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Review

Redefining the role of interferon in the treatment of malignant diseases

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ABSTRACT

Interferon (IFN) is a cytokine with a long history of use as immunotherapy in the treatment of various solid tumours and haematological malignancies. The initial use of IFN in cancer therapy was based on its antiproliferative and immunomodulatory effects, and it has been shown more recently to have cytotoxic and anti-angiogenic properties. These features make it a rational anticancer therapy; however, advances in our understanding of the molecular mechanisms involved in cancer development and growth and the availability of effective, alternative therapies have led to IFN therapy being superseded in many cancers. IFN is still commonly used in renal cell carcinoma (RCC), melanoma and myeloproliferative disorders, in which its optimal dose and treatment duration remain to be established despite extensive clinical experience. Preclinical studies of the mechanism of action of IFN suggest that different antitumour effects are relevant at different doses, providing a rationale to explore the use of different dose regimens of IFN, particularly when combined with other therapies. In particular, the advent of novel anti-angiogenic therapies in RCC means that the role of IFN needs to be re-examined with a focus on how best to maximise efficacy and minimise toxicity when used with these agents. This review will focus on the therapeutic use of IFN in these disorders, provide an overview of available data and consider what the data suggest regarding the potential optimal use of IFN in the future.

1. Introduction

Just over 50 years ago, Isaacs and Lindermann described a biological substance that inhibited or 'interfered' with viral replication and expression in virus-infected cells;[1] they named this molecule interferon (IFN). Subsequent research revealed that Isaac's and Lindermann's IFN molecule was just one of a number of different proteins or glycoproteins comprising the IFN family;[2] in addition to antiviral properties, IFNs have been found to be important in regulating antiproliferative

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and immunomodulatory pathways.[3] Also, IFN has a role in haematopoietic differentiation and inhibition of angiogenesis.[4–8]

IFNs are classified as either type I or type II according to the receptors through which they signal. All IFNs have overlapping, but distinct, biological activities: Type I IFNs induce antiproliferative and antiviral activity, whereas type II IFN-γ has weaker antiviral activity but more potent immunomodulatory properties. In humans, seven different IFN species have been identified to date: IFN-α (at least 13 subtypes transcribed), IFN-β, IFN-γ, IFN-ε, IFN-κ, IFN-ν and IFN-ω.[9] All of these are type I IFNs, with the exception of IFN-γ, which is type II.

Cells of most types appear to be able to produce IFN-α (and other type I IFNs), but the principal producers are T-cells, monocytes, macrophages, dendritic cells and natural killer (NK) cells. IFN-γ production is restricted to a subset of T-cells, dendritic cells and NK-cells.[2] The production and release of IFNs usually occur as part of an immune response to pathogens or foreign antigens. Subsequent IFN signalling is mediated through interaction with specific cell surface receptors, which activates a signal transduction cascade through the JAK-STAT pathways.[2]

Of the various subtypes of IFN, IFN-α and IFN-β are the most abundant and are the principal subtypes therapeutically. Subsequent discussion of IFN refers to these subtypes. Based on its antiproliferative and immunomodulatory effects, IFN has been investigated as an anticancer agent for many years, although not before early clinical trials in viral diseases such as chronic hepatitis B infection produced positive results.[10] IFN has been shown to have both direct and indirect effects on tumour cells (Table 1).[5] However, the broad spectrum of activity and the complex interactions of IFN make it difficult to pinpoint the antitumour activity of IFN, and it is likely a combination of these activities is responsible. The antiproliferative properties of IFN first attracted the attention of clinical oncologists.[11,12] IFN can directly affect the proliferation of tumour cells by down-regulating the activity of cyclin-dependent kinases (Cdk) active in the G1 phase of the cell cycle. Although the precise mechanism is not known, IFN can affect the expression or function of key regulators of the cell cycle such as Cdk2, cyclins, retinoblastoma protein and the transcription factor E2F.[13–15] Furthermore, Cdk inhibitors p15, p21 and p27 are upregulated as part of IFN-induced cell cycle arrest.[16]

Antiproliferative effects, however, are not the only activity of IFN of potential relevance in cancer. Endothelial cell proliferation is crucial in tumour-associated neoangiogenesis and it has recently been shown that under normoxic conditions IFN-α can inhibit the proliferation of human endothelial cells by up-regulating hypoxia-inducible factor (HIF)-1α expression (a transcription factor subunit normally upregulated by protein stabilisation in response to hypoxia).[6] However, IFN-α treatment fails to induce transcription of several prototypic HIF-responsive genes due to an insufficient increase in HIF-1α protein levels. IFN is also capable of downregulating basic fibroblast growth factor (bFGF), a pro-angiogenic factor, both at mRNA and protein level.[17] Other pro-angiogenic factors are inhibited by IFN and are discussed below.

IFNs can also exert direct cytotoxic effects on tumour cells via apoptosis. IFN is capable of up-regulating tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)[18,19] and activating the caspase cascade (initiator caspases-8 and -9 and the effector caspase-3, in addition to caspases-1 and -2) associated with the activation of the pro-apoptotic Bak and Bax, loss of mitochondrial membrane potential, and release of cytochrome c.[20] However, the ability of IFN to induce apoptosis appears to be dependent on an intact phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) signalling pathway.[21]

Furthermore, IFN has wide-ranging effects on the haematopoietic system: it can suppress the proliferation of erythroid progenitors and also antagonise the action of growth factors such as platelet-derived growth factor and transforming growth factor-beta.[22–24] In addition, IFN can directly increase the expression of tumour-associated surface antigens and other surface molecules, such as major histocompatibility class I and II molecules, modulating cellular differentiation and preventing tumour growth.[5]

Finally, indirect effects of IFN on tumour growth are mediated through regulation of the host immune system. IFN may enhance antitumour immunosurveillance by regulating and controlling cytotoxic lymphocytes such as cytotoxic CD8+ T-cells and NK-cells. Furthermore, the number of tumour infiltrating CD4+ T-cells has been correlated with response to IFN therapy.[4] Further, IFN might indirectly affect the process of immunologically induced angiogenesis, whereby IFN may modulate the expression of pro-angiogenic factors produced by tumour-associated immune cells,[25] which is important to tumour growth.[26] IFN may also inhibit

Table 1 – Proposed antitumour mechanisms of IFN.

Direct effects	Indirect effects
Antiproliferative	Stimulation of immunological functions
Cytotoxic/pro-apoptotic effects on tumour cells	Activation to cytotoxic T-lymphocytes
Abrogation of growth/survival factor signalling	Activation of NK-cells
Induction of differentiation	Activation of monocyctes
Altered expression of surface molecules recognised by non-malignant cells	Non-immunological host effects
	Anti-angiogenic effects
	Interaction between stroma and malignant cells
	Regulation of cytokine production

IFN, interferon.

angiogenesis by inhibiting endothelial cell proliferation,[6] and suppressing vascular endothelial growth factor (VEGF) transcription[7] and secretion.[8]

This broad range of potential effects of IFN led to the investigation of IFN in a range of malignant diseases, including renal cell carcinoma (RCC), melanoma, lymphomas and leukaemias.[5,27–29] However, the use of crude preparations of IFN purified from virus-infected leucocytes meant that the results of these studies could not differentiate whether activity was due to IFN or other factors present in the crude preparation. The subsequent availability of highly purified, recombinant IFN, first approved for use in clinical trials in 1981, helped clarify the true properties of IFN. As such, IFN has the longest record of use of all cytokine therapies and was approved for the treatment of numerous solid tumours and haematological malignancies, in addition to use as an antiviral treatment for chronic hepatitis B and C (Table 2).

Due to our increased understanding of the molecular mechanisms involved in cancer development and growth and the availability of newer, alternative treatments, IFN is not currently used in some of the cancers for which it was initially investigated. However, IFN is still commonly employed as therapy in RCC, melanoma and myeloproliferative disorders (MPDs). This review will focus on the role of IFN in treating these disorders, providing an overview of available data and considering what the data suggest regarding the potential optimal use of IFN in the future.

Table 2 – Clinical indications of IFN.

IFN type	Trade name	Indications
IFN-α2a	Roferon-A	Advanced RCC Follicular lymphoma Hairy cell leukaemia AIDS-related KS Chronic hepatitis B Chronic hepatitis C Carcinoid tumour Multiple myeloma CML Cutaneous T-cell lymphoma
	Pegasys (Peginterferon)	Chronic hepatitis B Chronic hepatitis C
IFN-α2b	Intron A	Follicular lymphoma Hairy cell leukaemia AIDS-related KS Malignant melanoma Condyloma accuminata Chronic hepatitis B Chronic hepatitis C Carcinoid tumour Multiple myeloma
	Pegintron	Chronic hepatitis C
	Viraferon (PEG)	Chronic hepatitis B Chronic hepatitis C
IFN alfacon-1	Infergen	Chronic hepatitis C
IFN-αn3	Alferon N	Condyloma accuminta
IFN-β1a	Avonex Rebif	Relapsing-remitting MS
IFN-β1b	Betaseron Betaferon Extavia	Relapsing-remitting MS
IFN-γ	Actimmune	Chronic granulomatous disease

IFN, interferon; RCC, renal cell carcinoma; KS, Kaposi's sarcoma; CML, chronic myeloid leukaemia; MS, multiple sclerosis.

2. Renal cell carcinoma

RCC represents 3% of all cancers, with more than 200,000 cases diagnosed annually worldwide.[30,31] Its incidence peaks in patients aged 60–80 years and affects men more than women at a ratio of approximately 2:1. Clear-cell RCC is the main histological subtype of RCC, accounting for approximately 85% of all RCC tumours and linked with mutations in the *von Hippel–Lindau* (*VHL*) *gene*.[32] Genetic or epigenetic alterations in the VHL gene occur in up to 82% of clear-cell RCC tumours.[33] Defects in VHL often result in overexpression of VEGF, a key mediator of angiogenesis. The result of VEGF overexpression is the high degree of vascularisation found in RCC.

Patients with localised RCC can be effectively treated with surgery, which, in a large percentage of patients, can be curative. However, prior to the availability of novel agents targeting VEGF, patients with advanced and/or metastatic RCC had very limited options and survival was poor: the 5-year survival rate for patients with stage I disease is >90%, 85% for stage II and 60% for stage III, whereas for stage IV disease it is only 10%.[34] Traditional approaches used to treat cancer such as chemotherapy, radiotherapy and hormone therapy have proven ineffective in RCC. Until recently, IFN-α and interleukin (IL)-2 were the mainstay of therapy for patients with metastatic RCC, although cure remained infrequent and a careful patient selection was required for those patients who could benefit from these therapies.[35,36]

A meta-analysis of efficacy of IFN therapy compared to non-immunotherapy controls in metastatic RCC reports that during the first 2 years of treatment, IFN is associated with an increase in median survival of 3.8 months, a reduction of 1 year mortality of 44%, and a risk reduction for death of 26%.[35] IFN was the accepted standard of care for first-line therapy in Europe for a number of years[37] and considered to be the standard comparator treatment for clinical trials of new therapies in advanced RCC.[38] A recent study reports the value of IFN therapy in RCC may be limited to a selected group of good-prognosis patients as determined using defined risk criteria.[38,39] However, the treatment protocol used in this study appears to differ to the currently accepted treatment model for IFN. Table 3 highlights key trials of IFN therapy in RCC.

3. Melanoma

In 2006 there were an estimated 59,700 cases of melanoma and 13,200 melanoma-related deaths in Europe.[30] The 10-year survival rate for patients with stage III A–C disease is between 15% and 63%,[48] with cure rates of up to 50%.[49] For patients with stage IV disease, prognosis is poor and 10-year survival rates drop to 2.5–15.7% (American Joint Committee on Cancer staging system).[48] A systematic review of 41 randomised trials

reported that response rates to systemic therapy in patients with disseminated melanoma range from 6% to 15%, with a median progression-free survival (PFS) of 2–3 months.[50]

Chemotherapy with single-agent dacarbazine is currently the only FDA-approved chemotherapeutic agent for metastatic melanoma. As a monotherapy it is associated with a poor response rate of 10–20% and a median overall survival (OS) of 8–10 months.[51] The European Society of Medical Oncology (ESMO) guidelines recommend that it should not be considered as 'standard of treatment' and ESMO clinical recommendations for the treatment of cutaneous malignant melanoma state that following resection there are no standard adjuvant therapies for patients with localised high-risk melanoma or locoregional metastatic melanoma.[48] Options for these patients include adjuvant immunotherapy with IFN at various doses. Table 4 lists the largest and most important adjuvant trials with high-dose IFN,[52–54] intermediate IFN,[55] low-dose IFN,[56–59] ultra-low dose IFN[60] and the adjuvant trial with pegylated-IFN.[61] The National Comprehensive Cancer Network clinical practice guidelines recommend three adjuvant treatment options for patients with stage III melanoma or resection stage IV disease: observation; entry into a clinical trial (both category 2A recommendations); or IFN-α (category 2B recommendation).[62]

Systematic reviews of trials of high-dose IFN in patients with metastatic melanoma consistently report a significant improvement in disease-free survival (DFS);[63–65] however, there is no statistically significant impact on OS. A recent

Table 3 – Selected studies of IFN in RCC.

Study	*n*	Treatment	Summary
Quesada and colleagues[40]	15	IFN: 2 MIU/m^2 i.m. daily	31% response rate, 3 months PFS, 50% required dose reduction due to toxicity following high-dose IFN. No response was achieved with low-dose IFN
	46	IFN: 20 MIU/m^2 i.m. daily	
Kirkwood and colleagues[41]	14	IFN: 1 MIU/m^2 i.m. daily	12% response rate in 10 MIU/m^2 arm, no response in 1 MIU/m^2 arm. Minimal grade 3–4 toxicity
	16	IFN: 10 MIU/m^2 i.m. daily	
Muss and colleagues[42]	51	IFN: 2–10 MIU/m^2 s.c. t.i.w	9% response rate in s.c.-treated arm, 6% response rate in i.v.-treated arm. Grade $\geqslant$3 toxicity more common in i.v.-treated group
	46	IFN: 30–50 MIU/m^2 i.v. day 1–5 q3w	
Kriegmair and colleagues[43]	41	IFN: 8 MIU/m^2 s.c. d 1–3 weekly + VBL 0.1 mg/kg i.v. q3w	20% response rate in IFN + VBL arm, no response in MPA arm
	35	MPA: 500 mg/week	
Pyrhonen and colleagues[44]	79	IFN: 3–18 MIU/m^2 s.c. t.i.w + VBL 0.1 mg/kg i.v. q3w	16.5% response rate in IFN + VBL arm, 2.5% response rate in VBL arm. 19% grade 4 toxicity in IFN + VBL arm, 2% in VBL arm
	81	VBL: 0.1 mg/kg i.v. q3w	
MRCRCC[45]	167	IFN: 10 MIU/m^2 s.c. t.i.w	14% response rate with IFN, 2% with MPA. IFN gave an improvement in 1-year survival of 12%
	168	300 mg/daily MPA	
Mickisch and colleagues[46]	42	IFN: 5 MIU/m^2 s.c. t.i.w + nephrectomy	19% response rate with IFN + nephrectomy, 12% with IFN alone. About 17 months median survival duration with IFN + nephrectomy, 7 months with IFN alone
	43	IFN: 5 MIU/m^2 s.c. t.i.w	
Flanigan and colleagues[47]	120	IFN: 5 MIU/m^2 s.c. t.i.w + nephrectomy	3.3% response rate with IFN + nephrectomy, 3.6% with IFN alone. 11.1 months median survival duration with IFN + nephrectomy, 8.1 months with IFN alone
	121	IFN: 5 MIU/m^2 s.c. t.i.w	
Negrier and colleagues[39]	122	IFN: 9 MIU s.c. t.i.w	In intermediate prognosis patient, PFS (95% CI) were 3.0 (2.9–3.6), 3.4 (3.0–5.6), 3.4 (2.9–5.8) and 3.8 (3.0–5.9) months in the MPA, IFN, IL-2, and combined treatment arms, respectively. OS (95% CI) were 14.9 (11.7–19.2), 15.2 (12.8–19.9), 15.3 (13.3–20.0) and 16.8 (14.0–18.9) months in the MPA, IFN, IL-2, and combined treatment arms, respectively. IL-2 was associated with highest incidences of grade 3/4 adverse events and lowest quality of life scores
	125	IL-2: week 1: 9 MIU s.c. b.i.d for 5 d (2 d rest); weeks 2–4: 9 MIU bid for 2 d then 9 MIU daily for 3 d; week 5: rest	
	122	IFN + IL-2: IFN 9 MIU s.c. t.i.w + IL-2 schedule as above	
	123	MPA: 200 mg daily	

IFN, interferon; RCC, renal cell carcinoma; i.m., intramuscular; PFS, progression-free survival; s.c., subcutaneously; t.i.w, three times a week; i.v., intravenous; q3w, every three weeks; VBL, vinblastine; b.i.d., twice a day; CI, confidence interval; MPA, medroxyprogesterone acetate; IL-2, interleukin-2; OS, overall survival.

Table 4 – Selected studies of IFN in high-risk or advanced melanoma.

Study	*n*	Treatment	Summary
ECOG1684 (Kirkwood and colleagues[52])	143	20 MIU/m^2/d i.v. 5 d/week for 4 weeks, then 10 MIU/m^2/d s.c. t.i.w for 48 weeks	The 5-year RFS was 37% in the IFN arm versus 26% in the observation arm. The 5-year OS was 46% IFN arm versus 37% in the observation arm
	137	Observation	
ECOG1690 (Kirkwood and colleagues[53])	215	20 MIU/m^2 i.v. 5 d/week for 4 weeks; 10 MIU/m^2 s.c. t.i.w for 48 weeks (HDI arm)	The 5-year RFS for the HDI, LDI and observation arms were 44%, 40% and 35%, respectively. By Cox analysis, the impact of HDI on RFS achieved significance ($p_2 = 0.03$). The 5-year survival rates for the HDI, LDI and observation arms were 52%, 53% and 55%, respectively. IFN treatment had no significant impact on OS compared with observation
	215	3 MIU s.c. t.i.w for 2 years (LDI arm)	
	212	Observation	
NCCTG 83705 (Creagan and colleagues[54])	131	20 MIU/m^2 i.m. t.i.w for 12 weeks	The 5-year RFS was 43% in the IFN arm versus 36% in the observation arm. The 5-year OS was 54% IFN arm versus 48% in the observation arm. However, neither differences reached statistical significance
	131	Observation	
EORTC 18952 (Eggermont and colleagues[55])	553	13 months IFN: 10 MIU s.c. t.i.w 5 d/week for 4 weeks, then 10 MIU s.c. t.i.w for 1 year	The 4.5-year OS was 48.3%, 53.1% and 47.7% in the 13 months IFN, 25 months IFN and surgery arms, respectively. The 4-year DMFI was 43.2%, 47.2% and 40.0% in the 13 months IFN, 25 months IFN and surgery arms, respectively
	556	25 months IFN: 10 MIU s.c. t.i.w 5 d/week for 4 weeks, then 5 MIU s.c. t.i.w for 2 years	
	279	Surgery	
WHO 16 (Cascinelli and colleagues[56])	218	3 MIU s.c. t.i.w for 3 years	The 5-year DFS was 28% and 28% in the IFN arm and observation arm, respectively ($p = 0.50$)
	208	Observation	
UKCCCR (Hancock and colleagues[57])	338	3 MIU s.c. t.i.w for 2 years or until recurrence	The 5-year RFS was 33% in the IFN arm versus 30% in the observation arm. The 5-year OS was 46% IFN arm versus 42% in the observation arm. However, neither differences reached statistical significance
	336	Observation	
French MG (Grob and colleagues[58])	244	3 MIU s.c. t.i.w for 18 months	The 3-year relapse rate was 32% and 44% for the IFN arm and the observation arm, respectively. This difference was significant. The 3-year OS was 85% and 79% for the IFN arm and the observation arm, respectively. This difference was not significant.
	245	Observation	
DeCOG (Garbe and colleagues[59])	148	IFN: 3 MIU s.c. t.i.w for 2 years	The 4-year OS was 59%, 45.2% and 42.4% in the IFN, IFN + DTIC and surgery arms, respectively. The 4-year RFS was 39%, 29.4% and 27.3% in the IFN, IFN + DTIC and surgery arms, respectively
	148	IFN + DTIC 850 mg/m^2 every 4–8 weeks for 2 years	
	148	Surgery	
EORTC 18871/ DKG 80-1 (Kleeberg and colleagues[60])	830	Post-surgery for stage II (>3 mm) or stage III patients: 1 year IFN-α2b (1 MIU, s.c., t.i.w), or IFN-γ (0.2 mg, s.c., t.i.w) or Iscador, versus observation	Overall DFI at 8 years was 32.4% and OS was 40%. No significant differences between the treatment arms for DFI or OS
EORTC 18991 (Eggermont and colleagues[61])	627	PEG IFN-α2b 6 μg/kg per week for 8 weeks (induction) then 3 μg/kg per week (maintenance) for an intended duration of 5 years	The 4-year RFS was 45.6% in the IFN arm versus 38.9% in the observation arm; no difference in OS
	629	Observation	

IFN, interferon; i.v., intravenous; s.c., subcutaneously; t.i.w, three times a week; RFS = relapse-free survival; OS, overall survival; HDI = high-dose IFN; LDI = low-dose IFN; i.m., intramuscular; DMFI = distant metastasis-free survival; DTIC = dacarbazine.

meta-analysis of individual patient data from all the available randomised trials evaluating adjuvant IFN therapy provides evidence that adjuvant IFN significantly reduces the risk of relapse and improves OS, although the absolute survival benefit is only 2–3%.[66] Proponents claim that high-dose IFN is the only available therapy to achieve relapse-free survival and OS benefits.[67] However, the use of high-dose IFN in patients with stage IIb/III disease remains controversial; some physicians do not consider high-dose IFN as the standard of care in patients with melanoma due to the absence of consistent evidence demonstrating a long-term OS benefit and the significant associated toxicity.[63–65,68]

Identifying the subgroup of patients that are IFN-sensitive is of great importance to limit IFN exposure to those who will benefit. The presence or emergence of autoimmune antibodies during IFN therapy was believed to be such a potential indicator.[69] When this was investigated in the EORTC 18952 and the Nordic adjuvant IFN trials in both patients that received IFN or no IFN it became clear that autoimmune antibodies are not a significant prognostic factor for outcome, nor a predictive factor for IFN-sensitivity.[70] Moreover, in the EORTC 18991 adjuvant pegylated-IFN trial in stage III patients, these results were corroborated.[71]

Recently, an analysis of the two largest adjuvant trials ever conducted, EORTC 18952 and EORTC 18991, revealed that the presence of ulceration of the primary melanoma was a very strong indicator for IFN sensitivity.[72] Patients with non-ulcerated primaries had virtually no benefit from adjuvant IFN therapy, whereas in patients with ulcerated primaries DFS, distant metastasis-free survival and OS were all similarly and significantly impacted, especially in patients who entered the trials with stage IIB or stage III-N1 (microscopically involved nodes only). An effect in patients with macroscopically involved nodes (stage III-N2) was still present, but greatly reduced and no longer significant.[72] This finding has led to the decision that the EORTC will conduct a randomised trial in this targeted population, i.e. only in patients with ulcerated primary melanomas. In this population it is expected that adjuvant pegylated-IFN therapy will impact on DFS as well as distant metastasis-free survival and OS. It is interesting to note the correlation between ulcerated primary melanomas and IFN sensitivity, especially as ulceration has also been correlated with increasing tumour vascularity.[73] It may be hypothesised that the sensitivity of ulcerated primaries to IFN could be due to the anti-angiogenic properties of IFN acting upon the neovasculature of the developing tumour.

4. Myeloproliferative disorders

The MPDs represent a range of clonal haematological diseases with overlapping features. The main entities are polycythaemia vera (PV), essential thrombocythaemia (ET), primary myelofibrosis (MF) and chronic myeloid leukaemia. These conditions are generally rare: the incidence of PV is 0.02–2.8 per 100,000 per year; ET 0.1–1.5 per 100,000 per year; and MF 0.4–0.9 per 100,000 per year. The common features of PV and ET are overproduction of one or more myeloid lineages in the bone marrow. While the exact pathogenesis of these disorders is unknown, myeloid proliferation is consistently linked to mutations in the Janus kinase 2 (JAK2) tyrosine kinase gene.[74,75]

Untreated PV has a dismal prognosis with a median survival in untreated patients of 18 months and, although thrombosis is the dominant cause of death,[76] there is an increased risk of death from acute myeloid leukaemia (AML).[77] Therapy is focused on reducing the risk of thrombosis and haemorrhage, and transformation to AML and myelofibrosis. Besides phlebotomy in PV and low-dose aspirin therapy in both PV and ET, hydroxyurea (HU) is commonly used to treat PV and ET, and has shown superior activity over pipobroman in PV[78] and over anagrelide in ET.[79] The most important point of concern is the potential leukaemogenicity of HU. The literature does not present any evidence for increased leukaemogenicity of HU given as a single-agent. However, long-term HU use in ET patients that have previously received alkylating agents[80] or in combination with ^{32}P in PV[81] has been shown to increase the risk of developing AML and other cancers. IFN-α is not implicated as a possible leukaemogenic agent.

In some 80% of patients, IFN-α therapy is able to rapidly normalise platelet and leucocyte counts, as well reducing the need for phlebotomies in PV, as recently shown by Kiladjian and colleagues, who summarised treatment results with different IFN-α preparations in 776 MPD patients.[82] Due to a high rate of side-effects, leading to discontinuation of therapy in approximately 25% of patients,[82] pegylated forms of IFN-α have been investigated. A recent study reports that pegylated IFN-α2a therapy is well tolerated, with only 5% of patients stopping therapy at 1 year, and has excellent clinical efficacy (95% complete responses).[83] Key trials of IFN in MPDs are highlighted in Table 5.

To date, IFN-α therapy is the only therapy that has been shown to modulate fundamental abnormal biological processes in PV (reversal of chromosome abnormalities,[90–92] restoration of polyclonal haematopoiesis, and the suppression of erythropoietin-independent erythroid colony growth).[92] This profound suppression of malignant cells may be responsible for the fact that long-term treatment with IFN-α is also able to induce normalisation of the bone marrow morphology, which may even be sustained after discontinuation of IFN-α for up to 20 months.[93]

5. Challenges with IFN in the treatment of malignant disease

5.1. *Dose and duration of therapy*

Despite decades of clinical experience with IFN in patients with RCC and melanoma, the optimal dose and treatment duration of IFN remains to be established. Interestingly, the dose may influence antitumour mechanism of action. In both RCC and melanoma, high-dose IFN regimens correlate with better responses, possibly due to greater exposure of tumour cells to IFN and an increase in direct cellular effects.[5] However, lower doses of IFN have been suggested to act via activation of the immune system and anti-angiogenic activity.[94–96]

The therapeutic dose of IFN varies according to the type of cancer being treated. High doses of IFN (~10–20 MIU various administration schedules) have traditionally been used in

Table 5 – Selected studies of IFN in MPDs.

Study	MPD	*n*	Treatment	Summary
Radin and colleagues[84]	PV/ET/MF	60	IFN 2 MIU/daily	Objective response rates: PV, 42%; ET, 88%; MF, 3%.
Langer and colleagues[85]	ET	36	PEG-IFN 50–150 mg/week	67% complete response rate at 12 months, 64% still receiving IFN after 23 months
Silver and colleagues[86]	PV	55	IFN-α2a/b 1 MIU s.c. t.i.w escalating to 3 MIU s.c. t.i.w	DFS of 10 years, 96% freedom from phlebotomy at 2 years, 15% discontinuation rate, no thrombohaemorrhagic events
Samuelsson and colleagues[87]	PV/ET	42	PEG-Intron 0.5–1.0 μg/kg/week	69% complete platelet response rate at 6 months, no thrombohaemorrhagic events, 36% achieved normalised PRV-1 expression
Jabbour and colleagues[88]	PV/ET/MF	38	PEG-IFN-α2b 2 μg/kg/week	45% objective response rates (34% complete response; 11% partial response), median duration of response 20 months, 26% discontinued therapy
Kiladjian and colleagues[83]	PV	37	PEG-IFN-α2a 90–135 μg/week	95% complete response rate at 12 months, 24% discontinuation rate for side-effects at 3 years, 90% had a reduction in JAK2V617F expression (19% achieved complete molecular response)
Quintas-Cardama and colleagues[89]	PV/ET	74	PEG-IFN-α2a 90 μg/week	85% overall response rate (81% complete response; 4% partial response) after a median follow-up of 23 months, 56% had a >10% reduction in JAK2V617F expression

IFN, interferon; MPD, myeloproliferative disorders; PV, polycythaemia vera; ET, essential thrombocythaemia; MF, myelofibrosis; PV, polycythaemia vera; DFS, disease-free survival; PRV-1, polycythaemia rubra vera-1.

the treatment of high-risk melanoma which contrasts with the lower 3 MIU three times a week doses used in MPD (Tables 4 and 5). In RCC, a number of doses of IFN have been investigated (Table 3). Early studies used very low doses of IFN (1 MIU) up to high doses of 18–20 MIU on a three times weekly regimen. Later studies, and subsequently clinical practice, commonly used an intermediate dose of IFN 9–10 MIU three times a week as this regimen provided a balance between efficacy and tolerability. The rationale for the IFN dose in RCC and other solid tumours appears to be based on the empirical approach adopted for chemotherapeutic agents, whereby dose is determined by the maximum tolerated dose. This approach may not be appropriate for immunotherapies such as IFN. The biological effects of immunotherapy may take several months to become apparent, in contrast to the early responses associated with cytotoxic chemotherapy agents. Furthermore, the goal of immunotherapy may also need to be re-evaluated. Davis and colleagues suggest that response rate is of reduced importance if improvements in PFS, OS and quality of life can be achieved with immunotherapy.[97] This is particularly relevant for an agent that historically has produced relatively low response rates.

Interestingly, no dose–response relationship was identified in terms of improved survival, although higher-dose regimens resulted in higher remission rates, in a meta-analysis of IFN dose in RCC.[35] However, there is a clear dose relationship with toxicity. This is echoed in melanoma treatment, with high doses of IFN being associated with improvements in DFS and a reduction in 2-year mortality but no significant survival benefit.[98] A recent meta-analysis was unable to clarify the optimal dosage for IFN;[66] thus, there is a need to determine optimal IFN dose for each tumour type and whether a maximum tolerated dose or a minimal effective dose approach is appropriate.

A limited number of trials have assessed the association of treatment duration with IFN with efficacy. The European Organisation for Research and Treatment of Cancer (EORTC) 18952 trial showed that intermediate doses of IFN administered for 13 or 25 months do not improve outcomes in patients with high-risk melanoma. However, following a subgroup analysis of patients by disease stage, the authors suggest that duration of IFN treatment may be more important than dose in early-stage disease (stage IIb and stage III with only microscopic nodal disease).[55] Duration of IFN therapy was also assessed by the EORTC 18991 study. This study assessed the efficacy and toxicity of long-term pegylated IFN-α (induction at 6 μg/kg/week for 8 weeks and maintenance at 3 μg/kg/week) administered for an intended duration of 5 years.[61] Pegylated-IFN had a significant and sustained effect on relapse-free survival in patients with stage III melanoma but not on distant metastasis-free survival or OS. Interestingly, a third study shows low dose-IFN (3 MIU three times a week) administered for 2 years provides significant improvements in DFS and also OS.[59] Taken together, these studies indicate the duration of IFN may influence efficacy, and potentially OS, but further studies are required to substantiate these observations.

5.2. *Safety considerations*

Although IFN treatment may be effective, many patients discontinue therapy due to its side-effects. The incidence and

severity of side-effects are dose-related, with intermediate-dose (5–10 MIU) and low-dose (3–5 MIU) regimens being associated with fewer events than the high-dose regimens (20 MIU) used in the treatment of malignant metastatic melanoma. The dose-dependent side-effects of IFN may be categorised as acute or chronic.[99] Acute toxicity includes fevers, chills, myalgia, headache and arthralgia, which may occur after each administration. Chronic toxicity, occurring as a result of repeated injections, includes fatigue, myelosuppression, anorexia, liver abnormalities and depression. Symptomatic treatment may be required to manage IFN-related side-effects. In severe cases, the side-effects may be managed by dose interruption, modification or discontinuation of treatment. However, long-term therapy (median 13 years) for PV with IFN-α is well tolerated, with gradual dose reductions to minimise toxicity (reduced from the target maximum dose of 3 MIU to 1 MIU), and long-term therapy with lower-dose IFN has been the key when the therapeutic goal was to maintain haematological remission.[86] Pegylated forms of IFN are now widely used in MPD therapy to achieve this goal as they have lower toxicity and increased drug stability, without affecting activity.[100] Theoretically of more importance, the pegylated forms of IFN has conferred a higher degree of molecular CR which may in the future lead to a diminished risk of myelofibrosis and leukaemia.

In addition, the route of IFN administration may also be important. Subcutaneous administration of IFN at a lower dose in patients with RCC retains efficacy but has lower toxicity than higher doses of IFN administered intravenously.[42]

5.3. *Predictors of response to IFN therapy*

Some tumour cells are resistant to IFN *in vitro*, which, if mirrored *in vivo*, may lead to treatment failure. The identification of surrogate markers of response to IFN or predictive assays might help to select those patients most likely to respond to IFN treatment and avoid the need for unnecessary toxic treatment in others. These markers may differ according to the type of cancer.

Initial studies in patients with MPDs have identified a number of potential molecular markers of IFN response. The vast majority of patients with PV have a mutation in JAK2.[101] JAK2 is a widely expressed cytoplasmic tyrosine kinase with a key role in signal transduction from multiple haemopoietic growth factor receptors. Almost 50% of patients with ET or chronic idiopathic myelofibrosis have this mutation. As mentioned above, pegylated IFN-α has been shown to decrease JAK2V617F mutant expression in patients with PV and ET.[87,102] and is at the moment the best marker for a molecular response to therapy in the MPDs.

The expression of methylthioadenosine phosphorylase (MTAP) has been identified as a potential marker of IFN response in malignant melanoma.[103] Evidence indicates that loss of MTAP results in inhibition of STAT signalling pathways regulated by IFN. In patients with RCC, a STAT3 polymorphism has been linked to IFN response.[104]

In summary, reassessment of the optimal dose and duration of IFN therapy in malignant diseases is required as it appears possible that lower IFN doses can maintain efficacy, particularly OS, which is still the gold standard measure of efficacy in many malignant diseases, while limiting toxicity.

6. Redefining the role of IFN in the treatment of malignant disease

Cancer does not usually have a single pathogenesis and combination therapy is commonly used in oncology. Dose modification to enable the use of combinations of agents is important both from a tolerability perspective and to maximise any potential synergy. To date, trials have failed to show that this approach is effective when IFN is combined with conventional chemotherapeutic agents.[35,105] However, better understanding of tumour biology and the development of agents inhibiting specific factors involved in tumour growth creates new opportunities for combinations that can be explored.

For example, the development of novel angiogenesis inhibitors has changed the therapeutic landscape of metastatic RCC. Angiogenesis is vital for tumour growth and VEGF is recognised as the key mediator of angiogenesis. As discussed earlier, the majority of patients with clear-cell RCC have mutations in the *VHL* tumour suppressor gene, leading to upregulation of the heterodimeric HIF transcription factor and overexpression of VEGF.[106] Bevacizumab is a humanised monoclonal antibody that inhibits VEGF and has demonstrated significant clinical benefit in several tumour types including metastatic colorectal,[107] breast[108] and non-small cell lung cancer.[109] It is approved in combination with IFN for the first-line treatment of advanced and/or metastatic RCC. Other novel agents approved for the treatment of RCC include the tyrosine kinase inhibitors sunitinib and sorafenib, and the mTOR inhibitors temsirolimus and everolimus. Given the differences between the mechanisms of action of IFN and these novel therapies, IFN has the potential to be an important combination partner.

Several combination studies have been conducted to determine whether the addition of IFN may improve further the efficacy of these novel agents in patients with metastatic RCC (Table 6). To date, the largest studies evaluating IFN in combination with a novel agent are the randomised phase III AVOREN trial and the US-based Cancer and Leukaemia Group B (CALGB) 90206 trial. Both trials show that combining bevacizumab with IFN significantly improves PFS compared with IFN (combined with placebo in AVOREN or alone in CALGB 90206) in patients with previously untreated metastatic RCC (AVOREN: hazard ratio [HR] = 0.63, $p = 0.0001$; CALGB 90206: HR = 0.71, $p < 0.0001$).[110,111] The OS data for these trials have recently been reported and also show a similar magnitude of benefit based on HR values: AVOREN, median OS with bevacizumab + IFN is 23.3 months compared with 21.3 months with placebo + IFN (HR = 0.86; $p = 0.1291$[stratified]);[117] CALGB 90206, median OS with bevacizumab + IFN is 18.3 months compared with 17.4 months with placebo + IFN (HR = 0.86; $p = 0.069$ [stratified]).[118] The difference in median OS between these two trials may be due to a number of factors, including differences in patient characteristics and familiarity with IFN use. It is interesting to note that patients in CALGB 90206 received shorter duration of IFN treatment

Table 6 – Trials of IFN combined with novel agents targeting angiogenesis in patients with RCC.

Trial	Phase	Treatment	*n*	PFS (months)	ORR (%)	SD (%)
Escudier and colleagues (AVOREN)[110]	III	Bevacizumab (10 mg/kg q2w) combined with IFN (9 MIU t.i.w)	649	10.2	31	46
Rini and colleagues (CALGB 90206)[111]	III	Bevacizumab (10 mg/kg q2w) combined with IFN (9 MIU t.i.w)	732	8.5	25.5	
Kondagunta and colleagues[112]	I	Sunitinib (50 or 37.5 mg daily; 4/2 schedule) plus IFN (dose escalation form 3 MIU t.i.w to 9 MIU t.i.w)	25	11.9[a]	12	
Ryan and colleagues[113]	II	Sorafenib (400 mg b.i.d) plus IFN (10 MIU t.i.w)	62	7	19	50[b]
Gollob and colleagues[114]	II	Sorafenib (400 mg b.i.d) plus IFN (10 MIU t.i.w)	40	10	33	40
Bracarda and colleagues (RAPSODY)[115]	II	Sorafenib (400 mg twice daily) plus IFN 9 MIU t.i.w (arm A) or IFN 3 MIU t.i.w (arm B)	Arm A: 51 Arm B: 49	6.4 8.5	17.6 34.7	45.1 44.9
Hudes and colleagues[116]	III	Temsirolimus (15 mg once weekly) plus IFN (3–6 MIU t.i.w)	626	4.7	8.1	

IFN, interferon; RCC, renal cell carcinoma; PFS, progression-free survival; ORR, overall response rate; SD, stable disease; q2w, every 2 weeks; t.i.w, three times a week; b.i.d, twice a day.

[a] Median time to progression.

[b] Achieved an unconfirmed partial response or SD as best response.

than that in AVOREN:[110,111] 5.5 months compared with 7.8 months in the bevacizumab + IFN arms of CALGB 90206 and AVOREN, respectively, and 2.8 months in the IFN monotherapy arm of CALGB 90206 compared with 4.6 months in the placebo + IFN arm of AVOREN.

An intermediate dose of IFN (9 MIU three times a week) was used as the standard IFN dose in these trials. This dose and regimen was selected as it was an acceptable dose in terms of the required efficacy and toxicity (Table 3), and is commonly used in this indication in Europe. IFN dose reduction was prespecified in these trials as means of managing grade 3/4 toxicity and a proportion of patients received either 3 or 6 MIU three times a week. Retrospective analysis of these patients suggests that the PFS benefit of bevacizumab combined with IFN is maintained (HR = 0.63, $p = 0.0026$) (Fig. 1) and PFS rates in the bevacizumab plus reduced dose IFN group is comparable with the total population (12-months Kaplan–Meier estimates of event-free rate at 1 year: 0.524 vs. 0.427).[119] Lowering the dose of IFN significantly reduces the incidence and severity of IFN-associated side-effects, allowing management of IFN-related side-effects while maintaining efficacy and enabling patients to remain on therapy.[119] A similar analysis has not been reported for CALGB 90206 but the data suggest patients stopped IFN therapy earlier than in AVOREN, regardless of whether dose reduction was performed.[110,111] This data could be interpreted to suggest that the lower exposure to IFN in CALGB 90206 could account for some of the differences in absolute median PFS and OS values compared with AVOREN.

The predictable tolerability profile of bevacizumab, consisting of events specifically related to VEGF inhibition, appears to be a requirement for effective combination with IFN. Combining IFN with agents with less-specific tolerability profiles appears to be more difficult because this may lead to overlapping or synergistic toxicity. A phase I dose-finding study demonstrated that sunitinib plus IFN was only tolerable

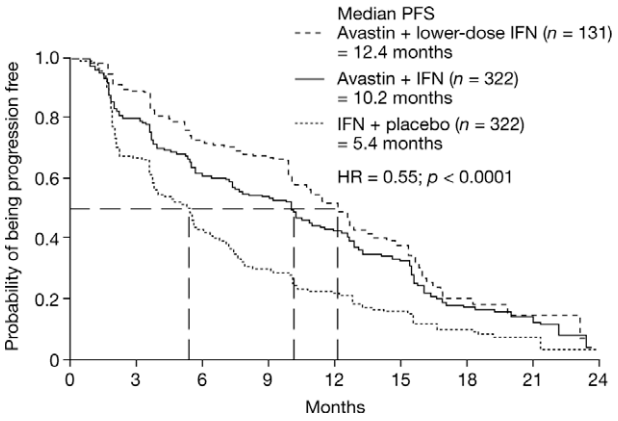

Fig. 1 – Kaplan–Meier analysis of PFS in patients receiving lower doses of IFN compared with the overall study populations.[110,119]

Reprinted from The Lancet, 370, Escudier B, et al., 2103–11, Copyright 2007, with permission from Elsevier. Melichar B, et al. First-line bevacizumab combined with reduced dose interferon-alpha2a is active in patients with metastatic renal cell carcinoma. Annals of Oncology 2008;19(8):1470–6, by permission of Oxford University Press.

at significantly reduced doses, which in turn impacts on the efficacy of the treatment regimen.[112] Patients receiving temsirolimus (25 mg weekly) had significantly longer OS (10.9 months) than patients receiving IFN monotherapy (18 MIU three times a week) (7.3 months; HR = 0.73, $p = 0.008$) and those receiving a combination of IFN (6 MIU three times a week) plus temsirolimus (15 mg weekly) (8.4 months; HR = 0.96, $p = 0.70$). The combination of temsirolimus and IFN was associated with greater toxicity than either agent used alone.[116] Two phase II studies have demonstrated that although sorafenib (400 mg twice daily) in combination

with IFN (10 MIU three times weekly) may be associated with an improved response rate compared with IFN-α or sorafenib alone, the combination is limited by toxicity.[113,114]

Intriguingly and similarly to AVOREN, the randomised phase II RAPSODY study of sorafenib in patients with RCC also showed that low-dose IFN is a suitable combination partner. Low-dose IFN (3 MIU five times a week) in combination with sorafenib (400 mg twice daily) was more effective and well tolerated in the first-line setting than the same combination with intermediate doses of IFN.[115] However, a study of a further lower dose of IFN (0.5 MIU three times a week) in combination with sorafenib (400 mg twice daily) did not show any efficacy benefit over sorafenib alone,[120] suggesting a minimum dose threshold for IFN may exist.

In patients with melanoma, a phase III trial of adjuvant low-dose IFN (3 MIU three times a week) for 2 years following surgery showed significantly improved OS and DFS in patients with melanoma that had spread to the lymph nodes compared with surgery alone.[59] However, the addition of dacarbazine to low-dose IFN reversed the beneficial effect of IFN. The authors suggested that this may be due to the immunosuppressive effects of dacarbazine counteracting the immunostimulatory effects of IFN. Other recent trials have investigated anti-angiogenic agents in combination with a reduced dose of IFN for metastatic melanoma. Low-dose IFN (1 MIU/m^2 daily) administered subcutaneously in combination with bevacizumab (15 mg/kg every 2 weeks) was well tolerated but had low activity, suggesting further optimisation of this dual anti-angiogenic regimen is required.[121]

What are the implications of these data suggesting that using lower doses of IFN in combination with novel agents are as effective as combinations involving higher IFN doses but better tolerated?[110,119] It has been hypothesised that anti-angiogenic agents may make tumours more vulnerable to the immune system and this may explain the potential synergistic effect when combining immunotherapy with these agents. In addition, IFN is used chronically and at relatively low doses in patients with haematological malignancies, suggesting that a number of different mechanisms could be relevant to its anticancer effects. The pleiotropic effects of IFN appear to be dependent on dose.[5] In RCC and melanoma, the intermediate and high doses of IFN may possibly result in tumours being directly exposed to IFN, resulting in effects such as growth inhibition and cytotoxicity. In contrast, the effectiveness of lower doses of IFN used in treatment of MPDs may rely on the myelosuppressive properties of IFN. Alternatively, the effects of lower doses of IFN have been suggested to stem from indirect effects such as activation of the immune system and anti-angiogenic activity. The anti-angiogenic properties of low-dose IFN may also explain the potential synergy observed in metastatic RCC when combined with therapy targeting VEGF such as bevacizumab or sorafenib. However, since not all low-dose combinations of IFN with other agents have demonstrated significant efficacy, a better understanding of the mechanism of action of IFN is needed to help determine which combination partners may be effective with low-dose IFN, which dose of IFN is best used and for how long, and which patients might be suitable for such therapy. What seems more certain is that IFN will continue to have a role in the management of malignant diseases and that further research to maximise its efficacy and minimise its toxicity is needed.

Conflict of interest statement

Dr. Bracarda has served on advisory boards for Bayer Schering-Pharma, Pfizer, F. Hoffmann-La Roche Ltd., Wyeth, Novartis and GlaxoSmithKline and has received honoraria for lectures from Novartis. Dr. Eggermont is a consultant and holds a research grant for Schering Plough. Dr. Samuelsson is a consultant for Swedish Orphan pharmaceuticals, has received honoraria for lectures from F. Hoffmann-La Roche Ltd., and has served on advisory boards for F. Hoffmann-La Roche Ltd. and GlaxoSmithKline.

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