Interferon and myeloproliferative neoplasm eradication

J-J. Kiladjian[1,2]
S. Giraudier[2,3,4]
B. Cassinat[2,5]

[1]Hopital Saint-Louis, APHP, Centre d'Investigations Cliniques, Paris ; [2]INSERM UMR-S 1131, Institut Universitaire d'Hématologie, Université Paris Diderot, Paris; [3]Hopital Henri Mondor, APHP, Laboratoire d'Hématologie, Créteil; [4]UPEC, Faculté de Médecine, Créteil; [5]Hopital Saint-Louis, APHP, Service de Biologie Cellulaire, Paris, France

Correspondence:
Jean-Jacques Kiladjian
E-mail: jean-jacques.kiladjian@aphp.fr

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A B S T R A C T

Interferon alfa (IFN-α) has been used for over 30 years to treat myeloproliferative neoplasms (MPN). IFN-α was shown to induce clinical, hematologic, molecular and histopathological responses in small clinical studies. Such combined efficacy has never been achieved with any other drug to date. However, toxicity remains a limitation to its broader use and several ongoing phase III studies of pegylated forms with better tolerance *versus* hydroxyurea will help to define its exact place in MPN management. IFN-α efficacy is likely the consequence of a broad range of biological properties, including enhancement of immune response, direct effects on malignant cells, and ability to force dormant malignant hematopoietic stem cell to enter the cell cycle. Indeed, recent mice models suggested that long-term treatment with IFN-α might eradicate JAK2V617F disease initiating cells. However, comprehensive elucidation of its mechanism of action is still lacking. Persistence of responses after IFN-α discontinuation raised the hope that this drug could eradicate MPN. The molecular complexity of these diseases could contradict this hypothesis, since some mutations, like in the TET2 gene, could be associated with resistance to IFN-α. Therefore, combined therapy with another targeted agent could be required to eradicate MPN, and the best IFN-α companion for achieving this challenge remains to be determined.

Learning goals

At the conclusion of this activity, participants should know about:
- the clinical and hematologic efficacy of IFN-α therapy in patients with MPNs;
- the impact of IFN-α therapy on MPN mutated clones and on bone marrow histopathology;
- the possible mechanism of action of IFN-α against MPNs.

Introduction

Interferon alpha (IFN-α) has been considered as a potential drug of interest in MPN treatment for more than 30 years.[1-3] It was used only in rare situations since demonstration of a possibly curative effect has only recently been clarified; this was due to its higher rate of short-term adverse reactions compared to conventional therapies like hydroxyurea (HU), and also because IFN-α is not approved for Philadelphia-negative MPN therapy.[4-7] However, with the development of pegylated (PEG) forms of IFN-α that induce better tolerance than classical forms of IFN-α, and the demonstration that IFN-α was the only drug able to reduce the MPN tumor burden by our group and others,[8-11] the use of IFN-α became more widely considered for MPN therapy, and was even recently quoted in experts' recommendations as a possible first-line therapy for patients with polycythemia vera (PV) and for younger patients with essential thrombocythemia (ET) or during pregnancy.[12]

Despite clinical and biological evidence of efficacy, the mechanisms of action of IFN-α in MPN have still not been well characterized. There is a clear and direct pro-apoptotic effect on myeloid progenitors that has been demonstrated for a long time in CML as well as in classical MPN, however, other effects have not been reliably determined.[5,13-15] IFN-α was initially demonstrated to be able to induce immunological changes and to stimulate immune mechanisms, suggesting that immunological effect on cancer cells could be the effective way to cure such disorders.[16,17] This hypothesis was reinforced by the finding that bone marrow transplantation (BMT), and more particularly after donor lymphocyte infusion, was able to cure MPN CML being a paradigm for such an immunological action, but this effect was also demonstrated more recently in post BMT-relapsed primary myelofibrosis (PMF).[18,19]

Clinical studies

The first evidence for a possible eradication of MPN after IFN-α therapy emerged from clinical studies. A review of the literature identified around 390 PV and ET, respectively, and 100 PMF patients reported in clinical trials using IFN-α with more than 10 patients included.[4] In almost all PV and ET studies, IFN-α was able to rapidly normalize platelet counts and leukocytosis, and reduce erythrocytosis, allowing a reduction in the need for phlebotomies within a few months. In both diseases, an objective hematologic response was observed in approximately 80% of patients, including complete freedom from phlebotomies in PV in 60% of patients.[20] In addition, a specific effect of IFN-α against the

MPN clone was previously suggested by occasional studies showing reversion from monoclonal to polyclonal patterns of hematopoiesis (using X-chromosome inactivation pattern studies) in IFN-α-treated patients.[21-24]

More recently, two phase II clinical studies of PEG–IFN-α2a that included the analyses of the JAK2V617F allele burden evolution during therapy demonstrated clinical hematologic response rates of 79%-100%, with complete response observed in 54%-95% of patients.[8,9] After median follow-up times of 21 and 31 months, complete molecular response (undetectable JAK2V617F) was achieved by 14% and 24% of patients, respectively. In addition, an ongoing phase II clinical trial of PEG–IFN-α2b in patients with PV reported an 89% clinic-hematologic response rate after 18 months on treatment, with 47% of patients achieving a complete response.[10] In that study, 35% of patients experienced a molecular response. Altogether these three studies provide consistent evidence for a clear ability of IFN-α to reduce and eventually eradicate the JAKV617F clone, a property that has never been shown in such proportions with any other drug to date. However, disappearance of JAK2V617F does not necessarily mean cure of the MPN. It has been shown that, in some cases, molecular relapse can rapidly occur after IFN-α discontinuation.[25]

Beyond the *JAK2* mutation, several studies have also reported that cytogenetic aberrations could also be eliminated after IFN-α therapy.[10,21-24] In these patients in clinical hematologic response and complete molecular or cytogenetic response, bone marrow histopathology can also be reversed to normal.[26,27] Thus, simultaneous achievement of clinical, hematologic, molecular and pathological complete responses suggests that the malignant clone(s) could be eradicated in selected patients.

Such a hypothesis is further supported by the persistence of responses even after discontinuation of IFN-α therapy.[27,28] Two important ongoing prospective randomized clinical trials comparing pegylated forms of IFN-α to HU in high-risk PV patients should provide the critical information needed to determine the exact role of IFN-α in this setting (*clinicaltrials.gov identifiers: 01259856 and 01949805*).

The role of immunity

Surprisingly, despite the fact that the immunological hypothesis of IFN-α action was suggested in the early 1980s, only limited data are available on the role of the immune system in the effects of IFN-α in MPNs.[29,30] These effects were mainly studied in CML and very few studies are available in classical MPNs. Three major mechanisms were hypothesized, according to the target cell among immune effectors: an effect on T or B cells, an effect on natural killer (NK) cells, and an effect on dendritic cells.

We and others have previously demonstrated that the oncogenic abnormality observed in PV and PMF, i.e. JAK2V617F or MPLW515 mutations, could be found in the B- and NK-cell compartments but also in rare cases in the T-cell compartment in the blood or in T cells obtained after fetal thymus organ cultures derived from MPN CD34-positive cells.[31,32] This illustrates the fact that the driver mutations are present in the lympho-myeloid lineages and, therefore, affect stem cells.[33] In addition, JAK2 is known to play a role in T- and B-lymphocyte functions and the presence of mutations in these lineages could result in a modification of their function.[34,35] However, to date, no clear functional abnormality was reported in the T- or B-cell populations harboring the JAK2V617F mutation. In the same way, no data were reported in the literature about the effects of IFN-α on T- or B-lymphocytes in classical MPN.

Another immunological effect of IFN-α was suggested on dendritic cells (DC). DC studies in MPN were also performed in CML.[36] Briefly, in this disorder, as well as in classical MPN, it has been demonstrated that DC can present the driver mutation. In CML, a significant decrease in DC population has been observed that may result in impaired antigen presentation and anergy induction. There have been few studies of DCs in classical MPNs and these monocyte-derived cells were reported as polyclonal in some studies while they were found to harbor the JAK2V617F mutation in others.[5] However, *in vitro*, monocyte-derived mature DC from 7 PV patients had a phenotype comparable to that of healthy donors. It has been suggested that IFN-α treatment resulted in an increased capacity of DCs to stimulate T-cell activation.[37] A strengthened T-cell proliferation and increased IFN-γ production of CD4+ and CD8+ T cells stimulated with IFN-α-activated DCs was observed, demonstrating a restoration of the immunogenic capacity of tolerogenic DCs in the presence of IFN-α. Re-stimulation experiments revealed also that IFN-α treatment of tolerogenic DCs abolished the induction of T-cell anergy.

A third immunological mechanism involves NK-cell stimulation. Recently, the effect of IFN-α on persistence of complete molecular remission in CML as well as in classical MPN focused on the role of NK cells.[38] As reported by us and others, the NK-cell compartment may be affected by the oncogenic process, since JAK2V617F as well as MPL W515 mutations can be found in MPN NK cells.[31,34] However, the consequences of such mutations (when DNA is expressed) on NK-cell functions were not reported. First reports were established on CML analysis and indicate that IFN-α-treated CML patients in remission have increased numbers of NK cells, clonal $\gamma\delta^+$ T cells, and a unique plasma cytokine profile.[38] These factors may relate to anti-leukemic effects of IFN-α in this specific group of patients and could account for prolonged therapy responses even after drug discontinuation. Riley *et al.* demonstrated that classical MPN untreated patients have low levels of circulating NK cells compared to healthy donors or patients treated with hydroxyurea or IFN-α and present also an expansion of circulating CD56 bright natural killer cells when patients receive IFN.[39] The authors then speculated that IFN-α may alter effects on immune cells involved in immune surveillance and might consequently enhance anti-tumor immune response against the JAK2-mutated clone. But other studies reported that NK-cytotoxic activity, as well as the percentage of NK cells, is decreased in PMF and increased in PV.[40,41]

In all, it is still difficult to draw definitive conclusion about the role of immune effectors in MPN pathophysiology,[29] and it is not clear whether curative effects of IFN-α in MPNs could be, at least in part, immune mediated.

Targeting leukemic stem cells

Another hypothesis to explain how IFN-α could eradicate MPN and cure some patients emerged with the demonstration that IFN-α may target leukemic stem cells (LSC). Again, CML was a paradigm when tyrosine kinase inhibitors (TKI) were shown to be unable to target LSCs in this disease. Therefore, a currently well accepted hypothesis proposes that a combined therapy is necessary to cure patients: a first drug would force LSCs to enter the cell cycle and make them sensitive to TKIs that target cycling cells. In this model, IFN-α appears as a hematopoietic stem cell (HSC) cycling agent that could be the drug of choice to be used in combination with TKIs.

Essers *et al*. made the first report of IFN-α action on normal HSC cycle in 2009.[42] Briefly, the authors demonstrated that IFN-α was able to force dormant hematopoietic stem cells to enter the cell cycle leading to a dual action of IFN-α on hematopoietic cells: a proliferative effect on HSC and a pro-apoptotic effect on more mature progenitor cells. The authors suggested that these antagonistic effects were related to the acute *versus* chronic exposure to IFN-α. Pietras *et al*. recently clarified the relationship between the proliferative and suppressive effects of IFN-α during acute *versus* chronic exposure to the drug.[43] They demonstrated that the cell cycle entry due to IFN acute exposure was transient and that HSC re-enter into quiescence during chronic exposure. They showed that the cell-cycle machinery is not directly activated by IFN-α *in vivo* and, instead, that multiple quiescence-enforcing mechanisms are transiently antagonized and subsequently reactivated during chronic IFN-α exposure. However, whereas returning into quiescence protects IFN-α-exposed HSC from apoptosis, forcing them to circulate again in the constant presence of IFN-α results in their depletion and subsequent inability to maintain blood homeostasis.

Animal models have been used to demonstrate a specific effect of IFN-α on MPN leukemic stem cells. The more recently published studies used knock-in (KI) models[44,45] and suggested that IFN-α may eradicate JAK2V617F disease initiating cells in long-term treatment, suppressing overall the proliferative advantage of oncogenic over wild-type (WT) HSC. In both studies, the authors used chimeric mice, i.e. mice reconstituted with a mixture of JAK2V617F and JAK2-WT cells, in which an MPN is constantly induced after few weeks. In both models, treatment with IFN-α led to a reduction in the blood parameters. Extramedullary hematopoiesis was also considerably reduced after IFN-α therapy, as shown by the decrease in the spleen weight and the reduction in the numbers of splenic hematopoietic progenitors. In contrast, the bone marrow cellularity was increased in IFN-α-treated mice with myelofibrosis, suggesting a normalization of the hematopoiesis.

Regarding the specificity of IFN-α activity against the malignant MPN clone, the authors have shown that the proportion of mutant to WT cells was highly reduced in IFN-α-treated mice suggesting a preferential targeting of the mutated cells. This hypothesis was reinforced by the analysis of secondary recipients after IFN-α therapy. Indeed, JAK2V617F long-term hematopoietic stem cells (LT-HSCs) have been identified as the reservoir of disease-initiating cells *in vivo,*[46] and it is now well accepted that the capacity to transplant the disease in a secondary recipient reflects the stem cell malignant profile. In the two animal studies mentioned above, mice transplanted with cells issued from IFN-α-treated mice (secondary transplants) did not develop the MPN, suggesting that IFN-α has the potential to eradicate the disease initiating cells in JAK2V617F-driven MPN.[44,45] This effect was abolished when JAK2V617F cells knocked out for the IFN-α receptor ($Ifnar1^{-/-}$) were tested in chimeric mice, showing that this result was due to a direct effect of IFN-α on JAK2V617F cells.[45]

From these results obtained in KI mice, it has been hypothesized that IFN-α could directly enhance the JAK2V617F HSC cell cycling, leading to a preferential exhaustion of these disease-initiating populations and their long-term disease eradication. However, the two studies analyzing the effects of IFN-α at the level of LT-HSCs differed in their results.[44,45] Hasan *et al*. did not observe any increase in cell cycle entry of JAK2V617F mutant HSCs in IFN-α treated mice, but a pro-apoptotic effect of IFN-α in these cells. In contrast, Mullally *et al*. observed a significantly reduced proportion of quiescent HSCs (and increased proportion of cycling HSCs) in IFN-α-treated JAK2V617F mice. Interestingly, they also had the opportunity to study the more mature JAK2V617F progenitor compartments and observed an induction of apoptosis in erythroid precursors. These results suggest that an IFN-α effect on HSCs may not be its sole mechanism of action in MPN.

Pitfalls in current models

Altogether, murine models confirmed the anti-MPN potential of IFN-α observed earlier in clinical studies in humans. These "post-clinical" animal models only suggest some clues for the mechanism of action of this drug. The direct effect of IFN-α on stem cells is demonstrated in mice only, and one should consider that mice models do not recapitulate the complexity of human disease. For example, in murine models of JAK2V617F or MPLW515 mutants, induced diseases are polyclonal and the vast majority of the animals rapidly develop myelofibrosis, the latter point being observed only in few human patients.

Furthermore, because of species specificity, only murine IFN-α can be used to treat mice, an alternative being polyinosinic-polycytidylic acid [poly(I:C)], a synthetic analog of double-stranded RNA (dsRNA), a molecular trigger associated with viral infection. Poly(I:C) is recognized by TLR3, inducing the activation of NF-kB and the production of cytokines including IFN-α. Thus, neither standard recombinant IFN-α nor a long-lasting pegylated form of IFN-α (commonly used nowadays to treat MPN patients) have been used in mice studies which represents an important difference when murine models are used to mimic the patients' behavior. Human therapeutic forms of IFN-α could be tested *in vitro* only against human cells. It has been reported that both standard and pegylated recombinant forms of IFN-α can inhibit colony formation from JAK2V617F CD34 positive cells from MPN patients.[47] This inhibition of colony formation was always more pronounced in cultures of JAK2V617F progenitors compared to WT progenitors, underlining the preferential activity of IFN-α against cells with an increased JAK-STAT pathway activation. Such findings may explain the potential of this

drug to rapidly normalize the blood cell counts in humans. However, colony assays do not necessarily reflect the behavior of LT-HSCs, which are very difficult to study in humans. One recent study addressed the question of a possible difference in JAK2V617F mutated clone sensitivity according to the heterozygous or homozygous status of the mutation.[48] Using an allele-specific PCR combining the 46/1 haplotype with the JAK2V617F, Hasan *et al*. have shown that IFN-α may preferentially target homozygous compared to heterozygous clones. These results suggest that the preferential effect of IFN-α on mutated cells compared to normal cells observed in mice models is also modulated in human cells according to the JAK2V617F allele burden.

Conclusion

In conclusion, a number of clinical studies with IFN-α in MPN have resulted in promising results in terms of clinical and hematologic response, especially in younger patients with PV and ET. In addition, molecular (clear decrease of the JAK2V617F mutant allele burden) and histological responses have also been repeatedly reported in these patients, suggesting that eradication of MPN could be achieved with IFN-α. However, there is still no definitive evidence for a curative effect.

The mechanism of action of IFN-α has not been completely elucidated in MPN and this is a new scientific challenge. So far it seems clear that IFN-α present a direct pro-apoptotic effect on myeloid progenitors, and more particularly on erythroid progenitors in humans as well as in mice models. The second effect, not as convincingly demonstrated as the first, is an effect on leukemic-initiating cells that could consist in relaxation of multiple HSC quiescence-enforcing mechanisms or in a direct proliferative effect of IFN-α on stem cells. It is still questionable whether this mechanism (so far only reported in mice) can be observed in human cells. A third effect could be related to the immunological effects of IFN-α therapy. However, there is no clear demonstration of such mechanisms in human MPN, despite modifications of the immune compartments induced by this potent immunomodulator.

Despite these scientific uncertainties, the story of IFN-α in MPN therapy is ongoing with several clinical studies that are expected to better define its role in the management of MPN patients. Whether or not this drug may eradicate MPN is still a matter of speculation. The molecular complexity of these diseases and the potential impact of some mutation on the response to IFN-α suggest that combining IFN-α with another drug would probably be the only way to eradicate all malignant clones. In that perspective, we still need to find the best suitor…

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