Expert Opinion

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Pegylated IFNs for chronic hepatitis C: an update

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For over a decade, IFN-α_2 has been the standard treatment for chronic hepatitis C. However, the drug's rapid clearance and short half-life have led to low rates of sustained virological response. Pegylation is a well-established method of modifying the pharmacological properties of IFNs, causing significant improvements in pharmacokinetics, which in turn lead to improved efficacy. Two pegylated forms of IFN-α_2 have been developed: PEG-IFN-α_{2b} and PEG-IFN-α_{2a}, and their efficacy has been established in randomised, controlled trials. However, the two differ significantly in structure, *in vitro* activity and pharmacological properties, and this may translate into differences in clinical efficacy. Comparative trials have been initiated that will provide insight into relative importance of pharmacokinetics, bioactivity and dosing regimen.

Keywords: chronic hepatitis C infection, IFN-α_2, pegylated IFN-α_{2a}, pegylated IFN-α_{2b}, ribavirin

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1. Introduction and overview of current status

Hepatitis C virus (HCV) is a leading cause of chronic liver disease worldwide and is estimated to have infected ≥ 170 million people [1]. HCV is the most common reason for liver transplantation and is also associated with an increased risk of hepatocellular carcinoma [2,3]. The virus is blood-borne and the primary mode of transmission is through intravenous drug abuse [4]. Due to the chronic nature of the disease, the number of infected patients is set to increase dramatically.

HCV is classified into six distinct genotypes and knowledge of the genotype is an important predictor of treatment response [5,6]. Genotype 1 is associated with a lower response to treatment, and accounts for 70 – 75% of HCV infections in the US [7]. Following genotype 1, genotypes 2 and 3 are the most common forms of HCV in the US and Western Europe [1]. Genotype 4 is more common in Egypt, genotype 5 in South Africa and genotype 6 in Southeast Asia. Genotypes 4, 5 and 6 have also been associated with a poor response to currently available therapies.

For more than a decade, IFN-α_2 has been the standard treatment for chronic HCV. However, efficacy has been limited by the drug's rapid clearance and short half-life of ~ 4 – 16 h [8]. IFN-α requires subcutaneous dosing three-times weekly to obtain adequate drug concentrations. This frequent dosing schedule increases the risk of peak-concentration-related adverse events and increasing patient inconvenience, thus compromising patient compliance.

The process of pegylation provides significant improvements in the pharmacokinetics of IFNs, allowing once-weekly dosing, improved adherence to treatment and an increased rate of sustained virological response (SVR). Two pegylated forms of IFN-α_2 have been developed: pegylated IFN-α_{2b} (PEG-IFN-α_{2b}) and PEG-IFN-α_{2a}. The addition of ribavirin to the treatment regimen has further improved efficacy, and PEG-IFN-α_2 in combination with ribavirin is, therefore, the current standard for treatment of chronic HCV infection [7].

Table 1. Differences between the two pegylated IFNs.

IFN	IFN-α_{2b}	IFN-α_{2a}
Polyethylene glycol	Single 12 kDa polymer	Two branched polymers
Positional isomers	His34 (> 50%) Cys1 (~ 13%) Lys121 (~ 7%) Lys31 (~ 5%) Lys49 (~ 5%) Remaining 20% consists of Lys83, Lys112, Lys161, Lys131, Lys133, His7, Tyr129, Ser163	Lys31 Lys121 Lys131 Lys134 Lys70, Lys83, Lys49, Lys112, Lys164, Lys23, Lys133
Antiviral activity	28% for mixture 37% for His34 isomer	1% for mixture

2. IFN therapy for chronic hepatitis C virus infection

2.1 IFN-α

IFN-α_{2b} and IFN-α_{2a} are recombinant DNA-derived products derived with amino acid sequence identity to endogenous IFNs. Both products have been shown to be effective for the treatment of HCV infection [5,6]. The initial treatment regimen consisted of IFN-α monotherapy. However, rates of SVR, defined as undetectable levels of HCV RNA in the serum for ≥ 6 months following completion of therapy, have been reported to be as low as ~ 10 – 20% following IFN-α monotherapy [9,10].

The addition of ribavirin nucleoside analogue with antiviral activity to the IFN treatment regimen improved SVR rates to ~ 38 – 43% [5,6]. Although the mechanism of action of ribavirin is unclear, its use with IFN has improved the efficacy of IFN-based therapy for HCV infection, and until recently IFN in combination with ribavirin was the standard for treatment of HCV infection [11]. However, major pharmacokinetic and pharmacodynamic limitations of the regimen remained.

2.2 Pegylated IFN-α

Conjugation of an inert polyethylene glycol (PEG) molecule to the IFN-α core protein is a well-established method of modifying the pharmacological characteristics of the core protein [12-14]. The chemical properties of PEG that are particularly useful for therapeutic applications are water solubility, lack of toxicity and immunogenicity [15-18]. The covalent bond created is an ester or amide bond at an amino acid residue on the surface of the protein. The type of bond and site of attachment varies with the PEG-linker and the chemistry conditions used for conjugation.

Pegylation creates a larger apparent molecule, with an increased molecular weight and apparent Stokes radius (the theoretical radius of the molecule). In fact, the Stokes radius for the conjugated protein is significantly larger than that predicted from the simple addition of the PEG mass to the core protein mass. This is due to the hydrophilic nature of PEG, in which each ethylene oxide unit is associated with two or three water molecules [19]. The increase in apparent size is believed to prolong drug absorption time and half-life, enabling once-weekly dosing. Several mechanisms may account for the increase in half-life of proteins linked to PEG. These include masking specific amino-acid sequences for which there are cellular receptors, interference with the interaction between carbohydrate chains and their specific receptors (both of which decrease receptor-mediated clearance), diminished proteolysis, and increasing the size of proteins above the limit for glomerular filtration [12].

In addition, pegylation has been suggested to reduce the immunogenicity and antigenicity of the core protein, which may result in a reduced incidence of hypersensitivity reactions [12]. However, pegylation has also been shown to result in decreased activity of the core protein in *in vitro* systems, which suggests that a perturbation in specific receptor interactions may occur [20,21].

Currently, two different pegylated forms of IFN-α_2 are approved and commercially available: PEG-IFN-α_{2b} (PEG-Intron®, Schering Corp, NJ, USA) and PEG-IFN-α_{2a} (Pegasys®, Hoffmann-La Roche, Basel, Switzerland).

3. Chemistry

3.1 PEG-IFN-α_{2b}

PEG-IFN-α_{2b} is produced by forming a covalent bond between a 12 kDa PEG linear molecule, monomethoxypolyethylene glycol (mPEG), and the IFN-α_{2b} core protein. Succinimidyl carbonate pegylation chemistry processes are used to create a product that is a mixture of biologically active monopegylated positional isomers [21,22]. The primary site of conjugation is on the histidine His34 amino acid residue (~ 50% of all the positional isomers) (**Figure 1**) [21,22]. The remaining positional isomer sites that make up the other 50% are distributed among the various lysine residues, another histidine residue, cysteine and serine residues (**Table 1**). In terms of antiviral activity, the His34-positional isomer has been shown to be the most active, possessing ~ 37% of the antiviral activity of recombinant IFN-α_{2b} [21]. It has been suggested that the bond formed by the succinimidyl carbonate conjugation chemistry with the imidazole ring of histidine is hydrolytically labile [19]. However, nuclear magnetic resonance studies have shown that the bond is 18-fold more stable in the context of the tertiary structure of the IFN-α_2 protein than in a simple peptide [23]. Additional studies in one laboratory have confirmed that the higher antiviral activity of the His34-positional isomer compared with other isomers occurs with the intact pegylated molecule [21,24]. Overall, the antiviral activity of PEG-IFN-α_{2b} is ~ 28% by weight of the IFN-α_{2b} core protein compared with IFN-α_{2b} (**Table 2**).

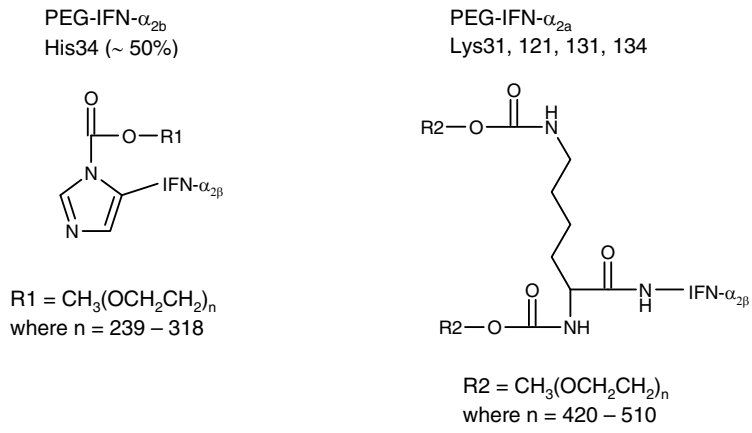

Figure 1. Chemical structure and linkages of PEG-IFN-α_{2b} and PEG-IFN-α_{2a}.
PEG: Polyethylene glycol.

Table 2. Specific activity of the two pegylated IFNs.

IFN	Specific activity	CPE antiviral assay
PEG-IFN-α_{2a}	1.1 x 10^6 IU/mg	MDBK cells/VSV virus infection [25]
PEG-IFN-α_{2b}	7.3 x 10^7 IU/mg	FS-71 cells/EMC virus infection [21]

The specific activity for IFN-α_{2b} in the FS-71/EMC assay has been reported to be 2.6 x 10^8 IU/mg.
CPE: Cytopathic effect; EMC: Encephalomyocarditis; MDBK: Madin–Darby kidney bovine; PEG: Polyethylene glycol; VSV: Vesicular stomatitis virus.

3.2 PEG-IFN-α_{2a}

With PEG-IFN-α_{2a}, a 40 kDa branched PEG molecule is attached by a covalent bond to the IFN-α_{2a} core protein. PEG-IFN-α_{2a} is synthesised using the *N*-hydroxysuccinimid pegylation chemistry and manufacturing process, which leads to a mixture of four major positional isomers at Lys31, Lys121, Lys131 and Lys134, and several minor positional isomers (**Figure 1**, **Table 1**) [20]. The antiviral activity of PEG-IFN-α_{2a} is ~ 1% of the antiviral activity of IFN-α_{2a} [25]. The Lys31 and Lys134-positional isomers have been shown to be more active than the mixture, possessing ~ 2% of the antiviral activity of IFN-α_{2b} (**Table 2**) [25].

4. Biological activity and molecular structure

Consistent with the modification of most biologically active proteins, pegylation of IFN-α core protein results in the loss of *in vitro* biological activity [12,26]. This does not appear to be due to a detectable alteration in the secondary or tertiary conformation of the core IFN-α protein [25,27,28]. Although additional physical–chemical studies are needed to rule out subtle changes in the core protein conformation, the respective size and distributions of the pegylated positional isomers appear to be the principal effector in reducing biological activity.

There are noted differences in the reported specific antiviral activity between PEG-IFN-α_{2a} (2%) and PEG-IFN-α_{2b} (37%), which have been compared and confirmed within one laboratory [24,29-31]. The loss in antiviral activity has been shown to be a result of differences in signal transduction and activator of transcription (STAT)-mediated signal transduction associated with the IFN-α_{2a} or -α_{2b} interaction with the receptor. Specifically, PEG-IFN-α_{2b} demonstrates higher STAT nuclear translocation, higher levels of IFN response gene mRNA and higher antiviral activity than PEG-IFN-α_{2a}.

The specific mechanisms that may account for lower signal transduction associated with PEG-IFN-α_{2a} are being investigated. One putative mechanism may be the effect of the higher molecular size of the pegylation conjugate on the interaction of IFN-α_{2a} with the receptor (**Figure 2**). A second mechanism may be the effect of the specific site of pegylation in the interaction with the core protein and the receptor. For instance, the highest antiviral activity of a PEG-IFN-α has been seen with the His34-positional isomer in PEG-IFN-α_{2b}, a positional isomer that is absent in PEG-IFN-α_{2a}. On the other hand, the lysine isomers have been shown to exhibit lower relative activity in both PEG-IFN-α_{2a} and PEG-IFN-α_{2b} [24].

Despite the loss in specific activity of the core protein associated with pegylation, PEG-IFN-α_{2b} has been reported to have an *in vitro* biological potency profile for both antiviral and immunotherapy activity that is highly comparable with IFN-α_{2b}. Thus, although pegylation reduces specific activity, it does not seem to alter the potential biotherapeutic potency of the antiviral response *in vitro* [22]. This is probably due to the loss in specific activity for PEG-IFN-α_{2b} being compensated for by adding equivalent antiviral activity back into the assay. However, this compensatory effect has not yet been demonstrated *in vitro* for PEG-IFN-α_{2a}, which has a significantly greater loss in specific activity associated with pegylation.

Table 3. Pharmacokinetic parameters of the two pegylated IFNs [14,33].

	IFN-α_{2b}	PEG-IFN-α_{2b}	IFN-α_{2a}	PEG-IFN-α_{2a}
Volume of distribution	1.4 l/kg	0.99 l/kg	31 – 73 l	8 – 12 l
Clearance	231 ml/h/kg	22 ml/h/kg	6600 – 29200 ml/h	60 – 100 ml/h
Absorption half-life	2.3 h	4.6 h	2.3 h	50 h
Half-life	4 – 7 h	27 – 37 h	3.8 h	65 h

PEG: Polyethylene glycol.

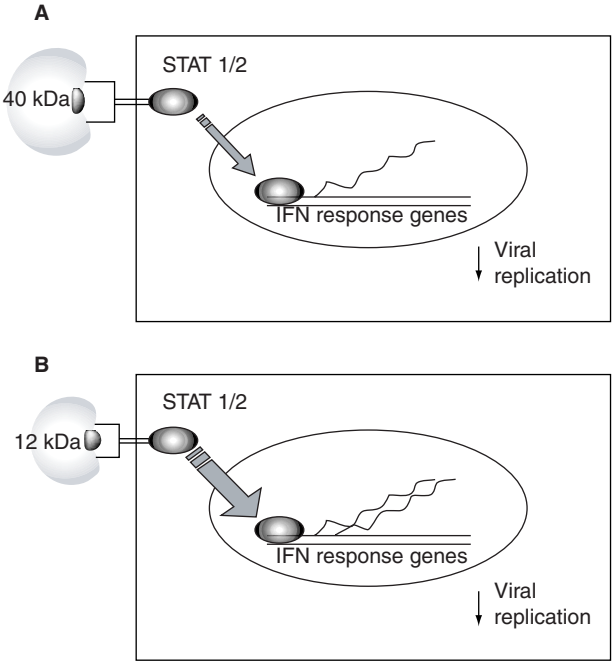

Figure 2. Schematic: reduced signal transduction with higher molecular size (A) and higher signal transduction with lower molecular size (B).

STAT: Signal transduction and activator of transcription.

5. Pharmacokinetics and pharmacodynamics

Pegylation has been shown in a number of studies to improve both the pharmacokinetic and pharmacodynamic properties of IFN-α (**Table 3**).

5.1 PEG-IFN-α_{2b}

In an open-label study, the mean apparent clearance of PEG-IFN-α_{2b} was substantially reduced compared with that of IFN-α_{2b} (22 versus 231 ml/h/kg). This study involved 58 patients with chronic HCV infection who were randomised to receive PEG-IFN-α_{2b} 0.035 – 2.0 µg/kg/week or IFN-α_{2b} 3 MIU three-times weekly, for 24 weeks. Pegylation had little or no effect on the rate of absorption or volume of distribution. However, whereas serum concentrations of IFN-α_{2b} declined rapidly and were undetectable 24 h after a single dose, maximal concentrations of PEG-IFN-α_{2b} were sustained for 48 – 72 h, before beginning a slow elimination phase. Pegylation did not alter the pharmacodynamic or tolerability profiles of IFN-α_{2b} in this study. Antiviral activity was increased in PEG-IFN-α_{2b} recipients, whereas changes in neutrophil, white blood cell and platelet counts were similar between treatment groups [32].

The addition of ribavirin to the treatment regimen has no effect on the pharmacokinetics of PEG-IFN-α_{2b}. In an open-label trial conducted by Glue *et al.*, 72 patients with chronic HCV infection were randomised to treatment with PEG-IFN-α_{2b} alone or in combination with ribavirin. Antiviral efficacy of PEG-IFN-α_{2b} was enhanced and this translated into increased SVR rates at each dose of PEG-IFN-α_{2b}. There was no significant difference in pharmacokinetic parameters between the two treatment groups and both were well tolerated [15].

5.2 PEG-IFN-α_{2a}

Improved pharmacokinetics have also been reported for PEG-IFN-α_{2a} compared with its nonpegylated equivalent. In two single-dose studies in volunteers, sustained absorption, reduced systemic clearance and an increased serum half-life were reported with PEG-IFN-α_{2a} compared with IFN-α_{2a} [33,34]. A multiple dosing study in patients with chronic HCV infection also reported sustained absorption and a reduced clearance with PEG-IFN-α_{2a} [35]. Serum 2′,5′-oligoadenylate synthetase (OAS) activity, a recognised marker of antiviral activity, was increased with both PEG-IFN-α_{2a} and IFN-α_{2a} in the study by Xu *et al.*, although the duration of activity was longer in PEG-IFN-α_{2a} recipients [33].

6. Clinical experience with pegylated IFNs

The increased efficacy of PEG-IFNs over IFNs has been well established. Moreover, the addition of ribavirin to the treatment regimen has further enhanced efficacy in a number of randomised, controlled clinical trials, while maintaining similar rates of adverse events to monotherapy.

6.1 PEG-IFN-α_{2b}

6.1.1 Monotherapy

The efficacy and tolerability of PEG-IFN-α_{2b} monotherapy was compared with IFN-α_{2b} in a multi-centre, randomised, double-blind, dose-finding study involving 1219 patients

with chronic HCV infection. IFN-α_{2b} was administered at a dosage of 3 MIU three-times weekly, whereas PEG-IFN-α_{2b} was given at dosages of 0.5, 1 or 1.5 µg/kg/week, both for 48 weeks. Patients were followed up at 72 weeks. All three PEG-IFN doses significantly improved both end-of-treatment and end-of-follow-up virological responses compared with IFN-α_{2b}. Interestingly, whereas end-of-treatment virological responses were dose-related in the PEG-IFN groups, there was no such effect with regard to SVRs; they were similar for the 1 and 1.5 µg/kg groups. No new or unexpected adverse events were reported with PEG-IFN-α_{2b}, and adverse event profiles were similar across all treatment groups. Moreover, changes to haematological parameters, which can be expected with IFN-based therapy, were similar in all three treatment groups [36].

6.1.2 *In combination with ribavirin*

The addition of ribavirin to the PEG-IFN-α_{2b} treatment regimen results in improved overall efficacy. In a multicentre, open-label trial involving 1530 patients with chronic HCV infection, combination therapy was reported to have greater efficacy than monotherapy. Patients were randomised to treatment with IFN-α_{2b} (3 MIU s.c. three-times weekly) plus ribavirin (1000 – 1200 mg/day p.o.), PEG-IFN-α_{2b} (1.5 µg/kg/week for 4 weeks, then 0.5 µg/week plus ribavirin 1000 – 1200 mg/day) or PEG-IFN-α_{2b} (1.5 µg/kg weekly) plus ribavirin (800 mg/day) for 48 weeks. In this trial, the dosage of ribavirin in the 1000 – 1200 mg/day groups was adjusted according to bodyweight (1000 mg/day for patients weighing < 75 kg and 1200 mg/kg for those ≥ 75 kg). Results indicated that the most effective therapy was PEG-IFN-α_{2b} at the higher dose of 1.5 µg/kg, in combination with ribavirin. Indeed, the SVR was significantly greater ($p = 0.01$ for both comparisons) in the higher-dose PEG-IFN group (54%) than in the lower-dose PEG-IFN (47%) or IFN groups (47%). In patients with HCV genotype 1, corresponding rates of SVR were 42, 34 and 33%, respectively. In patients with genotype 2 or 3, SVR rates were ~ 80% in all three groups. Treatment was similarly tolerated in all three treatment groups and, as in the PEG-IFN-α_{2b} monotherapy trials, no new or unexpected adverse events were reported with combined PEG-IFN plus ribavirin therapy. Although ribavirin monotherapy has previously been associated with anaemic effects [37-39], a greater fall in haemoglobin did not occur in the optimum PEG-IFN-α_{2b} 1.5 µg/kg group [40]. A recently completed randomised trial in 311 patients with HCV genotype 1 has confirmed the improved efficacy of PEG-IFN-α_{2b} plus ribavirin compared with IFN-α_{2b} plus ribavirin. Rates of SVR were 41 and 29%, respectively ($p < 0.05$) [41]. SVR rates of 93 and 79% have recently been reported with PEG-IFN-α_{2b} plus ribavirin treatment in a trial of patients with HCV genotype 2 and 3, respectively [42].

Confirmation of early virological response (EVR), defined as a reduction in HCV RNA of ≥ 2 logs after the first 12 weeks of therapy, is an important predictor of SVR. The majority of patients who achieve an EVR will achieve an SVR, provided that the remainder of the treatment course can be completed. Patients may, therefore, undergo a limited test period of treatment before undergoing a full course of treatment [40]. This is particularly useful in patients with HCV genotype 1.

Maintaining therapy until EVR is achieved can motivate patient adherence, which is critical to the success of PEG-IFN therapy [43,44]. Davis *et al.* reported that enhanced SVRs can be achieved in patients who can be maintained on > 80% of their PEG-IFN-α_{2b} plus ribavirin treatment regimen for the duration of therapy in the clinical trial setting [43].

However, a treatment strategy that involves EVR does not take into account the possible antifibrotic benefits of IFN-based therapy. Whether long-term treatment with PEG-IFN-α_{2a} or PEG-IFN-α_{2b} at lower dosages can prevent progression of liver fibrosis in the absence of a virological response is currently being investigated in several trials.

6.2 PEG-IFN-α_{2a}

6.2.1 *Monotherapy*

The greater efficacy of PEG-IFN-α_{2a} compared with that of nonpegylated IFN-α_{2a} has also been established. In a multicentre, randomised, open-label study, 531 patients with chronic HCV infection received either PEG-IFN-α_{2a} (180 µg/week s.c. for 48 weeks) or IFN-α_{2a} (6 MIU s.c. three-times weekly for 12 weeks, followed by 3 MIU three-times weekly for 36 weeks). The rates of SVR were greater in PEG-IFN-α_{2a} compared with IFN-α_{2a} recipients (39 versus 19%; $p = 0.001$), whereas the incidence of adverse events was similar between treatment groups [45].

Superior efficacy results with PEG-IFN-α_{2a} compared with IFN-α_{2a} were also reported in a recent study in patients with chronic HCV infection [46]. In this multi-centre, open-label trial, 639 patients were randomised to IFN-α_{2a} (3 MIU s.c. three-times weekly), PEG-IFN-α_{2a} 135 µg/week s.c. or PEG-IFN-α_{2a} 180 µg/week s.c. for 48 weeks. Rates of SVR were significantly greater in the PEG-IFN-α_{2a} 135 µg (28%) and 180 µg (28%) groups compared with that for the IFN-α_{2a} group (11%; $p = 0.001$). Rates of SVR in this study were lower compared with those reported above, by Zeuzem *et al.* This may have been due to the higher proportion of patients in the study who had poor prognostic factors (male, black or HCV genotype 1). Adverse event rates were similar in all three treatment groups [46].

6.2.2 *In combination with ribavirin*

As with PEG-IFN-α_{2b}, combination therapy with PEG-IFN-α_{2a} and ribavirin has also been shown to improve overall efficacy. In a multi-centre trial, 1211 patients with chronic HCV infection were randomised to 48 weeks of treatment with PEG-IFN-α_{2a} (180 µg/week s.c.) and ribavirin (1000 – 1200 mg/day p.o., depending on bodyweight), PEG-IFN-α_{2a} monotherapy (180 µg/week s.c.) and IFN-α_{2b} (3 MIU s.c. three-times weekly) in combination with

ribavirin (1000 – 1200 mg/day p.o.). A significantly greater rate of SVR ($p < 0.001$ for all comparisons) was reported in the PEG-IFN-α_{2a} plus ribavirin group (56%) compared with the IFN-α_{2b} plus ribavirin (44%) and PEG-IFN-α_{2a} monotherapy (29%) groups. In patients with HCV genotype 1, corresponding rates of SVR were 46, 36 and 21%, respectively, whereas in patients with genotype 2 or 3, SVR rates were 76, 61 and 45%, respectively. Tolerability profiles were similar across all patient groups [47].

7. Dosage and administration

In addition to differences in structure and biological activity, the two PEG-IFNs are also dosed and administrated differently. PEG-IFN-α_{2b} is administered at a dose of 1.5 µg/kg/week s.c. [36], and concentrations are maintained for ≥ 7 days, which is compatible with the approved dosing regimen [32]. PEG-IFN clearance has been shown to be dependent on patient weight; thus dosing using a weight-based regimen reduces differences in exposure owing to variations in patient mass. This allows consistent serum concentrations of PEG-IFN to be maintained.

PEG-IFN-α_{2a} is also administered by subcutaneous injection once-weekly, but at a fixed dose of 180 µg [45,48].

Treatment duration is longer in patients with HCV genotype 1 as response rates are lower in these patients compared with those with genotype 2 or 3 [5,6]. Patients with genotype 1 typically receive PEG-IFN-α_{2b} or -α_{2a} therapy for 48 weeks, whereas genotype 2 and 3 patients receive 24 weeks of therapy [42,49,50]. However, a recent study has found 12 weeks of therapy with PEG-IFN-α_{2b} in combination with ribavirin to be effective in genotype 2 or 3 patients who are HCV negative at 4 weeks on treatment, with an SVR of 89%. No correlation was found between baseline viraemia and relapse [51]. Zeuzem *et al.* found a higher relapse rate in PEG-IFN-α_{2b} plus ribavirin-treated HCV genotype 3 patients with a baseline HCV-RNA concentration of > 600,000 IU/ml; more prospective studies may need to be conducted in this area [42]. Limited data are available on treatment duration for patients with genotypes 4 – 6, but 48 weeks is recommended.

8. Ongoing and planned pegylated IFN studies

Although the efficacy of PEG-IFN-α_{2b} and PEG-IFN-α_{2a} has been established in randomised controlled trials, comparative efficacy is yet to be determined owing to the variability of patient populations and different treatment regimens used in each trial. Different patient demographics, geographical location of centres, criteria for dose reduction and stopping rules, and ribavirin dosages are just some of the factors that prevent a valid, accurate comparison of safety and efficacy.

Although the differences in antiviral activity of the two PEG-IFNs may be reflected in differences in efficacy outcomes, this has yet to be elucidated. An answer to this question is eagerly anticipated and there are several small trials and one major trial that may provide insight into this question. The Individualized Dosing Efficacy versus Flat Dosing to Assess Optimal Pegylated Interferon Therapy (IDEAL) study will include a direct comparison of the efficacy and tolerability of the two combination treatment regimens using PEG-IFN-α_{2b} (1.5 µg/kg plus ribavirin 800 – 1400 mg/day) and PEG-IFN-α_{2a} (180 µg plus ribavirin 1000 – 1200 mg/day) in US patients. Patients (n = 2880) with genotype 1 are currently being enrolled in 100 medical centres across the US. Initial results from this trial are expected in 2007. Meanwhile, the COMPARE trial has investigated the *in vivo* biological activity of PEG-IFN-α_{2b} and -α_{2a}.

9. Expert opinion

PEG-IFN-α_2, in combination with ribavirin, has rapidly become the standard of care for the treatment of HCV. The benefit to the patient of pegylation of IFNs is enhanced therapeutic efficacy, reduced acute toxicity and improved patient convenience, which may enhance patient compliance. In addition, the higher SVR rates achieved with PEG-IFNs lead to improved quality of life [52,53].

Two PEG-IFNs are available to the patient. PEG-IFN-α_{2a} and -α_{2b} have significantly different structures and exhibit differential biological activity *in vitro*. In particular, PEG-IFN-α_{2a} and -α_{2b} differ in the size of the PEG molecule attached to the core IFN-α_2 protein and the distribution of biologically active pegylated positional isomers. As a result, the *in vitro* biological activity profiles of PEG-IFN-α_{2a} and -α_{2b} differ significantly.

Preliminary studies have shown that the differential activity originates at or near the receptor–ligand interface. In particular, the most active antiviral pegylated positional isomer identified has been the 12 kDa isomer pegylated at the His34-position on the IFN-α_{2b} core protein in PEG-IFN-α_{2b}. The His34 pegylated isomer in PEG-IFN-α_{2b} retains high STAT translocation activity associated with receptor activation that translates downstream into high antiviral activity in cytopathic effect (CPE) assay.

The open question is how this diminution of activity at the receptor interface translates into clinical effect. Certainly, a principal purpose for pegylation is to improve the pharmacokinetics of the core protein, IFN-α_2. However, pegylation also impacts the biological activity at the receptor. Therefore, one must consider the balance: optimising the *in vivo* half-life of the core protein while preserving the receptor-mediated biological activity that the core protein can induce at the target cell.

Most previous clinical trials for PEG-IFN-α_{2a} and -α_{2b} have not been suitably designed to allow the scientific community to draw substantive conclusions about the effect of pegylation on the core protein for the balance between improved pharmacokinetics and receptor activity. The COMPARE and IDEAL studies have been designed to allow for a better understanding of this balance.

10. 5-year view

Over the next 5 years, the scientific and clinical communities will have comparative data, which will begin to answer the question about the best balance between pegylation for increased pharmacokinetics and receptor-mediated activity. These results will help the clinician in selecting from the available armamentarium of PEG-IFNs. The data, in combination with further *in vitro* studies on the direct receptor interaction, may also lead to design optimisation of the PEG-IFN molecule. Combination therapy with ribavirin and other small molecular weight anti-HCV compounds will also be investigated for antiviral synergism. Therefore, the continued elucidation of PEG-IFN receptor and cell biology will be required to lay a firm foundation on which future clinical studies can be designed.

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