

Roferon-A: A Biologic Product of Human Interferon Alpha 2a

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Abstract

Human interferon alpha 2a (hIFN α 2a) is a cytokine regulating immune system that has been used in hepatitis and cancer treatments. It has wide biological potency covering antiviral, antiproliferative and immunomodulative activities. This mini review discusses Roferon-A as a prominent commercial product of recombinant hIFN α 2a which is produced in bacterial system, *Escherichia coli*, as therapeutic protein for several diseases, such as chronic viral Hepatitis B, Hepatitis C, melanoma, hairy cell leukemia and renal cell carcinoma. The discussion focuses on the development process with regard to its manufacturing, preclinical and clinical studies, as well as therapeutic efficacy. In addition, we also discuss biosimilar development of hIFN α 2a and its potential future developments in the context of enhancing pharmacokinetic profiles.

Keywords: interferon alpha 2a, Roferon-A, therapeutic protein and biosimilar

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Introduction

Interferons (IFNs) are the primary line cytokine defence against the immune system regulating immunity with potential antiviral activity. Research has revealed that these cytokine families also have a potential for use in cancer treatment, including melanoma, hairy cell leukemia, and renal cell carcinoma. In 1980, the National Institute of Allergy and Infectious Diseases and the World Health Organization classified IFNs by three types, namely IFN- α , IFN- β , and IFN- γ . Currently, IFNs are classified by two major groups, type I IFN and type II IFN which are categorized by the difference in binding ability to common receptor types. Type I IFNs, known as viral IFNs, bind to type I IFN receptors. This type includes IFN- α (leukocyte), IFN- β (fibroblast), IFN- ω (leukocyte), and IFN- τ (ovine trophoblast) which have significant homologous structure. IFN- γ is only one of type II IFN which binds to type II IFN receptors. This IFN, known as immune IFN, is produced by T-cells and natural killer (NK) cells (Esperanza Gómez-Lucía, 2009; Jonasch & Haluska, 2001;

Nyman *et al.*, 1998; Plataniias, 2005; Samuel, 2001).

Human IFN- α 2a was found as the first pure human protein which has been approved as cancer therapeutic since 1986. It is used for the treatment of hairy cell leukemia and currently as many as 86 countries have been using recombinant human IFN- α 2a (rhIFN- α 2a) in hepatitis and cancer treatments (Jonasch & Haluska, 2001). It is also reported that rhIFN- α 2a has therapeutic effect for several skin diseases including chronic urticarial vasculitis with angioedema (Matteson, 1996), herpes virus associated inflammatory bowel disease (ulcerative colitis and Crohn's disease) (Ruther *et al.*, 1998) and cutaneous malignant melanoma (Wang *et al.*, 2007). In addition, a preliminary study in animal model revealed the potential therapy of rhIFN α -2a for the treatment for rabies infection (Roy *et al.*, 2015). rhIFN- α 2a is widely applied in monotherapy or in combination therapy with other drugs. rhIFN- α 2a is combined with ribavirin, lamivudine or adefovir in hepatitis treatment and combined with cytarabine, vinblastine, 5-fluorouracil, tamoxifen or interleukin-2 in cancer treatment (Golan *et al.*,

2008; Jonasch & Haluska, 2001; Buckle, 1996). Injectable rhIFN- α 2a solution is commonly used for therapy. However, a new research showed that injectable hyaluronic acid-tyramine hydrogels incorporating interferon- α 2a are more potential in apoptotic efficacy and less proliferating cells compared to rhIFN- α 2a solution in liver cancer cells and give a better pharmacokinetic profile in mice models (Xu *et al.*, 2013).

Molecular characteristics of IFN- α 2a

The first elucidation of the three-dimensional structure of monomeric hIFN- α 2a with high resolution NMR was reported in 1997 by F. Hoffman-La Roche (Klaus *et al.*, 1997) (Figure 1). Natural human IFN- α 2 is an O-glycosylated protein containing the disaccharide galactosyl-N-acetylgalactosamine (Gal-GalNAc) linked to Thr-106. The recognition site of asparagine linked-glycosylation (N-glycosylation) was not detected. Generally, character of IFN- α is slightly acidic (Adolf *et al.*, 1991). Another characteristic includes four cysteine residues forming two disulfide bonds between Cys 1-Cys 98 and Cys 29-Cys 138 (Baron & Narula, 1990). The structure of hIFN- α 2a is similar with the structure of hIFN- α 2b containing 165 amino acids with only one difference in amino acid residue at position 23 (Lys to Arg). In general, the dominant feature of hIFN- α 2a structure is a cluster of five α -helices, designated A to E. Four of them form the left-handed helix bundle. Related to four helical bundles of cytokines, the structure of hIFN- α 2a is similar with hIFN- α 2b and also murine IFN- β which are seen from the backbone fold, as they are belong to type I IFN. Based on NMR spectroscopy analysis, there are four putative domains of receptor binding site of hIFN- α 2a comprising domain A (Met16-Ser28), AB (Cys29-Phe36), C (Glu78-Asp95) and D (Tyr122-Ala139) (Klaus *et al.*, 1997).

Mechanism of IFN- α 2a

IFN- α 2a is a type I IFN, therefore, it is involved in the defense towards viral infections.

Type I IFNs play an important role to generate both adaptive and innate immune responses. Once a virus infects the cell, it induces the type I IFN regulated by two signal transduction pathways, the classical and the Toll-like receptors (TLR) pathways. The synthesized type I IFN will bind to their specific receptors, namely the interferon alpha receptor (IFNAR), formed by two subunits: IFNAR-1 and IFNAR-2 (Figure 2). This interaction produces the heterodimerization of both subunits. It will activate the tyrosine kinases TYK-2 and janus kinase JAK-1. The phosphorylation of IFN- α or IFN- β is involved in the signal transduction of several molecules, namely Signal Transduction and Activator of Transcription (STAT)-1 and STAT-2. They bind to IRF-9 then form a trimer (IFN-stimulated gene factor-3, ISGF-3). After translocation into the nucleus, ISGF-3 binds to Interferon-Stimulated Response Elements (ISRE) in IFN-inducible genes. This induction leads to mRNA translation and release of intracellular enzymes such as 2',5'-oligoadenylate synthetase and double-stranded RNA dependent protein kinase, resulting in degradation of viral messenger-RNA and inhibition of protein translation (Chawla-Sarkar *et al.*, 2003; Esperanza Gómez-Lucía, 2009; Samuel, 2001).

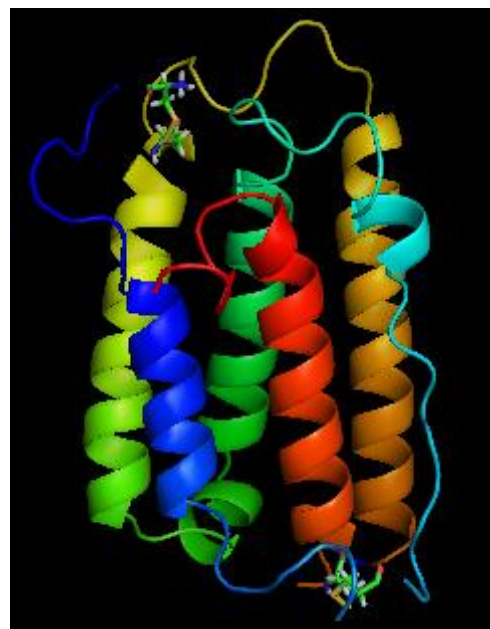


Figure 1. Three-dimensional structure of IFN- α 2a. The fold consists of five helices. Two disulfide bonds

stabilize the conformation of IFN- α 2a which differs in one amino acid residue with IFN- α 2b at position 23, lysine in place of arginine (the 3D structure was made using Pymol) (Ghasriani *et al.*, 2013; Klaus *et al.*, 1997).

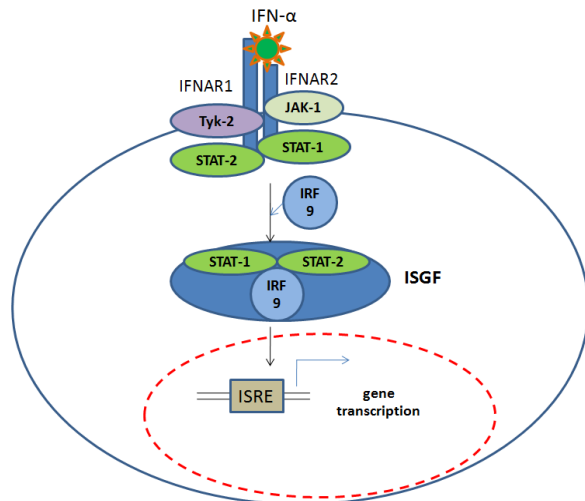


Figure 2. Induction mechanism of genes by type I interferons. Type I IFN signalling via JAK-STAT pathway (Ningrum, 2014).

Roferon-A

Roferon-A is recombinant protein of hIFN- α 2a with antiviral activity, which is similar to the natural substance produced by leukocyte of human body. This antiviral activity protects the body from the invasion of viral infections, tumours and other foreign materials (Roche, 2010). In general, the development of Roferon-A as an originator biologic product of rhIFN- α 2a covered the recombinant protein production, preclinical and clinical studies before the product got commercial approval from drug regulators.

Manufacturing process

The recombinant rhIFN- α 2a is manufactured by Hoffman LaRoche using recombinant DNA technology in *Escherichia coli* system under the trade name Roferon-A (Trown *et al.*, 1986). There are three major stages in manufacturing of IFN- α 2a expressed in *E. coli*, including cloning of hIFN- α 2a gene, expression of hIFN- α 2a in prokaryotic system, and production and large

scale recovery of rhIFN- α 2a expressed in *E. coli*. (Baron & Narulla, 1990)

1. Cloning of human IFN- α 2a

The first cDNA of IFN- α was generated in 1980 using 12S polyA RNA from stimulated human leukocyte (Nagata *et al.*, 1980).

2. Expression of human IFN- α 2a in prokaryotic system

The cDNA was cloned at the *Pst*I site of pBR322 plasmid. This restriction site lies within coding region which has ampicillin resistance gene encoding β -lactamase. IFN- α 2a was expressed under the control of tryptophan promoter. The rhIFN- α 2a ORF was expressed as inclusion bodies in *E. coli* strain K12. The expression level reached up to 1×10^{10} U/L using combination of bacterial strains and improvement of fermentation conditions (Henco *et al.*, 1985).

3. Production and large scale recovery of rhIFN- α 2a expressed in *E. coli*.

The combination of fermentation process and the use of sophisticated vector in protein expression can produce a large quantity of IFN- α 2a. However, in the downstream processing, microbial contamination interferes with the purity of protein. As a consequence, the purification steps play a key role for recovering the pure protein. Before purification steps, the first recovery of recombinant human IFN- α 2a involved isolation of the protein from *E. coli*. After harvesting, the cells were disrupted by deep freezing. The pellet containing recombinant inclusion bodies was stirred in buffer (Schmid & Dannert, 2016). Cell debris was removed by ultra-centrifugation and the clarified extract containing IFN was concentrated by ultrafiltration and ready for purification. In the production of rhIFN- α 2a, the purification process was improved by the use of immuno-affinity column chromatography. Monoclonal antibody to IFN- α 2a was used, followed by copper-chelating affinity chromatography to get the desired forms of IFN- α 2a. Gel filtration chromatography was incorporated as the polishing step to obtain pure protein before formulation and vialing processes (Kuitang *et al.*, 1988).

Preclinical studies

Recombinant DNA technology produces pure desired protein which is free from other substances and other subtypes of IFNs. The biological activity of Roferon-A was compared to IFNs from purified leukocyte which was used earlier. The preclinical studies were done to examine the activity of Roferon-A as antiviral, anti-proliferative or immunomodulating agent in both *in vitro* using cell lines and *in vivo*. These studies also include pharmacokinetics and toxicology testing (Trown *et al.*, 1986).

1. Antiviral activity

The antiviral activity of Roferon-A was not significantly different from purified leukocyte in several viruses, such as Rhinovirus, Herpes, and Encephalomyocarditis. Furthermore, antiviral activity of IFN- α 2a in guinea pigs can combat a human pathogen, Herpes Simplex Virus (HSV), which is similar to genital herpes in human. The administration of Roferon-A was given 3 times a day at 2×10^6 U/kg. This treatment was effective to suppress the lesions (Kramer *et al.*, 1983).

2. Antitumor activity

The result in antitumor activity was less successful than antiviral testing. In *in vitro* model, it was restricted only in human tumor cell grown or in nude mice. In cell line testing, the sensitivity of Roferon-A was less than 1000 U/mL in several cancer cell lines such as melanoma Hs294T and A101D, renal carcinoma A498, lung adeno carcinoma 549, T-cell acute lymphoblastoid leukemia CCRF-HSB-2 and Burkitt's lymphoma Daudi (Czarniecki *et al.*, 1984).

Based on the results, differing sensitivities were found in the human cell line for *in vitro* study. *In vivo* analysis in nude mice revealed that there was no inhibition in human melanoma and human colorectal carcinoma grafted in this animal. It seems that the host animal influences the effectiveness of antitumor activity of Roferon-A (Trown *et al.*, 1986).

3. Pharmacokinetic studies

The analysis of bioavailability, distribution and elimination was done in squirrel monkeys and African green monkeys. The ELISA method was used as bioassay. Monoclonal antibody was used to recognize the epitope of protein IFN- α 2a in serum. Pharmacokinetics data revealed that the elimination half life ranged from 1.8 to 4.8 hours. As described in previous research, the investigation of renal clearance revealed that there was a rapid proteolytic clearance during tubular reabsorption after the protein had totally filtered in glomeruli. Reappearance of protein in circular system was negligible (Bocci *et al.*, 1981).

4. Toxicology

Since the efficacy of IFN- α 2a is species-specific, toxicology testing was less useful, since the toxicological studies in several animals have failed to show the side effect in those animals similar to those that happened in human studies. However, the toxicology studies including acute parental toxicity and subchronic toxicity study were conducted to make sure that there were no unexpected results related to drug examination at clinical dosage in various animals. Furthermore, this study suggested that Roferon-A is not recommended to be administered to child-bearing women without consideration of the high risks of this drug (Trown *et al.*, 1986).

Clinical studies

Pharmacodynamics properties

rhIFN- α 2a as a therapeutic drug causes degradation of viral messenger RNA leading to protein synthesis inhibition. Furthermore, this recombinant protein has several immunomodulatory effects and can be used for treatment of patient with active hepatic diseases.

1. Antiviral effect

The mechanism of antiviral activity of IFNs is via indirect inhibition of virus replication. The interaction between IFN- α and its receptors can produce intracellular enzymes, such as 2'5-oligoadenylate synthetase and double-stranded RNA-dependent kinase. These enzymes are important in degradation of mRNA virus. In patients with Hepatitis C,

IFN- α therapy has shown clinical efficacy due to the inhibition of viral replication by increasing amounts of protein kinases (Cirelli & Tyring, 1995; Dorr, 1993).

2. Immunomodulatory effect

One of various immunomodulatory effects of IFN- α is the activation of T-cells and NK cells. Most patients with chronic liver diseases exhibit a reduction in the number of NK cells. IFN- α 2a therapy can activate NK cells for clearance of virus-infected cells. For example, the impaired NK cells in Hepatitis C patients can be restored up to 57 % after a 3-month cycle of IFN- α 2a 3 MU 3 times/week. Preliminary study suggested that it is the NK CD16+ cells that respond to IFN- α 2a in Hepatitis C patients. In addition, administration of IFN- α 2a 1-4.5 MU 3 times/week for 1 year could induce interleukine-2 by peripheral blood lymphocytes. Administration of IFN- α 2a (10×10^4 U/mL) increased the production of interferon- γ in pythohaemagglutinin-stimulated peripheral blood mononuclear cells from patient with chronic Hepatitis B. Furthermore, the significant reduction in IgG and IgM anty-idiotypic antibodies was found in patients with chronic Hepatitis B after administration of IFN- α 2a 10-20 MU 3 times/week during 6 months therapy (Haria & Benfield, 1995).

3. Hepatic effects

After 6 months of IFN- α 2a treatment at 6 MU/times a week, the progression of cirrhosis might be slowed in patients with Hepatitis B, C and D. The level of serum marker of liver fibroplasias, namely procollagen type III aminoterminal propeptida (PIIINP) was found to be lower than before therapy of IFN- α 2a late after 6 months (Haria & Benfield, 1995).

Pharmacokinetics properties

Recombinant human IFN- α 2a is commonly administered either via intra muscular (IM) or subcutaneous (SC) injection. There are a number of pharmacokinetics studies done with healthy volunteers (Table 1). After administration of 36 MIU via IM and SC, the concentration of serum peaked at 3.8 hours and 7.3 hours respectively. The area under curve showed that the

bioavailability of drugs was greater than 80 %. Multiple doses at IM injection increased the accumulated serum concentration from 2 to 4 times. The volume of distribution was 31.4 L when IFN- α 2a was administered via IV injection. Reabsorption of IFN- α 2a was near complete since there was small amount of radiolabeled IFN- α 2a in isolated rat kidney. IFN- α 2a is filtered through glomeruli and has a rapid clearance during reabsorption (Roche, 2010).

Pharmacokinetics profiles in patients with chronic Hepatitis B were similar with those found in healthy volunteers. The variation of IFN- α 2a administration including administration frequency of twice daily (0.5-36 MIU), once daily (1-54 MIU) and three times a week (1-136 MIU) during 28 days, where no changes were found in elimination and distribution profiles. However, in cases involving hairy cell leukemia and AIDS-related Kaposi's sarcoma patients these profiles remain unknown. Some neutralizing antibodies were found after administering of IFN- α 2a. At least one in five patients responded to these antibodies. Although there is still no clear relationship between neutralizing antibodies and the efficacy, in some small cases induction of these antibodies can block or neutralize the biological effect of IFN- α 2a (Roche, 2010).

Table 1. Mean pharmacokinetics parameters in healthy volunteers at a single dose of 36 MU IFN- α 2a (Haria & Benfield, 1995)

Route	t_{max} (h)	C_{max} (μ g/L)	$t_{1/2\beta}$ (h)	AUC (μ g/L \cdot h)	Vd (L)
IV ^b (n = 6)		13.9	5.1	17.6	31.4
IM (n = 6)	3.8	2.0	2.3	14.6	
SC (n = 6)	7.3	1.7	3.5	15.9	

a Serum concentrations of interferon- α -2a were measured using enzyme linked immunosorbent assay (ELISA) with a sensitivity of 20 ng/L.

b Administered as a short 40-minute infusion.

Abbreviations: AUC = area under the plasma concentration-time curve; C_{max} = peak plasma concentration; IM = intramuscular; IV = intravenous; SC = subcutaneous; t_{max} = time to C_{max} ; $t_{1/2\beta}$ = terminal elimination half-life; Vd = volume of distribution.

Therapeutic efficacy

1. Some clinical trials have shown therapeutic efficacy in chronic viral hepatitis, including Hepatitis B and C.

The clinical studies of IFN- α 2a in adult patients with chronic Hepatitis B virus (HBV) generally used IM or SC administration at fixed dose ranging from 2.5-10 MU/m² 3 times a week for up to 6 months. This treatment was also used in ongoing viral replication patients with positive HBeAg, HBsAg and HBV-DNA. Response serum normalisation from positive to negative HBeAg, and HBV-DNA was generally observed at 33-43 % at dose ranging from 4.5 -18 MU after 6 months therapy (Figure 3) (4.5 MU is equivalent with 2.5 MU/m² for an average body male surface are 1.8 m²). The complete loss of HBsAg was reported in less than 20% of individual patients (Haria & Benfield, 1995; Ryff, 1993). A small improvement was reported in mono-therapy IFN- α 2a beyond 6 months or at the dosage greater than 10 MU/m². Lower dosage (4.5 MU) gave better efficacy than higher dosage (18 MU) (Ryff, 1993). However, the preliminary studies suggested that combination therapy of IFN- α 2a with non-steroidal anti inflammatory such as indometaxyn may improve the efficacy therapy for chronic Hepatitis B patients (Findor *et al.*, 1994).

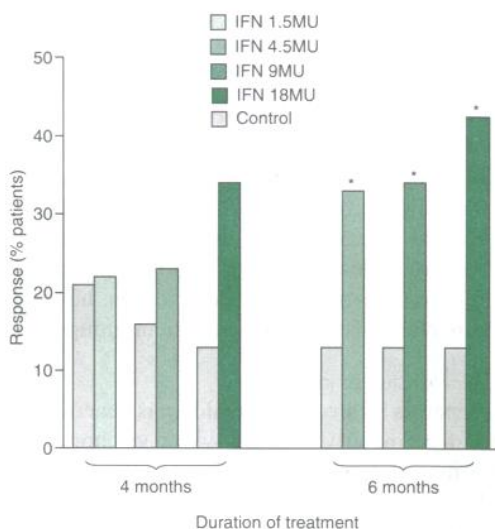


Figure 3. Response rate in patients with chronic hepatitis B after therapy IFN- α 2a by measuring clearance of HBV-DNA and HBeAg, and ALT normalisation. Dosages were administered 3 times a week in a total of 266 patients (115 individual no treatment) (Ryff, 1993).

In acute Hepatitis C patients, 3 months therapy with 6 MU 3 times a week reduced the chronicity. In 6 months therapy, six out of eight patients totally recovered (Haria & Benfield, 1995). The clinical studies in chronic Hepatitis C were conducted in both monotherapy and combination therapy. Roferon-A monotherapy has been shown to be efficacious in treating chronic Hepatitis C with several improvements after the completion of therapy, including the decrease in Hepatitis C viral load, hepatological liver inflammation, and biochemical markers (Roche, 2010).

In combination therapy, Roferon-A 3 MIU was administered via SC 3 times a week combined with daily 1,200 mg ribavirin. The virological response showed that it was of better efficacy when used in combination therapy (Table 2). Sustained virological response was determined as the undetectable RNA virus of HCV in patients' serum after 24 weeks treatment (Tatsuo *et al.*, 2013). Histological improvement was also observed in both groups. In general, there was no significant histological change in those treatments (Roche, 2010).

Table 2. Virological response in chronic hepatitis C therapy (Adapted from Roche, 2010)

Treatment	Roferon-A + Ribavirin, n = 21	Roferon-A, n = 19
Virological response, End of treatment (week 24)	90 %	42 %
Sustained virological response (week 48)	48 %	11 %
Sustained virological response (week 72)	43 %	6 %
Sustained virological response (week 96)	43 %	6 %

Recombinant human IFN- α 2a as a therapeutic agent for Hepatitis C is associated

with a high relapse rate although rapid normalisation of Alanine Amino transferase (ALT) indicating liver damage was usually observed after 4-12 weeks following treatment. This relapse happened especially with patients who obtained a low dosage of Roferon-A therapy of 1-1.5 MIU three times a week. Furthermore, there was a breakthrough phenomenon when the serum ALT increased after normalisation in almost 40 % patients within 2-9 months after therapy. To deal with this phenomenon, some suggestions were given as the solution including changing the therapy with other IFNs- α (Roffi *et al.*, 1995).

Another treatment for the patients who had relapsed after monotherapy was using combination therapy with ribavirin. Roferon-A at 4.5 MIU SC three times a week with daily 1000 mg ribavirin gave a significant virological response of up to 88 % after 24 weeks treatments in all genotypes. Fibrosis ranging from moderate to mild or absent was reported compared to monotherapy treatment. High or severe fibrosis indicated cirrhosis (Roche, 2010). The positive response of IFN- α 2a therapy was not solely for HCV patients, but also for HCV patients associated with type II cryoglobulinaemia, haemophilia, HIV infection, or chronic renal failure (Haria & Benfield, 1995).

2. Advance and/or metastatic renal cell carcinoma (RCC)

Combination therapy was used for advance and/or metastatic RCC treatment using Roferon-A with vinblastine or bevacizumab. Roferon-A was administered via SC at 3 MIU three times a week during the first week, then 18 MIU three times a week for the following weeks. Vinblastine was injected IV at 0.1 mg/kg once every three weeks. Treatment was continued until one year unless any side effect(s) was found. Treatment was discontinued after 3 months therapy for patients who showed the complete response. The survival rate of RCC patients was increased after this combination therapy compared to only monotherapy using vinblastine (Roche, 2010).

3. Low grade non-Hodgkyn's lymphoma

Clinical study of Roferon-A therapy in patients with low grade non-Hodgkyn's lymphoma was conducted in 2 clinical trials when it was used as adjunct to chemotherapy and radiotherapy and concomitantly with chemotherapy. In chemotherapy and radiotherapy patients, the additional treatment of IFN- α 2a at 3 MIU SC or IM three times a week reduced the relapsed rate from 87 weeks to 135 weeks. Furthermore, IFN- α 2a used in concomitant with chemotherapy reduced disease progression significantly but no differences were found in survival rate (Roche, 2010).

4. Chronic myelogenous leukaemia (CML)

IFN- α 2a treatment during 18 months produced haematological remission up to 60% in chronic phase CML patients (Roche, 2010).

5. Cutaneous T-Cell lymphoma (CTCL)

IFN- α 2a treatment during 6 months or up to one year therapy produced a complete tumour response in 60 % CTCL patients. Partial response was usually seen only in those receiving 3 months therapy (Roche, 2010).

6. Hairy Cell leukaemia

Around 61 % patients had complete or partial response after 16 weeks therapy. The positive response of IFN- α 2a in hairy cell leukaemia patients reduced the transfusion of red blood cells and platelet. The possibility of survival rate reached 94 % after 2 year therapy (Roche, 2010).

7. AIDS related Kaposi's sarcoma

The response rate in patient with AIDS-related Kaposi's sarcoma was around 28.6 % after 2-3 months therapy at 36 MIU. However, the response was only in 10 % of patients who had a history of opportunistic infection (Roche, 2010).

Tolerability and side effects

The commonly used dosage for administering Roferon-A is less than 5 MIU per day. After 2-8 hours injection at the first dose, patients experience an "influenza like" syndrome with fever, headache, chills, and diaphoresis. The severity is higher in SC or IM injection

compared to IV administration. However, after a few weeks treatment, better tolerance should be observed. Other reported side effects, including fatigue, anorexia, hair loss and weight loss (Figure 4) (Haria & Benfield, 1995).

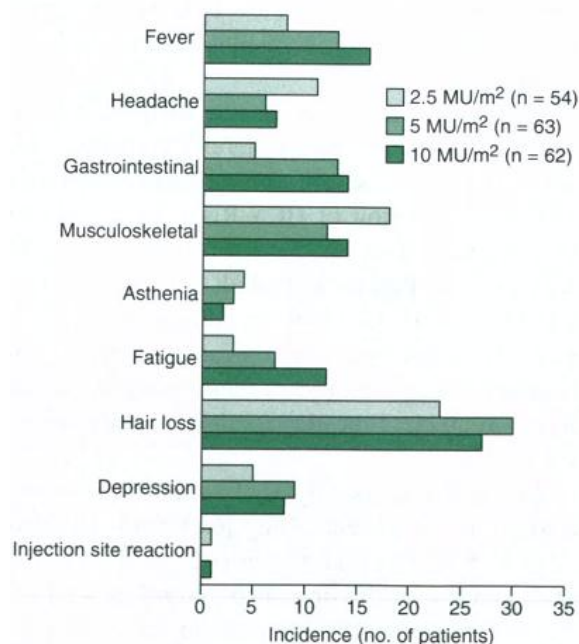


Figure 4. Adverse effects after IFN- α 2a therapy at 2.5-10 MU 3 times a week (Haria & Benfield, 1995).

Weaknesses

There are some studies on the comparison between Roferon-A and other related products:

1. Research revealed that Roferon-A was more immunogenic than Intron A (IFN- α 2b) in Chronic Myelogenous Leukemia patients (von Wussow *et al.*, 1991). Another study showed the similar effect in administration of IFN- α 2a for patient with chronic Hepatitis B (Antonelli *et al.*, 1991). A similar comparison was conducted in carcinoid tumor patients (Oberge *et al.*, 1989). All of those studies revealed that administering IFN- α 2a as therapeutic agent induced antibodies against IFN- α 2.
2. The modification of IFN- α 2a, PEG-IFN- α 2a (Pegasys) showed a higher terminal half life of approximately 60 hours compared to 3-4

hours for Roferon-A (Roche, 2012). As a consequence, the combination therapy between PEG-IFN- α 2a and Ribavirin became standard medication in the management of Hepatitis C (Keating & Curan, 2003).

3. In phase II clinical trial, administration of PEG-IFN- α 2a 450 μ g once weekly, compared to 9 MIU once daily, resulted in a higher haematological and cytogenetic response rates in CML patients (Lipton *et al.*, 2007).

Patent and Biosimilars

Hoffman-LaRoche as the company that produces the innovator biologic product of rhIFN- α 2a, has been in the possession of some patents related to it. The first approval was in June 4, 1986. However, most of the patents have expired except the patent for interferon solution that will be expired in 2016 (Drugbank, 2015). It is a big opportunity for other companies to make a biosimilar product of rhIFN- α 2a. Last decade, BioPartners GmbH submitted the biosimilar product of IFN- α 2a to European Medicines Agency (EMA) on 22 December 2003. The trade name was Alpheon, 6 million IU/ml solution for injection. *Saccharomyces cerevisiae* was used as the expression system of this product. European Medicine Agency (EMA) started to examine Alpheon as biosimilar of IFN- α 2a on 21 June 2004 and gave the result on 5 September 2006. Unfortunately, the Committee for Medicinal Products for Human Use (CHMP) refused the application for the Marketing Authorization for Alpheon (Schellekens, 2009; EMA, 2006).

There were several major objections related to this refusal. Firstly, Alpheon did not meet the comparability assessment. Both qualitative and quantitative impurity profiles were found in Alpheon, therefore, comparability of Alpheon versus Roferon-A as reference drugs could not be achieved. Secondly, the company failed to present sufficient stability data of drug substance and the shelf life. In addition, the manufacturing of drug product has not been sufficiently validated. Other inadequate comparability of Alpheon versus Roferon-A was related to significant difference in virological data, inconclusive data of response rate in genotype 1 patient, different rate between adverse and

laboratory-related events and insufficient immunogenicity documentation (EMA, 2006).

Development possibility

There are some related research developments aimed to diminish unsatisfying therapeutics outcome of rhIFNs with regard to rapid renal clearance and to increase biological activity. Pegylated IFNs, PEG-IFN- α 2a (Pegasys) and PEG-Intron (PEG-IFN- α 2b) have succeeded in increasing the half life in pharmacokinetics profiles (Roche, 2012). Other developments were reported in relation to increased pharmacokinetics profile in recombinant human IFNs. There was a new approach to production IFN- α 2a by modifying its structure around glycosylation site (Ghasriani *et al.*, 2013). The authors added a single glycan, *N*-Acetylgalactosamine residue, at Threonin 106 for biological activity studies. Previously, it had been shown that *O*-glycosylated IFN- α 2b produced in mammalian cell (HEK293 cells) exhibited higher antiviral activity than non-glycosylated IFN- α 2b produced in *E. coli* (Loignon *et al.*, 2008). The fusion with albumin can be an alternative strategy to increase pharmacology profiles. The fusion protein Albumin-IFN- α 2b (Albuferon) exhibited an extended half-life, was more efficacious and well tolerated than IFN- α 2b in Hepatitis C patients (Subramanian *et al.*, 2007). Another modification to increase half-life profile includes production of muteins. The novel IFN- α 2b mutein containing substitution of cysteine with aspartic acid showed longer plasma circulation than wild-type IFN- α 2b without diminishing the activity (Ningrum *et al.*, 2012). These developments might also be suitable in rhIFN- α 2a to enhance pharmacokinetic profiles.

Conclusion

Roferon-A, the innovator biologic product of hIFN- α 2a has widely been used as therapeutic agent for several diseases, including hairy cell leukemia, chronic myeloid leukemia, Kaposi's sarcoma in AIDS, renal cell cancer, chronic

active hepatitis B, and chronic active hepatitis C. The combination therapy with another drug such as Ribavirin is most effective in Hepatitis C treatment. With regard to some expiring patents of Roferon-A as prominent product of rhIFN- α 2a manufactured by Hoffman-LaRoche, there is an opportunity for other companies to develop biosimilar products. The case of Alpheon in 2006 should be a consideration for companies aiming to do so to ensure the safety and quality of the biosimilar products. They must fulfil the comparability assessment to meet the demands of healthcare markets. Strategies for development to enhance the biological activity and pharmacokinetic profile of rhIFN- α 2a should be addressed, which include the use of alternative expression system and drugs formulation.

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