Review article

The renaissance of interferon therapy for the treatment of myeloid malignancies

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IFNα has been used to treat malignant and viral disorders for more than 25 years. Its efficacy is likely the consequence of its broad range of biologic activities, including direct effects on malignant cells, enhancement of anti-tumor immune responses, induction of proapoptotic genes, inhibition of angiogenesis, and promotion of the cycling of dormant malignant stem cells. Because of the recent development of “targeted” therapies, the use of IFN has been dramatically reduced over the last decade. The increasing awareness of the multistep pathogenesis of many malignancies has suggested, however, that such an approach using target-specific agents is not universally effective. These observations have resulted in a number of recent clinical trials utilizing IFNα in patients with chronic myeloid leukemia (CML), systemic mast cell disease, hypereosinophilic syndrome and the Philadelphia chromosome-negative myeloproliferative neoplasms (MPN) with promising outcomes. These reports provide evidence that IFNα, alone or in combination with other agents, can induce surprisingly robust molecular response rates and possibly improve survival. Although IFNα at present remains an experimental form of therapy for patients with myeloid malignancies, these promising results suggest that it may become again an important component of the therapeutic arsenal for this group of hematologic malignancies.

Introduction

Almost 25 years after the approval of interferon-α (IFNα) for the treatment of patients with hairy cell leukemia, its use remains limited to a small numbers of patients with hematologic malignancies, a practice that is in contrast to its widespread use for the treatment of patients with a number of viral diseases. This restricted role of IFNα in the treatment of patients with hematologic malignancies can in part be attributed to limited patient tolerability and the recent availability of more effective chemotherapeutic agents such as the purine nucleoside analogs with which to treat patients with hairy cell leukemia, and tyrosine kinase inhibitors (TKI) to treat patients with chronic myeloid leukemia (CML). Furthermore, the promise of additional targeted small-molecule therapies for other forms of blood cancer has further dampened the enthusiasm for the use of IFNα. Recently, the potential limitations of relying solely on a single small molecule targeting a specific acquired genetic abnormality for the treatment of a number of cancers has become increasingly apparent. This change in strategy can be attributed to the development of resistance to TKIs and the growing understanding that the origins and progression of an increasing number of myeloid malignancies are likely the consequence not of a single but multiple genetic and epigenetic events acting in concert with a disordered tumor microenvironment. The administration of IFNα, which is associated with a broad range of therapeutic effects influencing multiple events affecting the biology of tumors, might, therefore, be valuable for use alone or in combination with other active drugs to effectively treat patients with several myeloid malignancies. Furthermore, the more recent availability of pegylated (peg) forms of IFNα with a more favorable toxicity and pharmacokinetic profiles has created renewed interest in the use of IFNα-containing regimens to treat patients with CML, the Philadelphia chromosome-negative myeloproliferative neoplasms (MPN), systemic mastocytosis, and the hypereosinophilic syndrome.

Mechanisms of Action of IFN

The mechanisms by which IFN therapy achieves therapeutic responses in patients with myeloid malignancies remains the subject of active investigation. The discovery of IFN more than 50 years ago was based on observations made concerning its role in antiviral innate immunity. IFN proteins are traditionally categorized as type 1 (α, β) or viral IFN and type 2 (γ) or immune IFN. Both type 1 and type 2 IFN exert their actions through cognate receptor complexes, IFNAR and IFNGR1 (CD119), respectively, which are present on the surface of cells belonging to numerous tissues. The genes that encode the type 1 IFNs are located on human chromosome 9, and the gene that encodes the single type 2 IFN is located on human chromosome 12. In addition, another family of IFNs, the type 3 IFNs, have been recognized and include 3 proteins, IFNA1, IFNA2, and IFNA3. Type 3 IFNs signal through the IFNα receptor, which has a specific ligand binding chain (IL-28R) and an IL-10R chain. The type 1 and type 3 IFNs have similar biologic activities and are illustrative of biologic redundancy among the members of the IFN system. The IFNα receptors are, however, not highly expressed by hematopoietic cells but act predominantly at epithelial surfaces. In this report, the type 1 IFNs will be discussed in greatest detail because recombinant forms of IFNα2 are exclusively approved to treat patients with myeloid malignancies.

The production of the type 1 IFNs is induced by viral infections whereas type 2 IFN production occurs in response to mitogenic or antigenic stimuli. Most types of virally infected cells are capable


The online version of this article contains a data supplement.

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of producing type 1 IFN, whereas type 2 IFN is synthesized exclusively by immune cells including natural killer cells, CD4+ T cells, and CD8+ T cells.1,15-17 A cell specialized for the production of large amounts of type 1 IFN, which is also necessary for NK cell–mediated killing of virally infected cells, has been recognized.19 These naturally occurring IFN-producing cells are characterized by their round appearance, eccentric nucleus, and abundant endoplasmic reticulum and are referred to as plasmacytoid dendritic cells (pDCs).19 These cells constitutively express IFN regulatory factors 7 and 8, which, once phosphorylated, drive the expression of the type I family of IFN genes, permitting the amplification of type 1 IFN production in response to single-stranded RNA and DNA viruses.17

IFNs are normally present at low levels in plasma, which presumably plays a role in antiviral and antitumor surveillance.17,20 The type 1 IFNs induce cell-autonomous antiviral immunity, whereas IFNγ acts predominately on macrophages to induce a microbicidal state against intracellular nonviral pathogens.17 The levels of the type 1 IFNs dramatically increase in response to viral infections.20 IFN inhibits several steps involved in viral multiplication by altering the synthesis of viral polypeptides.1,12 These antiviral effects have led to the successful use of IFN for the treatment of chronic hepatitis B and C, Kaposi sarcoma (human herpes virus 8), and genital warts (human papillomavirus).20 Although IFNγ has some antiviral activity, its structure differs from the type 1 IFNs.1,15-17 Patients with chronic granulomatous disease, a rare genetic disorder of superoxide generation, respond to therapy with IFNγ with increased superoxide generation resulting in fewer bacterial infections.21 Patients with severe congenital osteopetrosis have decreased bone resorption associated with defective leukocyte superoxide generation.22 IFNγ therapy in these patients also results in a reduced incidence of infections as well as a reduction in trabecular bone mass.22 IFNγ-1b is currently approved for the treatment of patients with these 2 genetic disorders.21,22

IFNα binds to a cell-surface complex consisting of 2 transmembrane type 1 IFN receptor subunits, IFNAR1 and IFNAR2, which are members of the class II cytokine receptor superfamily.16,17,23 The genes for these receptors are clustered on chromosome 21. IFNAR2 along with IFNAR1 form a high-affinity receptor complex that mediates the activities of the IFNα.16,17 IFNAR2 has a 1000-fold greater affinity for type I IFNs than IFNAR1. IFNAR1 appears to be primarily a signal-transducing unit whereas IFNAR2 mediates both ligand binding and signaling.1,5,17 There are 3 isoforms of IFNAR2: IFNAR2a, a soluble receptor; IFNAR2b, a short transmembrane form; and IFNAR2c, a long transmembrane form. IFNAR2b is usually expressed at lower levels than IFNAR2c and may exert a dominant negative effect on IFNα signaling by binding ligand but not transducing signal.16,17 IFN receptor lack tyrosine kinase activity and rely on Janus kinases (JAK) for their phosphorylation, and signal-transducing molecules such as STATs for the transmission of intracellular messages.17 IFNAR1 is constitutively associated with tyrosine kinase 2 (Tyk2) and IFNAR2c is associated with JAK1.17 The interaction of IFNα with these receptors results in their rearrangement, dimerization of their subunits followed by autophosphorylation, and activation of JAK proteins forming 2 transcriptional complexes. One complex is a homodimer of activated STAT1 that binds a distinct promoter sequence, the γ IFN–activated sites (GAS).16,17 The importance of GAS for IFNGR signaling is well established, whereas its importance for IFNα responses remains obscure.24 The other transcriptional complex results in the recruitment of STAT1 to receptor-bound STAT2 and the formation of STAT1-STAT2 heterodimers.16,17 These STAT1-STAT2 heterodimers associate with the IFN regulatory factor 9 (IRF9, p48) to form a heterotrimeric complex termed IFN stimulated gene (ISG) factor 3 (ISGF3).16,17 ISGF3 complexes migrate into the nucleus and bind to specific elements, termed IFN stimulated response elements (ISRE), which are located along the promotor region of the IFN-regulated genes leading to their transcription.16,17 Expression of the antiproliferative and proapoptotic genes associated with IFNα signaling are mainly under the control of the ISGF3 complex (STAT2 and IRF9).24,25 Further diversity of IFNAR signaling is achieved by activation of other pathways including other STAT and non-STAT proteins. Such alternative signaling pathways include CrKL, Rap1, MAP kinases, VAV, and PI3-kinases.16,25,26 IFNα can elicit multiple biologic functions depending on its binding affinity to receptors, the receptor composition, and the accessory molecules expressed by different cell types.27

Several biologic processes affected by IFNα have been shown to contribute to the therapeutic effectiveness of IFNα against a number of hematologic malignancies. These processes are discussed below.

The effects of IFNα on immune modulatory cells

A role for immune effector cells in mediating the antitumor responses associated with the IFNs was first suggested based on studies using a number of mouse models.1,15,28,29 IFNα augments the functional activity of T cells, macrophages, and natural killer cells, which may cooperate to target a number of malignancies including CML.29-31 IFNα induces dendritic cell differentiation of CML mononuclear cells both in vitro and in vivo. These dendritic cells serve as antigen-presenting cells for CML-specific peptides.30,31 This is in contrast to imatinib, which inhibits T-cell and dendritic cell activation providing evidence that these 2 drugs likely act against CML cells by different yet possibly complementary mechanisms.34 The dendritic cells that are favored by the IFNs are endowed with an ability to capture apoptotic bodies and promote CD8+ T-cell cross-priming, which may provide a possible explanation for the autoimmune reactions and antitumor effects associated with IFN therapy.31

IFN therapy also increases the expression of tumor-associated antigens and major histocompatibility complex antigens.28,31,35 Some of these gene products have been thought to be candidates for eliciting therapeutic immune responses. Major histocompatibility class I antigens present peptides to T cells. Proteinase 3 (P3) or myeloblastin is a myeloid tissue restricted serine protease that is abundantly expressed in the azurophilic granules of normal and leukemic myeloid cells.32,36-39 IFNα induces P3 expression by mononuclear cells, and increased P3 expression levels in CML cells has been shown to be associated with a favorable patient prognosis.33 Imatinib, by contrast, down-regulates the expression of P3.33 IFNα also promotes the maturation of monocytes to antigen-presenting cells with T-cell costimulatory potential, which likely contribute to the generation of PR1 cytotoxic T lymphocytes (CTL).33,36-38 CTLs specific for PR1, an HLA-A1-restricted peptide that is derived from P3 and neutrophil elastase, have been observed in virtually all CML patients who respond to IFNα therapy, although such CTLs are not detected in nonresponders and in most patients in remission following imatinib therapy.33,36-38 PR1-CTLs are capable of killing CML hematopoietic progenitor cells (HPCs).39 Burchert et al have demonstrated that these antileukemic PR1- CTLs persist in CML patients who continue to receive maintenance IFNα therapy after imatinib therapy is interrupted.32 Furthermore,
Kanodia and coworkers have reported that the numbers of PR1-CTLs are increased in CML patients who remain in complete cytogenetic remission after IFNα therapy is discontinued. This functional immunity can be eliminated by CML cells, which overexpress P3 by inducing deletion of high-avidity PR1-CTLs. IFNα treatment leads to continued proliferation of central memory T cells resulting in the expansion of the PR1-CTL compartment, which produces IFNγ following stimulation with PR1 peptide. Relapse in CML patients has been reported to be associated with the inability of PR1-CTLs to continue to produce IFNγ. Because imatinib and IFNα appear to affect CML cells by different mechanisms, regimens employing concurrent or sequential therapy of these agents have been evaluated to determine whether combination therapy might eliminate CML hematopoietic stem cells (HSC) by both cytotoxic and immunologic mechanisms.

The importance of immune effects associated with the therapeutic effectiveness of IFNα has also been reported in polycythemia vera (PV) patients who have achieved clinical and molecular responses following IFNα therapy. Expression of novel antigens by PV cells that are capable of eliciting a potent humoral immune response has been associated with the achievement of molecular remissions. IFNα therapy has been reported to up-regulate a putative tumor antigen termed MPD6, which is encoded by a cryptic open reading frame located in the 3’-untranslated region of myotrophin mRNA by a novel internal ribosome entry site upstream of the MPD6 reading frame. Furthermore, type 1 IFNs also enhance antibody responses to soluble antigens and promote immunoglobulin class switching.

**IFNα induces the expression of proapoptotic genes**

IFNα promotes apoptosis of a variety of tumor cell types. The induction of apoptosis by IFNα is frequently delayed (by 48 hours), indicating that this effect involves the activation of a number of ISGs that mediate this response. Gene-expression studies have identified more than 15 ISGs with proapoptotic functions. Although these ISGs alone are probably not sufficient to induce apoptosis, their cumulative effects likely result in apoptosis. These mediators of apoptosis include caspase 4, caspase 8, tumor necrosis–related apoptosis-inducing ligand (TRAIL), Fas/CD95, the X-linked inhibitor of apoptosis (XIAP), death-activating protein kinases, IFN regulatory factors, dsRNA-activated protein kinase, and PML (acute promyelocytic leukemia gene). The induction of caspase 8 and caspase 4 by IFN may sensitize tumor cells to death receptor (TRAIL and Fas L)-mediated apoptosis. The ability of IFN to induce apoptosis is independent of cell-cycle arrest, the presence of wild-type p53, or expression of Bcl-2 family members but appears to always involve the FAS-associated death domain (FADD)/caspase 8 signaling, activation of the caspase cascade, which leads to disruption of mitochondrial potential and eventual DNA fragmentation.

Crosstalk between IFN activation of the P3K/mTOR pathway and the JAK/STAT pathways have been shown to play a role in IFNα-induced apoptosis. Furthermore, there has been conflicting evidence as to the role that STAT1 and STAT2 play in IFN-induced apoptosis. It appears most likely that the P3K and JAK-STAT pathways act independently of each other in mediating IFNα-induced apoptosis, and that the particular pathway operating is dependent on the target cell type. Several of these pathways may actually act together to contribute to IFNα-induced apoptosis of tumor cells.

**Interferon is an inhibitor of angiogenesis**

The anti-angiogenic properties of type 1 IFN has been attributed, in part, to inhibition of basic fibroblast growth factor, IL-8, or vascular endothelial cell growth factor gene expression. In addition, IFNα directly impairs endothelial cell proliferation and migration. Furthermore, IFNα can also act by up-regulating the production of the angiostatic chemokines, CXCL9, CXCL10, and CXCL11. Whether these properties contribute to the effects of the IFNs against human hematologic malignancies has yet to be determined. Collectively, these studies suggest that IFNα therapy may also be altering the microenvironment of tumors, thereby contributing to the achievement of therapeutic responses.

**Interferon suppresses the proliferation of hematopoietic progenitor cells and promotes the cycling of hematopoietic stem cells**

Type 1 IFNs suppress the ability of normal human HPCs to form colonies in vitro in the presence of a variety of cytokine combinations or conditioned media. IFNα acts directly against HPCs, as this inhibitory activity has been documented using CD34+ cell populations. This inhibitory role of IFNα has been shown to synergize with the inhibitory activities of IFNγ, which is likely produced by marrow auxiliary cells in response to IFNα. CML and PV CD34+ cells are more sensitive to the inhibitory effects of IFNα than normal HPCs. CML CD34+ cells are characterized by greater expression of IFNAR2 but lower expression of IFNAR1. The greater degree of expression of IFNAR2 by CML CD34+ cells has been shown to correlate with a clinical response to IFNα. Peschel and coworkers have also reported that the myelosuppressive effects of IFNα could also be in part attributed to the inhibition of the paracrine production of several stimulatory hematopoietic growth factors including GM-CSF and IL-11 and inhibition of the production of IL-1 receptor antagonist by marrow stromal cells.

**IFNα therapy of hepatitis patients is frequently complicated by dose-limiting thrombocytopenia.** This has led to a more careful examination of the effects of IFNα on normal megakaryocyte development. IFNα directly inhibits colony forming unit-megakaryocyte proliferation and differentiation by blunting the JAK/STAT signaling responses that occur in response to thrombopoietin (TPO), and impairs TPO-induced intracellular signaling by up-regulating SOCS-1 expression. IFNα reduces TPO-induced activation of the TPO receptor, MPL, as well as phosphorylation of JAK2, JAK3, and STAT5. Furthermore, IFNα directly inhibits cytoplasmic maturation and platelet production by megakaryocytes but not the proliferation of megakaryocyte progenitor cells or endomisiosis of human megakaryocytes. These dramatic effects of IFNα on thrombopoiesis likely account for the effectiveness of IFNα in treating patients with MPN with extreme degrees of thrombocytosis.

The ability of type 1 IFNs to inhibit HPC proliferation and maturation has been documented to occur independently of the STAT pathway. Type 1 IFNs activate the p38 MAPK, a proline-directed serine/threonine kinase that is required for ISG transcription through IRSE. The pharmacologic blockade of p38 in IFN-treated CML and PV HPCs reverses the inhibitory effects of IFNα. The inhibitory activities of type 1 IFN against normal and malignant HPCs involve several additional downstream signaling pathways including the Crkl adaptor protein, the MAPK-interacting kinase 1 (Mnk), a MAPK-regulated kinase that phosphorylates the eukaryotic initiation factor 4e, and the Schlafen family of proteins that include several members which regulate cell-cycle...
progression and growth arrest.\textsuperscript{61,62} The relative contribution of each of these pathways to the antiproliferative effects of IFN\textalpha on HPC requires further investigation. P38 MAP kinase activation by IFN\textalpha has been reported to promote apoptosis of PV CD34\textsuperscript{+} cells.\textsuperscript{49} Activation of p38 MAP kinase results in mitochondrial translocation of the proapoptotic protein Bax, leading to the induction of apoptosis.\textsuperscript{63}

Recently 2 groups have independently reported that IFN\textalpha has a surprising effect on murine marrow HSCs.\textsuperscript{66,67} High levels of IFN\textalpha induce murine HSCs to exit from a normally quiescent state and to transiently proliferate.\textsuperscript{66,67} In these studies, the HSC proliferative response was not observed in HSCs that lacked IFNAR or STAT1. However, HSCs that lacked the IFNAR did respond to IFN\textalpha if they were mixed with wild-type cells, suggesting that other cytokines such as IFN\gamma that are elaborated by marrow auxiliary cells may be responsible for this biologic activity. In support of this hypothesis is the recent report that IFN\gamma is also capable of inducing HSC cycling.\textsuperscript{68} The effect of IFNs on HSC cycling appears to occur by a pathway that is distinct, from which mediates their antiproliferative effect on HPC. Essers et al reported that STAT1 was required for the IFN\textalpha-mediated exit of HSC from dormancy, suggesting that this effect is mediated by canonical type 1 IFN signaling.\textsuperscript{66} It remains uncertain whether human HSCs respond in a similar fashion to IFN\textalpha, and clonal stem cells from MPN patients cycle at a higher rate than the small reservoir of normal HSCs that persist in such patients. If MPN HSCs are characterized by such a proliferative advantage, this might serve as an additional rationale for the use of combined modality therapy with IFN\textalpha and a TKI to treat patients with MPNs.\textsuperscript{69} These effects of IFN\textalpha on MPN HSCs are of potential importance because imatinib therapy alone does not affect the fate of CML stem cells, which likely accounts for the persistence and relapse that frequently occur following the cessation of TKI therapy.\textsuperscript{70}

Current uses of interferon in the treatment of myeloid malignancies

IFN\textalpha has been shown to have therapeutic activity against a broad variety of both myeloid and lymphoid neoplasms. As previously mentioned, the availability of several effective treatment options (such as imatinib in CML–chronic phase\textsuperscript{71}) has relegated the uses of IFN\textalpha to being mainly that of a second-line therapeutic option. Disorders in which IFN\textalpha has been found to be therapeutically active but where its use has been dramatically curtailed include patients with multiple myeloma\textsuperscript{72,73} and hairy cell leukemia.\textsuperscript{74} However, it is particularly in myeloid neoplasms where the greatest activity is being demonstrated.

The experience with targeted therapies has, however, demonstrated that (1) targeted therapeutics are sometimes active but not always capable of inducing complete eradication of malignant clone and that (2) there remains a subset of patients in whom such therapy is not sufficiently effective. Given the historical knowledge of IFN\textalpha therapeutic activity in many of these disorders, clinical investigators are now re-evaluating its use in conjunction with targeted agents or as maintenance therapy after remission has been obtained. Areas of potential benefit showing incremental benefits are CML, systemic mast cell disease, hypereosinophilic syndromes, and finally classic MPN (PV and essential thrombocytopenia [ET]; Table 1).\textsuperscript{32,41,46,75-79}

The role of interferon therapy in CML

The therapy of CML has evolved significantly over the past 2 decades. IFN\textalpha treatment had a significant impact on the outcome of patients with CML.\textsuperscript{30} Before the availability of imatinib, IFN\textalpha was used in high doses and was associated with the achievement of hematologic and even molecular remissions in individuals with CML; however, significant toxicities were frequently encountered including depression and neurologic disturbances. The development of targeted therapy with imatinib was a watershed moment in the history of cancer treatment, and imatinib was found to be superior for achieving cytogenetic and molecular responses and prolonging event-free survival as compared with IFN\textalpha (in combination with low-dose Ara-C).\textsuperscript{81} The subsequent development of additional TKIs including dasatinib\textsuperscript{82} and nilotinib\textsuperscript{83} has further impacted the utilization of IFN\textalpha. However, the long-term follow-up of participants in the IRIS study continue to show that not all patients remain in long-term molecular remissions with imatinib therapy and that as many as one-quarter to one-third of patients become resistant or intolerant.\textsuperscript{84} Given these findings, there has been increased interest in reincorporating IFN\textalpha into treatment strategies for CML patients based on the rationale previously discussed and the clinical outcomes of patients treated with combined modality therapy. Utilization of IFN\textalpha is now being investigated in 3 clinical settings in patients with chronic phase (CP) CML: combination therapy including a TKI and IFN\textalpha, as maintenance after TKI therapy, and during pregnancy.

Combination therapy with IFN\textalpha and imatinib in CML

A seminal phase 3 randomized multicenter clinical trial of 636 patients was recently completed in France using 2 different forms of combination therapy in CML patients.\textsuperscript{81} The outcomes of imatinib mesylate alone at doses of 400 mg/d or 600 mg/d were compared with a combination regimen of imatinib plus cytarabine (20 mg/m\textsuperscript{2}d, days 15-28) or a second combination regimen of imatinib plus pegylated (peg)-IFN\textalpha-2a (90 µg weekly). The end points of this study were based on data that showed that CML patients receiving imatinib therapy who underwent monitoring of the BCR-ABL transcript burden by real-time quantitative polymerase chain reaction and had a reduction of 3log\textsubscript{10} or more of BCR-ABL transcripts had a negligible risk of disease progression.

### Table 1. Uses of interferon-\textalpha (alone or in combination) for myeloid malignancies in 2011

<table>
<thead>
<tr>
<th>Disease</th>
<th>Situation</th>
<th>Interferon use (+ combination)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Chronic myeloid leukemia</td>
<td>Chronic phase:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. Combined with imatinib as front line therapy</td>
<td>Imatinib</td>
<td>Preudhomme et al\textsuperscript{41}</td>
</tr>
<tr>
<td></td>
<td>2. Maintenance after imatinib plus interferon</td>
<td>Interferon-\textalpha alone</td>
<td>Burchert et al\textsuperscript{32}</td>
</tr>
<tr>
<td>Systemic mast cell disease</td>
<td>Aggressive systemic mast cell disease (ASM)</td>
<td>Single agent</td>
<td>Kuin-Nelemans et al,\textsuperscript{75} Lim et al\textsuperscript{78}</td>
</tr>
<tr>
<td>Myeloproliferative neoplasms</td>
<td>Polycythemia vera and essential thrombocytopenia</td>
<td>Single agent</td>
<td>Quintas-Cardama et al,\textsuperscript{46} Kiladjian et al\textsuperscript{76}</td>
</tr>
<tr>
<td>Hypereosinophilic syndrome</td>
<td>Resistance to steroids, hydroxyurea, and TKI-insensitive</td>
<td>Single agent</td>
<td>Butterfield and Gleich,\textsuperscript{35} Ceretelli et al\textsuperscript{26}</td>
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This degree of reduction in BCR-ABL transcripts was considered to be a major molecular response. A superior molecular response was defined as a 4-log₁₀ reduction in BCR-ABL transcripts. The independent safety and monitoring board for this study recommended that patient enrollment in the groups receiving 600 mg of imatinib alone and imatinib plus cytarabine be stopped because of the superiority of the imatinib plus peg-IFNα-2a therapy and the toxicity associated with the administration of cytarabine. Compared with imatinib alone at the 2 doses tested or imatinib in combination with cytarabine, treatment with imatinib plus peg-IFNα-2a was associated with a significantly higher rate of major and complete molecular responses. To achieve these rates of molecular responses, combination therapy for more than 12 months was required, which was frequently difficult because of toxicity of peg-IFNα-2a, leading these investigators to reduce the dose of peg-IFNα-2a. More impressive was the doubling of the number of patients with undetectable residual disease in the group of patients receiving the combination of imatinib and peg-IFNα-2a compared with patients randomized in the other treatment arms. By contrast, the use of second-generation TKIs (nilotinib and dasatinib) has been associated with lower rates of major molecular remissions after 12 months of therapy than those reported with imatinib plus peg-IFNα-2a. Whether these superior molecular responses achieved with the combination of imatinib plus peg-IFNα-2a will significantly improve long-term survival or reduce the rate of evolution to blast crisis will require further study.

Interferon as maintenance therapy in CML-CP

Although cessation of imatinib does not necessarily results in relapse in patients with CP CML, recent studies suggest that likely up to 61% of patients will relapse even after achieving an initial complete molecular response. A pilot study performed in Germany focusing individuals who had received combination imatinib and IFNα-2a therapy for a median of 2.4 years and then received IFNα-2a as maintenance therapy showed that 75% (15 of 20) of patients remained on complete molecular remission on IFNα-2a alone after discontinuing imatinib. In addition, the number of individuals with a complete molecular response increased from 2 to 5 after 2 years of IFNα-2a maintenance therapy. The 5 individuals who relapsed off imatinib restarted imatinib and were able to again achieve a molecular remission. The response observed with IFNα has been attributed to the induction of a proteinase-3–specific CTL response as previously discussed. However, because second-generation TKIs may be superior to imatinib for front-line therapy, the next step of evaluating IFNα in CML may require combinations with second-generation TKIs.

Interferon use during pregnancy in CML-CP and other MPNs

The uses of TKIs are believed to be relatively contraindicated during pregnancy because of a small but real risk of teratogenicity. IFNα has never been tested in randomized trials, nor approved by the Food and Drug Administration for use during pregnancy. With those reservations aside, pregnant women with CML, particularly after the first trimester, have successfully been managed by an approach of selective leukapheresis and IFNα. In Philadelphia chromosome–negative MPNs, current guidelines recommend the use of IFNα when cytotherapy is indicated during pregnancy. Although the drug is not approved for this indication, many case reports have shown that its use is safe for both the mother and the fetus.

Systemic mast cell disease and hypereosinophilic syndrome

Aggressive systemic mast cell disease represents an MPN where the degranulation of mast cells leads to damage to a variety of end organs. IFNα has been shown to be quite effective in individuals with imatinib-insensitive disease (53% overall response rate, 18% complete responses), improving symptoms because of mast cell degranulation, bone marrow mast cell infiltration and ameliorating mastocytosis-related ascites and hepatosplenomegaly, cytopenias, skin changes, and osteoporosis. The use of IFNα for systemic mast cell disease should, however, be restricted to individuals with aggressive disease, lacking an imatinib-sensitive target, and likely having failed therapies such as corticosteroids.

Similarly, IFNα has been shown to be effective in suppressing the aberrant proliferation of eosinophils observed in patients with hypereosinophilic syndromes. However, the published literature includes the experience with a limited number of patients. Presently, IFNα therapy in this setting should likely be limited to patients refractory to steroids and hydroxyurea and who are not responsive to TKI therapy.

Myeloproliferative neoplasms: PV, ET, and PMF

In the United States, the pioneering study of Silver in 1988 documented the clinical effectiveness of IFNα in controlling erythrocytosis as well as pruritus and other constitutional symptoms in PV patients, while Austrian and French groups reported during the same period evidence that IFNα was also effective in reducing thrombocytosis in ET patients. A number of clinical trials have been performed subsequently, using several different commercial preparations of IFN which are summarized in Tables 2-4. Recent reviews of the literature found more than 400 PV and ET patients and more than 100 primary myelofibrosis (PMF) patients reported who participated in clinical trials. A meta-analysis of these studies has proven problematic. First, various forms of IFN were used, and one cannot exclude the possibility that each of these preparations of IFN might have different effects that might influence response rates and/or disease evolution. Furthermore, heterogeneous response criteria were used to evaluate efficacy. The recently proposed response criteria for use in MPNs clinical trials provided by the European LeukemiaNet might be helpful to avoid such dilemma in the future.

In almost all PV and ET trials, IFNα therapy rapidly normalized platelet numbers and corrected the degree of leukocytosis and erythrocytosis, allowing reduction in the requirement for phlebotomies within a few months (Tables 2-3). In both diseases, an objective hematologic response was observed in about 80% of patients, including complete freedom from phlebotomies in PV in 60% of patients. In addition, IFNα was also able to reduce PV-associated pruritus in a significant number of cases, and appears to be an effective drug for this purpose. However, toxicity associated with IFNα therapy was not trivial, leading to the discontinuation of treatment in almost one-quarter of patients.

In contrast to trials of PV and ET, the clinical trials of IFNα therapy in PMF have been disappointing, although in vitro data suggested that IFNα might be effective in correcting bone marrow fibrosis (PMF clinical trials that included more than 10 patients are listed in Table 4). Virtually no objective response were observed, and toxicity in these studies was much higher than that recorded in PV or ET patients, leading to the rapid discontinuation of treatment (usually after 3-6 months) in more than 50% of the patients. One of the major limiting toxicities of IFNα therapy in PMF was the worsening of cytopenias. However, the French Intergroup of MPN
(FIM) recently reported promising results with an acceptable degree of toxicity in PMF using peg-IFN-α-2a. This retrospective study requires confirmation in a larger cohort of patients. Such results with peg-IFN-α-2a confirm the earlier findings with low-dose standard IFN, suggesting that IFN-α therapy should be considered as an effective therapy of PMF in the hypercellular phase of the disease (reviewed in Hasselbalch et al99).

Recently, investigators have performed 2 independent phase 2 studies using peg-IFN-α-2a in PV and ET46,78 and showed similarly impressive hematologic response rates compared with standard IFN-α, but with less associated toxicity (less than 10% of patients discontinued therapy during the first year of therapy). In addition, these studies showed for the first time evidence of significant molecular responses as documented by a clear reduction in the JAK2V617F allele burden following IFN-α treatment.45 A specific effect of IFN-α against the MPN clone had previously been suggested by occasional studies showing reversion from monoclonal to polyclonal patterns of hematopoiesis (based on X-chromosome inactivation pattern studies) or disappearance of a marker chromosomal abnormality present before treatment.103-106

The discovery of the JAK2V617F mutation provides a tool with which to measure molecular response to a variety of therapeutic approaches in MPN, an end point not available before the discovery of this mutation in 2005. Overall, the 2 clinical trials with peg-IFN-α-2a46,78 showed a meaningful and progressive reduction in the JAK2V617F allele burden in about 70% of PV and 40% of ET patients. Importantly, the JAK2V617F mutation became undetectable (with 1% sensitivity PCR assays) in 24% of PV patients in the French “PVN-1” study after about 3 years’ median follow-up, and in 14% and 6% of PV and ET patients, respectively, in the US

### Table 2. Clinical trials of interferon in polycythemia vera

<table>
<thead>
<tr>
<th>First author, year</th>
<th>No. of patients</th>
<th>Reduction of PHL, n (%)</th>
<th>Freedom from PHL, n (%)</th>
<th>Discontinuations, 1st year, n (%)</th>
<th>Discontinuations, total</th>
<th>Type of IFN</th>
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<td>9 (82)</td>
<td>5 (45)</td>
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<td>NA</td>
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<tr>
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<td>4 (31)</td>
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</tr>
<tr>
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<td>11 (85)</td>
<td>11 (85)</td>
<td>3 (23)</td>
<td>NA</td>
<td>α2a</td>
</tr>
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<td>Turri, 1991</td>
<td>11</td>
<td>7 (64)</td>
<td>4 (36)</td>
<td>0</td>
<td>0</td>
<td>α2a</td>
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<td>8 (73)</td>
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<tr>
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<td>21 (95)</td>
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<td>Muller, 1995</td>
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<td>NA</td>
<td>4 (27)</td>
<td>6 (40)</td>
<td>α2b</td>
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<tr>
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<td>9 (53)</td>
<td>2 (12)</td>
<td>6 (35)</td>
<td>NA</td>
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<td>11 (29)</td>
<td>11 (29)</td>
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<td>α2a</td>
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<tr>
<td>Gilbert, 1998</td>
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<td>2 (11)</td>
<td>α2b</td>
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<td>Radin, 2003</td>
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<td>53 (96)</td>
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<td>8 (14)</td>
<td>α2a, α2b</td>
</tr>
<tr>
<td>Silver, 2006</td>
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<td>79 (78)</td>
<td>49 (44)</td>
<td>7/3 (30)</td>
<td>peg-α2b</td>
<td>NA</td>
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<tr>
<td>Samuelsson, 2006</td>
<td>37</td>
<td>37 (100)</td>
<td>36 (97)</td>
<td>3 (8)</td>
<td>13 (35)</td>
<td>peg-α2a</td>
</tr>
<tr>
<td>Quintas-Cardama, 2009</td>
<td>40</td>
<td>32 (80)</td>
<td>29 (70)</td>
<td>10%</td>
<td>22%</td>
<td>peg-α2a</td>
</tr>
</tbody>
</table>

For complete references, see supplemental Table 2 (available on the Blood Web site; see the Supplemental Materials link at the top of the online article).

PHL indicates phlebotomy; IFN, interferon; NA, data not available; and hl-IFN, human leukocyte interferon.

### Table 3. Clinical trials of interferon in essential thrombocytopenia

<table>
<thead>
<tr>
<th>First author, year</th>
<th>No. of patients</th>
<th>Response rate, %</th>
<th>Discontinuation, n (%)</th>
<th>Type of IFN</th>
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</thead>
<tbody>
<tr>
<td>Giles, 1988</td>
<td>18</td>
<td>100%</td>
<td>0</td>
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</tr>
<tr>
<td>Bellucci, 1988</td>
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<td>NA</td>
<td>4 (33)</td>
<td>α2a</td>
</tr>
<tr>
<td>Gugliotta, 1989</td>
<td>10</td>
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<td>α2a</td>
</tr>
<tr>
<td>Lazzarino, 1989</td>
<td>26</td>
<td>88</td>
<td>9 (35)</td>
<td>α2b</td>
</tr>
<tr>
<td>Giralt, 1991</td>
<td>21</td>
<td>69</td>
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<td>α2b</td>
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<tr>
<td>Gisslinger, 1991</td>
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<td>85</td>
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<td>Sacchi, 1991</td>
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</tr>
<tr>
<td>Turri, 1991</td>
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<td>α2a</td>
</tr>
<tr>
<td>Seewann, 1991</td>
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<td>80</td>
<td>6 (30)</td>
<td>α2b</td>
</tr>
<tr>
<td>Kasparu, 1992</td>
<td>14</td>
<td>86</td>
<td>0</td>
<td>α2b</td>
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<tr>
<td>Rametta, 1994</td>
<td>25</td>
<td>92</td>
<td>NA</td>
<td>α2b</td>
</tr>
<tr>
<td>Berte, 1996</td>
<td>12</td>
<td>83</td>
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<td>α2a and α2b</td>
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<tr>
<td>Sacchi, 1998</td>
<td>11</td>
<td>100</td>
<td>1 (9)</td>
<td>α</td>
</tr>
<tr>
<td>Radin, 2003</td>
<td>17</td>
<td>88</td>
<td>NA</td>
<td>α2</td>
</tr>
<tr>
<td>Alvarado, 2003</td>
<td>11</td>
<td>100</td>
<td>2 (18)</td>
<td>peg-α2b</td>
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<tr>
<td>Saba, 2005</td>
<td>20</td>
<td>75</td>
<td>3 (15)</td>
<td>α2a</td>
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<tr>
<td>Langer, 2005</td>
<td>36</td>
<td>75</td>
<td>13 (36)</td>
<td>peg-α2b</td>
</tr>
<tr>
<td>Samuelsson, 2006</td>
<td>21</td>
<td>70</td>
<td>11 (55)</td>
<td>peg-α2b</td>
</tr>
<tr>
<td>Jabbour, 2007</td>
<td>13</td>
<td>70</td>
<td>NA</td>
<td>peg-α2b</td>
</tr>
<tr>
<td>Quintas-Cardama, 2009</td>
<td>39</td>
<td>81</td>
<td>NA</td>
<td>peg-α2a</td>
</tr>
</tbody>
</table>

For complete references, see supplemental Table 3.

IFN indicates interferon; and NA, data not available.
study after about 2 years’ median follow-up. Combining both studies, 12/64 (19%) of PV patients achieved a molecular complete response (MCR). Of note, each of these MCRs occurred after the 12th month of treatment whereas hematologic responses occurred within a few weeks. Prolonged exposure (>12 months) to peg-IFNα-2a seems to be an important factor in achieving MCR. The reduction in the JAK2V617F allele burden was not influenced by the cumulative dose of peg-IFNα-2a in the PVN-1 study, but toxicity was clearly dose-dependent in the US study. Taken together, these results indicate that peg-IFNα-2a therapy is best initiated at very low doses that are gradually increased until hematologic response is achieved; this strategy avoids cessation of therapy and allows sufficient exposure to the drug to occur to allow for the achievement of a molecular response. These results are in agreement with studies from Denmark showing major molecular responses in PV after long-term treatment with IFNα-2b, that could persist after IFNα was discontinued.107 Finally, 5/7 patients who achieved MCR in the PVN-1 study have maintained this response after peg-IFNα-2a was discontinued (up to 30 months after discontinuation), with none experiencing hematologic relapse (J.-K.K., personal unpublished data). Such long-term clinical and molecular responses after the discontinuation of treatment have previously been reported after IFNα therapy in MPN patients,107,108 an effect that has not been reported with other currently available therapies.

It is important to emphasize that the elimination of JAK2V617F in this setting is not necessarily indicative of cure of the MPN, and that clinical implications of these molecular responses remain uncertain and require validation. It has been shown that molecular relapse can rapidly occur after IFNα discontinuation in some cases.109 In one patient with a biclonal JAK2V617F/TET2-mutated PV, peg-IFNα-2a treatment did not affect the TET2-mutated cells while the JAK2V617F clone was eradicated.110 Nevertheless, prolonged periods of time (up to 40 months, personal unpublished data) during which the patient remained in complete hematologic remission without any cytotherapeutic intervention after peg-IFNα-2a withdrawal was observed in several patients of the PVN-1 study, even though low levels of the JAK2V617F allele (around 5%) were still detected. Such results suggests that despite the disease not being eradicated, long-term exposure to peg-IFNα-2a was sufficient to modify the clinical expression of the MPN for a sustained period of time.99

In addition to clinical, hematologic, and molecular complete responses achieved in a significant proportion of PV and ET patients, IFNα therapy has also been shown recently to reverse bone marrow histopathologic abnormalities in selected cases of both PV107 and PMF.111 Thus, IFNα seems to be the uniquely available drug able to induce complete resolution of all clinical, biologic, and morphologic abnormalities in selected MPN patients, raising the hope that a curative outcome might be possible in a subset of such patients.99 These observations lead one to question the validity of the current therapeutic strategies for PV and ET patients in which myelosuppressive therapy is exclusively used in subpopulations of patients at high risk for developing additional thrombotic events. If IFNα is shown in a large prospective trial to eliminate clinical, histologic, cytogenetic, and molecular evidence of disease with acceptable toxicity, one might also consider early treatment of low-risk patients, when the tumor burden is still low.99

Interestingly, the incidence of new thrombotic events in IFNα-treated MPN patients was consistently lower than that expected in patients receiving the standard of care.112,113 In the PVN-1 study, no vascular events were observed in a cohort of 37 PV patients after a median follow-up of 55 months (J.-K.K., personal unpublished data), although about 8 cases would have been expected, given the cumulative rate of 5.5 events per 100 patients per year reported in the literature.114 Silver also reported an absence of vascular events in 55 PV patients treated with standard IFNα, with an average follow-up of 53 months.113

Finally, it is of note that currently published national guidelines for PV115 (British Society of Hematology) and ET116 (Italian Society of Hematology) recommend the off-label use of IFNα as first-line therapy in younger patients, arguing that this drug is not leukemogenic. The updated MPN management recommendations recently published by international experts on behalf of the European LeukemiaNet even propose IFNα as first-line therapy in PV patients regardless of their age.117 Evolution to myelofibrosis, myelodysplastic syndrome, or acute leukemia is indeed a matter of concern in such patients who are expected to live for several decades, although the exact incidence of such transitions is still debated.118,119 If IFNα has an impact on the disease natural history as suggested by the decrease of JAK2V617F allele burden, reversion to normal features of the bone marrow histopathology, or the reduced incidence of vascular events on IFNα therapy, a delay or actual elimination in the evolution to myelofibrosis or leukemia might be anticipated.

Conclusions

The novel use of IFNα as a therapeutic option (eg, lower doses, in combination with other active agents, pegylated forms) have led to promising results in the treatment of various myeloid malignancies including CML and Philadelphia chromosome–negative MPN. We conclude that IFNα represents an important component of the therapeutic arsenal that has the potential to improve not only response rates but also the number of patients with these disorders achieving sustained molecular remissions. As opposed to targeted

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**Table 4. Clinical trials of interferon in myelofibrosis**

<table>
<thead>
<tr>
<th>First author, year</th>
<th>No. of patients</th>
<th>Response rate, %</th>
<th>Spleen size reduction, % of patients</th>
<th>Discontinuation, %</th>
<th>Type of IFN</th>
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<tr>
<td>Gilbert, 1998</td>
<td>22</td>
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<td>58</td>
<td>46</td>
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<tr>
<td>Tefferi, 2001</td>
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<td>3</td>
<td>33</td>
<td>NA</td>
<td>α2</td>
</tr>
<tr>
<td>Jabbour, 2007</td>
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<td>peg-α2a</td>
</tr>
<tr>
<td>Silver, 2009</td>
<td>13</td>
<td>38</td>
<td>38</td>
<td>8</td>
<td>α2b</td>
</tr>
</tbody>
</table>

For complete references, see supplemental Table 4. IFN indicates interferon; and NA, data not available.
therapies, the broad range of biologic properties of this drug (including direct toxic effects on transformed cells, enhancement of immune response against malignant cells, induction of proapoptotic genes, inhibition of angiogenesis, ability to cycle dormant malignant stem cells) may paradoxically render IFNα a candidate agent with which to treat a number of these malignancies. Recent clinical trials have, however, included limited numbers of patients, and the poor patient tolerability of IFNα may still be a limiting factor when used by clinicians not familiar with the use of this drug. A large, randomized, multicenter trial (Myeloproliferative Disorders Research Consortium Clinical Trial -112), which is being performed jointly in Europe and the United States, will compare treatment with peg-IFNα-2a to the standard of care (hydroxyurea) in high-risk patients with ET and PV. This study will document whether the recently proposed low-dose sustained utilization strategies of peg-IFNα-2a are truly more tolerable and effective therapies resulting in a favorable change in the natural history of patients with MPNs. At present, IFNα, despite the promising trials summarized here, remains an experimental therapy in most myeloid malignancies, thereby emphasizing the importance of completing such large clinical trials in this setting. IFNα has previously been used as a single agent, which is at odds with the manner in which most hematologic malignancies are treated with combinations of drugs. It is hoped that future clinical trials with IFNα in MPN patients will involve combinations of this cytokine with chromatin-modifying agents, JAK2 inhibitors, or inhibitors of antipapoptotic proteins to improve tolerability and increase efficacy.

Authorship

Contribution: All authors equally contributed to the article.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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The renaissance of interferon therapy for the treatment of myeloid malignancies

Jean-Jacques Kiladjian, Ruben A. Mesa and Ronald Hoffman