New Advances in Interferon Therapy of Cancer

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ABSTRACT

Substantial increases in both the understanding of the cellular mechanisms of actions of interferon (IFN) and in its clinical use in cancer have occurred in recent years. The efficacy of interferon for the treatment of select malignancies has been established, and IFN-α and IFN-β have been approved by the Food and Drug Administration for multiple clinical indications. IFN-α increased median survival and relapse-free survival in patients with locally advanced melanoma when used as adjuvant therapy and had modest activity against advanced disease. In other tumors where studies indicated that IFN lacked direct therapeutic activity, clinical trials suggested that it increased the antitumor activity of cytotoxic chemotherapeutic agents when used in combination therapy. IFN has substantial activity in chronic myelogenous leukemia, increasing survival in patients in early chronic phase when compared with conventional chemotherapy, and has some activity in non-Hodgkin’s lymphoma in combination with cytotoxic agents. Recent molecular and pharmacologic studies defining cellular receptor activation, signal transduction pathways, and biochemical modulating activities of interferon have yet to be fully incorporated into clinical development. Further preclinical advances along with the expanding identification of potentially clinically sensitive tumors make it likely that the use of IFN in cancer chemotherapy will continue to grow. The Oncologist 1997;2:254-267

INTRODUCTION

The interferons (IFNs) were discovered nearly 40 years ago and first approved for commercial use in the United States more than 10 years ago; however, for most of this period, they had only limited use in the treatment of cancer. In recent years, their use in cancer chemotherapy has grown, as IFN-α and IFN-β have been approved by the Food and Drug Administration (FDA) for multiple clinical indications (Table 1). This has been due, in part, to a developing understanding about the biology of the IFNs. It is anticipated that the expansion of knowledge about IFN signal transduction pathways and increased understanding of how the IFNs function at the cellular level will lead to the further incorporation of the IFNs into chemotherapy, particularly the identification of specific cellular targets for IFN therapy. Large, randomized clinical trials have led to a better understanding of the role of the IFNs in the treatment of cancer. Finally, there has been a recent effort to understand the biologic basis for IFN-mediated fatigue, the most significant barrier to wider adoption of IFN-based therapies. This review will provide an update on these issues in an attempt to delineate future directions for IFN research in the laboratory and the clinic.

Table 1. FDA-approved indications for IFN

<table>
<thead>
<tr>
<th>IFN-α2b</th>
<th>IFN-α2a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hairy cell leukemia</td>
<td>1985</td>
</tr>
<tr>
<td>Condylomata acuminata</td>
<td>1988</td>
</tr>
<tr>
<td>AIDS-related Kaposi’s sarcoma</td>
<td>1988</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>1991</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>1992</td>
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<tr>
<td>Melanoma</td>
<td>1995</td>
</tr>
<tr>
<td>Hairy cell leukemia</td>
<td>1989</td>
</tr>
<tr>
<td>AIDS-related Kaposi’s sarcoma</td>
<td>1988</td>
</tr>
<tr>
<td>CML</td>
<td>1995</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>1996</td>
</tr>
</tbody>
</table>

CML = chronic myelogenous leukemia.

CLASSIFICATION AND RECEPTORS

The current classification of the IFNs is based mainly on sequence, chromosomal location, and receptor specificity. Type I IFNs include at least 18 IFN-α genes and pseudogenes, one IFN-β gene, and six IFN-ω genes and pseudogenes [1]. The type I IFN genes all lack introns and are clustered on the short arm of human chromosome 9. The type I IFNs have

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secretory signal peptide signal sequences, which are removed prior to their secretion, and the mature forms of type I IFNs are 165 to 172 amino acids long. Type II IFN consists of a single IFN-γ gene, which has three introns and is located on chromosome 12. The mature IFN-γ is 166 amino acids long.

Both type I and type II recombinant IFNs have been studied clinically; these include IFN-α2a, IFN-α2b, IFN-β1a, IFN-β1b, and IFN-γ. Other IFNs, including hybrid species made by molecular recombination, have been studied experimentally. Although the type I IFNs have a high level of species specificity, one human hybrid, IFN-αA/D, was found to be active on both human and mouse cells [2]. It was of interest that neither of the parental IFNs from which IFN-αA/D was derived had this property. Different species of IFN-α and their recombinant hybrids have been found to exhibit subtle differences in biologic activities, for example, having relatively greater or less antiviral or antiproliferative activity.

The IFNs exhibit substantial overlap in their cellular and biologic activities, with differences in their immunomodulatory actions being the most notable feature distinguishing type I from type II IFNs [3]. The type I IFNs also have a different cellular receptor than does type II IFN [4]. Experiments demonstrating competition among the human type I IFNs for binding to cell surface proteins led to the suggestion that the type I IFNs have a common receptor [4-5]. However, other studies clearly demonstrated differences between both different IFN-α subtypes and between IFN-α and IFN-β in their interactions with the IFN receptor, subsequent signal transduction pathways, and cellular actions [6-7]. In particular, the subunit components of the type I IFN receptor differ on IFN-β binding compared with IFN-α binding. Comparison of IFN-α- and IFN-β-induced protein tyrosine phosphorylation indicated that there are IFN-β-specific signals, and a gene that was induced by IFN-β but not by IFN-α has been identified [8-12].

**The IFNs exhibit substantial overlap in their cellular and biologic activities.**

**Signal Transduction Via JAK-STAT Pathways**

Over the past decade, attempts to elucidate the transmembrane signal transduction pathways for the IFNs have resulted in the discovery of a novel signaling pathway that is shared not only by the IFNs but also by other cytokines and growth factors as well. This pathway, the Janus kinase family signal transducers and activators of transcription proteins, termed “JAK-STAT,” couples the transmembrane glycoproteins, which function as cytokine receptors, with the specific DNA binding sites, termed IFN-stimulated response elements, or IFN-γ-activated sites (GAS), in the promoters of IFN-responsive genes [13].

The JAK family are nonreceptor protein tyrosine kinases that are located proximate to the membrane-bound cytokine receptor and are activated by phosphorylation following receptor-ligand binding [1, 14-15]. Three members of the JAK family that have been found to mediate the activity of the IFNs are JAK1, JAK2, and tyk2, and they also mediate the activity of other cytokines, such as interleukins (IL)-2, -3, -4, -5, -6, -7, and -12, GM-CSF, and erythropoietin, among others. Thus, binding of one ligand to its specific receptor offers the possibility of cross-activation of other pathways and may explain in part the functional pleiotropism of a number of cytokines. Nevertheless, specificity may be maintained in part by the pattern of phosphorylation; for example, IFN-α binding results in phosphorylation of tyk2 and JAK1 but not JAK2, while IFN-γ binding results in phosphorylation of JAK1 and JAK2 but not tyk2 [14].

STAT proteins are preformed cytoplasmic proteins that are activated by phosphorylation on specific tyrosine residues as a result of JAK action [16]. The activated proteins form homodimers or heterodimers mediated by an Src Homology 2 (SH2) domain and migrate to the nucleus where they function as transcription factors. Different STATs are activated by different receptors, despite phosphorylation of similar JAKs by these receptors [17]; thus, the specificity of action of separate ligands may lie in the particular coupling of latent STATs to the intracellular domain of their cognate receptors [18]. It is also possible that the SH2 domains may contribute to the specificity of action of specific STAT mediators.

**IFN-Stimulated Response Elements (ISREs)**

On activation, STAT proteins assemble in the cytoplasm, forming multimeric protein aggregates that migrate to the nucleus as IFN-stimulated gene factors (ISGFs) [19]. These complexes confer specificity on the action of the ligand at the nuclear level. Gel shift mobility assays have allowed determination of the specific components of ISGFs; for example, ISGF-3, associated with binding of IFN-α, is composed of p113, p91, p84, and p48 [20]. The protein p91 also plays a central role in the signal transduction pathway for IFN-γ. These individual proteins have been isolated and sequenced, confirming their identity as DNA binding proteins [21].

Two DNA binding sites for the STAT complexes have been identified: ISRE for IFN-α and GAS for IFN-γ. These multimeric complexes have distinct DNA binding activities capable of distinguishing between these two sites. Although ISRE and GAS are clearly different, the binding sites for
epithelial growth factor, IL-6, and prolactin elements are similar to the GAS site [22-23]. The significance of this homology remains unclear. ISGF-2, also known as IFN regulatory factor (IRF)-1, is induced by IFN or virus treatment and activates both IFN and IFN-responsive genes, although it is not the sole activator of these genes [24]. IRF-2 is also induced by IFN but only after the induction of IRF-1; furthermore, IRF-2 appears to repress the expression of IFN-induced genes. Once bound, these complexes regulate transcription of a number of IFN-stimulated genes in a transacting fashion. Overexpression of IRF-2 in NIH 3T3 cells caused the cells to become transformed and increased their tumorigenicity in nude mice; the transformed phenotype was reversed by coexpression of the IRF-1 gene [25]. In view of the oncogenic effect of IRF-1, it was of interest that the IRF-1 gene was found to be deleted in human leukemia and preleukemic myelodysplasia [26]. Similarly, the IFN-α genes were also found to be deleted in human leukemias [27].

**IFN Activity at the Cellular Level: Clinical Implications**

The IFNs have been found to produce a large array of molecular and cellular actions of potential relevance to their use in cancer. These include direct growth inhibition of tumor cells, with IFN-β exhibiting greater activity than IFN-α [28-29]. The IFNs have well-described actions to induce or inhibit the expression of specific genes [1, 3]. Two IFN-inducible genes that have been best characterized are the 2'-5' oligoadenylate synthetase and a double-stranded RNA-dependent protein kinase. The activation of these genes leads to increased RNA degradation and inhibition of protein synthesis, respectively. Although the potential role of these genes in the antiviral effects of the IFNs is more clearly defined than in their antitumor actions, expression of a functional defective mutant of human double-stranded RNA-dependent protein kinase in NIH 3T3 cells resulted in malignant transformation [30]. Potentially of greater relevance to the use of IFN in cancer are its modulatory effects on the expression of oncogenes and tumor suppressor genes. Both IFN-α and IFN-γ reduced the expression of the Her-2 proto-oncogene in human breast and ovarian carcinoma cells [31], suppressed the phosphorylation of the retinoblastoma protein [32], reduced expression of the c-myc gene [33-35] and reduced the overexpression of the p53 gene [36]. IFN has been shown to antagonize the growth stimulatory effects of serum, epidermal growth factor, and platelet-derived growth factor, in part because of the downregulation of cell surface receptors for the growth factors and the inhibition of other early growth factor-mediated events [37-40]. These and related mechanisms may be particularly relevant in the actions of IFN-α in hairy cell leukemia, a rare malignancy that is particularly responsive to IFN therapy. Studies suggest that IFN induces the differentiation of the leukemic cells toward a stage less responsive to growth factor stimulation [41].

There are numerous reports describing the ability of the IFNs to enhance the efficacy of many of the cytotoxic cancer chemotherapy drugs in several different tumor models [42], and recent studies have begun to explore potential mechanisms for these interactions. IFN-α has been reported to reverse cellular resistance to doxorubicin and potentiate the reversal of multidrug resistance (MDR) by other agents through a complex mechanism that may include increasing the ability of MDR-inhibitory agents to bind to p-glycoprotein [43]. IFN has been shown to cause the induction of tryptophan degradation and the depletion of intracellular nicotinamide adenine dinucleotide in cells with consequent increased generation of reactive oxygen species and DNA strand breaks [44-45].

**The IFNs have been found to produce a large array of molecular and cellular actions of potential relevance to their use in cancer.**

Our studies have focused on the mechanism by which IFN potentiated the antitumor activity of the pyrimidine antimetabolite 5-fluorouracil (5-FU) in colon carcinoma cells. IFN was found to increase the levels of FdUMP, an active metabolite of 5-FU, in human colon carcinoma cells. This effect was selective for FdUMP, as neither the uptake of 5-FU nor the formation of fluoropyrimidine ribonucleotides was increased with IFN treatment. Further investigation demonstrated that the effect on FdUMP levels was due to the induction of expression of the enzyme thymidine phosphorylase (TP), which catalyzes the reversible conversion of thymine to thymidine and is also a component of the pathway for the direct conversion of 5-FU to FdUMP [46]. IFN-β, which was more active than IFN-α as a modulator of 5-FU in vitro, also produced a larger increase in TP expression. To confirm the relevance of these observations to the cytotoxic actions of 5-FU, an expression vector containing the TP cDNA was stably transfected into the HT-29 cells; transfectants with increased TP expression had higher levels of FdUMP formation and demonstrated increased sensitivity to 5-FU [47]. Induction of TP expression was also seen in peripheral blood mononuclear cells from patients treated with IFN, indicating that this biochemical mechanism may also contribute to the antitumor interaction of IFN and 5-FU in vivo [48].
ANTIANGIOGENESIS ACTIVITY

The process by which blood supply is established, angiogenesis, is a critical component for the growth of malignant tumors and for the establishment of metastases [49]. The neovascularization process is complex and involves factors that either stimulate or inhibit new vessel growth. These factors are derived both from the tumor cells themselves and from tumor-associated macrophages. The angiogenic phenotype of tumors appears to be acquired as cells undergo sequential genetic changes that ultimately lead to malignancy.

These observations have led to the hypothesis that antiangiogenesis could be used as a therapeutic modality to control tumor growth, either by inhibiting the formation of new blood vessels in tumors or by preventing their further growth [50]. An increasing number of antiangiogenesis agents are under investigation. The IFNs were one of the first group of active agents described, demonstrating antitumor effects in a variety of rodent tumor models [51-52]. A combination of three angiogenesis inhibitors, including IFN, markedly attenuated tumor growth in a transgenic mouse model, apparently exclusively, by inhibiting angiogenesis [53]. The IFNs also proved to be useful as antiangiogenic agents in animal models when they were combined with other angiogenesis inhibitors and with cytotoxic chemotherapeutic agents [54]. Finally, IFN-α also had dramatic clinical activity against proliferative disorders of the blood vessels [55-56].

Mechanisms by which the IFNs may act at the cellular level have been sought. Inhibition of the production of the angiogenesis promoter, basic fibroblast growth factor, has been identified as a potential mechanism of IFN-α and IFN-β action [57]. Another target in the extracellular matrix includes urokinase-type plasminogen activator [58]. Thus, the IFNs potentially will play an important role in evolving antiangiogenesis strategies both in tumor models and in the clinical setting.

CLINICAL TRIALS WITH THE IFNS

Melanoma

Adjuvant Therapy

Patients with early-stage melanoma (lesions less than 1.5 mm in depth) will have an 85% survival rate at 10 years. The prognosis for patients with lesions greater than 4 mm is substantially worse, with a survival rate of less than 50%. Based on the results of a randomized trial conducted by the Eastern Cooperative Oncology Group (ECOG) [EST 1684], IFN-α2b has recently been approved for the adjuvant treatment of melanoma by the FDA. In this trial, patients with locally advanced melanoma at high risk for recurrence were randomized to high-dose adjuvant therapy with IFN-α2b for one year or to observation [59]. Patients at high risk included those with lesions of Breslow thickness greater than 4 mm or patients with primary or recurrent lymph node involvement. Treatment consisted of high-dose daily intravenous therapy for one month at 20 MU/m², followed by s.c. maintenance therapy, t.i.w., for 11 months at 10 MU/m². With a median follow-up of more than seven years, there was an increase in the median survival duration for patients receiving IFN-α2b of 12.4 months (33.4 months versus 45.8 months, p = 0.02) and an increase in the median relapse-free survival of 8.8 months (11.8 months versus 20.6 months, p = 0.002). Despite this significant clinical improvement, there were substantial treatment delays and dose reductions, which potentially could compromise the efficacy of the treatment in specific patients. In particular, 24% of patients failed to complete treatment, and 81% of patients experienced toxicities of grade 3 or greater. This was primarily secondary to IFN-α2b-mediated constitutional symptoms, as well as some hematologic and neurologic symptoms.

Quality-of-life assessments were performed by applying the Quality-Adjusted Time Without Symptoms or Toxicity instrument to the ECOG trial data [60]. This instrument calculates treatment effects on quality of life by comparisons with periods when patients have no toxic events and no relapse and with periods when patients have relapsed. With 84 months of follow-up, the IFN-α2b-treated group, which gained a mean of 8.9 months without relapse and a mean of 7 months of overall survival, also had a mean of 5.8 months with severe treatment-related toxicities. Nevertheless, the IFN-α2b-treated group had a gain in quality-of-life-adjusted time (p < .05), and this was greatest in the node-positive strata, suggesting that the clinical benefits of the treatment could significantly offset the side effects of treatment.

Locally Advanced Disease

Patients with locally advanced melanoma may be candidates for intra-arterial therapies. Impressive results have been achieved with combinations of intra-arterial IFN-γ, tumor necrosis factor-α, melphalan, and hyperthermia. Furthermore, the IFN appeared to have been an essential component of the combination [61-63].

Advanced Disease

Patients with melanoma beyond the scope of surgical excision generally have a poor prognosis, with a five-year survival rate of approximately 15%. Few single agents have shown substantial activity. Single-agent IFN has been reported to produce objective responses in about 15% of patients with few complete responders and few long-term survivors [64]. Cytotoxic drugs with single-agent activity include dacarbazine...
this approach is clearly of interest in ameliorating the toxicities observed in administering these regimens in the outpatient setting, the severe toxicities observed in some studies do suggest caution with IFN-activated tumor-infiltrating lymphocytes. Although no demonstrable advantage, and the use of cytokine therapy s.c. immunotherapy in combination with chemotherapy, without tamoxifen. Other trials have also added IL-2, using the low- or intermediate-dose, s.c., or continuous-infusion schedules. In many of these trials, therapy was well tolerated, with higher-than-expected complete response rates and some long-term responders. The ECOG is currently comparing single-agent DTIC with a more complex, multi-agent regimen to determine the effectiveness of combination therapy. The addition of IFN to these regimens is still of unknown benefit.

Renal Cell Carcinoma

Single-agent IFN has modest activity in the treatment of renal cell carcinoma [64], with objective response rates in the 15% to 20% range and acceptable toxicities. Response durations have generally been short, however, and therefore alternative therapies have been explored. With the approval of IL-2 by the FDA for the treatment of renal cell carcinoma, combinations of IL-2 and IFN have been explored. Of interest has been the use of low- or intermediate-dose combinations of the two in outpatient regimens. Most of these have been well tolerated, with response rates that have been higher than expected in some cases (Table 3) [69, 81-89]. Modifications of this combination have included the use of low-dose infusional or s.c. immunotherapy in combination with chemotherapy, without a demonstrable advantage, and the use of cytokine therapy with IFN-activated tumor-infiltrating lymphocytes. Although the severe toxicities observed in some studies do suggest caution in administering these regimens in the outpatient setting, this approach is clearly of interest in ameliorating the toxicities observed with high-dose IL-2 and with improving quality of life in this patient population.

Colon Cancer and Other Gastrointestinal Malignancies

Based on in vitro data that demonstrated that IFN synergistically augments the effects of 5-FU against human cancer cell lines (as discussed above) and on early phase II trials that demonstrated higher than expected objective response rates in patients with advanced colorectal cancer, several U.S. and international randomized trials were conducted to determine the efficacy of the combination of 5-FU plus IFN. Most of these attempted to duplicate the methodology of the earlier studies, although it has been noted that the administration of the IFN was not sequenced identically to that of the prior studies; because of the importance of the modulatory effects of IFN on thymidine phosphorylase, this calls into question whether these larger trials have truly duplicated the earlier methodology. Nevertheless, the majority of these trials have been negative or equivocal, demonstrating no benefit for the combination of 5-FU plus IFN versus either 5-FU alone or 5-FU plus leucovorin (Table 4) [90-95]. One trial from France demonstrated a threefold increase in response rate (6% to 18%, \( p < 0.05 \)) and a significant improvement in relapse-free survival (\( p = 0.01 \)), but not overall survival for the combination. Furthermore, a small phase III trial from Spain of 5-FU plus IFN-\( \beta \) versus 5-FU alone demonstrated a survival benefit for the combination therapy.

In patients with biliary tract cancer, the combination of 5-FU plus IFN-\( \alpha \) has been demonstrated to be active. Among 35 patients, there were 11 responders (34%) with a median survival of one year and acceptable toxicities [96]. Further studies are being performed with the regimen of cisplatin, IFN, doxorubicin, and 5-FU at the M.D. Anderson Cancer Center. The combination of 5-FU, IFN, and the pyrimidine inhibitor N-(phosphonacetyl)-L-aspartic acid has been studied in patients with gastric cancer with some durable remissions noted [97]. Following preclinical studies that demonstrated synergy for the combination of 5-FU plus IFN and the ribonucleotide reductase inhibitor hydroxyurea plus IFN, clinical trials were initiated in patients with gastric, pancreatic, and hepatobiliary tumors; these are ongoing [98]. Finally, the combination of 5-FU, IFN, and cisplatin has been shown to be highly active in patients with advanced and locally advanced squamous cell carcinoma of the esophagus [99]. Radiation therapy is being incorporated into this regimen in the treatment of localized esophageal carcinoma at the Albert Einstein College of Medicine.

Non-Hodgkin’s Lymphoma

IFN as a single agent has demonstrated clinical activity against follicular lymphomas in patients who have failed prior
Table 2. Clinical trials with IFN in melanoma

<table>
<thead>
<tr>
<th>Institution/Group</th>
<th>n</th>
<th>Regimen</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milan [65]</td>
<td>266</td>
<td>DTIC + IFN-α 9 MU/day</td>
<td>Phase III trial; increased response duration with IFN; no improvement in OS, ORR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ IFN-α 3 MU t.i.w.</td>
<td></td>
</tr>
<tr>
<td>Princess Alexandra [66]</td>
<td>170</td>
<td>DTIC ± IFN-α</td>
<td>Phase III trial; no advantage for combination therapy</td>
</tr>
<tr>
<td>M.D. Anderson Cancer Center [67]</td>
<td>102</td>
<td>CDDP/VBL/DTIC/IFN-α/IL-2</td>
<td>ORR, 60% to 69%; improved survival versus chemotherapy alone</td>
</tr>
<tr>
<td>Pretoria [68]</td>
<td>73</td>
<td>DTIC ± IFN-α</td>
<td>Phase III trial; ORR, 20% versus 50%; OS, 8 months versus 16.7 months; combination &gt; single agent</td>
</tr>
<tr>
<td>Hannover [69]</td>
<td>67</td>
<td>CBDCA/DTIC IFN-α/IL-2; or DTIC/BCNU/CDDP/Tam IFN-α/IL-2</td>
<td>ORR, 35% to 55%; CR, 8% to 11%; some long-term responders; well tolerated</td>
</tr>
<tr>
<td>Helsinki [70]</td>
<td>48</td>
<td>DTIC/VCR Bleo/CNU IFN-α</td>
<td>ORR, 62%; some unmaintained complete remissions</td>
</tr>
<tr>
<td>Chicago [71]</td>
<td>42</td>
<td>BCNU/CDDP/DTIC IL-2/IFN-α</td>
<td>10 CR; 14 PR</td>
</tr>
<tr>
<td>City of Hope [72]</td>
<td>42</td>
<td>CDDP IFN-α</td>
<td>ORR, 34%; 3 CR, 5 to 31+ months; 7 PR; well tolerated</td>
</tr>
<tr>
<td>Salpetriere [73]</td>
<td>39</td>
<td>CDDP IL-2 (CIVI) IFN-α</td>
<td>79% pretreated; ORR, 54%; 5/39 CR, 16/39 PR</td>
</tr>
<tr>
<td>Miami [74]</td>
<td>33</td>
<td>BCNU/DTIC CDDP/Tam IFN-α</td>
<td>ORR, 42%; OS, 5 months</td>
</tr>
<tr>
<td>Torino [75]</td>
<td>32</td>
<td>DTIC/BCNU/CDDP/Tam IFN-α</td>
<td>ORR, 47%; 5 CR</td>
</tr>
<tr>
<td>Salpetriere [76]</td>
<td>22</td>
<td>CDDP/Tam IL-2 IFN-α</td>
<td>Tam did not improve ORR</td>
</tr>
<tr>
<td>Groningen [77]</td>
<td>20</td>
<td>DTIC/IFN-α/filgrastim/ondansetron</td>
<td>ORR, 20%; OS, 8 months</td>
</tr>
<tr>
<td>Tel Aviv [78]</td>
<td>16</td>
<td>DTIC/CBDCA IL-2 IFN-α</td>
<td>ORR, 38%</td>
</tr>
<tr>
<td>Heidelberg [79]</td>
<td>12</td>
<td>DTIC or CDDP IFN-α IL-2</td>
<td>2/12 PR; no impairment of secondary immune mediators; all had failed IL-2/IFN</td>
</tr>
<tr>
<td>NCI [80]</td>
<td>NS</td>
<td>CDDP IFN-α IL-2 (LD, CIVI)</td>
<td>ORR, 45%; with responses 2 to 14+ months; moderate to severe toxicity</td>
</tr>
</tbody>
</table>

Abbreviations: BCNU = carmustine; Bleo = bleomycin; CBDCA = carboplatin; CCNU = lomustine; CDDP = cisplatin; CIVI = continuous intravenous infusion; CR = complete response; DTIC = dacarbazine; IFN = interferon; IL-2 = interleukin-2; LD = low-dose; NCI = National Cancer Institute; NS = not specified; ORR = objective response rate; OS = overall survival; PR = partial response; Tam = tamoxifen; VBL = vinblastine; VCR = vincristine.
chemotherapy, with response rates of 30% to 50% [100-101]. Additional trials have demonstrated improved response rates with IFN in combination with chemotherapy. Combination regimens employing IFN plus alkylating agents and doxorubicin were therefore introduced in the 1980s, with the results of several mature phase III trials available now.

### Table 3. Combination IFN/IL-2 regimens in renal cell carcinoma

<table>
<thead>
<tr>
<th>Institution/Group</th>
<th>n</th>
<th>Regimen</th>
<th>Schedule</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>FNCLCC [81]</td>
<td>425</td>
<td>IL-2, IFN-α</td>
<td>ID, CIVI</td>
<td>Phase III; improved ORRs and event-free survival with combination treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-2 + IFN-α</td>
<td>HD, SC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-FU + IFN-α</td>
<td>ID, CIVI + ID, SC</td>
<td></td>
</tr>
<tr>
<td>Hannover [69]</td>
<td>67</td>
<td>Chemotherapy</td>
<td>ID, SC</td>
<td>Two phase II platinum-based regimens followed by immunotherapy; 35% to 55% response rate; ? advantage over immunotherapy alone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-2, IFN-α</td>
<td>ID, SC</td>
<td></td>
</tr>
<tr>
<td>New York Medical College [82]</td>
<td>43</td>
<td>IL-2, IFN-α</td>
<td>ID, CIVI</td>
<td>Phase I; ORR, 22% (4/18 RCC); well tolerated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-FU + LV</td>
<td>ID, SC</td>
<td></td>
</tr>
<tr>
<td>Chicago [83]</td>
<td>42</td>
<td>IL-2, IFN-α</td>
<td>LD, SC</td>
<td>ORR, 12% (5/42 RCC); well tolerated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-FU + IFN-α</td>
<td>ID, SC</td>
<td></td>
</tr>
<tr>
<td>Pisa [84]</td>
<td>42</td>
<td>IFN-α, Flox</td>
<td>IM</td>
<td>ORR, 33%; some long-term responders</td>
</tr>
<tr>
<td>Hershey [85]</td>
<td>39</td>
<td>IL-2, IFN-α</td>
<td>CIVI</td>
<td>ORR, 33%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-FU + IFN-α</td>
<td>ID, IM</td>
<td></td>
</tr>
<tr>
<td>Frankfurt [86]</td>
<td>35</td>
<td>IL-2, IFN-α</td>
<td>ID, IV</td>
<td>ORR, 26% (9/35); well tolerated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-FU + IFN-α</td>
<td>ID, SC</td>
<td></td>
</tr>
<tr>
<td>MSKCC [87]</td>
<td>34</td>
<td>IL-2, IFN-α</td>
<td>LD, CIVI</td>
<td>ORR, 12% (4/34); severe toxicity (2 deaths)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-FU + IFN-α</td>
<td>ID, SC</td>
<td></td>
</tr>
<tr>
<td>UCLA [88]</td>
<td>30</td>
<td>IL-2, IFN-α</td>
<td>LD, CIVI</td>
<td>ORR, 30% (9/30); well tolerated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-FU + IFN-α</td>
<td>ID, SC</td>
<td></td>
</tr>
<tr>
<td>UCLA [89]</td>
<td>11</td>
<td>IL-2, IFN-α</td>
<td>LD, CIVI</td>
<td>ORR, 30% (3/10 RCC); well tolerated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-FU + IFN-β</td>
<td>ID, SC</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** CIVI = continuous intravenous infusion; Flox = floxuridine; FNCLCC = French Immunotherapy Group; HD = high-dose; ID = intermediate dose; IFN = interferon; IL = interleukin; IM = intramuscular; IV = intravenous; LD = low dose; MSKCC = Memorial Sloan-Kettering Cancer Center; ORR = objective response rate; RCC = renal cell carcinoma; SC = subcutaneous; TIL = tumor-infiltrating lymphocyte; UCLA = University of California at Los Angeles.

### Table 4. Combination trials in colorectal cancer

<table>
<thead>
<tr>
<th>Institution/Group</th>
<th>n</th>
<th>Regimen</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corfu-A Study Group [90]</td>
<td>496</td>
<td>5-FU + IFN-α</td>
<td>Equal response and survival</td>
</tr>
<tr>
<td>UK MRC [91]</td>
<td>260</td>
<td>5-FU + IFN-α</td>
<td>Equal response and survival; greater toxicity with IFN</td>
</tr>
<tr>
<td>Roferon [92]</td>
<td>245</td>
<td>5-FU + IFN-α</td>
<td>Equal response and survival; greater toxicity with IFN</td>
</tr>
<tr>
<td>Royal Marsden [93]</td>
<td>106</td>
<td>5-FU + IFN-α</td>
<td>Equal response and survival; greater toxicity with IFN</td>
</tr>
<tr>
<td>Strasbourg [94]</td>
<td>105</td>
<td>5-FU + IFN-α</td>
<td>Improved response rate and event-free survival with IFN</td>
</tr>
<tr>
<td>Valencia [95]</td>
<td>48</td>
<td>5-FU + IFN-β</td>
<td>Improved response rate and overall survival with IFN</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-FU + IFN-β</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** 5-FU = 5-fluorouracil; IFN = interferon; LV = leucovorin; UK MRC = United Kingdom Medical Research Council.
One early trial conducted at M. D. Anderson Cancer Center [102] added IFN-α1, 3 MU/m² t.i.w. for 24 months, in patients with stage IV, low-grade lymphoma who had undergone a complete remission to cyclophosphamide, doxorubicin, vincristine, and prednisone plus bleomycin induction therapy. There were 127 previously untreated patients enrolled in this trial; 64% achieved a complete remission after induction therapy, and this rose to 73% after the addition of radiotherapy to bulk disease. Sixty-seven patients received IFN; these patients had a five-year survival rate of 74%, not significantly different from historical controls. The failure-free survival rate, 60%, was significantly better, however.

In a trial conducted by the ECOG [103], the combination of cyclophosphamide, vincristine, prednisone, and doxorubicin was compared with the combination plus IFN-α, 6 MU/m² in the last five days of a 28-day course. Patients were required to have stage III or IV intermediate-grade lymphoma. There were 291 patients enrolled, but 42 were ineligible. Although response rates for the two arms were not different, there was a significantly longer duration of response (p = .03) and time to failure (p = .0013), with a trend favoring survival in the IFN group [104].

The European Organization for the Research and Treatment of Cancer performed a randomized trial of cyclophosphamide, vincristine, prednisone, and doxorubicin followed by either observation or IFN-α, 3 MU/m², as maintenance therapy. There were 331 patients enrolled, 231 of whom were randomized. Although there was no difference in overall survival noted at a preliminary analysis, there was a significant improvement in progression-free survival from 86 weeks to 135 weeks (p = 0.02) among patients receiving IFN [105].

The Groupe d’Etude des Lymphomes de l’Adulte performed a randomized trial in patients with advanced low-grade follicular lymphoma [106]. There were 242 patients entered. All patients received induction therapy with cyclophosphamide, doxorubicin, teniposide, and prednisone monthly for six cycles, then every two months for one year. At randomization, 123 patients received IFN-α2b, 5 MU t.i.w. for 18 months. There was a significantly higher response rate (85% versus 69%, p = 0.006) and significantly longer event-free survival (34 months versus 19 months, p < 0.01) and overall survival at three years (34 months versus 19 months, p < 0.001) for the group receiving IFN-α2b therapy.

Although these trials demonstrated a benefit for IFN, there were several confounding factors. First, the patient populations were often heterogeneous, with both low-grade and intermediate-grade patients and some patients having nonfollicular lymphomas. Second, only one trial demonstrated a clear survival advantage for the IFN group; there was no clear plateau in the survival curves. Third, the toxicities of IFN were formidable in most trials, with a relatively large dropout rate. Thus, additional studies are warranted to confirm these findings and to attempt to improve the constitutional symptoms associated with IFN. Further trials adding newer antilymphoma agents, such as fludarabine and cladribine, are also warranted.

Multiple Myeloma

Combined Modality Treatment

Based on the modest single-agent activity of IFN in the setting of refractory myeloma, IFN was incorporated into induction regimens. Several randomized trials have tested the role of chemotherapy with or without the addition of IFN. Cancer and Leukemia Group B employed a melphalan/prednisone regimen with or without the addition of low-dose, s.c. recombinant IFN-α, 2 MU/m² t.i.w., with no advantage for the combined modality regimen [107]. The Myeloma Group of Central Sweden employed a similar trial design using leukocyte-derived IFN at a higher dose than that employed by the Cancer and Leukemia Group B, and found higher response rates for the combination treatment and prolonged survival among patients with immunoglobulin A or light-chain myeloma [108]. Based on encouraging results from a phase II trial [109], the ECOG has recently completed a trial of combination chemotherapy with vincristine, carmustine, melphalan, cyclophosphamide, and prednisone with or without recombinant IFN-α; the study is currently being analyzed.

Maintenance Therapy

IFN has been studied in the maintenance setting following induction chemotherapy in patients with multiple myeloma. An early trial from the Italian Myeloma Study Group initially suggested improved survival with IFN maintenance; however, longer follow-up only demonstrated an improved response duration for patients receiving IFN, with no overall survival benefit [110]. Three other trials have demonstrated improved response durations for patients receiving IFN maintenance therapy [111-113], with one suggesting a survival benefit. In a randomized trial of IFN maintenance following high-dose melphalan/prednisone followed by autologous bone marrow transplantation, selected patients, those achieving a complete response, survived longer with IFN treatment [114]. Thus, there appears to be an advantage to maintenance therapy with IFN in some patients following induction therapy for multiple myeloma.

Chronic Myelogenous Leukemia

IFN has substantial clinical activity against chronic myelogenous leukemia (CML). This may result from IFN’s effects on integrins, which restore normal progenitor-stromal interactions in the bone marrow [115]. Response is highly dependent
on the stage of disease, however. In patients in blast crisis, a major cytogenetic response is almost never achieved with virtually any therapeutic intervention, whereas in early chronic phase, IFN treatment results in a major cytogenetic response in 20% to 30% of patients, with responses in late chronic phase and accelerated phase being intermediate. In comparison with conventional chemotherapy, IFN treatment has demonstrated a survival advantage in several trials [116-120]. In all of these trials, IFN treatment resulted in improved rates of cytogenetic and hematologic remission. In an Italian trial [117], treatment with IFN resulted in a six-year survival rate of 50% compared with 29% for patients receiving chemotherapy ($p = 0.002$). Furthermore, low-dose therapy may be as effective as high-dose therapy [121].

IFN combination therapy has been employed in an attempt to improve on the relatively poor survival of patients with CML. Combination therapy with IFN-α and IFN-γ failed to improve on results with single-agent IFN [122]. In a single-arm trial, the combination of IFN and cytarabine resulted in a complete hematologic remission rate of 55%, which was better than that of historical controls [123]. In early-phase CML, the combination of IFN and cytarabine resulted in a complete hematologic response rate of 30% [124]. Combinations of IFN and hydroxyurea appear less active, and maintenance therapy with IFN does not appear to offer a survival advantage [125].

The cost effectiveness of IFN therapy versus therapy with oral hydroxyurea in the treatment of CML has been analyzed by a multi-institutional panel of physicians [126]. The marginal cost effectiveness of IFN therapy (incremental discounted cost compared with conventional therapy) was $34,800 per quality-adjusted year of life saved. This was considered to be favorable compared with hydroxyurea.

**IFN-MEDIATED FATIGUE**

IFN-mediated fatigue is the major barrier to wider acceptance of IFN-based therapies. The greatest impediment to treating IFN-mediated fatigue remains the absence of knowledge about specific mediators of fatigue. Neuropsychiatric, endocrine, and neuromuscular mechanisms have been identified and are currently being studied. Preliminary data have demonstrated nonspecific changes on magnetic resonance imaging scans, with a decrease in various objective measurements in cognitive function. Other preliminary studies have suggested that IFN-induced fatigue may be mediated via impaired endocrine function, either by dysregulated thyroid function, insufficiency of the pituitary-adrenal axis, or suppression of gonadal function. Early studies, for example, have demonstrated autoimmune thyroiditis in about 10% of patients receiving partially purified IFNs, with decreases in free triiodothyronine and thyroid-stimulating hormone levels and decreased conversion of thyroxine to triiodothyronine peripherally [127]. IFN-mediated fatigue has been reviewed in a recent editorial [128].

**FUTURE DIRECTIONS**

The role of the IFNs in the treatment of neoplastic diseases continues to expand as large clinical trials continue to mature. Based on the ECOG adjuvant trial of high-dose IFN in patients with high-risk, resected melanoma, current clinical trials are exploring the utility of lower, more clinically tolerable dosing schedules for IFN as well as the role of other immune adjuvant therapies, which may have greater specificity for melanoma cell surface proteins (Table 5). For patients with metastatic melanoma, the utility of IFN is being explored in combination with cytotoxic drugs and with other biologic agents, including IL-2. Similar themes are being explored in patients with advanced renal cell carcinoma. Based on impressive data in patients with advanced cervical cancer studied in Mexico and on preliminary data from the Memorial Sloan-Kettering Cancer Center, combinations of IFN and the retinoid 13-cis retinoic acid are being investigated.

In patients with gastrointestinal malignancies, a variety of approaches are currently being explored. Several studies are examining the utility of IFN in combination with other biologic agents, such as radiolabeled monoclonal antibodies, specifically to determine the feasibility of such an approach and to determine whether IFN augments the targeting of the monoclonals. Based on extensive data that IFN augments the clinical activity of 5-FU, several studies are investigating the modulatory role of IFN in combination with 5-FU and other agents against refractory gastrointestinal tumors.

The role of IFN in hematologic malignancies has also continued to expand. Based on the activity demonstrated for IFN in combination with standard alkylating agent-based therapies, combinations of IFN and the purine nucleoside analogs are being investigated. Furthermore, because of potential antiviral as well as antiproliferative activity, IFN in combination with other biologic agents is being studied in patients with virally mediated lymphomas, such as human T cell leukemia/lymphoma virus-1 and HIV. Finally, the
Table 5. Selected current protocols studying IFN-related questions

<table>
<thead>
<tr>
<th>Institution/Group</th>
<th>Disease state/protocol number</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOG</td>
<td>Melanoma/2696</td>
<td>Phase II randomized study of vaccination with GM-2 ganglioside conjugated to keyhole limpet hemocyanin plus the immunologic adjuvant QS21 with versus without high-dose IFN-α in high-risk melanoma</td>
</tr>
<tr>
<td>ECOG</td>
<td>Melanoma/E1690</td>
<td>Phase III randomized study of adjuvant intermediate high-dose IFN-α versus intermediate low-dose IFN-α versus observation following definitive resection of thick primary and/or regional lymph node metastases in high-risk stage III melanoma</td>
</tr>
<tr>
<td>EORTC</td>
<td>Melanoma/18952</td>
<td>Phase III randomized study of DTIC/CDDP/IFN-α with versus without IL-2 for metastatic melanoma</td>
</tr>
<tr>
<td>SWOG</td>
<td>RCC/9338</td>
<td>Phase II study of 5-FU/IFN-α/IL-2 for advanced RCC</td>
</tr>
<tr>
<td>EORTC</td>
<td>RCC/30951</td>
<td>Phase II randomized study of IFN-α with versus without 13-CRA for metastatic RCC</td>
</tr>
<tr>
<td>ECOG</td>
<td>RCC/2894</td>
<td>Phase III randomized study of IFN-α with versus without 13-CRA in patients with metastatic RCC</td>
</tr>
<tr>
<td>University of Nebraska</td>
<td>GI/11796</td>
<td>Phase IA/IB study of IFN-γ plus 90Y-labeled mAb CC-49 for metastatic or unresectable GI adenocarcinomas</td>
</tr>
<tr>
<td>Albert Einstein Cancer Center</td>
<td>GI/T94-0025</td>
<td>Phase II study of 5-FU/IFN-α/HU in advanced cancer of the pancreas, stomach, or biliary system and hepatocellular carcinoma</td>
</tr>
<tr>
<td>EORTC</td>
<td>GI/40924</td>
<td>Phase II/III randomized study of CDDP/5-FU versus CDDP/5-FU/IFN-α for metastatic pancreatic cancer</td>
</tr>
<tr>
<td>University of Nebraska</td>
<td>GI/11796</td>
<td>Phase IA/IB study of IFN-γ plus 90Y-labeled mAb CC-49 for metastatic or unresectable GI adenocarcinomas</td>
</tr>
<tr>
<td>Fox Chase Cancer Center</td>
<td>Lymphoma/94060</td>
<td>Phase II study of alternating FAMP and IFN-α in low-grade non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>NCI</td>
<td>Lymphoma/94-C-0070E</td>
<td>Phase II study of ZDV/IFN-α in patients with HTLV-1-associated adult T-cell leukemia/lymphoma</td>
</tr>
<tr>
<td>ECOG</td>
<td>Lymphoma/1494</td>
<td>Phase II study of CDE (CTX/DOX/VP-16) plus ddl for HIV-related intermediate- and high-grade non-Hodgkin’s lymphoma</td>
</tr>
<tr>
<td>EORTC</td>
<td>CML/06941</td>
<td>Phase II study of IFN-α/TRA for chronic-phase adult CML</td>
</tr>
<tr>
<td>MSKCC</td>
<td>CML/94070</td>
<td>Phase II study of induction with high-dose DHAD/ara-C, consolidation with high-dose CTX/VP-16, and maintenance with IFN-α in CML in myeloid blast crisis</td>
</tr>
<tr>
<td>M.D. Anderson Cancer Center</td>
<td>CML/DM-93151</td>
<td>Phase II study of homoharringtonine with IFN-α in chronic-phase CML</td>
</tr>
</tbody>
</table>

Abbreviations: ara-C = cytarabine; CDDP = cisplatin; CML = chronic myelogenous leukemia; CTX = cyclophosphamide; ddl = didanosine; DHAD = mitoxantrone; DOX = doxorubicin; DTIC = decarbazine; ECOG = Eastern Cooperative Oncology Group; EORTC = European Organization for the Research and Treatment of Cancer; 5-FU = 5-fluorouracil; FAMP = 5-FU, doxorubicin, mitomycin C, prednisone; GI = gastrointestinal; HTLV = human T-cell leukemia/lymphoma virus; HU = hydroxyurea; IFN = interferon; IL = interleukin; mAb = monoclonal antibody; MSKCC = Memorial Sloan-Kettering Cancer Center; NCI = National Cancer Institute; RCC = renal cell carcinoma; SWOG = Southwest Oncology Group; TRA = all-trans retinoic acid; VP-16 = etoposide; ZDV = zidovudine.

The role of IFN in CML continues to expand, including studies of combinations of IFN and cytotoxic agents or IFN plus differentiating agents, such as all-trans retinoic acid.

IFN-mediated fatigue remains a major barrier to wider incorporation of this agent into clinical regimens. Current trials are being directed at strategies to understand and treat this phenomenon. One major problem has been the absence of a reproducible schema for grading and classifying IFN-mediated fatigue. Quality-of-life studies will be an integral part of future IFN clinical trials.

Future advances in IFN therapy are likely to be based on emerging information about the cellular actions of IFN and the IFN-related signal transduction pathways. As the components of these pathways become more clearly understood, potential targets for IFN-mediated effects will likely be identified. One gene therapy strategy to enhance IRF-1 levels in order to sensitize cells to the effects of IFN has been proposed as a model [129].

Acknowledgments

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REFERENCES


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