Recombinant Interferon Alfa in BCR/ABL-Negative Chronic Myeloproliferative Neoplasms

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Abstract: The treatment landscape for BCR/ABL-negative myeloproliferative neoplasms (MPNs), driven by *JAK2, CALR,* and *MPL* mutations, has evolved significantly over the last decade. Recent regulatory approvals in polycythemia vera (PV) include the JAK inhibitor ruxolitinib, and more recently, a novel recombinant interferon alfa-2 (IFN-α) therapeutic agent. Many clinical trials have documented the safety and efficacy of IFN-α therapy in PV and essential thrombocythemia, the classical BCR/ABL-negative MPNs. Used off-label for more than 30 years as a cytoreductive agent, IFN-α therapy promotes significant clinical, hematologic, and molecular responses. In some IFN-α–treated patients, partial or complete reduction of the mutant *JAK2* allele burden may lead to a durable measurable residual disease state, owing to the ability of long-term IFN-α therapy to selectively deplete mutant *JAK2*–harboring hematopoietic stem cells. Pegylated IFN-α forms were developed to improve the drug stability and tolerability of first-generation IFN-α therapeutics. More recently, a novel pegylated IFN-α, ropeginterferon alfa-2b, received approval for PV by the European Medicines Agency and the US Food and Drug Administration in 2019 and 2021, respectively. This article reviews the clinical research and recent advances that led to the first regulatory approval of IFN-α in a BCR/ABL-negative MPN and its future promise as a disease-modifying therapeutic agent.

Keywords
Essential thrombocythemia, *JAK2* V617F mutation, myelofibrosis, myeloproliferative neoplasm, polycythemia vera, recombinant interferon

Introduction

Chronic myeloproliferative neoplasms (MPNs) are heterogeneous, clonal disorders of hematopoietic stem cells (HSCs) that lead to the uncontrolled proliferation and expansion of hematopoietic progenitors in the bone marrow, abnormal peripheral blood counts, thrombohemorrhagic complications, microvascular disturbances, and potential transformation to acute leukemia. The classical Philadelphia chromosome–negative (or BCR/ABL-negative) MPNs include polycythemia vera (PV), essential thrombocythemia (ET), and primary

myelofibrosis (PMF). The discovery in 2005 of the *JAK2* V617F driver mutation, followed by the discovery of additional driver mutations in *MPL* and *CALR,* represented a major breakthrough.[1] These driver mutations are ubiquitously encountered in BCR/ABL-negative chronic MPNs, leading to direct or indirect abnormal activation of the JAK/STAT signaling pathway, a central theme of MPN pathogenesis.[2]

The clinical manifestations and course of PV and ET range widely from asymptomatic alterations in peripheral blood cell counts to the emergence of microvascular or constitutional symptoms, pruritus, symptoms related to increased red blood cell (RBC) mass in PV, splenomegaly, cardiovascular and thrombohemorrhagic complications, and hematologic transformation to myelofibrosis with myeloid metaplasia and acute myeloid leukemia (AML) or blast phase. Although several studies reported a median overall survival for ET and PV patients in excess of 20 and 10 years, respectively, overall life expectancy was inferior compared with the general population.[3-6] Several other studies have shed light on the negative impact on the quality of life of patients living with MPNs.[7,8] Cardiovascular thrombohemorrhagic complications and second malignancies, such as acute leukemia and solid tumors, constitute major causes of death in PV and ET.[9,10]

Given the long clinical trajectory of PV and ET over decades, the choice of and indication for therapeutic interventions should take into account both short-term and long-term goals, including alleviation of disease-related symptoms such as headaches or microvascular manifestations, control of increased RBC mass in PV via therapeutic phlebotomy, recognition and management of progressive splenomegaly, a risk-stratified approach to embarking on antiplatelet and/or cytoreductive therapy in carefully selected patients, and prevention of cardiovascular thrombotic or hemorrhagic complications while minimizing the potential adverse effects of specific therapies on quality of life, second malignancy, and risk of hematologic transformation to acute leukemia.[11-14] Consensus expert panel guidelines are available and periodically updated to guide risk-stratified indications for embarking on cytoreductive therapy in patients with ET and PV.[15-17]

In the 1980s, recombinant forms of interferon alfa-2 (IFN-α) were first used off-label in ET and PV. These agents represent a nonchemotherapy alternative to the ribonucleotide reductase inhibitor hydroxyurea (HU), the most commonly used oral cytoreductive chemotherapy agent. Early studies revealed that IFN-α therapy could normalize blood counts, improve disease-related symptom burden, control splenomegaly, and promote long-term remissions. IFN-α therapy has become an attractive option, particularly in younger patients, as a nonleukemogenic cytoreductive agent. However, adverse events (AEs) associated with frequent administration of first-generation IFN-α forms limited the tolerability of these agents owing to common flu-like symptoms, fatigue, and injection site reactions. Historically, in addition to tolerability concerns, other challenges to more widespread clinical use of IFN-α in PV and ET included the fact that clinicians were less familiar with IFN-α agents than with HU, the requirement of parenteral administration, the high cost of the agent, the need for authorization for off-label use, and the sparse availability of clinical trial data. Fortunately, randomized, controlled trials have recently been completed and there has been further publication regarding the cumulative experience with IFN-α.

The introduction of first-generation pegylated forms of IFN-α, PEG-IFN, improved the stability and tolerability of IFN-α, allowing less frequent dose administration. In addition to clinical and hematologic responses, IFN-α therapy was reported to reduce the mutant *JAK2* V617F allele burden, with long-term molecular responses in some patients with PV and ET.[18-23] Following the completion of the phase 1/2 PEGINVERA[24] and phase 3 PROUD-PV/CONTINUATION-PV trials[25,26] reporting long-term safety, efficacy, and superiority to HU, the novel next-generation agent ropeginterferon alfa-2b, also known as ROPEG (Besremi, PharmaEssentia), gained European Medicines Agency approval in 2019. This was followed by US Food and Drug Administration (FDA) approval of ROPEG in 2021 for use in PV. ROPEG therapy was reported to induce a sustained reduction of the mutant *JAK2* V617F allele burden to less than 10% at 5 years in more than half (54.3%) of patients with PV, who may be potential candidates for treatment discontinuation.[26] This article reviews the large body of clinical research involving IFN-α in BCR/ABL-negative chronic MPNs, leading ultimately to the development and regulatory approval of the first recombinant IFN-α agent in PV.

Mechanisms of Action of IFN-α in MPNs

Interferons are part of a large family of cytokines with diverse antiviral, immunomodulatory, and antiproliferative effects. The long-recognized antitumor activity of IFN-α and its efficacy in hairy cell leukemia and chronic myeloid leukemia (CML) provided a rationale for its therapeutic development in BCR/ABL-negative MPNs. IFN-α was shown to be a potent suppressor of myeloid colony formation by markedly reducing the colony-forming ability of erythroid, granulocytic, and megakaryocytic progenitors in PV and PMF.[27-31] Other studies focusing on IFN-α stimulatory effect on the immune system demonstrated enhanced surveillance and targeting of the mutant clone in CML by natural killer cells, macrophages, and T cells.[32,33] In HSCs, IFN-α was shown to impair HSC

Table 1. Summary of Clinical Trials of ROPEG in PV

Clinical Trial (Arms)	PV Population (n)	Trial Design	ROPEG Dose	Median Follow-up	Hematologic Response	Molecular Response	Discontinuation Rate and AEs	Thromboembolic Events
Phase 1/2 PEGINVERA trial[24,62] (ROPEG)	Newly diagnosed and previously treated, high- or low-risk (51)	Multicenter, prospective, open-label, 1-arm	50-540 µg q2wk (mean, 263 µg)	5.1 y	ORR 90%, CHR 47%, PHR 43%	ORR 73.8%, CMR 28.6%, PMR 45.2%	20%; fatigue, arthralgia, headache, influenza-like illness	1 TIA, 1 DVT (first treatment cycle)
Phase 2 Low-PV trial[68,69] (ROPEG + PHL vs PHL)	Low-risk PV (127)	Multicenter, open-label, 2-arm	100 µg q2wk	12 mo, 24 mo	ORR at 12 mo: ROPEG + PHL 84%, PHL 60%; ORR at 24 mo: ROPEG + PHL 83%, PHL 59%	ORR: ROPEG 22%, PHL 0%	ROPEG 6%	ROPEG: no events; PHL: 1 splenic vein thrombosis
Phase 2 trial[79] (ROPEG)	First- or second-line therapy (29)	Open-label, 1-arm	100-500 µg q2wk	52 wk	CHR 51.7%	80%	1 patient, silent thyroiditis	
Phase 3 PROUD-PV trial[25] (ROPEG vs HU)	<3 y of cytoreduction (257)	Randomized, controlled, open-label, 2-arm	ROPEG 100 µg q2wk, median 426 µg; HU 500 mg/d, median 1000 mg	12 mo	ROPEG 43%, HU 46% (CHR without spleen criterion)	ROPEG 34%, HU 42%	ROPEG 8%, HU 4%; low platelets, hypothyroidism, anxiety, rheumatoid arthritis	
Phase 3 CONTINUATION-PV trial[25,26] (ROPEG vs HU)	Rollover from PROUD-PV trial (171)	Randomized, controlled, open-label, 2-arm	ROPEG 100 µg q2wk, median 425 µg; HU 500 mg/d; median 1000 mg	60 mo	ROPEG CHR 72.6% (5 y); HU CHR 52.6% (5 y)	ROPEG: 44% (1 y), 68% (3 y), 69.1% (5 y); HU: 51% (1 y), 33% (3 y), 21.6% (5 y)	ROPEG 10.2%, HU 3.1%	ROPEG 3.1%, HU 3.9%

AEs, adverse events; CHR, complete hematologic response; CMR, compete molecular response; CR, complete response; DVT, deep venous thrombosis; HU, hydroxyurea; mo, months; NR, not reported; ORR, overall response rate; PHL, phlebotomy; PHR, partial hematologic response; PMR, partial molecular response; PV, polycythemia vera; ROPEG, ropeginterferon alfa-2b; TIA, transient ischemic attack; wk, weeks; y, years.

function associated with long-term IFN-α exposure.[34,35] Acute IFN-α treatment was reported to induce cell cycle entry of dormant HSCs, which in turn increased susceptibility to elimination by an antiproliferative chemotherapy agent, whereas long-term IFN-α pathway activation markedly impaired HSC function.[34] Subsequent studies revealed that IFN-α–exposed HSCs were susceptible to apoptosis upon reentry into the cell cycle.[36]

Several studies demonstrated the selective effects of IFN-α on *JAK2*-mutant HSCs and progenitor cells.[37-40] In a murine model of *JAK2* V617F–positive MPN, IFN-α induced cell cycle activation of dormant *JAK2* V617F–mutant HSCs, which may be associated with long-term depletion of the mutated clone.[37] In a study conducted in human CD34+ cells and hematopoietic progenitors, IFN-α was shown to exert more selective and direct suppression of *JAK2* V617F–mutant hematopoietic cells, acting through the p38 mitogen activated protein

kinase (MAPK) pathway.[41] In a mouse model of *JAK2* V617F–expressing MPN vs wild-type controls, increased frequency of a subpopulation of HSCs expressing elevated CD41 (CD41hi) was observed, exhibiting bias for differentiation toward megakaryocytes.[40] Bone marrow from patients with MPN also exhibited elevated CD41hi HSCs that correlated with *JAK2* V617F allele burden. IFN-α treatment further increased the frequency and percentage of CD41hi HSCs and reduced the number of *JAK2* V617F–positive HSCs in mice and patients with MPN. This IFN-α–mediated shift toward CD41hi HSCs, which display less self-renewing capability, provided a possible mechanism by which IFN-α may preferentially target and deplete the *JAK2* V617F–mutant, MPN-sustaining clone.

The *JAK2* V617F mutation has been shown to generate reactive oxygen species (ROS) that may contribute to thrombosis and pathogenesis of MPNs.[42] Gene expression profiling in blood samples from patients with MPNs revealed that IFN-α promotes an expression signature that corresponds to decreased oxidative stress and enhancement of antioxidative defense genes, suggesting that IFN-α may favorably impact disease progression by decreasing genomic instability.[43] Increasing *JAK2* V617 allele burden may be associated with accumulation of genomic instability and disease transformation.[44] In a mouse model of *JAK2* V167F–mutant MPN, chronic IFN-α treatment induced ROS production and DNA damage preferentially in *JAK2* V617F–positive HSCs.[45] In another study utilizing MPN patient cells and a mouse model of MPN, arsenic trioxide (ATO) treatment potentiated IFN-α–induced growth suppression of *JAK2* V617F–mutant patient and hematopoietic progenitors. Combination treatment with IFN-α and ATO enhanced and accelerated responses leading to the eradication of MPN in most mice by targeting the MPN-sustaining clone.[46] In a recent study, IFN-α–mediated activation of p38 MAPK was shown to require PKC-δ–dependent phosphorylation of ULK1.[47] Higher messenger RNA levels of ULK1 in blood and bone marrow cells of MPN patients before PEG-IFN therapy correlated with better PEG-IFN response. IFN-α treatment activated ULK1-interacting ROCK1/2 proteins to trigger a negative feedback loop that suppressed IFN responses. ROCK1/2 was found to be overexpressed in the blood cells of MPN patients and its inhibition enhanced antineoplastic IFN-α responses in vitro and in vivo, suggesting a clinical potential for combining IFN-α therapy with pharmacologic inhibition of ROCK1/2.[47]

Distinct molecular responses to IFN-α therapy have been suggested in *JAK2* V617F–positive patients as compared with *CALR*-mutated patients with MPN.[48,49] Earlier studies demonstrated the ability of IFN-α to induce molecular responses in some patients with *CALR*-mutated MPNs while raising the question of whether higher doses were required for induction of molecular response in *CALR*-mutated as compared with *JAK2* V617F–positive patients.[50,51] A mechanism for this differential response was suggested by the demonstration of constitutive activation of JAK2/STAT1 in *JAK2* V617F–harboring cells but not in *CALR*-mutated cells. It was proposed that crosstalk between *JAK2* V617F could lead to JAK2/STAT1 priming toward increased IFN-α response as a mechanism for differential sensitivity.[48] Consistent with these findings, a subsequent study showed that *JAK2* V617F–mutated progenitor cells were more sensitive than *CALR*-mutated progenitors to IFN-α.[39] In another study, genomic profiling on patient samples assessed the molecular response to IFN-α therapy at 24 months. This study revealed a significant reduction in *JAK2* mutant allele frequency yet no significant reduction in *CALR* mutant allele frequency, despite the achievement of complete clinical and hematologic responses.[52] This study also revealed treatment-emergent mutations in *DNMT3A*, which were more prevalent in IFN-α–treated patients not achieving a complete response.

Early Clinical Trials of IFN-α in ET and PV

In the 1980s, pioneering studies by Linkesch[53] and Silver[54] reported on the efficacy of IFN-α in controlling myeloproliferation in MPNs with severe thrombocytosis and in PV, respectively. As reviewed previously,[55] a series of early studies in PV and ET followed, utilizing recombinant IFN-α-2a (Roferon-A, Roche) and IFN-α-2b (Intron-A, no longer available) commercial forms (at the time approved for use in hairy cell leukemia and CML) injected subcutaneously several times a week, leading to objective response rates of 80% with improvement of blood counts, decreased phlebotomy requirements in PV, and improved MPN-related symptoms. The AE profile raised concerns regarding tolerability and included flu-like symptoms, mood changes, fatigue, injection site reactions, and cytopenias; less common AEs included liver function abnormalities, hair loss, rare autoimmune abnormalities such as hypothyroidism and hemolytic anemia, cardiac effects, and neurologic effects. In early studies, average discontinuation rates of 25% to 30% were observed in PV and ET. Even poorer tolerability was reported in initial studies of patients with PMF, with high discontinuation associated with worsening cytopenias.[56]

Clinical Trials of First-Generation PEG-IFN in PV and ET

First-generation pegylated forms of IFN-α, including IFN-α-2b (PegIntron, Merck) and IFN-α-2a (Pegasys, pharma&), were developed and FDA approved in 2001 and in 2002, respectively, for the treatment of chronic

hepatitis C. These agents exhibited increased half-life and drug stability, which allowed less frequent subcutaneous administration (once a week) and improved tolerability. As reviewed previously,[56,57] clinical trials of off-label PEG-IFN therapy in PV and ET patients reported high hematologic remission rates, freedom from phlebotomy, and significant molecular response rate, with a decline in the *JAK2* V617F variant allele frequency. PEG-IFN was also shown to be effective in PV and ET patients refractory to or intolerant of HU in the phase 2 Myeloproliferative Diseases Research Consortium (MPD-RC) 111 trial, involving 65 patients with ET and 50 patients with PV, with few vascular events.[58]

The MPD-RC-112 phase 3 trial prospectively randomized 168 high-risk ET or PV patients to first-line treatment with either PEG-IFN or HU, with a median treatment duration of 81 weeks.[59] The primary endpoint was the complete response (CR) rate at 12 months, as defined by European LeukemiaNet (ELN) criteria.[60] The planned accrual of 300 patients could not be achieved after the decision by the manufacturer of PEG-IFN to halt the drug supply. The CR rate at 12, 24, and 36 months was 37%, 20%, and 17%, respectively, for HU and 35%, 29%, and 33%, respectively, for PEG-IFN. PEG-IFN therapy required a longer time to produce a full response but was as effective as HU in achieving a CR at 1 year (P=.80). In patients with PV, hematocrit control (without phlebotomy) was achieved in 65% of those randomized to PEG-IFN vs 43% of those on HU (P=.04).

The MPD-RC-112 trial assessed molecular and bone marrow histopathologic responses. The median change in the *JAK2* V617F allele burden was approximately 5.3% in the patients treated with HU and approximately 10.7% in the PEG-IFN arm. The median *JAK2* V617F allele burden continued to decrease through month 24 with PEG-IFN, but increased with HU after month 12. The presence of concomitant, nondriver *ASXL1* mutations was associated with decreased odds of achieving hematologic CR on multivariable analysis (P=.055), consistent with prior reports of poorer clinical responses associated with additional mutations in *ASXL1* and *TET2*.[23,50,61] Bone marrow histopathologic responses were significantly more frequent with HU (33% at best response) compared with PEG-IFN (17% at best response, P=.05), contrasting with the molecular response findings that revealed better molecular response rate with PEG-IFN. The reason for the incongruence of histopathologic and molecular responses in this trial was not clear. One limitation of this study was the absence of a central pathology review committee. Achieving histopathologic bone marrow response was dose-dependent with HU but not with PEG-IFN (P=.04), suggesting a predominant myelosuppressive effect rather than a disease-modifying property of HU.

Clinical Trials of ROPEG

ROPEG, a next-generation monopegylated IFN-α-2b, was developed to further improve the tolerability of IFN-α and safety-related patient adherence. The extended half-life allows for subcutaneous administration every 2 weeks, with a 4-week maintenance schedule. The phase 1/2, open-label, prospective PEGINVERA clinical trial[24] enrolled patients with PV for ROPEG therapy, with long-term follow-up results published in abstract form (Table 1).[62] A hematologic response was achieved in 90% of patients.[62] A median of 10 weeks of therapy was required to achieve any hematologic response. Common adverse reactions were mild, including arthralgias, influenza-like illness, and fatigue. Importantly, a complete molecular response (CMR) was achieved in 28.6% of patients at a median of 84 weeks. A median of 34 weeks was required to achieve any molecular response. Molecular responses tended to increase over time, irrespective of whether the dosing regimen was every 2 or 4 weeks.[62]

The PROUD-PV trial and its extension study, CONTINUATION-PV, enrolled patients with PV with no history of prior cytoreductive treatment or less than 3 years of previous HU treatment (Table 1).[25] After 12 months, 171 of the 217 patients who completed the PROUD-PV study rolled over to the extension part of the trial, wherein 95 patients continued to receive ROPEG and 76 patients received the best available treatment (66 patients received HU). The primary endpoint of PROUD-PV was noninferiority of ROPEG vs HU at 12 months in achieving a complete hematologic response (CHR), defined as a hematocrit level or less than 45% with no phlebotomy in the preceding 3 months, normal platelet and white blood cell counts, and normal spleen size. In the CONTINUATION-PV study, the coprimary endpoints were CHR with normalization of spleen size and with improved disease burden (splenomegaly, microvascular disturbances, pruritus, and headache). Median overall follow-up was 182.1 weeks in the ROPEG arm and 164.5 weeks in the standard therapy arm.

In the PROUD-PV trial, the composite endpoint of CHR with normal spleen size was met in 21% of 122 patients in the ROPEG group and 28% of 123 patients in the HU arm at 12 months; noninferiority was not shown. However, for CHR without the spleen criterion at 12 months, responses were similar between the groups (43% ROPEG vs 46% HU). In CONTINUATION-PV, CHR was significantly higher in the ROPEG group, as the proportion of patients with response gradually increased up to 24 months and remained high at 36 months. In contrast, the response in the HU group was highest at 12 months and decreased thereafter. The number of patients achieving a molecular response

steadily increased on ROPEG (Table 1), with the *JAK2* V617F allele burden at 36 months decreasing to less than half the baseline level, from 42.8% to 19.7%. In contrast, the mutant allele burden reduction in the HU group was transient and was lost by month 36. The mean *JAK2* V617F allele burden at 24 and 36 months was significantly lower in the ROPEG group compared with the HU group (*P*<.0001). The molecular responses correlated with the results of ancillary ex vivo bone marrow colony formation assays showing that ROPEG increased the proportion of wild-type to mutant colonies.[63] Discontinuation owing to drug toxicity was rare (Table 1). Quality-of-life data showed no differences between the groups.[25] In the elderly patient subset aged 60 years and older (46 patients in ROPEG and 37 patients in the control group), ROPEG was well tolerated and efficacious, with a trend toward fewer and less serious treatment-related AEs compared with HU.[64] Based on the results of the PEGINVERA and PROUD-PV/CONTINUATION-PV trials, ROPEG was granted regulatory approval by the European Medicines Agency in 2019 and by the FDA in 2021. The current approved and recommended regimen of ROPEG consists of a starting dose of 100 μg subcutaneously every 2 weeks, with dose escalation by 50 μg every 2 weeks as tolerated, to a maximum dose of 500 μg.

Long-term outcomes in the PROUD-PV/CONTINUATION-PV trials after 5 years were reported.[26] The CHR rate was significantly higher in the ROPEG group than in the control arm at 60 months, at 72.6% vs 52.6%, respectively (*P*=.004). The molecular response rate at 5 years was also significantly higher in the ROPEG arm than in the control arm, at 69.1% vs 21.6%, respectively (*P*<.0001). At 60 months, the *JAK2* V617F allele burden decreased to less than 1% in 19.6% (18 of 92) of patients on ROPEG therapy compared with 1.4% (1 of 72) of patients in the control arm (*P*=.0002). There were 5 major thromboembolic events in 4 patients in the ROPEG arm and 5 events in 5 patients in the standard therapy arm, for an incidence rate of 1.0% per patient year and 1.2% per patient year, respectively. The incidence of disease progression (secondary myelofibrosis or leukemia) was 0.2% per patient year among ROPEG-treated patients (1 case of myelofibrosis) vs 1.0% per patient year in the control treatment arm (2 cases of myelofibrosis and 2 cases of acute leukemia). Although there was a 5-fold lower incidence of disease progression with ROPEG, these events were too rare for statistical comparison. The possibility of a disease-modifying effect of ROPEG is consistent with previous findings that in patients with PV and ET, a higher *JAK2* V617F allele burden at diagnosis or during follow-up may be predictive of progression to secondary MF.[65,66]

The final results of the PROUD-PV/CONTINUATION-PV trials, which analyzed at least 6 years of ROPEG treatment and confirmed the superiority of ROPEG therapy, have been published in abstract form.[67] No phlebotomies were required to maintain a hematocrit level of less than 45% in 81.4% of the patients in the ROPEG group compared with 60% of the patients in the control group (*P*=.005). At 6 years, the *JAK2* V617F allele burden reduction to less than 1% was achieved in 19 of the 92 patients (20.7%) in the ROPEG arm vs just 1 of the 74 patients (1.4%) in the control arm (most on HU; *P*=.0001). The analysis of event-free survival (risk events: disease progression, death, thromboembolic events) over 6 or more years of treatment was significantly superior in the ROPEG group compared with the control group (risk events were observed in 5/95 patients in the ROPEG group and in 12/74 patients in the control group; *P*=.04).

The Low-PV trial randomized patients aged 18 to 60 years with conventionally-defined low-risk PV to receive either phlebotomy and low-dose aspirin as standard therapy or fixed-dose ROPEG at 100 μg every 2 weeks in addition to standard therapy in an open-label, 2-arm design.[68] The primary endpoint was treatment response, defined as the maintenance of median hematocrit values of no more than 45% over 12 months and in the absence of progressive disease. At the 1-year interim analysis, the accrual of new patients was halted owing to the superiority of experimental treatment with ROPEG, wherein 84% of patients in the ROPEG group exhibited a response compared with 60% of patients in the standard therapy group (*P*=.0075). In the extension phase,[69] 91% of patients rolled over, continuing the treatment assigned at randomization or crossing over to the alternative group. A combined treatment response from randomization to 24 months was reached in 67% of patients treated with ROPEG vs 30% of patients on phlebotomy-only therapy (odds ratio, 4.74; *P*=.02). Disease progression was observed in 8% of patients in the standard therapy group (3 cases of symptomatic thrombocytosis and 1 case of splenic vein thrombosis) and none of the patients in the ROPEG group. No hemorrhagic events were observed and only 1 thrombosis (splenic vein) was reported in the standard therapy group vs none in the ROPEG group. ROPEG was well tolerated, with numbers of grade 3 and 4 AEs that were similar to those reported in the standard therapy group. Symptom scores revealed worsening fatigue and fever with ROPEG, but improvement of all other symptoms such as pruritus and night sweats. Among the secondary endpoints, ROPEG therapy was associated with a significant reduction in the number of phlebotomies, higher ferritin levels, lower leukocyte and platelet counts, reduction of palpable splenomegaly, and reduction of the *JAK2* V617F allele burden in the experimental group. None of the patients

Table 2. Summary of Ongoing Clinical Trials of IFN-α in MPNs

Clinical Trial, Phase	MPN Type, Estimated Enrollment (n)	Trial Design	Patient Population	Treatment	Outcome Measures
NCT05482971, phase 2	ET (64)	1-arm	First-line or second-line treatment	ROPEG	Safety, efficacy, and tolerability of ROPEG in patients with ET
NCT04285086 (SURPASS-ET trial),[74] phase 3	ET (160)	Randomized, controlled, open-label, multicenter, international	Second-line in HU-intolerant or -resistant patients	ROPEG vs anagrelide	Safety, efficacy, tolerability, and pharmacokinetics of ROPEG vs anagrelide
NCT05485948, phase 2	PV (49)	1-arm, open-label, multicenter	Second-line in HU-intolerant or -resistant patients	ROPEG	Phlebotomy-free CHR
NCT05481151 (ECLIPSE PV trial), phase 3b	PV (100)	Randomized, open-label, parallel group, multicenter	PV patients requiring cytoreductive therapy	ROPEG, starting dose 250-350-500 μg vs 100 μg with 50-μg dose increments	Safety, efficacy, and tolerability of 2 dosing regimens; CHR at week 24
NCT04116502 (MITHRIDATE trial), phase 3	PV (586)	Randomized, controlled, open-label, multicenter, international	First-line, high-risk PV	Ruxolitinib vs best available therapy (IFN-α or HU)	Event-free survival (major thrombosis/hemorrhage, death, MDS, AML, post-PV MF)
NCT02742324, (RUXOPEG trial, recruitment completed),[80] phase 1/2	MF (37)		First-line in primary or secondary MF	Ruxolitinib in combination with PEG-IFN-α-2a	Efficacy, safety, spleen response, molecular response

Source: Clinical Trials.gov database, accessed August 2023.

AML, acute myeloid leukemia; CHR, complete hematologic response; ET, essential thrombocythemia; HU, hydroxyurea; IFN-α, interferon alfa-2; MF, myelofibrosis; MDS, myelodysplastic syndrome; MPNs, myeloproliferative neoplasms; PEG-IFN-α, pegylated interferon alfa; PV, polycythemia vera; ROPEG, ropeginterferon alfa-2b.

in the standard therapy group compared with 22% of patients in the ROPEG group were molecular responders, according to ELN criteria (P=.0070).[60] Adding ROPEG to standard therapy resulted in 80% of patients remaining at their hematocrit target of less than 45%. The collective results of the PEGINVERA, PROUD-PV, CONTINUATION-PV, and Low-PV trials, documenting the favorable long-term safety profile, tolerability, efficacy, and superiority to HU of ROPEG, have led to change in clinical practice guidelines for PV.[16] Several ongoing studies of ROPEG in PV and other MPNs (ET and MF) are detailed in Table 2.

Systematic Reviews of IFN-α in PV and ET

The cumulative experience with IFN-α therapy in PV and ET over more than 3 decades was reported in 2 systematic reviews and meta-analyses, each including more than 1000 patients.[70,71] Bewersdorf and colleagues[70] reviewed 44 studies through March 2019 including 1359 patients (730 with ET, 629 with PV) treated with short-acting IFN-α (31 studies), first generation PEG-IFN (12 studies), and ROPEG (1 study). The overall response rate (ORR) in patients with ET was 80.6% (30 studies), with a CHR rate of 59%. In patients with PV, the ORR was 76.7% (23 studies) and the CHR rate was 48.5%. Freedom from phlebotomy occurred in 58.1% of patients (11 studies). The rate of thromboembolic events was 1.2% per patient year in ET patients (13 studies) and 0.5% per patient year for PV patients (11 studies). There was a statistically nonsignificant trend toward a higher rate of thromboembolic events in studies with an older patient population for both ET and PV. The annualized discontinuation rate was 8.8% per patient year for ET patients and 6.5% for PV patients. A formal assessment of molecular response, spleen size reduction, AEs, and symptom outcomes could not be performed in this meta-analysis.

Gu and colleagues[71] reviewed 37 studies through

March 2021 including 1794 patients with ET and PV treated with short acting IFN-α (14 studies), first-generation PEG-IFN (15 studies), and ROPEG (3 studies). The pooled overall hematologic response (OHR) rate was 86% (33 studies), the CHR rate was 53%, and the partial hematologic response (PHR) rate was 27%. Response rates were higher in ET (60% CHR, 22% PHR) than in PV (45% CHR, 32% PHR). The highest OHR was achieved with PEG-IFN (P<.001). Being age 60 years or older was associated with a lower OHR rate (P=.038). Patients on PEG-IFN benefited from higher doses of more than 100 μg per week (P<.001), whereas short-acting IFN-α did not exhibit a dose-related effect. The overall molecular response rate (12 studies including 543 patients) was 48% (51% for PV, 42% for ET), comprising a CMR rate of 16% and a partial molecular response (PMR) rate of 33%. The pooled overall molecular response rate was better for younger patients (P=.009). The overall incidence of thrombosis was very low, at 0.42 per 100 person years. The incidence of thrombosis increased with the patient's age (P=.01). The rate of hematologic transformation to MF was 16 events in 834 patients (average rate, 0.21/100 person years). The pooled incidence of AML (7 among 796 patients) was even lower, at 0.08 per 100 person years. AEs were reported in 84% of patients on IFN-α, most of which were mild. Grade 3/4 AEs were experienced by 10% of patients. Treatment outcomes with HU were reported in 8 studies. Meta-analysis demonstrated noninferiority of IFN-α compared with HU for hematologic response, but a significantly better outcome of IFN-α for molecular response (CMR, P=.01; PMR, P<.01).

The long-term outcomes of IFN-α therapy vs HU or therapeutic phlebotomy as the only therapy were reported in a single-center, longitudinal study of a large cohort of 470 patients with PV.[72] Although nonrandomized and retrospective, the median follow-up was 10 years. The adverse effects limiting IFN-α therapy were most commonly musculoskeletal and constitutional symptoms, with a discontinuation rate of 13% for IFN-α vs 16% for HU-treated patients. There was infrequent transformation to AML (4%), precluding assessment of significant differences between groups. Importantly, longer time on IFN-α therapy was associated with significantly improved MF-free and OS compared with HU or phlebotomy-only groups, independent of age and thrombosis history in multivariable analysis.[72] In contrast, longer time on HU therapy was not associated with a lower risk of MF transformation or all-cause mortality. These findings are consistent with the superior long term treatment results achieved with ROPEG in the PROUD-PV and CONTINUATION-PV trials[25,26,67] suggesting that long term IFN-α therapy may exert a disease-modifying effect that might favorably alter the natural course of PV.

Conclusions and Future Directions

Conventional treatment strategies for patients with PV and ET have long focused on primary or secondary prevention of thrombohemorrhagic events by controlling blood counts and platelet function inhibition when indicated. Evidence-based integration of IFN-α as a potentially disease-modifying therapy provides an impetus for the implementation of a comprehensive, long-term treatment strategy to achieve a measurable residual disease state to prevent or delay disease progression and to improve long-term outcomes such as MF-free survival, leukemia-free survival, and overall survival.

In an update based on results of recent IFN-α randomized trials, the ELN investigators and expert panel specified recombinant IFN-α (either ROPEG or PEG-IFN-α-2a) rather than HU as the recommended cytoreductive treatment choice for low-risk patients with PV (age <60 years and no prior thrombotic event) with an indication for cytoreductive therapy, including strictly defined intolerance to phlebotomy, symptomatic progressive splenomegaly, significant/progressive leukocytosis, extreme thrombocytosis, inadequate hematocrit control requiring phlebotomies, persistently high cardiovascular risk, and persistently high symptom burden.[16] The updated ELN 2021 recommendations also provided guidance for second-line cytoreductive therapy options in patients with PV on treatment with HU who require therapy change, either to ruxolitinib or IFN-α based on individual clinical features. The revised ELN guidelines also recognized the challenge of a uniform definition of therapeutic phlebotomy intolerance and failure for adequate hematocrit control, as an issue that requires validation in future longitudinal studies. Current National Comprehensive Cancer Network (NCCN) Guidelines for PV list either HU or IFN-α (PEG-IFN-α-2a or ROPEG) as the preferred agents in high-risk patients or low-risk patients with an emerging indication for cytoreductive therapy such as the development of new thrombosis or progressive splenomegaly.[17]

In high-risk patients with ET (history of thrombosis at any age or age >60 years with a *JAK2* mutation), the current version of NCCN guidelines recommends HU as the preferred cytoreductive therapy, with PEG-IFN-α-2a or anagrelide as other recommended upfront options.[17] ELN investigators recently focused on unmet clinical needs in the management of the subset of *CALR*-mutated ET, and recommended cytoreduction for extreme thrombocytosis (>1500 × 10^9/L), with PEG-IFN being the preferred option for younger patients.[73] The efficacy and safety of ROPEG in ET is currently under study.[74] Ongoing clinical trials that are investigating ROPEG in patients with MPN are summarized in Table 2. The findings of these ongoing

studies may lead to evidence-based changes in expert panel treatment guidelines for patients with ET in the future.

Several important questions are likely to be addressed by ongoing and future studies of IFN-α in PV and ET, including: (1) further evaluation of durable molecular remissions on IFN-α therapy; (2) optimal timing to embark on IFN-α therapy and its duration; (3) evaluation of the potential of treatment-free remissions; (4) feasibility, safety, and efficacy of combination therapies in conjunction with IFN-α[75]; (5) the clinical impact of molecular responses on long-term outcomes such as MF-free and leukemia-free survival; and (6) the ability of IFN-α therapy to change the natural clinical course of PV or ET. Reduction of the *JAK2* V617F allele burden has been emerging as a surrogate marker that not only may correlate with clinical and hematologic response but also is associated with a reduction of thrombosis risk and improved long-term outcomes.[65,66,76-78] Future studies of IFN-α signaling mechanisms in MPNs may continue to identify biomarkers predictive of IFN-α response[46,47] and further elucidate the molecular and cellular basis of IFN-α resistance. In addition, future studies are expected to characterize novel targets and therapeutic agents that may be used to enhance the efficacy of and reduce resistance to IFN-α therapy, optimizing the potential to achieve deep and durable molecular responses and, in turn, potentially delay and prevent disease progression.

Disclosures

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