclax (ABT-263) + Ruxolitinib	MF	2	NCT03222609
NEDD-8 inhibitor (ubiquitin) + JAK inhibitor	Pevonedistat + Ruxolitinib	MF	1	NCT03386214
PIM and LEE kinase inhibitor + JAK inhibitor	PIM447 + LEE011 + Ruxolitinib	MF	1	NCT02370706**
Hsp90 inhibitor + JAK inhibitor	PU-H71 + Ruxolitinib	MF	I	NCT03373877
PI3K delta inhibitor + JAK inhibitor	PI3K delta inhibitor (TGR-1202) + Ruxolitinib	MF, MDS/MPN	1/2	NCT02493530
BET inhibitor + JAK inhibitor	BET inhibitor (CPI-0610) +/− Ruxolitinib	MF	1	NCT02158858
IMIDs + JAK inhibitor	Thalidomide + Ruxolitinib	MF	2	NCT03069326
IMIDs + JAK inhibitor	Pomalidomide + Ruxolitinib (POMINC)	MF	1/2	NCT01644110
MDM2 inhibitor +/− PEG-INF	Idasanutlin (RG7388) +/− PEG-INF-α	PV, ET	1	NCT02407080 **
PD-1 inhibitor	Pembrolizumab	MF	2	NCT03065400
	Durvalumab	MF	1	NCT02871323 **
PD-1/CTLA4 inhibitor	Nivolumab or Ipilimumab	MF after SCT	1	NCT01822509
Anti CD123 MoAb	SL-401	MF (SM, HES)	1/2	NCT02268253
Peptide vaccine	CALR Exon 9 Peptide Vaccine	MF, ET –CALR (+)	1	NCT03566446**

¶ Only eligible diagnoses among MPNs; ** active, but currently not recruiting studies; SCT stem cell transplant

not only potentiate efficacy of INF-α, but also reduce its toxicity. Similarly, INF-α might increase efficacy of ruxolitinib by depleting dormant *JAK2V617F* MPN stem cells [167, 168••]. The feasibility of ruxolitinib and PEG-INF in MF has been shown in phase 1 study ($n = 15$), with no dose-limiting toxicity observed at the highest tested dose of ruxolitinib 15 mg twice daily + PEG-INF 135 mcg/week. Among 10 patients, 7 achieved hematologic clinical improvement (CI) and 3 achieved partial response [169]. The combination is currently being evaluated in PV and MF patients in the phase 2 COMBI trial in Denmark (Table 4). Recently reported results after the median follow-up of 12 months are promising. The study enrolled 32 PV and 18 MF patients of low/intermediate-risk; 94% of them were previously treated with PEG-INF monotherapy. Overall CHR and sustained CHR (≥ 3 months) was achieved in 81% and 44% of PV, and 92% and 58% of MF patients, respectively. Complete remission was observed in 17% of MF patients. A decline in *JAK2V617F* allele burden was noted in 88% and 50% of PV and MF patients, respectively. Although the most frequent AEs were hematologic, there was only one instance of grade ≥ 3 thrombocytopenia. Non-hematologic AEs (arthralgia 48%, flu-like symptoms 48%, and gastrointestinal symptoms 54%) and discontinuation rates (20%) were similar to those reported with single agents [82•].

Another combinatorial approach includes IMIDs. Although lenalidomide with ruxolitinib ($n = 31$) showed clinical improvement in 55% of MF patients, this study failed to meet predetermined efficacy, and the combination was very myelosuppressive [170]. Currently, thalidomide is being evaluated with ruxolitinib in a phase 2 study in MF patients, either ruxolitinib-naïve or on ruxolitinib for at least 3 months but not in complete or partial remission (43% pretreated with JAK inhibitors). Among 12 evaluable patients, the ORR was 59.3% ($n = 7$) with CI in anemia, symptoms, spleen, and major response in platelets observed in 3, 4, 2, and 6 patients, respectively. Furthermore, there was a significant increase in platelet count and hemoglobin in those with baseline thrombocytopenia and anemia [171].

Immunotherapy

Given the fact that immune dysregulation and a pro-inflammatory state are central features of MPNs, immunotherapy targeting disease-specific antigens or immune regulating cells should represent one of the most effective treatment approaches. Enhancement of antitumor immunity represents one of the most exciting areas in oncology. However, data on immunotherapy in patients with MPN are very limited and its role remains to be defined [172]. Tumors have evolved many mechanisms by which they avoid immune recognition; thus, the role of immunotherapy is to activate the patient's immune system to recognize and attack the tumor cells—by either increasing the function of immune effector cells or reducing the capacity of tumors to suppress the immune response. Antigen-specific immunotherapies include monoclonal antibodies (MoAb; directed against disease specific antigens or immune checkpoints), tumor-specific vaccines, or chimeric antigen receptor-modified T cells (CAR-T cells).

Proper T cell–mediated immune response is a stepwise process involving initial presentation of antigen, peptide bound to major histocompatibility complex on antigen-presenting cells, to T cells, and a co-stimulatory signal between CD80 or CD86 on antigen-presenting cells and CD28 on T cells. All the steps of this pathway are carefully regulated by numerous co-stimulatory (e.g., CD28, 4-1BB/CD137, CD27, CD80, CD86) and co-inhibitory (e.g., lymphocyte activation gene-3 [LAG-3], cytotoxic T lymphocyte–associated protein 4 [CTLA4], and programmed cell-death protein [PD-1]; called the immune checkpoints) signals and receptors [173].

Under normal circumstances, immune checkpoints regulate self-tolerance and protect tissues from immune-mediated destruction. Upregulation of negative co-stimulatory receptors or downregulation of positive co-stimulatory receptors represent an important mechanism of tumor-mediated immune evasion and T cell anergy [174, 175].

Targeting immune checkpoints by using MoAb against CTLA4 and PD-1/PD-L1 has become a major breakthrough in cancer therapy in numerous solid tumors, and more recently in hematologic malignancies. Data suggest the susceptibility of MPN to immune checkpoint inhibition (especially PD-1/PD-L1). Chronic inflammation typical for MPN patients has been shown to facilitate activation of T cells and upregulation of inhibitory checkpoint molecules, particularly PD-1 and PD-L1 in tumor cells and the tumor microenvironment [173]. The overexpression of the immune checkpoints PD-1 and CTLA4 have been connected to a more aggressive type of leukemia [176]; and PD-1 positive T cells were shown to be significantly increased in the splenic hematopoietic cells (including megakaryocytes and myeloid cells) of patients with MF [177]. Furthermore, Prestipino et al. recently reported that the *JAK2V617F* mutation enhances the expression of PD-L1 via STAT3 activation and that patients with this mutation display increased levels of PD-L1 on *JAK2V617F*-mutated cells and in the bone marrow [43•].

Despite the obvious rationale for using immunotherapeutic approaches relying on checkpoint inhibition in patients with MPN, only a few studies so far have evaluated this option in these patients. As of the date of this writing, no data from these studies were reported, although one study (clinicaltrials.gov identifier NCT02421354) evaluating the PD1 inhibitor nivolumab (*Opdivo*®) in patients with MPN was terminated for lack of efficacy (unpublished data). Ongoing trials are summarized in Table 4 and include the PD1 inhibitor pembrolizumab

(*Keytruda*®) and the PDL1 inhibitor durvalumab (*Imfinzi*®), and the combination of the PD1 inhibitor nivolumab with the CTLA4 inhibitor ipilimumab (*Yervoy*®) in patients with relapsed MF following allogeneic SCT.

Another MoAb currently evaluated in patients with MF with existing preliminary clinical data is anti-CD123 MoAb tagraxofusp conjugated to diphtheria toxin (*Elzonris*™; SL-401). This is a MoAb directed to the interleukin-3 receptor-α (CD123), which has been shown to be overexpressed in myeloid malignancies, including MPNs, and was associated with enhanced cell survival [178]. In a phase 1/2 trial in 23 patients with MF refractory or intolerant to ruxolitinib, tagraxofusp showed ≥ 29% and ≥ 45% spleen reduction in 43% and 21% of patients with palpable splenomegaly, respectively. Safety was acceptable with grade ≥ 3 hematologic toxicity (thrombocytopenia) in 2% of patients [179].

The use of MPN mutations as targets for personalized vaccines and adoptive cell-based therapy represents an exciting therapeutic approach. As mentioned previously, Holmstrom et al. showed the presence of spontaneous T cell responses against PD-L1-derived *JAK2V617F* or *CALR* exon 9 mutation epitopes in 71% of MPN patients [46••, 47••] and launched a clinical trial with an anti-mutant *CALR* peptide vaccine (Table 4). Similarly, Schischlik et al. [180] identified 149 unique neo-antigens in 62% of MPN patients (*CALR*, *MPL*, and *SF3B1* mutations). Lately, the laboratory of Constantinescu et al. [49••] revealed groundbreaking research that mutant *CALR* also acts as a "rogue cytokine"—secreted outside of cells, thereby directly activating neighboring MPN cells via the STAT5 pathway, promoting progression of the malignant clone. Therefore, vaccination against mutant *CALR* and other targetable antigens appears highly intriguing and might represent an effective way to enhance tumor-specific immune responses.

Conclusions

Over the past years, tremendous progress has been made in our understanding of the pathogenesis of the Philadelphia chromosome negative MPNs. Disease phenotype is not only driven by the effect of the malignant clone but also by chronic inflammation and significant immune dysfunction. From this perspective, treatment with agents targeting the immune system and/or having anti-inflammatory effect appears rational.

Interferon has been used in MPNs for decades and robust data support its ability to alter MPN stem cells. So far, interferon use in broad clinical practice has been hampered by significant toxicity, which might be overcome by the novel monopegylated isoform just recently approved for PV patients in Europe.

JAK1/JAK2 inhibitors, particularly ruxolitinib, significantly reduce inflammatory cytokines in MF patients, which result in clinical improvement of symptoms, spleen size, and quality of life and ultimately prolongation of survival.

More potent and selective JAK inhibitors, some of them "resurrected" after their failure for various reasons, are under clinical investigation. Significant work still remains to be done to fully understand the potential and proper use of immunotherapy and cancer-directed vaccines within MPNs. A severely deregulated immune system, defective surveillance, and dysfunctional effector immune cells, which could even originate from the malignant clone itself, represent just a few challenges to be addressed in the years ahead. Combinatorial approaches of various agents may lower toxicity and provide more effective disease control, yet more preclinical data are needed to define the most effective and synergistic combinations.

In summary, our success in treating MPNs is increasing, and the current pace of drug development is exciting. The phenotypic heterogeneity of MPNs necessitates a more individualized, risk-adapted treatment approach. Development of more effective and targeted medical therapies, which could prolong survival and alter the natural history of these diseases, will lead MPN research in the near future. The entire potential of immunotherapy has not yet been utilized in MPN, but it is clear that the next few years will bring further developments in this rapidly expanding field.

Compliance with Ethical Standards

Conflict of Interest Lucia Masarova reports no conflict of interest.

Prithviraj Bose reports research support from Incyte Corp., Celgene, Astellas, Pfizer, Blueprint Pharmaceuticals, Kartos Therapeutics, Constellation Pharmaceuticals, CTI BioPharma, NS Pharma, and Promedior, and honoraria from Incyte Corp., Celgene, and Blueprint Pharmaceuticals.

Srdan Verstovsek reports research support and grants from Incyte Corp.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Cervantes F, Passamonti F, Barosi G. Life expectancy and prognostic factors in the classic BCR/ABL-negative myeloproliferative disorders. Leukemia. 2008;22(5):905–14. https://doi.org/10.1038/leu.2008.72.

2. Barosi G, Rosti V, Bonetti E, Campanelli R, Carolei A, Catarsi P, et al. Evidence that prefibrotic myelofibrosis is aligned along a clinical and biological continuum featuring primary myelofibrosis. PLoS One. 2012;7(4):e35631. https://doi.org/10.1371/journal.pone.0035631.
3. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352(17):1779–90. https://doi.org/10.1056/NEJMoa051113.
4. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379–90. https://doi.org/10.1056/NEJMoa1311347.
5. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006;3(7):e270. https://doi.org/10.1371/journal.pmed.0030270.
6. Milosevic Feenstra JD, Nivarthi H, Gisslinger H, Leroy E, Rumi E, Chachoua I, et al. Whole-exome sequencing identifies novel MPL and JAK2 mutations in triple-negative myeloproliferative neoplasms. Blood. 2016;127(3):325–32. https://doi.org/10.1182/blood-2015-07-661835.
7. Viny AD, Levine RL. Genetics of myeloproliferative neoplasms. Cancer J. 2014;20(1):61–5. https://doi.org/10.1097/ppo.0000000000000013.
8. Rumi E, Pietra D, Pascutto C, Guglielmelli P, Martinez-Trillos A, Casetti I, et al. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. Blood. 2014;124(7):1062–9. https://doi.org/10.1182/blood-2014-05-578435.
9. Kralovics R, Teo SS, Li S, Theocharides A, Buser AS, Tichelli A, et al. Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. Blood. 2006;108(4):1377–80. https://doi.org/10.1182/blood-2005-11-009605.
10. Lundberg P, Karow A, Nienhold R, Looser R, Hao-Shen H, Nissen I, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood. 2014;123(14):2220–8. https://doi.org/10.1182/blood-2013-11-537167.
11. Barbui T, Vannucchi AM, Buxhofer-Ausch V, De Stefano V, Betti S, Rambaldi A, et al. Practice-relevant revision of IPSET-thrombosis based on 1019 patients with WHO-defined essential thrombocythemia. Blood Cancer J. 2015;5:e369. https://doi.org/10.1038/bcj.2015.94.
12. PharmaEssentia. PharmaEssentia and AOP orphan receive EU approval of Besremi™ (ropeginterferon alfa-2b) for treatment of polycythemia Vera (PV) in EU. 2018. Available from: https://www.prnewswire.com/news-releases/pharmaessentia-and-aop-orphan-receive-eu-approval-of-besremi-ropeginterferon-alfa-2b-for-treatment-of-polycythemia-vera-pv-in-eu-300800079.html. Accessed April 2019.
13. Gupta V, Hari P, Hoffman R. Allogeneic hematopoietic cell transplantation for myelofibrosis in the era of JAK inhibitors. Blood. 2012;120(7):1367–79. https://doi.org/10.1182/blood-2012-05-399048.
14. Kroger NM, Deeg JH, Olavarria E, Niederwieser D, Bacigalupo A, Barbui T, et al. Indication and management of allogeneic stem cell transplantation in primary myelofibrosis: a consensus process by an EBMT/ELN international working group. Leukemia. 2015;29(11):2126–33. https://doi.org/10.1038/leu.2015.233.
15.• Bose P, Alfayez M, Verstovsek S. New concepts of treatment for patients with myelofibrosis. Curr Treat Options in Oncol. 2019;20(1):5. https://doi.org/10.1007/s11864-019-0604-y **Comprehensive reviews of novel therapeutic approaches in MPN.**
16.• Pettit K, Odenike O. Novel therapies for myelofibrosis. Curr Hematol Malig Rep. 2017;12(6):611–24. https://doi.org/10.1007/s11899-017-0403-0 **Comprehensive reviews of novel therapeutic approaches in MPN.**
17.• Bose P, Verstovsek S. JAK2 inhibitors for myeloproliferative neoplasms: what is next? Blood. 2017;130(2):115–25. https://doi.org/10.1182/blood-2017-04-742288 **Comprehensive reviews of novel therapeutic approaches in MPN.**
18.•• Hasselbalch HC. Perspectives on chronic inflammation in essential thrombocythemia, polycythemia vera, and myelofibrosis: is chronic inflammation a trigger and driver of clonal evolution and development of accelerated atherosclerosis and second cancer? Blood. 2012;119(14):3219–25. https://doi.org/10.1182/blood-2011-11-394775 **Excellent review about inflammation and deregulated immune system in MPNs.**
19.•• Hasselbalch HC. The role of cytokines in the initiation and progression of myelofibrosis. Cytokine Growth Factor Rev. 2013;24(2):133–45. https://doi.org/10.1016/j.cytogfr.2013.01.004 **Excellent review about inflammation and deregulated immune system in MPNs.**
20.•• Lussana F, Rambaldi A. Inflammation and myeloproliferative neoplasms. J Autoimmun. 2017;85:58–63. https://doi.org/10.1016/j.jaut.2017.06.010 **Excellent review about inflammation and deregulated immune system in MPNs.**
21.•• Barosi G. An immune dysregulation in MPN. Curr Hematol Malig Rep. 2014;9(4):331–9. https://doi.org/10.1007/s11899-014-0227-0 **Excellent review about inflammation and deregulated immune system in MPNs.**
22.•• Hasselbalch HC. Chronic inflammation as a promotor of mutagenesis in essential thrombocythemia, polycythemia vera and myelofibrosis. A human inflammation model for cancer development? Leuk Res. 2013;37(2):214–20. https://doi.org/10.1016/j.leukres.2012.10.020 **Excellent review about inflammation and deregulated immune system in MPNs.**
23. Delhommeau FDS, Tonetti C, Massé A, et al. Evidence that the JAK2 G1849T (V617F) mutation occurs in a lymphomyeloid progenitor in polycythemia vera and idiopathic myelofibrosis. Blood. 2007;109:71–7.
24. Pourcelot E, Trocme C, Mondet J, Bailly S, Toussaint B, Mossuz P. Cytokine profiles in polycythemia vera and essential thrombocythemia patients: clinical implications. Exp Hematol. 2014;42(5):360–8. https://doi.org/10.1016/j.exphem.2014.01.006.
25. Tefferi A, Vaidya R, Caramazza D, Finke C, Lasho T, Pardanani A. Circulating interleukin (IL)-8, IL-2R, IL-12, and IL-15 levels are independently prognostic in primary myelofibrosis: a comprehensive cytokine profiling study. J Clin Oncol Off J Am Soc Clin Oncol. 2011;29(10):1356–63. https://doi.org/10.1200/jco.2010.32.9490.
26. Tefferi A. Pathogenesis of myelofibrosis with myeloid metaplasia. J Clin Oncol Off J Am Soc Clin Oncol. 2005;23(33):8520–30. https://doi.org/10.1200/jco.2004.00.9316.
27. Le Bousse-Kerdiles MC, Chevillard S, Charpentier A, Romquin N, Clay D, Smadja-Joffe F, et al. Differential expression of transforming growth factor-beta, basic fibroblast growth factor, and their receptors in CD34+ hematopoietic progenitor cells from patients with myelofibrosis and myeloid metaplasia. Blood. 1996;88(12):4534–46.
28. Bock O, Hoftmann J, Theophile K, Hussein K, Wiese B, Schlue J, et al. Bone morphogenetic proteins are overexpressed in the bone marrow of primary myelofibrosis and are apparently induced by fibrogenic cytokines. Am J Pathol. 2008;172(4):951–60. https://doi.org/10.2353/ajpath.2008.071030.
29. Flamant L, Toffoli S, Raes M, Michiels C. Hypoxia regulates inflammatory gene expression in endothelial cells. Exp Cell Res. 2009;315(5):733–47. https://doi.org/10.1016/j.yexcr.2008.11.020.

30. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;44(7203):436–44. https://doi.org/10.1038/nature07205.
31. Levy DE, Darnell JE Jr. Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol. 2002;3(9):651–62. https://doi.org/10.1038/nrm909.
32. Boissinot M, Cleyrat C, Vilaine M, Jacques Y, Corre I, Hermouet S. Anti-inflammatory cytokines hepatocyte growth factor and interleukin-11 are over-expressed in polycythemia vera and contribute to the growth of clonal erythroblasts independently of JAK2V617F. Oncogene. 2011;30(8):990–1001. https://doi.org/10.1038/onc.2010.479.
33.•• Kleppe M, Kwak M, Koppikar P, Riester M, Keller M, Bastian L, et al. JAK-STAT pathway activation in malignant and nonmalignant cells contributes to MPN pathogenesis and therapeutic response. Cancer Discov. 2015;5(3):316–31. https://doi.org/10.1158/2159-8290.cd-14-0736 **Excellent paper about JAK/STAT pathway in MPN pathogenesis.**
34. Zhan H, Ma Y, Lin CH, Kaushansky K. JAK2(V617F)-mutant megakaryocytes contribute to hematopoietic stem/progenitor cell expansion in a model of murine myeloproliferation. Leukemia. 2016;30(12):2332–41. https://doi.org/10.1038/leu.2016.114.
35. Lataillade JJ, Pierre-Louis O, Hasselbalch HC, Uzan G, Jasmin C, Martyre MC, et al. Does primary myelofibrosis involve a defective stem cell niche? From concept to evidence. Blood. 2008;112(8):3026–35. https://doi.org/10.1182/blood-2008-06-158386.
36. Corre-Buscail I, Pineau D, Boissinot M, Hermouet S. Erythropoietin-independent erythroid colony formation by bone marrow progenitors exposed to interleukin-11 and interleukin-8. Exp Hematol. 2005;33(11):1299–308. https://doi.org/10.1016/j.exphem.2005.07.002.
37. Skov V, Larsen TS, Thomassen M, Riley CH, Jensen MK, Bjerrum OW, et al. Molecular profiling of peripheral blood cells from patients with polycythemia vera and related neoplasms: identification of deregulated genes of significance for inflammation and immune surveillance. Leuk Res. 2012;36(11):1387–92. https://doi.org/10.1016/j.leukres.2012.07.009.
38. Zhao WB, Li Y, Liu X, Zhang LY, Wang X. Involvement of CD4+ CD25+ regulatory T cells in the pathogenesis of polycythaemia vera. Chin Med J. 2008;121(18):1781–6.
39. Skov V, Riley CH, Thomassen M, Larsen TS, Jensen MK, Bjerrum OW, et al. Whole blood transcriptional profiling reveals significant down-regulation of human leukocyte antigen class I and II genes in essential thrombocythemia, polycythemia vera and myelofibrosis. Leuk Lymphoma. 2013;54(10):2269–73. https://doi.org/10.3109/10428194.2013.764417.
40. Marty C, Lacout C, Droin N, Le Couedic JP, Ribrag V, Solary E, et al. A role for reactive oxygen species in JAK2 V617F myeloproliferative neoplasm progression. Leukemia. 2013;27(11):2187–95. https://doi.org/10.1038/leu.2013.102.
41. Bjorn ME, Hasselbalch HC. The role of reactive oxygen species in myelofibrosis and related neoplasms. Mediat Inflamm. 2015:648090. https://doi.org/10.1155/2015/648090.
42. Wang JC, Kundra A, Andrei M, Baptiste S, Chen C, Wong C, et al. Myeloid-derived suppressor cells in patients with myeloproliferative neoplasm. Leuk Res. 2016;43:39–43. https://doi.org/10.1016/j.leukres.2016.02.004.
43.• Prestipino A, Emhardt AJ, Aumann K, O'Sullivan D, Gorantla SP, Duquesne S, et al. Oncogenic JAK2(V617F) causes PD-L1 expression, mediating immune escape in myeloproliferative neoplasms. Sci Transl Med. 2018;10(429). https://doi.org/10.1126/scitranslmed.aam7729 **Interesting paper about the role of JAK2 in PD-L1 overexpression.**
44.•• Holmstrom MO, Hjortso MD, Ahmad SM, Met O, Martinenaite E, Riley C, et al. The JAK2V617F mutation is a target for specific T cells in the JAK2V617F-positive myeloproliferative neoplasms. Leukemia. 2017;31(2):495–8. https://doi.org/10.1038/leu.2016.290 **Very interesting data about the potential use of JAK2 and CALR as neo-antigens for cancer vaccines.**
45.•• Holmstrom MO, Riley CH, Svane IM, Hasselbalch HC, Andersen MH. The CALR exon 9 mutations are shared neoantigens in patients with CALR mutant chronic myeloproliferative neoplasms. Leukemia. 2016;30(12):2413–6. https://doi.org/10.1038/leu.2016.233 **Very interesting data about the potential use of JAK2 and CALR as neo-antigens for cancer vaccines.**
46.•• Holmstrom MO, Riley CH, Skov V, Svane IM, Hasselbalch HC, Andersen MH. Spontaneous T-cell responses against the immune check point programmed-death-ligand 1 (PD-L1) in patients with chronic myeloproliferative neoplasms correlate with disease stage and clinical response. Oncoimmunology. 2018;7(6):e1433521. https://doi.org/10.1080/2162402x.2018.1433521 **Very interesting data about the potential use of JAK2 and CALR as neo-antigens for cancer vaccines.**
47.•• Holmstrom MO, Hasselbalch HC, Andersen MH. The JAK2V617F and CALR exon 9 mutations are shared immunogenic neoantigens in hematological malignancy. Oncoimmunology. 2017;6(11):e1358334. https://doi.org/10.1080/2162402x.2017.1358334 **Very interesting data about the potential use of JAK2 and CALR as neo-antigens for cancer vaccines.**
48.•• Elf SAN, Chen E, et al. Mutant calreticulin requires both its mutant C-terminus and the thrombopoietin receptor for oncogenic transformation. Cancer Discov. 2016;6(4):368–81 **Very interesting data about the potential use of JAK2 and CALR as neo-antigens for cancer vaccines.**
49.•• Pecquet Ch BT, Chachoua I, Roy A, Vertenoeil G, et al. Secreted mutant calreticulins as rogue cytokines trigger thrombopoietin receptor activation specifically in CALR mutated cells: perspectives for MPN therapy. Blood. 2018;132:4 **Very interesting data about the potential use of JAK2 and CALR as neo-antigens for cancer vaccines.**
50.•• Mesa RA, Niblack J, Wadleigh M, Verstovsek S, Camoriano J, Barnes S, et al. The burden of fatigue and quality of life in myeloproliferative disorders (MPDs): an international Internet-based survey of 1179 MPD patients. Cancer. 2007;109(1):68–76. https://doi.org/10.1002/cncr.22365 **Important study about symptoms burden in patients with MPN.**
51. Mughal TI, Vaddi K, Sarlis NJ, Verstovsek S. Myelofibrosis-associated complications: pathogenesis, clinical manifestations, and effects on outcomes. Int J Gen Med. 2014;7:89–101. https://doi.org/10.2147/ijgm.s51800.
52. Mesa R, Jamieson C, Bhatia R, Deininger MW, Gerds AT, Gojo I, et al. Myeloproliferative neoplasms, version 2.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw. 2016;14(12):1572–611.
53. Mesa RA, Jamieson C, Bhatia R, Deininger MW, Fletcher CD, Gerds AT, et al. NCCN guidelines insights: myeloproliferative neoplasms, version 2.2018. J Natl Compr Cancer Netw. 2017;15(10):1193–207. https://doi.org/10.6004/jnccn.2017.0157.
54. Kiladjian JJ, Mesa RA, Hoffman R. The renaissance of interferon therapy for the treatment of myeloid malignancies. Blood. 2011;117(18):4706–15. https://doi.org/10.1182/blood-2010-08-258772.
55. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol. 2005;5(5):375–86. https://doi.org/10.1038/nri1604.
56. Chawla-Sarkar M, Lindner DJ, Liu YF, Williams BR, Sen GC, Silverman RH, et al. Apoptosis and interferons: role of interferon-stimulated genes as mediators of apoptosis. Apoptosis. 2003;8(3):237–49.
57. Wang Q, Miyakawa Y, Fox N, Kaushansky K. Interferon-alpha directly represses megakaryopoiesis by inhibiting thrombopoietin-

induced signaling through induction of SOCS-1. Blood. 2000;96(6):2093–9.
58. Peschel C, Aulitzky WE, Huber C. Influence of interferon-alpha on cytokine expression by the bone marrow microenvironment–impact on treatment of myeloproliferative disorders. Leuk Lymphoma. 1996;22(Suppl 1):129–34. https://doi.org/10.3109/10428199609074370.
59. Dai CHPJ, et al. Fas ligand is present in human erythroid colony-forming cells and interacts with Fas induced by interferon gamma to produce erythroid cell apoptosis. Blood. 1998;91(4):1235–42.
60. Kanfer EJ, Price CM, Gordon AA, Barrett AJ. The in vitro effects of interferon-gamma, interferon-alpha, and tumour-necrosis factor-alpha on erythroid burst-forming unit growth in patients with non-leukaemic myeloproliferative disorders. Eur J Haematol. 1993;50(5):250–4.
61. Aman MJ, Bug G, Aulitzky WE, Huber C, Peschel C. Inhibition of interleukin-11 by interferon-alpha in human bone marrow stromal cells. Exp Hematol. 1996;24(8):863–7.
62. Carlo-Stella C, Cazzola M, Gasner A, Barosi G, Dezza L, Meloni F, et al. Effects of recombinant alpha and gamma interferons on the in vitro growth of circulating hematopoietic progenitor cells (CFU-GEMM, CFU-Mk, BFU-E, and CFU-GM) from patients with myelofibrosis with myeloid metaplasia. Blood. 1987;70(4):1014–9.
63. Indraccolo S. Interferon-alpha as angiogenesis inhibitor: learning from tumor models. Autoimmunity. 2010;43(3):244–7. https://doi.org/10.3109/08916930903510963.
64. Essers MA, Offner S, Blanco-Bose WE, Waibler Z, Kalinke U, Duchosal MA, et al. IFNalpha activates dormant haematopoietic stem cells in vivo. Nature. 2009;458(7240):904–8. https://doi.org/10.1038/nature07815.
65. Lu M, Zhang W, Li Y, Berenzon D, Wang X, Wang J, et al. Interferon-alpha targets JAK2V617F-positive hematopoietic progenitor cells and acts through the p38 MAPK pathway. Exp Hematol. 2010;38(6):472–80. https://doi.org/10.1016/j.exphem.2010.03.005.
66. Riley CH, Jensen MK, Brimnes MK, Hasselbalch HC, Bjerrum OW, Straten PT, et al. Increase in circulating CD4(+)CD25(+)Foxp3(+) T cells in patients with Philadelphia-negative chronic myeloproliferative neoplasms during treatment with IFN-alpha. Blood. 2011;118(8):2170–3. https://doi.org/10.1182/blood-2011-03-340992.
67. Riley CH, Hansen M, Brimnes MK, Hasselbalch HC, Bjerrum OW, Straten PT, et al. Expansion of circulating CD56bright natural killer cells in patients with JAK2-positive chronic myeloproliferative neoplasms during treatment with interferon-alpha. Eur J Haematol. 2015;94(3):227–34. https://doi.org/10.1111/ejh.12420.
68. Rizza P, Moretti F, Belardelli F. Recent advances on the immunomodulatory effects of IFN-alpha: implications for cancer immunotherapy and autoimmunity. Autoimmunity. 2010;43(3):204–9. https://doi.org/10.3109/08916930903510880.
69. Xiong Z, Yan Y, Liu E, Silver RT, Verstovsek S, Yang F, et al. Novel tumor antigens elicit anti-tumor humoral immune reactions in a subset of patients with polycythemia vera. Clin Immunol. 2007;122(3):279–87. https://doi.org/10.1016/j.clim.2006.10.006.
70. Skov V, Riley CH, Thomassen M, Kjaer L, Stauffer Larsen T, Bjerrum OW, et al. The impact of interferon-alpha2 on HLA genes in patients with polycythemia vera and related neoplasms. Leuk Lymphoma. 2017;58(8):1914–21. https://doi.org/10.1080/10428194.2016.1262032.
71. Skov VRC, Thomassen M, Lasse Kjær L, et al. Interferon-alfa2 treatment of patients with polycythemia vera and related neoplasms impacts deregulation of oxidative stress genes and antioxidative defence mechanisms. Potential implications of IFN-alfa induced changes in TP53, NRF2 and CXCR4 for genomic instability and CD34+ mobilisation. Blood. 2018;132:4326.
72. Radin AI, Kim HT, Grant BW, Bennett JM, Kirkwood JM, Stewart JA, et al. Phase II study of alpha2 interferon in the treatment of the chronic myeloproliferative disorders (E5487): a trial of the Eastern Cooperative Oncology Group. Cancer. 2003;98(1):100–9. https://doi.org/10.1002/cncr.11486.
73. Gowin K, Thapaliya P, Samuelson J, Harrison C, Radia D, Andreasson B, et al. Experience with pegylated interferon alpha-2a in advanced myeloproliferative neoplasms in an international cohort of 118 patients. Haematologica. 2012;97(10):1570–3. https://doi.org/10.3324/haematol.2011.061390.
74. Gowin KLJT, Kosiorek HE, Camoriano J, Tibes R, Palmer J, Mesa RA. Pegylated interferon alpha-2a in 75 patients with myeloproliferative neoplasms: a single center experience. Blood. 2015;126:2818.
75. Stauffer Larsen T, Iversen KF, Hansen E, Mathiasen AB, Marcher C, Frederiksen M, et al. Long term molecular responses in a cohort of Danish patients with essential thrombocythemia, polycythemia vera and myelofibrosis treated with recombinant interferon alpha. Leuk Res. 2013;37(9):1041–5. https://doi.org/10.1016/j.leukres.2013.06.012.
76. Larsen TS, Moller MB, de Stricker K, Norgaard P, Samuelsson J, Marcher C, et al. Minimal residual disease and normalization of the bone marrow after long-term treatment with alpha-interferon2b in polycythemia vera. A report on molecular response patterns in seven patients in sustained complete hematological remission. Hematology. 2009;14(6):331–4. https://doi.org/10.1179/102453309x12473408860587.
77. Yacoub AMJ, Kosiorek HE, et al. Single-arm salvage therapy with pegylated interferon alfa-2a for patients with high-risk polycythemia vera or high-risk essential thrombocythemia who are either hydroxyurea-resistant or intolerant: final results of the myeloproliferative disorders-research consortium (MPD-RC) protocol 111 global phase II trial. Blood. 2017;130:321.
78. Masarova L, Patel KP, Newberry KJ, Cortes J, Borthakur G, Konopleva M, et al. Pegylated interferon alfa-2a in patients with essential thrombocythaemia or polycythaemia vera: a post-hoc, median 83 month follow-up of an open-label, phase 2 trial. Lancet Haematol. 2017;4(4):e165–75. https://doi.org/10.1016/s2352-3026(17)30030-3.
79. Masarova L, Yin CC, Cortes JE, Konopleva M, Borthakur G, Newberry KJ, et al. Histomorphological responses after therapy with pegylated interferon alpha-2a in patients with essential thrombocythemia (ET) and polycythemia vera (PV). Exp Hematol Oncol. 2017;6:30. https://doi.org/10.1186/s40164-017-0090-5.
80. Knudsen AT HD, Ocias LF, Bjerrum OW, Brabrand M, et al. Three-year analysis of the DALIAH trial - a randomized controlled phase III clinical trial comparing recombinant interferon alpha-2 vs. hydroxyurea in patients with myeloproliferative neoplasms. EHA learning center S1609. 2019.
81. Mascarenhas JKH, Prchal J, Rambaldi A, et al. Results of the myeloproliferative neoplasms - research consortium (MPN-RC) 112 randomized trial of pegylated interferon alfa-2a (PEG) versus hydroxyurea (HU) therapy for the treatment of high risk polycythemia vera (PV) and high risk essential thrombocythemia (ET). Blood. 2018;132:577.
82.• Mikkelsen SU, Kjaer L, Bjorn ME, Knudsen TA, Sorensen AL, Andersen CBL, et al. Safety and efficacy of combination therapy of interferon-alpha2 and ruxolitinib in polycythemia vera and myelofibrosis. Cancer Med. 2018;7(8):3571–81. https://doi.org/10.1002/cam4.1619 **Interesting study of combination of ruxolitinib and interferon in PV and MF.**
83.•• Silver RT. Long-term effects of the treatment of polycythemia vera with recombinant interferon-alpha. Cancer. 2006;107(3):451–8. https://doi.org/10.1002/cncr.22026 **Important study of interferon in PV.**

84. Kiladjian JJ, Cassinat B, Chevret S, Turlure P, Cambier N, Roussel M, et al. Pegylated interferon-alfa-2a induces complete hematologic and molecular responses with low toxicity in polycythemia vera. Blood. 2008;112(8):3065–72. https://doi.org/10.1182/blood-2008-03-143537.
85. Kiladjian JJ, Cassinat B, Turlure P, Cambier N, Roussel M, Bellucci S, et al. High molecular response rate of polycythemia vera patients treated with pegylated interferon alpha-2a. Blood. 2006;108(6):2037–40. https://doi.org/10.1182/blood-2006-03-009860.
86. Gisslinger H, Zagrijtschuk O, Buxhofer-Ausch V, Thaler J, Schloegl E, Gastl GA, et al. Ropeginterferon alfa-2b, a novel IFNalpha-2b, induces high response rates with low toxicity in patients with polycythemia vera. Blood. 2015;126(15):1762–9. https://doi.org/10.1182/blood-2015-04-637280.
87. Gisslinger HB-AV, Josef Thaler J, Forjan E, et al. Long-term efficacy and safety of ropeginterferon alfa-2b in patients with polycythemia vera – final phase I/II Peginvera Study results. Blood. 2018;132:3030.
88.•• Gisslinger HKC, Georgiev P, et al. Evidence for superior efficacy and disease modification after three years of prospective randomized controlled treatment of polycythemia vera patients with ropeginterferon alfa-2b vs. HU/BAT. Blood. 2018;130:579 **Important study showing superiority of long term monopegylated interferon to hydrea.**
89. Gisslinger HKC, Georgiev P, et al. Final results from PROUD-PV a randomized controlled phase 3 trial comparing ropeginterferon alfa-2b to hydroxyurea in polycythemia vera patients. Blood. 2016;128(22):475.
90. Verger E, Cassinat B. Clinical and molecular response to interferon-alpha therapy in essential thrombocythemia patients with CALR mutations. 2015;126(24):2585–91. https://doi.org/10.1182/blood-2015-07-659060.
91. Silver RT, Vandris K. Recombinant interferon alpha (rIFN alpha-2b) may retard progression of early primary myelofibrosis. Leukemia. 2009;23(7):1366–9. https://doi.org/10.1038/leu.2009.90.
92. Silver RT, Barel AC, Lascu E, Ritchie EK, Roboz GJ, Christos PJ, et al. The effect of initial molecular profile on response to recombinant interferon-alpha (rIFNalpha) treatment in early myelofibrosis. Cancer. 2017;123(14):2680–7. https://doi.org/10.1002/cncr.30679.
93. Ianotto JC, Kiladjian JJ, Demory JL, Roy L, Boyer F, Rey J, et al. PEG-IFN-alpha-2a therapy in patients with myelofibrosis: a study of the French Groupe d'Etudes des Myelofibroses (GEM) and France Intergroupe des syndromes Myeloproliferatifs (FIM). Br J Haematol. 2009;146(2):223–5. https://doi.org/10.1111/j.1365-2141.2009.07745.x.
94. Ianotto JC, Chauveau A, Boyer-Perrard F, Gyan E, Laribi K, Cony-Makhoul P, et al. Benefits and pitfalls of pegylated interferon-alpha2a therapy in patients with myeloproliferative neoplasm-associated myelofibrosis: a French Intergroup of Myeloproliferative neoplasms (FIM) study. Haematologica. 2018;103(3):438–46. https://doi.org/10.3324/haematol.2017.181297.
95. Gisslinger HGB, Schalling M, Krejcy K, et al. Effect of Ropeginterferon alfa-2b in Prefibrotic primary myelofibrosis. Blood. 2018;132:3029.
96. Samuelsson J, Hasselbalch H, Bruserud O, Temerinac S, Brandberg Y, Merup M, et al. A phase II trial of pegylated interferon alpha-2b therapy for polycythemia vera and essential thrombocythemia: feasibility, clinical and biologic effects, and impact on quality of life. Cancer. 2006;106(11):2397–405. https://doi.org/10.1002/cncr.21900.
97. Silver RT, Vandris K, Goldman JJ. Recombinant interferon-alpha may retard progression of early primary myelofibrosis: a preliminary report. Blood. 2011;117(24):6669–72. https://doi.org/10.1182/blood-2010-11-320069.
98. Turlure PCN, Roussel M, Bellucci S, et al. Complete hematological, molecular and histological remissions without cytoreductive treatment lasting after Pegylated-interferon α-2a (peg-IFNα-2a) therapy in polycythemia vera (PV): long term results of a phase 2 trial. Blood. 2011;118:280.
99. Knudsen ATHD, Ocias LF, Bjerrum OW, Brabrand M, et al. Long-term efficacy and safety of recombinant interferon Alpha-2 vs. hydroxyurea in polycythemia Vera: preliminary results from the three-year analysis of the Daliah trial - a randomized controlled phase III clinical trial. Blood. 2018;132:580.
100. Muller GW, Chen R, Huang SY, Corral LG, Wong LM, Patterson RT, et al. Amino-substituted thalidomide analogs: potent inhibitors of TNF-alpha production. Bioorg Med Chem Lett. 1999;9(11):1625–30.
101. Galustian C, Meyer B, Labarthe MC, Dredge K, Klaschka D, Henry J, et al. The anti-cancer agents lenalidomide and pomalidomide inhibit the proliferation and function of T regulatory cells. Cancer Immunol Immunother. 2009;58(7):1033–45. https://doi.org/10.1007/s00262-008-0620-4.
102. Dredge K, Marriott JB, Macdonald CD, Man HW, Chen R, Muller GW, et al. Novel thalidomide analogues display anti-angiogenic activity independently of immunomodulatory effects. Br J Cancer. 2002;87(10):1166–72. https://doi.org/10.1038/sj.bjc.6600607.
103. Elliott MA, Mesa RA, Li CY, Hook CC, Ansell SM, Levitt RM, et al. Thalidomide treatment in myelofibrosis with myeloid metaplasia. Br J Haematol. 2002;117(2):288–96.
104. Marchetti M, Barosi G, Balestri F, Viarengo G, Gentili S, Barulli S, et al. Low-dose thalidomide ameliorates cytopenias and splenomegaly in myelofibrosis with myeloid metaplasia: a phase II trial. J Clin Oncol Off J Am Soc Clin Oncol. 2004;22(3):424–31. https://doi.org/10.1200/jco.2004.08.160.
105. Barosi G, Grossi A, Comotti B, Musto P, Gamba G, Marchetti M. Safety and efficacy of thalidomide in patients with myelofibrosis with myeloid metaplasia. Br J Haematol. 2001;114(1):78–83.
106. Mesa RA, Elliott MA, Schroeder G, Tefferi A. Durable responses to thalidomide-based drug therapy for myelofibrosis with myeloid metaplasia. Mayo Clin Proc. 2004;79(7):883–9. https://doi.org/10.1016/s0025-6196(11)62154-x.
107. Thapaliya P, Tefferi A, Pardanani A, Steensma DP, Camoriano J, Wu W, et al. International working group for myelofibrosis research and treatment response assessment and long-term follow-up of 50 myelofibrosis patients treated with thalidomide-prednisone based regimens. Am J Hematol. 2011;86(1):96–8. https://doi.org/10.1002/ajh.21892.
108. Weinkove R, Reilly JT, McMullin MF, Curtin NJ, Radia D, Harrison CN. Low-dose thalidomide in myelofibrosis. Haematologica. 2008;93(7):1100–1. https://doi.org/10.3324/haematol.12416.
109. Tefferi A, Cortes J, Verstovsek S, Mesa RA, Thomas D, Lasho TL, et al. Lenalidomide therapy in myelofibrosis with myeloid metaplasia. Blood. 2006;108(4):1158–64. https://doi.org/10.1182/blood-2006-02-004572.
110. Mesa RA, Yao X, Cripe LD, Li CY, Litzow M, Paietta E, et al. Lenalidomide and prednisone for myelofibrosis: Eastern Cooperative Oncology Group (ECOG) phase 2 trial E4903. Blood. 2010;116(22):4436–8. https://doi.org/10.1182/blood-2010-05-287417.
111. Chihara D, Masarova L, Newberry KJ, Maeng H, Ravandi F, Garcia-Manero G, et al. Long-term results of a phase II trial of lenalidomide plus prednisone therapy for patients with myelofibrosis. Leuk Res. 2016;48:1–5. https://doi.org/10.1016/j.leukres.2016.06.007.
112. Tefferi A, Verstovsek S, Barosi G, Passamonti F, Roboz GJ, Gisslinger H, et al. Pomalidomide is active in the treatment of

anemia associated with myelofibrosis. J Clin Oncol Off J Am Soc Clin Oncol. 2009;27(27):4563–9. https://doi.org/10.1200/jco.2008.21.7356.

113. Mesa RA, Pardanani AD, Hussein K, Wu W, Schwager S, Litzow MR, et al. Phase1/-2 study of Pomalidomide in myelofibrosis. Am J Hematol. 2010;85(2):129–30. https://doi.org/10.1002/ajh.21598.
114. Daver N, Shastri A, Kadia T, Newberry K, Pemmaraju N, Jabbour E, et al. Phase II study of pomalidomide in combination with prednisone in patients with myelofibrosis and significant anemia. Leuk Res. 2014;38(9):1126–9. https://doi.org/10.1016/j.leukres.2014.06.015.
115. Begna KH, Pardanani A, Mesa R, Litzow MR, Hogan WJ, Hanson CA, et al. Long-term outcome of pomalidomide therapy in myelofibrosis. Am J Hematol. 2012;87(1):66–8. https://doi.org/10.1002/ajh.22233.
116. Masarova LDN, Kadia T, Pemmaraju N, et al. Efficacy and safety of pomalidomide in combination with prednisone in patients with myelofibrosis and anemia — final results of a prospective phase 2 study. Blood. 2018;132:1764.
117. Tefferi A, Al-Ali HK, Barosi G, Devos T, Gisslinger H, Jiang Q, et al. A randomized study of pomalidomide vs placebo in persons with myeloproliferative neoplasm-associated myelofibrosis and RBC-transfusion dependence. Leukemia. 2016. https://doi.org/10.1038/leu.2016.300.
118.•• Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med. 2012;366(9):799–807. https://doi.org/10.1056/NEJMoa1110557 **COMFORT studies of ruxolitinib in patients with MF.**
119.•• Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med. 2012;366(9):787–98. https://doi.org/10.1056/NEJMoa1110556 **COMFORT studies of ruxolitinib in patients with MF.**
120. Verstovsek S, Mesa RA, Gotlib J, Gupta V, DiPersio JF, Catalano JV, et al. Long-term treatment with ruxolitinib for patients with myelofibrosis: 5-year update from the randomized, double-blind, placebo-controlled, phase 3 COMFORT-I trial. J Hematol Oncol. 2017;10(1):55. https://doi.org/10.1186/s13045-017-0417-z.
121. Passamonti FKJ, Vannucchi AM, Reiter A, et al. ReTHINK: a randomized, double-blind, placebo-controlled, multicenter, phase 3 study of ruxolitinib in early myelofibrosis patients. J Clin Oncol. 2016;34:TPS7080.
122. Al-Ali HK, Griesshammer M, le Coutre P, Waller CF, Liberati AM, Schafhausen P, et al. Safety and efficacy of ruxolitinib in an open-label, multicenter, single-arm phase 3b expanded-access study in patients with myelofibrosis: a snapshot of 1144 patients in the JUMP trial. Haematologica. 2016;101(9):1065–73. https://doi.org/10.3324/haematol.2016.143677.
123. Harrison CN, Vannucchi AM, Kiladjian JJ, Al-Ali HK, Gisslinger H, Knoops L, et al. Long-term findings from COMFORT-II, a phase 3 study of ruxolitinib vs best available therapy for myelofibrosis. Leukemia. 2016;30(8):1701–7. https://doi.org/10.1038/leu.2016.148.
124.•• Vannucchi AM, Kiladjian JJ, Griesshammer M, Masszi T, Durrant S, Passamonti F, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. N Engl J Med. 2015;372(5):426–35. https://doi.org/10.1056/NEJMoa1409002 **RESPONSE studies of ruxolitinib in patients with PV.**
125.•• Passamonti F, Griesshammer M, Palandri F, Egyed M, Benevolo G, Devos T, et al. Ruxolitinib for the treatment of inadequately controlled polycythaemia vera without splenomegaly (RESPONSE-2): a randomised, open-label, phase 3b study. Lancet Oncol. 2017;18(1):88–99. https://doi.org/10.1016/s1470-2045(16)30558-7 **RESPONSE studies of ruxolitinib in patients with PV.**
126. Kiladjian JJPZP, Hino M, Pane F, et al. Long-term efficacy and safety (5 years) in RESPONSE, a phase 3 study comparing ruxolitinib (rux) with best available therapy (BAT) in hydroxyurea (HU)-resistant/intolerant patients (pts) with polycythemia vera (PV). Blood. 2018;132:1753.
127. Passamonti FPF, Saydam G, Benevolo G, et al. Ruxolitinib for the treatment of inadequately controlled polycythemia vera without splenomegaly: 156-week follow-up from the phase 3 response-2 study. Blood. 2018;132:1754.
128. Foltz LPGM, Zerazhi H, Droogenbroeck JV, et al. Updated results from an open-label, multicenter, expanded treatment protocol (ETP) phase (Ph) 3b study of Ruxolitinib (RUX) in patients (pts) with polycythemia vera (PV) who were hydroxyurea (HU) resistant or intolerant and for whom no alternative treatment (Tx) was available. Blood. 2018;132:1774.
129. Mesa R, Vannucchi AM, Yacoub A, Zachee P, Garg M, Lyons R, et al. The efficacy and safety of continued hydroxycarbamide therapy versus switching to ruxolitinib in patients with polycythaemia vera: a randomized, double-blind, double-dummy, symptom study (RELIEF). Br J Haematol. 2017;176(1):76–85. https://doi.org/10.1111/bjh.14382.
130. Verstovsek S, Passamonti F, Rambaldi A, Barosi G, Rumi E, Gattoni E, et al. Ruxolitinib for essential thrombocythemia refractory to or intolerant of hydroxyurea: long-term phase 2 study results. Blood. 2017;130(15):1768–71. https://doi.org/10.1182/blood-2017-02-765032.
131. Harrison CN, Mead AJ, Panchal A, Fox S, Yap C, Gbandi E, et al. Ruxolitinib vs best available therapy for ET intolerant or resistant to hydroxycarbamide. Blood. 2017;130(17):1889–97. https://doi.org/10.1182/blood-2017-05-785790.
132. Singer JW, Al-Fayoumi S, Ma H, Komrokji RS, Mesa R, Verstovsek S. Comprehensive kinase profile of pacritinib, a nonmyelosuppressive Janus kinase 2 inhibitor. J Exp Pharmacol. 2016;8:11–9. https://doi.org/10.2147/jep.s110702.
133. Mesa RA, Vannucchi AM, Mead A, Egyed M, Szoke A, Suvorov A, et al. Pacritinib versus best available therapy for the treatment of myelofibrosis irrespective of baseline cytopenias (PERSIST-1): an international, randomised, phase 3 trial. Lancet Haematol. 2017;4(5):e225–e36. https://doi.org/10.1016/s2352-3026(17)30027-3.
134. Mascarenhas J, Hoffman R, Talpaz M, Gerds AT, Stein B, Gupta V, et al. Pacritinib vs best available therapy, including ruxolitinib, in patients with myelofibrosis: a randomized clinical trial. JAMA Oncol. 2018;4(5):652–9. https://doi.org/10.1001/jamaoncol.2017.5818.
135. Asshoff M, Petzer V, Warr MR, Haschka D, Tymoszuk P, Demetz E, et al. Momelotinib inhibits ACVR1/ALK2, decreases hepcidin production, and ameliorates anemia of chronic disease in rodents. Blood. 2017;129(13):1823–30. https://doi.org/10.1182/blood-2016-09-740092.
136. Mesa RA, Kiladjian JJ, Catalano JV, Devos T, Egyed M, Hellmann A, et al. SIMPLIFY-1: a phase III randomized trial of momelotinib versus ruxolitinib in Janus kinase inhibitor-naive patients with myelofibrosis. J Clin Oncol Off J Am Soc Clin Oncol. 2017;35(34):3844–50. https://doi.org/10.1200/jco.2017.73.4418.
137. Harrison CN, Vannucchi AM, Platzbecker U, Cervantes F, Gupta V, Lavie D, et al. Momelotinib versus best available therapy in patients with myelofibrosis previously treated with ruxolitinib (SIMPLIFY 2): a randomised, open-label, phase 3 trial. Lancet Haematol. 2018;5(2):e73–81. https://doi.org/10.1016/s2352-3026(17)30237-5.
138. Pardanani A, Harrison C, Cortes JE, Cervantes F, Mesa RA, Milligan D, et al. Safety and efficacy of fedratinib in patients with

primary or secondary myelofibrosis: a randomized clinical trial. JAMA Oncol. 2015;1(5):643–51. https://doi.org/10.1001/jamaoncol.2015.1590.

139. Harrison CN, Schaap N, Vannucchi AM, Kiladjian JJ, Tiu RV, Zachee P, et al. Janus kinase-2 inhibitor fedratinib in patients with myelofibrosis previously treated with ruxolitinib (JAKARTA-2): a single-arm, open-label, non-randomised, phase 2, multicentre study. Lancet Haematol. 2017;4(7):e317–e24. https://doi.org/10.1016/s2352-3026(17)30088-1.
140. Zhang Q, Zhang Y, Diamond S, Boer J, Harris JJ, Li Y, et al. The Janus kinase 2 inhibitor fedratinib inhibits thiamine uptake: a putative mechanism for the onset of Wernicke's encephalopathy. Drug Metab Dispos. 2014;42(10):1656–62. https://doi.org/10.1124/dmd.114.058883.
141. Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Franco J, Thomas DA, et al. Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis. N Engl J Med. 2010;363(12):1117–27. https://doi.org/10.1056/NEJMoa1002028.
142. Harrison CN, Mesa RA, Kiladjian JJ, Al-Ali HK, Gisslinger H, Knoops L, et al. Health-related quality of life and symptoms in patients with myelofibrosis treated with ruxolitinib versus best available therapy. Br J Haematol. 2013;162(2):229–39. https://doi.org/10.1111/bjh.12375.
143. Mesa RA, Gotlib J, Gupta V, Catalano JV, Deininger MW, Shields AL, et al. Effect of ruxolitinib therapy on myelofibrosis-related symptoms and other patient-reported outcomes in COMFORT-I: a randomized, double-blind, placebo-controlled trial. J Clin Oncol Off J Am Soc Clin Oncol. 2013;31(10):1285–92. https://doi.org/10.1200/jco.2012.44.4489.
144. Mesa RA, Shields A, Hare T, Erickson-Viitanen S, Sun W, Sarlis NJ, et al. Progressive burden of myelofibrosis in untreated patients: assessment of patient-reported outcomes in patients randomized to placebo in the COMFORT-I study. Leuk Res. 2013;37(8):911–6. https://doi.org/10.1016/j.leukres.2013.04.017.
145. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. The clinical benefit of ruxolitinib across patient subgroups: analysis of a placebo-controlled, phase III study in patients with myelofibrosis. Br J Haematol. 2013;161(4):508–16. https://doi.org/10.1111/bjh.12274.
146. Mesa RA, Scherber RM, Geyer HL. Reducing symptom burden in patients with myeloproliferative neoplasms in the era of Janus kinase inhibitors. Leuk Lymphoma. 2015;56(7):1989–99. https://doi.org/10.3109/10428194.2014.983098.
147. Mesa RAVS, Gupta V, et al. Improvement in weight and total cholesterol and their association with survival in ruxolitinib-treated patients with myelofibrosis from COMFORT-I. Blood. 2012;120(21):1733.
148. Mascarenhas J, Hoffman R. A comprehensive review and analysis of the effect of ruxolitinib therapy on the survival of patients with myelofibrosis. Blood. 2013;121(24):4832–7. https://doi.org/10.1182/blood-2013-02-482232.
149. Kvasnicka HM, Thiele J, Bueso-Ramos CE, Sun W, Cortes J, Kantarjian HM, et al. Long-term effects of ruxolitinib versus best available therapy on bone marrow fibrosis in patients with myelofibrosis. J Hematol Oncol. 2018;11(1):42. https://doi.org/10.1186/s13045-018-0585-5.
150. Vannucchi AM, Verstovsek S, Guglielmelli P, Griesshammer M, Burn TC, Naim A, et al. Ruxolitinib reduces JAK2 p.V617F allele burden in patients with polycythemia vera enrolled in the RESPONSE study. Ann Hematol. 2017;96(7):1113–20. https://doi.org/10.1007/s00277-017-2994-x.
151. Deininger M, Radich J, Burn TC, Huber R, Paranagama D, Verstovsek S. The effect of long-term ruxolitinib treatment on JAK2p.V617F allele burden in patients with myelofibrosis. Blood. 2015;126(13):1551–4. https://doi.org/10.1182/blood-2015-03-635235.
152. Verstovsek SGV, Gotlib JR, et al. A pooled overall survival (OS) analysis of 5-year data from the COMFORT-I and COMFORT-II trials of ruxolitinib for the treatment of myelofibrosis (MF). Blood. 2016;128(22):3110–311.
153. Heine A, Held SA, Daecke SN, Wallner S, Yajnanarayana SP, Kurts C, et al. The JAK-inhibitor ruxolitinib impairs dendritic cell function in vitro and in vivo. Blood. 2013;122(7):1192–202. https://doi.org/10.1182/blood-2013-03-484642.
154. Rudolph J, Heine A, Quast T, Kolanus W, Trebicka J, Brossart P, et al. The JAK inhibitor ruxolitinib impairs dendritic cell migration via off-target inhibition of ROCK. Leukemia. 2016;30(10):2119–23. https://doi.org/10.1038/leu.2016.155.
155. Massa M, Rosti V, Campanelli R, Fois G, Barosi G. Rapid and long-lasting decrease of T-regulatory cells in patients with myelofibrosis treated with ruxolitinib. Leukemia. 2014;28(2):449–51. https://doi.org/10.1038/leu.2013.296.
156. Keohane C, Kordasti S, Seidl T, Perez Abellan P, Thomas NS, Harrison CN, et al. JAK inhibition induces silencing of T helper cytokine secretion and a profound reduction in T regulatory cells. Br J Haematol. 2015;171(1):60–73. https://doi.org/10.1111/bjh.13519.
157. Parampalli Yajnanarayana S, Stubig T, Cornez I, Alchalby H, Schonberg K, Rudolph J, et al. JAK1/2 inhibition impairs T cell function in vitro and in patients with myeloproliferative neoplasms. Br J Haematol. 2015;169(6):824–33. https://doi.org/10.1111/bjh.13373.
158. Schonberg K, Rudolph J, Vonnahme M, Parampalli Yajnanarayana S, Cornez I, Hejazi M, et al. JAK inhibition impairs NK cell function in myeloproliferative neoplasms. Cancer Res. 2015;75(11):2187–99. https://doi.org/10.1158/0008-5472.can-14-3198.
159. Colomba C, Rubino R, Siracusa L, Lalicata F, Trizzino M, Titone L, et al. Disseminated tuberculosis in a patient treated with a JAK2 selective inhibitor: a case report. BMC Res Notes. 2012;5:552. https://doi.org/10.1186/1756-0500-5-552.
160. Wysham NG, Sullivan DR, Allada G. An opportunistic infection associated with ruxolitinib, a novel janus kinase 1,2 inhibitor. Chest. 2013;143(5):1478–9. https://doi.org/10.1378/chest.12-1604.
161. Caocci G, Murgia F, Podda L, Solinas A, Atzeni S, La Nasa G. Reactivation of hepatitis B virus infection following ruxolitinib treatment in a patient with myelofibrosis. Leukemia. 2014;28(1):225–7. https://doi.org/10.1038/leu.2013.235.
162. Goldberg RA, Reichel E, Oshry LJ. Bilateral toxoplasmosis retinitis associated with ruxolitinib. N Engl J Med. 2013;369(7):681–3. https://doi.org/10.1056/NEJMc1302895.
163. Wathes R, Moule S, Milojkovic D. Progressive multifocal leukoencephalopathy associated with ruxolitinib. N Engl J Med. 2013;369(2):197–8. https://doi.org/10.1056/NEJMc1302135.
164. Weinacht KG, Charbonnier LM, Alroqi F, Plant A, Qiao Q, Wu H, et al. Ruxolitinib reverses dysregulated T helper cell responses and controls autoimmunity caused by a novel signal transducer and activator of transcription 1 (STAT1) gain-of-function mutation. J Allergy Clin Immunol. 2017;139(5):1629–40.e2. https://doi.org/10.1016/j.jaci.2016.11.022.
165. Porpaczy ETS, Hoelbl-Kovacic A, et al. Aggressive B-cell lymphomas in patients with myelofibrosis receiving JAK1/2 inhibitor therapy. Blood. 2018;132(7):694–706. Blood. 2019;133(7):768. https://doi.org/10.1182/blood-2019-01-895136.
166. Pemmaraju N, Kantarjian H, Nastoupil L, Dupuis M, Zhou L, Pierce S, et al. Characteristics of patients with myeloproliferative neoplasms with lymphoma, with or without JAK inhibitor therapy. Blood. 2019. https://doi.org/10.1182/blood-2019-01-897637.

167. Bjorn ME, de Stricker K, Kjaer L, Ellemann K, Hasselbalch HC. Combination therapy with interferon and JAK1-2 inhibitor is feasible: proof of concept with rapid reduction in JAK2V617F-allele burden in polycythemia vera. Leuk Res Rep. 2014;3(2):73–5. https://doi.org/10.1016/j.lrr.2014.05.003.

168.•• Koschmieder S, Mughal TI, Hasselbalch HC, Barosi G, Valent P, Kiladjian JJ, et al. Myeloproliferative neoplasms and inflammation: whether to target the malignant clone or the inflammatory process or both. Leukemia. 2016;30(5):1018–24. https://doi.org/10.1038/leu.2016.12 **Excellent review of immunological dysregulation in MPNs.**

169. Kiladjian JJ, Soret-Dulphy J, Resche-Rigon M, et al. Ruxopeg, a multi-center Bayesian phase 1/2 adaptive randomized trial of the combination of ruxolitinib and pegylated interferon alpha 2a in patients with myeloproliferative neoplasm (MPN)-associated myelofibrosis. Blood. 2018;132:581.

170. Daver N, Cortes J, Newberry K, Jabbour E, Zhou L, Wang X, et al. Ruxolitinib in combination with lenalidomide as therapy for patients with myelofibrosis. Haematologica. 2015;100(8):1058–63. https://doi.org/10.3324/haematol.2015.126821.

171. Rampal R VS, Devlin S, Stein E, et al. Early results of he phase II study of combined ruxolitinib and thalidomide in patients with myelofibrosis. EHA Learning Center PS1467. 2019.

172. Masarova L, Verstovsek S, Kantarjian H, Daver N. Immunotherapy based approaches in myelofibrosis. Expert Rev Hematol. 2017;10(10):903–14. https://doi.org/10.1080/17474086.2017.1366853.

173. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. https://doi.org/10.1016/j.cell.2011.02.013.

174. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252–64. https://doi.org/10.1038/nrc3239.

175. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med. 2002;8(8):793–800. https://doi.org/10.1038/nm730.

176. Mumprecht S, Schurch C, Schwaller J, Solenthaler M, Ochsenbein AF. Programmed death 1 signaling on chronic myeloid leukemia-specific T cells results in T-cell exhaustion and disease progression. Blood. 2009;114(8):1528–36. https://doi.org/10.1182/blood-2008-09-179697.

177. Craig RTS, Deininger M, Salama ME. Programmed death ligand (PD-L1) expression is increased in spleens of myelofibrosis patients United States and Canadian Academy of 2016 Annual meeting, Abstract 1353.

178. Lasho TFC, Kimlinger TK, Zblewski D, Chen D, Patnaik MM, Hanson CA, et al. Expression of CD123 (IL-3R-alpha), a therapeutic target of SL-401, on myeloproliferative neoplasms. Blood. 2014;124:5577.

179. Pemmaraju NAH, Gupta V, Schiller GJ, et al. Results from ongoing phase 1/2 clinical trial of tagraxofusp (SL-401) in patients with intermediate or high risk relapsed/refractory myelofibrosis. J Clin Oncol. 2019;37(15_suppl):7058.

180. Schischlik FJR, Rosebrock F, et al. Mutational landscape of the transcriptome offers a rich neoantigen resource for immunotherapy of myeloproliferative neoplasms. Blood. 2018;132:3058.