New Perspectives of Interferon-alpha2 and Inflammation in Treating Philadelphia-negative Chronic Myeloproliferative Neoplasms

Hans C. Hasselbalch¹, Richard T. Silver²

Correspondence: Hans C. Hasselbalch (hans.hasselbalch@gmail.com).

In recent years, the use of recombinant interferon-alpha (rIFNα) as the initial treatment of the myeloproliferative neoplasms (MPNs), essential thrombocythemia, polycythemia vera and myelofibrosis, has been increasing. In a subset of patients, treatment with rIFNα for approximately 5 years may result in minimal residual disease (MRD) characterized by hematologic remission, a low JAK2V617F allele burden, and normal bone marrow morpholgy. The important role of chronic inflammation in the driving force for clonal evolution and disease progression and the impact of chronic inflammation upon symptom burden have been substantiated. Here, we highlight timely research questions regarding the use of rIFNα in the future MPN landscape and underscore the importance of early diagnosis and treatment with it to achieve MRD. Based upon the highly encouraging results from combination therapy of stem cell-targeted therapy with rIFNα and the potent anti-inflammatory drug, ruxolitinib, we also place in perspective studies of combinations with older, inexpensive agents (eg, statins, N-acetylcysteine, and colchicine), which have well-established anti-inflammatory and antithrombotic capabilities. Mathematical modeling studies have substantiated the concept that chronic inflammation is a trigger and driver of MPN development, and stress the importance of initiating rIFNα treatment as early as possible. Studies of the impact of rIFNα in individuals carrying the JAK2V617F or the CALR mutation as clonal hematopoiesis of indeterminate potential (CHIP) are urgently needed to determine whether rIFNα treatment at this early CHIP stage may eradicate the malignant clone. We foresee a bright future for patients with an MPN, in whom early intervention with stem cell-targeted therapy, rIFNα, alone or in combination with drugs targeting the chronic inflammatory state, may allow many to achieve MRD, thus becoming candidates for clinical trials employing vaccines leading to the possibility of cure.

Interferon-alpha2 in the myeloproliferative neoplasms

In 1985, Linkesch et al reported for the first time that rIFNα controlled myeloproliferation in patients with an MPN accompanied with severe thrombocytosis.¹² A few years later, Silver³ demonstrated the safety and efficacy of rIFNα treatment in patients with polycythemia vera (PV) and afterward, its value in the proliferative phase of myelofibrosis (MF) was reported, resulting in normalization of marrow architecture and cellularity, and reduction in degree of fibrosis to normal.⁴ Many subsequent studies in more than a thousand patients have confirmed that rIFNα is safe and effective for treating essential thrombocythemia (ET), PV, and early-stage MF patients: in ET, it normalizes elevated platelet counts within weeks to months in the large majority of patients; in PV, it reduces or eliminates the phlebotomy requirement and the degree of pruritus, normalizes elevated leukocyte and platelet counts and reduces spleen size; in MF patients, it reduces or normalizes elevated leukocyte- and platelet counts and—as noted above—may also induce regression of bone marrow fibrosis in some patients after long-term treatment.⁵ All these studies have been thoroughly described in several recent reviews.⁶–¹⁵ In a single-arm study of 55 patients with PV, rIFNα therapy resulted in significant reduction in need for phlebotomy and in thrombotic events.¹⁶ In the largest retrospective study of 470 PV patients from the same institution, improved myelofibrosis-free survival and probably overall survival were observed in rIFNα-treated patients compared to those treated with hydroxyurea (HU) or phlebotomy only (PHL-O).¹⁷ Recent studies have elucidated novel mechanisms of action of rIFNα therapy in the MPNs, which basically and simplistically depends on physiological stem cell exhaustion and/or depletion. In MPN mice, rIFNα can directly eradicate malignant disease-initiating cells by inducing changes in the cell cycle and apoptosis.¹⁷–¹⁹ Tong et al, by single-cell transcriptomic profiling coupled with mutation detection, showed that in patients with ET, JAK2V617F megakaryocytic stem cells had elevated interferon signaling. Upon treatment, homozogous mutant HSCs had a quiescent signature in comparison to heterozygous stem cells, which underwent enhanced apoptosis.²⁰

The interest in using rIFNα long-term was abetted by the reports of it decreasing the JAK2V617F allele burden in PV,²¹–²⁶ MRD, noted in a subset of patients, was defined as clinical and hematologic remission, a JAK2V617F allelic burden <1% and normalization of marrow morphology.²³,²⁴,²⁷ These results could be sustained after discontinuation of rIFNα for more than 2–3 years.²¹,²³,²⁴,²⁷ The long-term impact of rIFNα in patients following discontinuation of therapy may reflect rIFNα reprogramming defective immune cells and restoring competent “tumor immune surveillance.”²¹–²³,²⁴,²⁷

Despite these impressive results, these were primarily based upon phase 2 or single-arm studies and did not satisfy regulatory requirements.¹¹,¹³,¹⁴ Accordingly, rIFNα was used off label and in the United States, required tedious insurance company approval prior to its use. This has recently changed in Europe because of the licensing of ropeg-rIFNα-2b (Besremi) for the treatment
of European LeukemiaNet (ELN) defined high-risk PV patients without symptomatic splenomegaly. The safety and efficacy of this novel drug characterized by a proline pegylated bond have been demonstrated in several studies; it has the advantage of administration every second or third week. Its toxicity profile may be less than with either pegylated rIFNα-2a (Pegasys) or pegylated rIFNα-2b (PegIntron). However, there have been no comparative trials to verify this presumption.

The future interferon-MPN landscape

In the future, several research questions regarding the use of rIFNα will hopefully be addressed:

How does chronic inflammation, caused by smoking, impact the response to rIFNα?

Smoking elicits a massive systemic inflammatory stimulus, causing leukocytosis and, sometimes, thrombocytosis. The JAK-STAT and NF-kappaB signaling pathways are activated in both smokers and in patients with MPNs. Both share elevated levels of several pro-inflammatory cytokines, in vivo activation of leukocytes and platelets, endothelial cell dysfunction, and increased systemic oxidative stress. In this context, it has been suggested that smoking may trigger MPN development and may also enhance clonal evolution as a consequence of inflammation-mediated genomic instability. Indeed, the concept of smoking as a risk factor for the development of an MPN has been substantiated in recent studies. Since smoking may be a likely trigger and driver of clonal evolution in patients with an MPN and since smoking, per se, gives rise to erythrocytosis, leukocytosis, and sometimes thrombocytosis, it increases the thrombotic risk associated with an MPN. A recent study has shown that smoking impairs molecular response and reduces overall survival in MPN patients treated with rIFNα.

What are the reasons for rIFNα resistance or intolerance in the MPNs?

In some patients rIFNα may elicit a sustained “inflammatory syndrome,” characterized by fatigue and muscle and joint pain, necessitating its dose reduction, thereby perhaps leading to its discontinuation because of diminishing efficacy. Currently, it is unknown which mechanisms are responsible for the emergence of this “inflammatory syndrome,” but several may be operative. First, our clinical experience indicates that patients with advanced MPN-disease and a large tumor burden, for example, patients with myelofibrosis and massive splenomegaly, do not tolerate rIFNα well, owing to its side effects. Perhaps, this intolerance might be explained by a rIFNα-induced cytokine storm. This increase may be temporary and may decline in concert with rIFNα-mediated reduction in tumor burden. In this time-frame, adding a potent anti-inflammatory drug (eg, ruxolitinib or prednisolone) might be a rational approach as addressed below. Second, studies are ongoing to explore whether such autoimmunity and inflammatory side effects may be associated with a particular human leukocyte antigen (HLA) tissue type. In this regard, it is worth considering whether MPN patients intolerant to rIFNα may have a predisposition for developing autoimmunity which then is elicited or exacerbated during treatment with rIFNα. There are reports that patients with TET2-mutations have impaired response to treatment with rIFNα. Recently, Stetka et al demonstrated that genetic loss of DNMT3A confers resistance to treatment with rIFNα in a JAK2V617F-driven MPN mouse model. An association between DNMT3A-mutations and impaired response to rIFNα is supported by the Danish DALIATH-trial, in which DNMT3A-mutations emerged on treatment more frequently than non-DNMT3A-mutations among patients not achieving complete hematological remission (CHR). Third, as alluded to previously, inflammatory signaling is associated with a diminished effect of rIFNα. All rIFNα effects are elicited through interaction with type I IFN receptors, the IFNα-2AR1 and IFNα-2AR2 chains. Inflammation-mediated downregulation of IFNα-2AR1 is associated with refractoriness to rIFNα. Noteworthy in this context is that the inflammatory cytokines interleukin 1-alpha (IL-1α) and tumor necrosis factor alpha (TNF-α) stimulate IFNα-2AR1 degradation and accordingly attenuate IFNα-2a signaling. Similarly, unresponsiveness to rIFNα-2a in hepatitis patients may be explained by oxidative stress, also impairing IFNα-2a signaling. MPNs are associated with increased levels of several inflammatory cytokines, including IL-1α and TNF-α, the highest levels have been reported in patients with advanced myelofibrosis. Thus, treating patients with rIFNα at the earliest disease stage possible, when inflammation is less pronounced, seems a more rational approach rather than a “watch and wait policy,” which permits the malignant clone to expand, thus increasing its inflammatory load. The early intervention with rIFNα has recently been supported by mathematical modeling studies. These show that the earlier rIFNα is started in PV and related neoplasms, the more rapid the decline in the JAK2V617F allele burden. This results in a shorter treatment period in order to obtain a major molecular remission. Early rIFNα treatment of patients with primary and secondary myelofibrosis may result in regression of bone marrow fibrosis and improved marrow architecture and cellularity. Recently, germ-line genetic factors have been shown to influence rIFNα-response in patients with PV, which may affect rIFNα resistance or intolerance.

How does rIFNα-2a impact the chronic inflammatory state and defective tumor immune surveillance in the MPNs?

By normalizing elevated leukocyte and platelet counts, rIFNα helps minimize the sustained release of inflammatory cytokines and chemokines and concurrently improves immune cell function which is important for intact tumor immune surveillance. Patients with MPNs are subject to an increased risk of second cancers, which have an inherently worse prognosis compared to the same cancer as in an MPN-naive person. Thrombocytosis is a worse prognostic factor in several cancers, and platelets enhance cancer invasiveness and metastatic potential. Thus, leukocytosis and thrombocytosis in patients with MPNs may contribute to the increased risk of second cancers and inferior survival, both by eliciting defective tumor immune surveillance and by increasing cancer invasiveness. rIFNα may restore normal tumor surveillance by increasing the number of several types of immune cells, including dendritic cells, T-cells and natural killer (NK)-cells. In addition, rIFNα upregulates previously downregulated HLA-genes, thereby improving tumor cell killing. Furthermore, rIFNα also downregulates or normoregulates JAK2V617F-induced expression of the immune check point programmed-cell-death-ligand 1 (PD-L1), thereby impairing PD-L1 mediated immune escape. Whole blood gene expression studies indicate that rIFNα treatment decreases expression of genes involved in regulation of inflammation and enhances expression of genes of importance for immune cell function. Whole blood transcriptional profiling studies have also shown that rIFNα has a major impact upon deregulated oxidative stress genes and antioxidative defense genes. Importantly, down-regulation of several upregulated thromboinflammatory genes, including the PAD4 gene has been demonstrated. This gene is required for neutrophil extracellular trap (NET) formation and thrombosis development.
Interferon-alpha2 combination therapies: combination with ruxolitinib

In PV, rIFNα-2a monotherapy, together with targeted therapeutic phlebotomy, normalizes elevated blood cell counts within a few months, often accompanied by a decrease in the JAK2V617F allele burden. However, major molecular remissions are rare within the first 2 years of therapy and a minority of patients with PV may require a few phlebotomies per year despite 2–3 years of treatment. We prefer to gradually increase the dose of rIFNα, starting with a low dose of pegIFNα-2a 45 μg/week; if no normalization of peripheral cell counts after 1–2 months, we increase the dose to 90 μg/week. Rarely, patients need 135 or 180 μg/week. About 15%–40% of patients show a response after 4–5 weeks because of symptoms of toxicity, usually because the doses used have been too high.

However, even with low-dose pegIFNα-2a, 45 μg/week, the discontinuation rate in the DALIAH-trial reached 50%. Since intolerance may be partly explained by rIFNα-exacerbated inflammation, combination therapy of rIFNα with an anti-inflammatory drug such as ruxolitinib may dampen inflammation and restore its sensitivity and enhance efficacy. Taking into account that ruxolitinib inhibits canonical type 1 IFN-signaling through JAK1 inhibition, such a combination therapy might theoretically have antagonistic effects. However, our clinical trials in PV and MF patients who had been previously intolerant or refractory to rIFNα-2a monotherapy have shown this combination therapy to be both safe and effective. These highly interesting and encouraging findings may be explained by several mechanisms, including the fact that ruxolitinib has a half-life of only a few hours leaving an open window of several hours per day for IFN-signaling. Other mechanisms might be that JAK/STAT inhibition dampens inflammation, which has been reported to impair IFN-signaling by degradation of the IFN-receptor as alluded to above. The rationale for this combination has been substantiated by in vivo murine studies of JAK2V617F hematopoietic stem cells, demonstrating distinct effects of ruxolitinib and rIFNα. However, the results require validation in both newly diagnosed PV and MF patients.

Since statins may enhance the efficacy of ruxolitinib and rIFNα, triple therapy of rIFNα + ruxolitinib + statin may be a highly effective triplet, but obviously requires evaluation in future trials. A recent study indicates hypoxia-inducible factor 1 (HIF-1) as a new therapeutic target in JAK2V617F-positive MPNs, demonstrating the potential of the peptide arginase, echinomycin, alone and in combination with ruxolitinib, to selectively target JAK2V617F-positive cells inducing apoptosis and cell cycle arrest. In this context, it may be interesting to combine a HIF-1-inhibitor and JAK1-2 inhibitor with rIFNα, which might further enhance the synergistic effects of combining ruxolitinib and rIFNα.

Combination with HU

HU is the drug most often used in the treatment of patients with MPNs. However, concern has been raised regarding its leukemogenic potential for treatment exceeding 10–15 years. Therefore, physicians at many MPN centers are cautious about using HU in patients <60 years. Theoretically, combination therapy of rIFNα with HU might nevertheless be a relevant approach. By inducing so-called immunogenic cell death, HU may expose tumor antigens to the immune system. Studies have shown that HU upregulates the immunoreceptor, natural-killer group 2, member D (NGK2D), originally identified in NK cells, and inhibits an immunosuppressive arginase, thereby exposing tumor antigens to the immune system. Thus, the combination of rIFNα and HU might exert a synergistic immune killing effect on the malignant clone in excess of their direct cell killing effects. HU potentially lowers elevated levels of inflammatory cytokines in patients with sickle cell anemia (SCA), thereby decreasing the inflammatory state and reducing the risk of thrombosis. Although the impact of HU upon increased inflammatory cytokines has not been studied systematically in patients with MPNs, HU could reduce cytokines in MPN patients, and enhance the efficacy of rIFNα, dampened by concurrent inflammation. HU might also alleviate the inflammation-mediated flu-like symptoms elicited by rIFNα. Preliminary data indicate that fluctuating cell counts during treatment of PV with HU may contribute to an increased thrombotic risk within the first 3–6 months after starting the drug. Since rIFNα causes normalization of elevated cell counts without such oscillations, a combination of both drugs during the first months after diagnosis might offer less toxicity than single drug treatment and perhaps reduce further the increased risk of thrombosis.

Combination with vaccination and immune checkpoint inhibitor strategies

Recently, the CALR and the JAK2V617F mutations, present in >90% of MPN patients, have been shown to be immunogenic neo-antigens. Importantly, the immune responses in JAK2V617F-positive patients are minor compared to those of CALR-positive patients. This small discrepancy may be related to the single amino acid difference between the mutant JAK2V617F epitope and the wild type JAK2 epitope, whereas the mutant CALR C-terminus spans 36 amino acids. Furthermore, patients with MPN display frequent and strong T-cell responses against the PD-L1 and arginase-1. Thus, peptide vaccination with either JAK2 mutant or CALR mutant epitopes in combination with vaccination against PD-L1 and/or arginase may be a new and potentially curable treatment modality for MPN patients. This requires pretreatment with rIFNα, either as monotherapy or in combination with ruxolitinib, to achieve MRD, a prerequisite for eliminating the residual clone by vaccination strategies. Studies of the safety and efficacy of immune checkpoint inhibitors, for example, blocking PD-L1, are currently under investigation in patients with myelofibrosis. PD-L1 is upregulated on JAK2V617F mutated cells, prohibiting a tumor-specific immune response against the malignant JAK2V617F-mutated cells by binding to tumor-specific T cells, resulting in their inactivation. The JAK2V617F mutation also generates reactive oxygen species, which in turn impairs T-cell function, as mentioned above.
Discussion

The impact of chronic inflammation as an important driving force for clonal expansion and evolution in patients with MPNs opens a new horizon for combination studies. Such studies preferentially should include rIFNα, which is the only disease-modifying drug that can induce deep molecular remission and normalization of marrow morphology in a subset of patients. We believe these beneficial effects are likely attributed to the stem-cell targeting potential of rIFNα which boosts virtually all immune cells engaged in “tumor immune surveillance.” The encouraging results of combining rIFNα with ruxolitinib75,76 may introduce combination studies with currently available and inexpensive drugs, such as statins, and N-acetylcysteine, which all have shown potent anti-inflammatory, antithrombotic, and anticancer capabilities.82–84,104 The intriguing combination of rIFNα and arsenic may have the potential to eradicate the JAK2V617F clone.103 Since HU does not induce sustained normalization of elevated cell counts in PV patients, it may be rational to combine lower doses of HU with rIFNα, thereby reducing the increased thrombotic risk in PV and reducing rIFNα toxicity. Mathematical modeling studies have shown that the earlier treatment with rIFNα is insti- tuted the more likely the chance of obtaining rapid and deep molecular responses.35 It would be interesting to study the impact of rIFNα treatment in the CHIP phase to determine whether inhibiting JAK2V617F would also inhibit prodromal thrombotic events and overt MPN disease development. Similarly, studies of the impact of IL-1b or IL-6R blockade upon the kinetics of the JAK2V617F mutation in the CHIP phase might unravel the important role of chronic inflammation for abetting clonal expansion. Future research should also focus on the use of colchicine. This old and inexpensive drug has recently been shown to decrease the risk of cardiovascular events,106 likely owing to its impact upon circulating inflammatory cyto-
kines, the inflammasome, and subsequently NETosis generation.107 Studies on the impact of colchicine on the kinetics of the driver mutations, JAK2V617F and CALR, and blood cell counts both in the CHIP stage and in MPN patients are urgently needed.

In conclusion, MPNs are not truly orphan diseases because they are frequently underdiagnosed.103 MPNs carry an inherently early and increased risk of life-threatening thrombotic events109,110 and an increased risk of second cancers,69,61 underscoring the urgent need for their earlier detection. Fortunately, at last, our early intervention concept with rIFNα is also an effective therapy for patients with PV (or ET) previously refractory and/or intolerant of HU.15,112 Pegylated rIFNα is also an effective therapy for patients with PV (or ET) previously refractory and/or intolerant of HU,15,112 Importantly, as previously discussed a recent study of 470 PV patients has shown that rIFNα yields improved myelofibrosis-free and overall survival,66 as does a recent meta-analy-
sis.114 These data, together with those generated from a large number of single-arm studies which enrolled more than 1,000 patients over the past 30 years,15,16,35,114-116 will result in more MPN patients who will be fortunately treated with rIFNα in the future.

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References

1. Linkesch W, Gisslinger H, Ludwig H, et al. Therapy with interferon (recombinant IFN-alpha-2C) in myeloproliferative diseases with severe thrombocytoses. Acta Med Austriaca. 1985;12:123–127.
2. Ludwig H, Linkesch W, Gisslinger H, et al. Interferon-alfa corrects thrombocytosis in patients with myeloproliferative disorders. Cancer Immunol Immunother. 1987;25:266–273.
3. Silver RT. Recombinant interferon-alpha for treatment of polycythe-
mia vera. Lancet. 1988;2:40.
4. Gilbert HS. Long term treatment of myeloproliferative dis-
ease with interferon-alpha-2b: feasibility and efficacy. Cancer. 1998;83:1205–1213.
5. Silver RT, Vandris K, Goldman JJ. Recombinant interferon-α may retard progression of early primary myelofibrosis: a preliminary report. Blood. 2011;117:8669–8672.
6. Kiladjian JJ, Chomienne C, Fenaux P. Interferon-alpha therapy in bcr-abl-negative myeloproliferative neoplasms. Leukemia. 2008;22:1990–1998.
7. Hasselbalch HC, Larsen TS, Riley CH, et al. Interferon-alpha in the treatment of Philadelphia-negative chronic myeloproliferative neo-
plasms. Status and perspectives. Curr Drug Targets. 2011;12:392–419.
8. Kiladjian JJ, Mesa RA, Hoffman R. The renaissance of inter-
feron therapy for the treatment of myeloid malignancies. Blood. 2011;117:4706–4715.
9. Silver RT, Kiladjian JJ, Hasselbalch HC. Interferon in the treatment of essential thrombocythemia, polycythemia vera and myelofibrosis. Expert Rev Hematol. 2013;6:49–58.
10. Stein BL, Tiu RV. Biological rationale and clinical use of interferon in the classical BCR-ABL-negative myeloproliferative neoplasms. J Interferon Cytokine Res. 2013;33:145–153.
11. Hasselbalch HC, Silver RT. Interferon in polycythemia vera and related neoplasms. It can become the treatment of choice without a random-
ized trial? Expert Rev Hematol. 2015;8:439–445.
12. Kiladjian JJ, Giraudier S, Cassinat B. Interferon-alpha for the ther-
apy of myeloproliferative neoplasms: targeting the malignant clone. Leukemia. 2016;30:776–781.
13. Hasselbalch HC, Holmström MO. Perspectives on interferon-alpha in the treatment of polycythemia vera and related myeloproliferative neoplasms: minimal residual disease and cure? Semin Immunopathol. 2019;41:5–19.
14. How J, Hobbs G. Use of interferon alfa in the treatment of myeloprolif-
erative neoplasms: perspectives and review of the literature. Cancers (Basel). 2020;12:E1954.
15. Silver RT. Long-term effects of the treatment of polycythemia vera with recombinant interferon-alpha. Cancer. 2006;107:451–458.
16. Abu-Zeinah G, Krichevsky S, Cruz T, et al. Interferon-alpha for treat-
ing polycythemia vera yields improved myelofibrosis-free and overall survival. Leukemia. 2021;35:2592–2601.
17. Lane SW, Mullaly A. Jak2V617F myeloproliferative neoplasm stem cells and interferon-alpha. OncoTargets. 2013;4:500–501.
18. Hasan S, Lacout C, Marty C, et al. JAK2V617F expression in mice amplifies early hematopoietic cells and gives them a competitive advan-
tage that is hampered by IFNα. Blood. 2013;122:1464–1477.
19. Austin RJ, Straube J, Bruedigam C, et al. Distinct effects of ruxolitib and interferon-alpha on murine JAK2V617F myeloprolifer-
ative neoplasm hematopoietic stem cell populations. Leukemia. 2020;34:1075–1089.
20. Tong J, Sun T, Ma S, et al. Hematopoietic stem cell heterogeneity is linked to the initiation and therapeutic response of myeloproliferative neoplasms. Cell Stem Cell. 2021;28:502–513.
21. Kiladjian JJ, Cassinat B, Turlure P, et al. High molecular response rate of polycythemia vera patients treated with pegylated interferon alpha-2a. Blood. 2006;108:2037–2040.
22. Kiladjian JJ, Cassinat B, Chevret S, et al. Pegylated interferon-alpha-2a induces complete hematologic and molecular responses with low toxicity in polycythemia vera. Blood. 2008;112:3065–3072.
23. Larsen TS, Pedersen RK, Moller MB, et al. Complete molecular remission of polycythemia vera during long-term treatment with pegylated interferon alpha-2b. Ann Hematol. 2008;87:847–850.
24. Larsen TS, Iverson KF, Hansen E, 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;37:1041–1045.
25. Utke Rank C, Weis Bjerrum O, Larsen TS, 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;14:331–334.
26. Utke Rank C, Kantarjian H, Manshouri T, et al. Pegylated interferon alpha-2a yields high rates of hematologic and molecular response in patients with advanced essential thrombocythemia and polycythemia vera. J Clin Oncol. 2009;27:5418–5424.
27. Larsen TS, Pedersen KM, Moeller MB, et al. Complete molecular remission of polycythemia vera during long-term treatment with pegylated interferon alpha-2b. Ann Hematol. 2008;87:847–850.
28. Larsen TS, Moeller MB, de Stricker K, et al. Increase in circulating blood cells and myeloproliferative neoplasms: meta-analysis and red blood cells. Eur J Haematol. 2015;39:1137–1145.
29. Riley CH, Jensen MK, Brimnes MK, et al. Increase in circulating CD55bright natural killer cells in patients with JAK2-α2-positive chronic myeloproliferative neoplasms during treatment with IFN-α. Blood. 2011;118:2170–2173.
30. Riley CH, Hansen M, Brimnes MK, et al. Expansion of circulating CD55bright natural killer cells in patients with JAK2-α2-positive chronic myeloproliferative neoplasms during treatment with interferon-α. Eur J Haematol. 2015;94:227–234.
31. Silver RT, Hasselbalch HC. Optimal therapy for polycythemia vera and essential thrombocythemia. Preferred use of interferon therapy based on phase 2 trials. Hematology. 2016;21:387–391.
32. Them NG, Bagienski K, Berg T, et al. Molecular responses and chromosomal aberrations in patients with polycythemia vera treated with peg-proline-interferonalpha-2b. Am J Hematol. 2015;90:288–294.
33. Gisslinger H, Zagrijschuk O, Buxhofer-Ausch V, et al. Roquefinterferon alpha-2b, a novel IFNα-2b, induces high response rates with low toxicity in patients with polycythemia vera. Blood. 2015;126:1722–1729.
34. Verger E, Soret-Dulphy J, Maslah N, et al. Roquefinterferon alpha-2b targets JAK2V617F-positive polycythemia vera cells in vitro and in vivo. Blood Cancer J. 2018;8:94.
35. Gisslinger H, Klaade C, Georgiev P, et al; PROUD-PV Study Group. Roquefinterferon alpha-2b versus standard therapy for polycythemia vera (PROUD-PV and CONTINUATION-PV): a randomised, non-inferiority, phase 3 trial and its extension study. Lancet Haematol. 2020;7:e196–e208.
36. Hasselbalch HC. Smoking as a contributing factor for development of polycythemia vera and related neoplasms. Leuk Res. 2015;39:1137–1145.
37. Pedersen KM, Colak Y, Ellervik C, et al. Smoking and increased white and red blood cells. Arterioscler Thromb Vasc Biol. 2019;39:965–977.
38. Lindholm Sorensen A, Hasselbalch HC. Smoking and Philadelphia-negative chronic myeloproliferative neoplasms. Eur J Haematol. 2016;97:63–69.
39. Jayasuriya NA, Kjaergaard AD, Pedersen KM, et al. Smoking, blood cells and myeloproliferative neoplasms: meta-analysis and Mendelian randomization of 2·3 million people. Br J Haematol. 2020;189:323–334.
40. Pedersen KM, Bak M, Sorensen AL, et al. Smoking is associated with increased risk of myeloproliferative neoplasms: a general population-based cohort study. Cancer Med. 2018;7:5796–5802.
41. Sorensen AL, Knudsen TA, Skov V, et al. Smoking impairs molecular response, and reduces overall survival in patients with chronic myeloproliferative neoplasms: a retrospective cohort study. Br J Haematol. 2021;193:83–92.
42. Kiladjian JJ, Massé A, Cassinat B, et al; French Intergroup of Myeloproliferative Neoplasms (FIM). Clonal analysis of erythroid progenitors suggests that pegylated interferon-alpha-2a treatment targets JAK2V617F clones without affecting TET2 mutant cells. Leukemia. 2015;29:1519–1523.
43. Quintás-Cardama A, Abdel-Wahab O, Manshouri T, et al. Molecular analysis of patients with polycythemia vera or essential thrombocythemia receiving pegylated interferon α2a. Blood. 2013;122:893–901.
44. Silver RT, Barel AC, Lascu E, et al. The effect of initial molecular profile on response to recombinant interferon (rIFN) treatment in early myelofibrosis. Cancer. 2017;123:2680–2687.
45. Hasselbalch HC. Molecular profiling as a novel tool to predict response to interferon-α2 in MPNs: the proof of concept in early myelofibrosis. Cancer. 2017;123:2600–2603.
46. Stetka J, Hansen N, Kubovcakova L, et al. Loss of DNmt3a confers resistance to PegIFN in JAK2 -V617F mouse model. Blood. 2020;136(Supplement 1):8–9.
47. Knudsen TA, Skov V, Stevenson KE, et al. Genomic profiling of a randomized trial of interferon-α versus hydroxyurea in MPN reveals mutation-specific responses. Blood Adv. 2021 September 10. [Epub ahead of print].
48. Routaung WC, Qian J, Liu C, et al. Inflammatory signaling compromises cell responses to interferon alpha. Oncogene. 2012;31:161–172.
49. Messina JL, Yu H, Riker AI, et al. Activated Stat-3 in melanoma. Cancer Control. 2008;15:196–201.
50. Di Bona D, Cippitelli M, Fionda C, et al. Oxidative stress inhibits IFN-alpha- induced antiviral gene expression by blocking the JAK-STAT pathway. J Hepatol. 2006;45:271–279.
51. Hasselbalch HC. The role of cytokines in the initiation and progression of myelofibrosis. Cytokine Growth Factor Rev. 2013;24:133–145.
52. Pedersen RK, Andersen M, Knudsen TA, et al. Data-driven analysis of JAK2V617F kinetics during interferon-alpha2 treatment of patients with polycythemia vera and related neoplasms. Cancer Med. 2020;9:2039–2051.
53. Pizzi M, Silver RT, Barel A, et al. Recombinant interferon-α in myelofibrosis reduces bone marrow fibrosis, improves its morphology and is associated with clinical response. Mod Pathol. 2015;28:1315–1323.
54. Völler RA, Gisslinger H, Fuchs E, et al. Germline genetic factors influence the outcome of interferon-α therapy in polycythemia vera. Blood. 2011;118:4066–4073.
55. Lindgren M, Samuelsson J, Nilsson L, et al. Genetic variation in IL28B (IFNa-2aL3) and response to interferon-alpha treatment in myeloproliferative neoplasms. Eur J Haematol. 2018; 100:419–425.
56. Dong M, Blobe GC. Role of transforming growth factor-beta in hematologic malignancies. Blood. 2006;107:4589–4596.
57. Yang L, Pang Y, Moses HL. TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. Trends Immunol. 2010;31:220–227.
58. Johnson BF, Clay TM, Hobeika AC, et al. Vascular endothelial growth factor and immunosuppression in cancer: current knowledge and potential for new therapy. Expert Opin Biol Ther. 2007;7:449–460.
59. Frederiksen H, Farkas DK, Christiansen CF, et al. Chronic myeloproliferative neoplasms and subsequent cancer risk: a Danish population-based cohort study. Blood. 2011;118:6515–6520.
60. Lindgren M, Samuelsson J, Nilsson L, et al. Genetic variation in IL28B (IFNa-2aL3) and response to interferon-alpha treatment in myeloproliferative neoplasms. Eur J Haematol. 2018; 100:419–425.
61. Dong M, Blobe GC. Role of transforming growth factor-beta in hematologic malignancies. Blood. 2006;107:4589–4596.
62. Yang L, Pang Y, Moses HL. TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. Trends Immunol. 2010;31:220–227.
63. Johnson BF, Clay TM, Hobeika AC, et al. Vascular endothelial growth factor and immunosuppression in cancer: current knowledge and potential for new therapy. Expert Opin Biol Ther. 2007;7:449–460.
65. Skov V, Riley CH, Thomassen M, 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;54:2269–2273.
66. Skov V, Riley CH, Thomassen M, et al. The impact of interferon-alpha2 on HLA genes in patients with polycythemia vera and related neoplasms. Leuk Lymphoma. 2017;58:959–967.
67. Skov V, Riley CH, Thomassen M, et al. Interferon-Alpha2 downregulates expression of PD-L1 in patients with polycythemia vera and related neoplasms. Potential implications for tumor immune escape? (In preparation).
68. Prestipino A, Emhardt AJ, Aumann K, et al. Oncogenic JAK2V617F causes PD-L1 upregulation, mediating immune escape in myeloproliferative neoplasms. Sci Transl Med. 2018;10:eam7729.
69. Skov V, Riley C, Thomassen M, et al. Interferon-alpha2 treatment of patients with polycythemia vera and related neoplasms influences deregulated inflammation and immune genes in polycythemia vera and allied neoplasms. Blood. 2018;132:5490.
70. Hasselbalch HC, Thomassen M, Riley CH, et al. Whole blood transcriptional profiling reveals deregulation of oxidative and antioxidative defence genes in myelofibrosis and related neoplasms. Potential implications of downregulation of Nrf2 for genomic instability and disease progression. PLoS One. 2014;9:e112786.
71. Skov V, Riley C, Thomassen M, et al. Significantly upregulated thromboxane B2 immunomarkers are normoregulated or significantly downregulated during treatment with interferon-alpha2 in patients with Philadelphia-negative chronic myeloproliferative neoplasms. Blood. 2019;134(Supplement 1):2978.
72. Knudsen TA, Hansen DL, Ociás LF, et al. A three-year analysis of the DALI4A trial – a randomized controlled phase III clinical trial comparing recombinant interferon-α versus hydroxyurea in patients with MPNs. HemaSphere. 2019;3(3):741–742.
73. Bjørn ME, de Stricker K, Kjaer L, et al. 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;7:33–75.
74. Bjørn ME, Hasselbalch HC. Minimal residual disease or cure in myeloproliferative neoplasms: Perspectives and perspectives on combination therapy with interferon-alpha2 and ruxolitinib. Expert Rev Hematol. 2017;10:393–404.
75. Mikkelsen SU, Kjaer L, Bjørn ME, et al. Safety and efficacy of combination therapy of interferon-α2 and ruxolitinib in polycythemia vera and myelofibrosis. Cancer Med. 2018;7:3571–3581.
76. Sørensen AL, Mikkelsen SU, Knudsen TA, et al. Ruxolitinib and interferon-α2 combination therapy for patients with polycythemia vera or myelofibrosis: a phase II study. Haematologica. 2020;105:2262–2272.
77. Silver RT. Combination therapy with interferon and ruxolitinib for polycythemia vera and myelofibrosis: are two drugs better than one? Haematologica. 2016;101:2190–2195.
78. Kolewiedder S, Mugnier TI, Hasselbalch HC, et al. Myeloproliferative neoplasms and inflammation: whether to target the malignant clone or the inflammatory process or both. Leukemia. 2016;30:1018–1024.
79. Griner LN, McGraw KL, Johnson JO, et al. JAK2-V617F-mediated signalling is dependent on lipid rafts and statins inhibit JAK2-V617F-dependent cell growth. Br J Haematol. 2013;160:177–187.
80. Kozloski M, Sommerfeld S, Krüger C, et al. Effects of ruxolitinib in polycythemia vera and related neoplasms. Leukemia. 2013;27:2187–2195.
81. Bjørn ME, Hasselbalch HC. The role of reactive oxygen species and inflammatory genes in the inflammatory process or both. Haematologica. 2021;106:e112786.
82. Gruner H, Gruter H, Helm T, et al. Thrombosis in a murine model of myeloproliferative neoplasm. Blood. 2015;126:2152–2160.
83. Gruner H, Helm T, Helm H, et al. Thrombosis in a murine model of myeloproliferative neoplasm. Blood. 2015;126:2152–2160.
84. Liu X, Ohtaka K, Kondo Y, et al. Hydroxyurea upregulates NGK2D ligand expression in myeloid leukemia cells synergistically with valproic acid and potentially enhances susceptibility of leukemic cells to lncRNA Killer cell-membrane receptor. Cancer Sci. 2010;101:609–615.
85. Martinovic KM, Milicic M, Larsen AK, et al. Effect of cytokines on NK cell activity and activating receptor expression in high-risk cutaneous melanoma patients. Eur J Cancer. 2013;180:160–167.
86. Zahran AM, Nafady A, Saad K, et al. Effect of hydroxyurea treatment on the inflammatory markers among children with sickle cell disease. J Clin Res Pediatr Endocrinol. 2020;12:230–239.
87. Spivak JL, Hasselbalch H. Hydroxycarbamide: a user’s guide for chronic myeloproliferative disorders. Expert Rev Anticancer Ther. 2011;11:403–414.
88. Lu X, Ohtaka K, Kondo Y, et al. Hydroxyurea upregulates NGK2D ligand expression in myeloid leukemia cells synergistically with valproic acid and potentially enhances susceptibility of leukemic cells to interferon. Cancer Res. 2010;70:6096–6105.
111. Kiladjian JJ, Barbui T. From leeches to interferon: should cyto-reduction be prescribed for all patients with polycythemia vera? *Leukemia*. 2020;34:2837–2839.

112. Barbui T, Vannucchi AM, De Stefano V, et al. Ropeginterferon alfa-2b versus phlebotomy in low-risk patients with polycythaemia vera (Low-PV study): a multicentre, randomised phase 2 trial. *Lancet Haematol*. 2021;8:e175–e184. Erratum in: *Lancet Haematol*. 2021 Mar;8:e170.

113. Yacoub A, Mascarenhas J, Kosiorek H, et al. Pegylated interferon alfa-2a for polycythemia vera or essential thrombocythemia resistant or intolerant to hydroxyurea. *Blood*. 2019;134: 1498–1509.

114. Bewersdorf JP, Giri S, Wang R, et al. Interferon alpha therapy in essential thrombocythemia and polycythemia vera—a systematic review and meta-analysis. *Leukemia*. 2021;35:1643–1660.

115. Bewersdorf JP, Giri S, Wang R, et al. Interferon therapy in myelofibrosis: systematic review and meta-analysis. *Clin Lymphoma Myeloma Leuk*. 2020;20:e712–e723.

116. Gu W, Yang R, Xiao Z, Zhang L. Clinical outcomes of interferon therapy for polycythemia vera and essential thrombocythemia: a systematic review and meta-analysis. *Int J Hematol*. 2021;114:342–354.