

## Human Interferon Alpha2a as Anti Hepatitis B and C

Ratih A. Ningrum

Research Center for Biotechnology Indonesian Institute of Sciences,  
Cibinong Science Center, Bogor, Indonesia

### Abstract

Hepatitis is an inflammation of the liver mainly caused by hepatitis viruses. There are 5 different types of hepatitis based on the infecting virus; A, B, C, D and E. Hepatitis B and C are chronic diseases that potentially develop into hepatocarcinoma and cirrhosis on inappropriate treatments. World Health Organization (WHO) stated that currently 350 million people worldwide are living with chronic hepatitis B and 150 million people are living with Hepatitis C. The mortality rate in the world due to hepatitis is about 1.5 million people per year. The human interferon alpha2a (hIFN $\alpha$ 2a) is a therapeutic protein used as therapeutic protein for hepatitis B and C. This review discusses the hepatitis B (HBV) and C (HCV) viruses, mechanisms of hIFN $\alpha$ 2a as antiviral through signal transduction pathway and improvement of hIFN $\alpha$ 2a properties by protein modification. The application of recombinant hIFN $\alpha$ 2a (rhIFN $\alpha$ 2a) in the treatment of hepatitis B and C that recommended by European Association for The Study of Liver (EASL) and the viral resistance mechanism are also included. The status of hepatitis B and C and the development of rhIFN $\alpha$ 2a is also described as well.

**Keywords:** Antiviral, hepatitis, human interferon alpha2a, protein modification, viral resistance

## Protein Interferon Alfa-2a Manusia sebagai Anti Hepatitis B dan C

### Abstrak

Hepatitis merupakan kondisi inflamasi pada hati yang terutama disebabkan oleh virus hepatitis. Berdasarkan tipe virus yang menginfeksi, terdapat lima jenis penyakit hepatitis yaitu A, B, C, D dan E. Hepatitis B dan C merupakan penyakit kronis yang berpotensi menjadi kanker hati dan sirosis jika tidak ditangani dengan baik. *World Health Organization* (WHO) menyatakan bahwa saat ini terdapat 350 juta orang yang terinfeksi hepatitis B dan 150 juta orang terinfeksi hepatitis C di seluruh dunia. Angka kematian yang disebabkan hepatitis mencapai 1,5 juta orang per tahun. Protein interferon alfa-2a manusia (hIFN $\alpha$ 2a) adalah protein terapeutik yang digunakan sebagai obat hepatitis B dan C. *Review* ini mendiskusikan mengenai virus hepatitis B (HBV) dan C (HCV), mekanisme hIFN $\alpha$ 2a sebagai antivirus melalui sistem transduksi sinyal dan peningkatan sifat hIFN $\alpha$ 2a melalui modifikasi protein. *Review* ini juga membahas aplikasi bentuk rekombinan hIFN $\alpha$ 2a (rhIFN $\alpha$ 2a) dalam penanganan hepatitis B dan C yang direkomendasikan oleh *European Association for The Study of Liver* (EASL) dan mekanisme resistensi virus. Status hepatitis B dan C serta perkembangan rhIFN $\alpha$ 2a juga didiskusikan lebih lanjut.

**Kata kunci:** Antivirus, hepatitis, interferon alfa-2a manusia, modifikasi protein, resistensi virus

**Correspondence:** Dr. Ratih A. Ningrum, Research Center for Biotechnology Indonesian Institute of Sciences, Cibinong Science Center Bogor, Bogor, Jawa Barat 16911, Indonesia, *email:* [ratih.asmana@gmail.com](mailto:ratih.asmana@gmail.com)/[rati004@lipi.go.id](mailto:rati004@lipi.go.id)

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## Introduction

Interferon (IFN) is a group of cytokine secreted by almost all eukaryotic cells as response to virus, bacteria, antigen or mitogen exposure.<sup>1</sup> IFN is classified into two types based on the type of receptors that are recognized on the cell membrane surface, namely type I and II. Type I recognizes IFNAR-1 and IFNAR-2 receptors whereas type II recognizes IFNGR-1 and IFNGR-2. IFN type I consists of IFN $\alpha$ , IFN $\beta$ , IFN  $\tau$  and IFN  $\omega$  sub-types. Type II consists of IFN  $\gamma$ .<sup>1-3</sup> IFN type I has amino acid sequence similarity from 30 to 80%. IFN $\alpha$  is a monomer and IFN  $\beta$  is a homodimer. The receptors that recognized by IFN type I were grouped into two sub-units, namely IFNAR-1 and IFNAR-2 which consists of three types, namely IFNAR-2a, IFNAR-2b, and IFNAR-2c. IFNAR-2c has an important role in ligand binding and signal transduction process, while IFNAR-2a and 2b are competitive inhibitors that prevent IFN $\alpha$  binding into IFNAR-2c.<sup>3,4</sup>

hIFN $\alpha$ 2 is produced by leukocytes and consists of 16 types. hIFN $\alpha$ 2a, as one of hIFN $\alpha$ 2 member, is widely known to have a very broad biological activity as antiviral, antiproliferative, and immunomodulative therapeutic. The protein is almost similar with hIFN $\alpha$ 2b in biological activity and amino acid sequence with more than 99% of similarity. The difference between hIFN $\alpha$ 2a and hIFN $\alpha$ 2b is amino acid residue at position 23 which is lysine in hIFN $\alpha$ 2a and arginine in hIFN $\alpha$ 2b. hIFN $\alpha$  coding genes located on human chromosome 9 and the genes were classified into two groups: the immediate-early response group (hIFN $\alpha$ 4) and slower response group (comprised of hIFN $\alpha$ 2, hIFN $\alpha$ 5, hIFN $\alpha$ 6 and hIFN $\alpha$ 8). As previously mentioned, hIFN $\alpha$ 2a gene located on chromosome 9 and classified as slower response group. Almost all of hIFN $\alpha$ s are glycoproteins that consist of single polypeptide chain with 165–166 amino acids. The use of rhIFN $\alpha$ 2a as recombinant

protein therapeutic has been approved by the United States Food and Drug Administration (US FDA) since 1986.<sup>5-7</sup> This mini review is focused on the use of hIFN $\alpha$ 2a as therapeutic protein against hepatitis B and C.

## Methods

This review obtained data from international and national publications indexed by Scopus, NCBI and also Google Scholar. Additional informations were searched from websites and regulations as well.

## Results and Discussion

### Hepatitis B and C

Chronic liver disease is mainly caused by hepatitis B virus (HBV) and hepatitis C virus (HCV). Hepatitis B and C that are not handled properly can develop into cirrhosis, liver failure, as well as hepatoma.<sup>1</sup> WHO stated that HBV and HCV can cause acute and chronic infections. The incidence of HBV infection is very high in childhood by 90% at birth, 25–50% at the age of 1–5 years and 1–5% at 6 years and above. Acute hepatitis C occurs without symptoms and non-lethal. Approximately 15–45% of people who are infected can eliminate the virus spontaneously within 6 months without any specific treatment and the remaining 55–85% can develop into chronic infection (Figure 1). Chronic infection has become a possible risk of cirrhosis by 30–50% within 20 years and liver cancer by 5–15%.<sup>5,8</sup>

HBV is hepadnavirus with 3.2 kb DNA genome in size.<sup>9,10</sup> HBV performs DNA replication in hepatocytes cells and produces virion that contains viral DNA into the blood. It secretes protein of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg). Chronic HBV infection is identified by two parameters: the presence of HBV DNA and HBeAg in the blood serum. The elimination

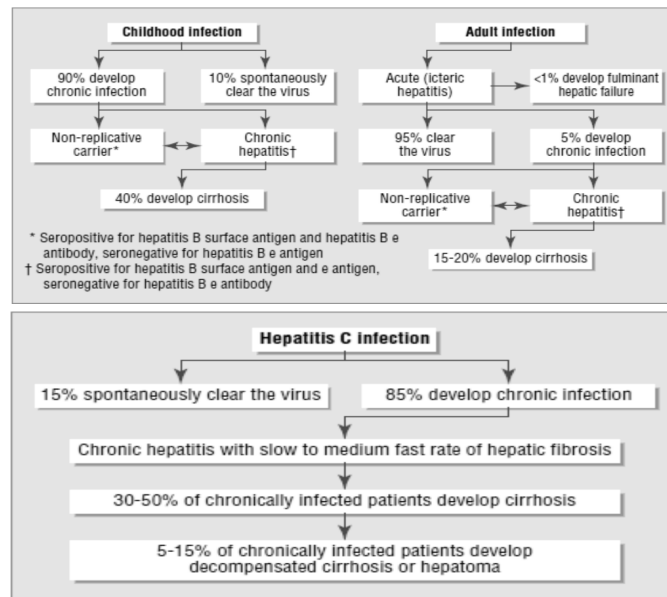


Figure 1 The Development of Hepatitis B and C<sup>5</sup>

of infection is determined by the absence of the two parameters although the clearance also declared whereas the presence of HBsAg in low titer is still detected.<sup>10-12</sup> The HBV viral genome encodes four open reading frame (ORF) that are overlapping encoding reverse transcriptase, viral core protein (HbcAg), HBeAg antigen, surface protein (HBsAg) and X antigen (HbxAg). The ORF encoding DNA polymerase is overlapping

with that of encoding the envelope so a mutation in one ORF will give the effect to another (Figure 2A). Currently, there are 8 genotypes of HBV that have been identified, namely genotypes A to H.<sup>13</sup> HCV is RNA virus that has nucleocapsid and envelope with 9.4 kb RNA genome in size. The genome is encoding structural (core, E1, E2) and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, NS5B) (Figure 2B). HCV detection is

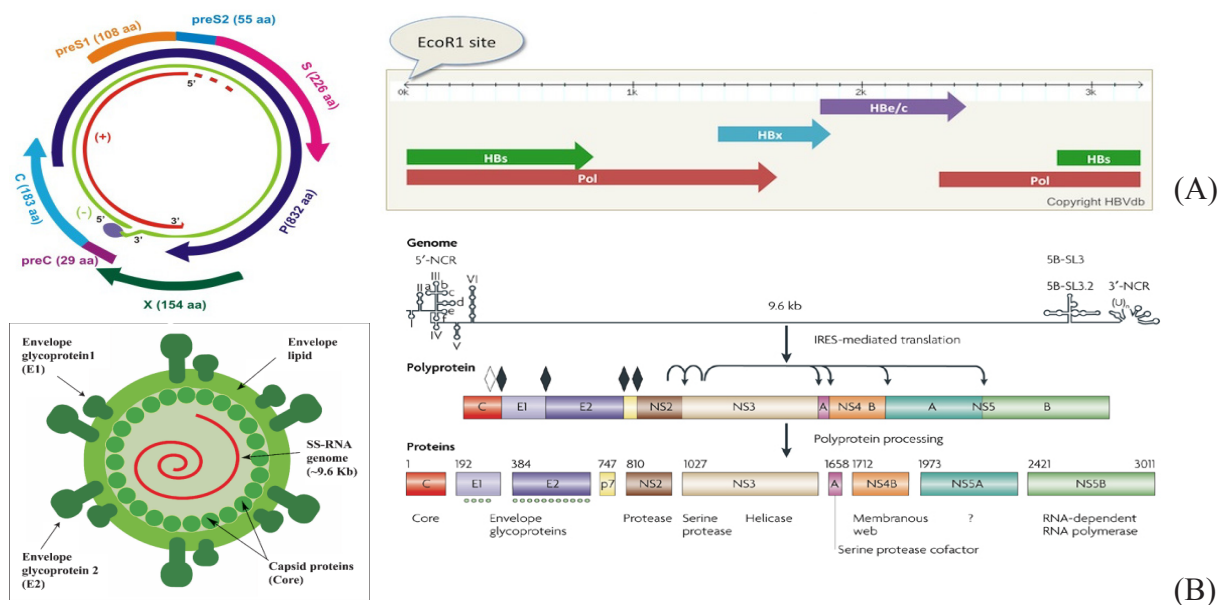


Figure 2 The Genome Structure of (A) HBV and (B) HCV<sup>15-18</sup>

performed carried out based on the presence of antibodies against HCV and HCV RNA. The viral genome has untranslated region at 5' and 3' as well as the internal parts of the entry site (Ires) at the 5' end which can bind to 40S ribosomal subunit. HCV has classified into 6 genotypes that consist of 80 sub-types.<sup>14</sup>

The antiviral activity of hIFN $\alpha$ 2a against HBV and HCV in the treatment of hepatitis B and C occurred by direct/indirect mechanism. Direct mechanism occurs through production of antiviral proteins, such as MxA and GBP (inhibitor of the viral transport process and inhibitor of the synthesis of viral genetic material), 2'-5' OAS (degrading viral genetic material protein) and PKR (inhibitor of viral protein production). Indirect mechanism occurs through the production of proteins involved in immune cell recognition, the activation of T lymphocytes, the movement of lymphocytes and effectors in immune system. The antiviral activity is activated as a result of gene expression regulation which initiated by signal transduction and transcriptional activation of JAK-STAT signaling pathway.<sup>3</sup>

The JAK-STAT signaling pathway

The JAK-STAT signaling is activated by receptors binding on the cell surface (Figure 3). The janus activated kinase (JAK), a tyrosine

kinase enzyme, activates signal transducer and activator of transcription (STAT) through tyrosine phosphorylation. STAT family consists of seven proteins, namely STAT-1, STAT-2, STAT-3, STAT-4, STAT-5a, STAT-5b, STAT-6 respectively and JAK family consists of 4 proteins, namely JAK-1, JAK-2, JAK-3 and tyrosine kinase-2 (TYK-2). JAK-1 and TYK-2 that are activated by IFN  $\alpha$  will result in phosphorylation and dimerization of STAT-1 (P91) and STAT-2 (p113) which subsequently translocated with interferon regulating factor-9 (IRF-9 or P48) to the cell nucleus. The protein complex known as IFN-Stimulated Gene Factor 3 (ISGF-3) activates interferon stimulating response element (ISRE). These induce transcription of hundreds of interferon-stimulated genes (ISGs) that involved in anti viral activity. Study of gene expression in melanoma cell lines (WM9), fibrosarcoma (HT1080), embryonic fibroblasts and human dendritic cells reported that hIFN $\alpha$ s regulate more than 300 genes of signal transduction pathways in cells.<sup>1,3,19-21</sup>

Anti hepatitis mechanism

hIFN $\alpha$ 2a prevents viral replication from the infected into the surrounding cells. Prevention is performed by protein binding on the cell

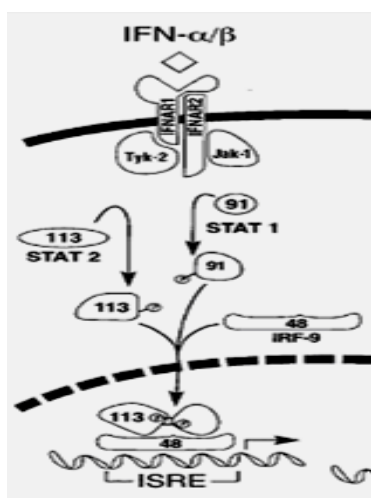


Figure 3 The JAK-STAT Signaling Pathway<sup>3</sup>

membrane surface receptors that activate JAK-STAT signaling. The mechanism of action of IFN $\alpha$ 2a as IFN family member is summarized in Figure 4. The inducers of IFN will trigger the cells to activate IFN gene (A) so result in mRNA (B) which is translated into IFN (C). The IFN is secreted by cells (D) and it will recognize receptors on the surrounding cell (E). This binding process will stimulate the genes producing effector proteins which can inhibit viral replication (F). Activated cells also produce activator protein that activated other cells to produce effectors (G).<sup>3</sup>

Mechanism of hIFN $\alpha$ 2a against hepatitis virus replication occurs through 2 activities: replication inhibition and multifunctional immunomodulator (see Figure 5). The signal transduction process results in 2 phenomena: first, it produces antiviral proteins such as MxA and GBP (nucleocapsid transport and RNA synthesis inhibitors), 2'-5' OAS (RNA degrading protein), PKR (inhibitor of translation) and ADAR1 that can inhibit the viral replication process in hepatocytes cells. More detailed activation of the proteins described in Figure 5. Second, it produces proteins involved in antigen processing and

presentation, T cell activation, lymphocytes movement and the effectors in immune cells. This enhances innate immunity (Natural Killer cells) and adaptive immunity (T cells CD4 + CD8 +) against viral hepatitis. IFN $\alpha$  signaling can be inhibited by several proteins, namely suppressor of cytokine signaling (SOCS), Protein Tyrosine Phosphatase (PTPs), protein inhibitor of activated STAT (PIAS1) and protein kinase.<sup>3,19</sup>

Improving the Properties by Protein Modification  
The use of hIFN $\alpha$ 2a as therapeutic protein in hepatitis B and C treatments still remains a great challenge. hIFN $\alpha$ 2a has short elimination half-life due to rapid renal clearance. Property of hIFN $\alpha$ 2a which has 19 kDa molecular weight and isoelectric point at 6 causes major elimination route of protein occurs via renal clearance by glomerular filtration process. Glomerular filtration by kidneys is selective to the size and charge. Glomerular filter consists of three layers, namely the endothelial layer, the basal membrane, and the slit diaphragm. Glomerular basement membrane consists of large proteins such as laminin, collagen type IV, entaktin and sulfate proteoglycans. Sulfate proteoglycans have negative charge

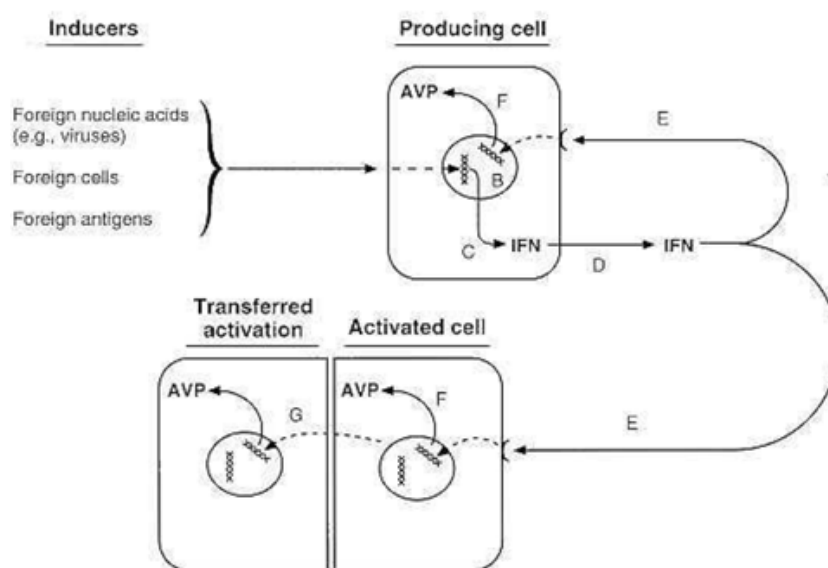


Figure 4 The Activation Process of IFN<sup>22</sup>

that contributes to the charge selectivity. Slit diaphragm has 4x14 nm pore size which important on size selectivity.<sup>23-25</sup>

Plasma elimination half-life of unmodified rhIFN $\alpha$ 2a (Roferon®, Roche) and that administered intravenously is five hours. As a consequence of short elimination half-life the mean residence time of the protein is low so that the therapy frequency should be increased. The effective drug concentration can be achieved by three times administration a week for 24-48 weeks. High frequency and long duration of therapy may generate viral relapse and side effects, such as toxicity into the skin, nerve, endocrine as well as immune system. Improvement of hIFN $\alpha$ 2a elimination half life has been created by several approaches based on protein modification.<sup>26,27</sup>

The most successful protein modification is pegylation based on molecular size improvement. The improvement is conducted by polyethylene glycol (PEG) conjugation to targeted amino acid in the protein molecule to result in bigger molecular weight. Commercial product of pegylated hIFN $\alpha$ 2a is PEGASYS (Hoffman La Roche) that conjugated with 40 kDa of PEG with 70 hours elimination half-life. The pegylated form of hIFN $\alpha$ 2a is able to decrease the therapy frequency into once a week. The most disadvantageous of pegylation is product heterogeneity caused by different binding position of PEG. Other disadvantages are higher production process due to after translational modification. and lower protein biological activity from 7% to 28% only comparing to unmodified form.

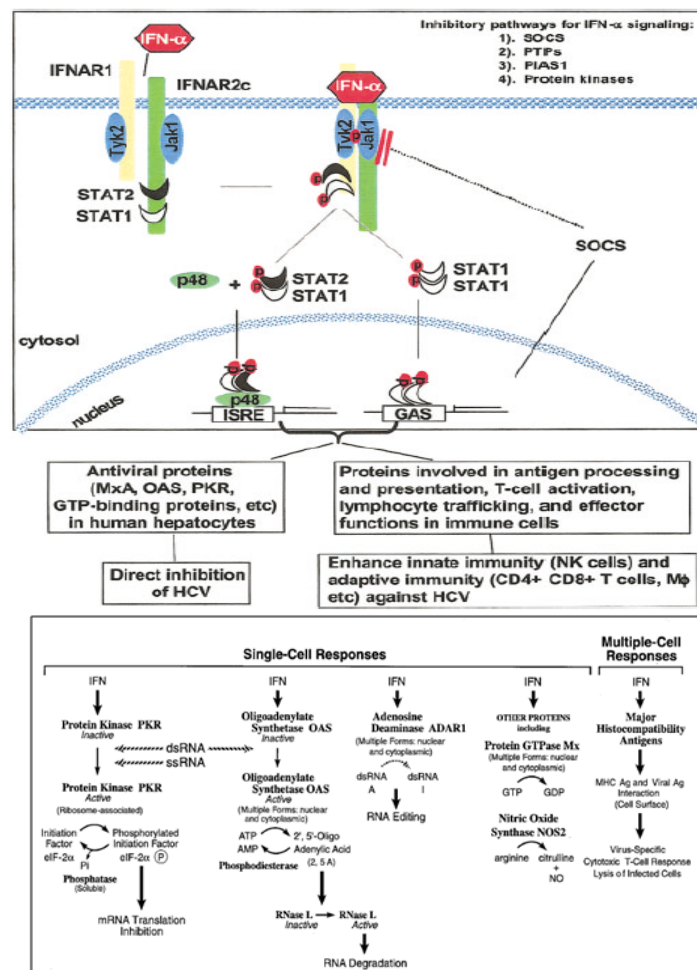
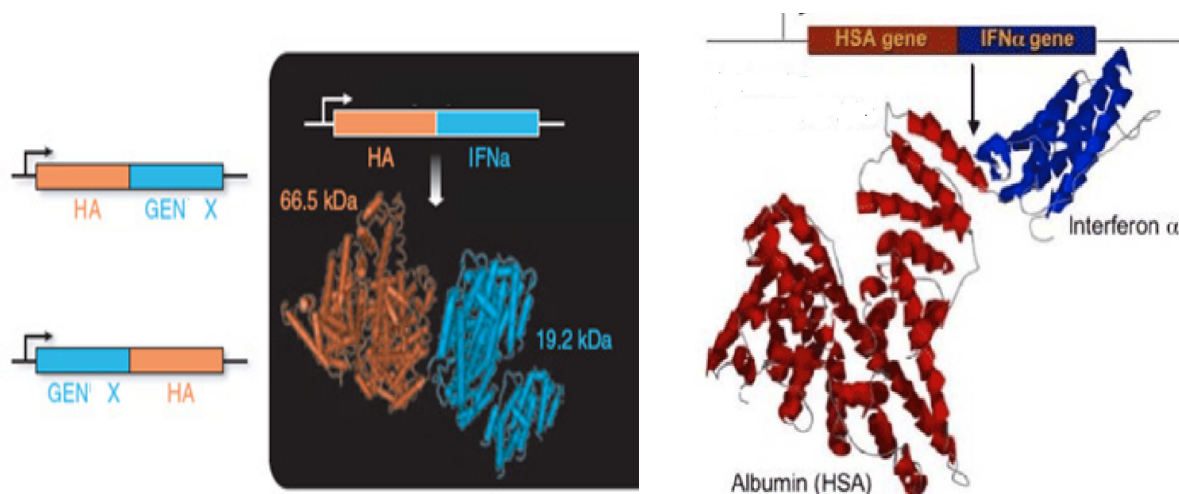


Figure 5 Antiviral Mechanism of hIFN $\alpha$ 2a<sup>3,19</sup>



**Figure 6 Albumin Fusion Technology in hIFN $\alpha$ 2b<sup>37</sup>**

Protein modification by glycosylation can be performed *in vitro* and *in vivo* to improve protein molecular weight and to obtain more negatively charge. The commercial product of this approach is not available yet. The disadvantage of *in vitro* glycosylation is almost similar with pegylation whereas *in vivo* glycosylation brings out the antigenicity problem due to different glycosylation pattern of host. Other modification is certain amino acid substitution by negative charge amino acid to modify total charge of protein. The substituted amino acids are restricted to maintain protein biological activity.<sup>26–34</sup>

Albumin fusion technology is one rational alternative approach to improve protein molecular weight that applied on DNA level. Human Serum Albumin (HSA) that used in this approach is major protein in human blood plasma with 65 kDa in size and produced by liver. It has a role to preserve blood osmolarity and transport small molecules. The advantage of albumin fusion technology is the properties of HSA that has 9 days elimination half-life, wide distribution ability in the body and non-immunogenic. HSA is elected due to its capability to increase protein stability and solubility. There were some proteins using HSA on molecular weight modification,

such as growth hormone, granulocyte colony stimulating factor, interferon alpha-2b, interleukin 2, glucagon like peptide and  $\beta$ -natriuretic peptide.<sup>35–37</sup>

The HSA fusion protein form of hIFN $\alpha$ 2a is not available yet whereas HSA fusion hIFN $\alpha$ 2b is known as Albuferon (HGSI, Novartis). HSA fusion is conducted at DNA level to construct hIFN $\alpha$ 2b containing HSA at its N or C terminal. Previous research stated that N terminal fusion caused protein heterogeneity due to incomplete disulfide bond formation between cysteine at position 1 and 98. This problem can be solved by using linker between the two proteins alteration of orientation of HSA fusion.<sup>35,36</sup> Albuferon has 159 hours elimination half-life that decreases the therapy frequency into once in two weeks for hepatitis C treatment (Figure 6).<sup>37</sup>

#### Anti hepatitis and viral resistance

The recommended therapeutics that used in treatment for chronic hepatitis B with HbeAg positive or negative are pegylated interferon alpha (PEG-IFN $\alpha$ : PEG-IFN $\alpha$ 2a or PEG-IFN $\alpha$ 2b) and nucleos(t)ide analogues (NA: lamivudine, telbivudine, entecavir, adefovir or tenofovir). There are two different strategies for the treatment; finite duration (48

weeks) with PEG-IFN $\alpha$  and long term (48 or 52 weeks) treatment with NA(s). Entecavir and tenofovir are potent HBV inhibitors with high barrier to resistance so that they can be used as first line monotherapies, while the other three may only used if more potent drugs with are not available. PEG-IFN $\alpha$ 2 (180  $\mu$ g of PEG-IFN $\alpha$ 2a or 100  $\mu$ g of PEG-IFN $\alpha$ 2b) is given as percutaneous once a week and NA as oral tablets one daily. Main advantages of PEG-IFN $\alpha$  comparing to NA are the absence of virus resistance, potential immune-mediated control, opportunity of sustained virological response off treatment and change of HBSAg loss in patients who achieve and maintain undetectable HBV DNA.<sup>38</sup>

Therapeutics recommended in hepatitis C treatment are pegylated interferon alpha (PEG-IFN $\alpha$ : PEG-IFN $\alpha$ 2a or PEG-IFN $\alpha$ 2b) and nucleos(t)ide analogues (NA: ribavirin, sofosbuvir, simeprevir, daclatasvir, sofosbuvir/ledipasvir, paritaprevir/ombitasvir/ritonavir and dasabuvir. The doses of PEG-IFN $\alpha$  are once weekly subcutaneous injection of 180  $\mu$ g or less for PEG-IFN $\alpha$ 2a and 1.5  $\mu$ g/kg or less for PEG-IFN $\alpha$ 2b. The doses of NA is once to twice daily. The duration of the therapy is 12 to 24 weeks depends on the genotype of infecting virus.<sup>39</sup> The use of PEG-IFN $\alpha$  potentially may cause side effects which the severity depends on the administered dose and the duration of therapy. A common side effect is fatigue, anorexia, decreased body weight, fever and headache. More severe side effect is neuropsychiatric, such as emotional depression and vertigo.<sup>32</sup>

Hepatitis virus has various strategies to eliminate antiviral activity. It can inhibit the synthesis of hIFN $\alpha$ 2a directly, inhibit signal transduction pathways, or interfere with the activity of antiviral proteins. HCV as RNA virus is able to form quasispecies phenomenon, a strain that closely related but genetically distinct. Quasispecies heterogeneity is caused by RNA-dependent RNA polymerase during

virus replication. It derives from mutations in the NS5A gene encoding 5A protein that play the role as interferon sensitivity determining region (ISDR). The mutation in NS5A causes resistance to IFN $\alpha$ 2a.<sup>40</sup> HCV can also produces NS3/4A protease which is able to inhibit phosphorylation of IRF-3 in the JAK-STAT pathway so the synthesis of IFN is obstructed. The virus also interrupts the signal transduction pathways to degrade and inactivate STAT proteins as well as other antiviral proteins. HCV can generate E2 envelop protein as pseudosubstrate of PKR. Mutations in the gene encoding E2 also contribute to viral resistance.<sup>41</sup>

HBV resistance can occur via overexpression of calreticulin (CRT). CRT is a chaperone involved in the homeostatic regulation of calcium ions. It also has a role in protein refolding as well as other cellular functions. CRT can suppress IFN $\alpha$ 2 antiviral activity by inhibiting the phosphorylation of STAT1 and decreasing the expression of PKR and 2'-5' oligo adenylate synthase.<sup>42</sup> HBV also increases the expression of protein phosphatase 2A (PP2A). PP2A may inhibit protein arginine methyltransferase1 (PRMT1) that can catalyze the methylation of STAT1. Unmethylated STAT1 is less active so that the JAK-STAT pathway is hampered.<sup>43</sup>

#### Current status in Indonesia

According to Indonesian Health Ministry, Indonesia is the second country in South East Asian Region (SEAR) with the highest hepatitis B endemy. A study in 2013 predicted that 10 from 100 persons were infected by hepatitis B or C. Currently, there are 28 millions of people that are hepatitis B or C positive, with 14 millions are potential to be chronic status and 1.4 millions potentially develop to liver cancer. The comparison of hepatitis incidence showed that there was a twofold increase of patients in 2013 comparing to 2007. In 2007, top five provinces with highest



hepatitis prevalence were Nusa Tenggara Timur, Sulawesi Tengah, Aceh, Gorontalo and Papua Barat. In 2013, there were 13 provinces with high prevalence of hepatitis; Nusa Tenggara Timur, Papua, Sulawesi Selatan, Sulawesi Tengah, Maluku, Sulawesi Tenggara, Sulawesi Utara, Aceh, Nusa Tenggara Barat, Maluku Utara, Kalimantan Tengah, Sumatera Utara and Kalimantan Selatan as shown in Figure 7.<sup>44</sup> This strongly indicated that Indonesia has serious problem on hepatitis.

Study in 2015 reported that the risk of hepatitis B infection in Indonesia is high in hemodialysis (HD) patients, men having sex with men, and health care workers. The most common HBV subgenotype in Indonesia is B3 followed by C1. Various novel subgenotypes of HBV have been identified with the novel HBV subgenotypes C6-C16 and D6. HCV report in 2010 claimed that the most common genotype was HCV1b, followed by 1c, 3k, 2a, 1a, 2e 2f and 3a.<sup>45,46</sup> Hepatitis management have been started since 1997 in Indonesia.<sup>45</sup> The national hepatitis B programme was launched in 1997. From 2011, the number of hepatitis B immunization is higher than 80% as percentage of targeted population. The hepatitis control guidance was applied in

2012 and early detection of hepatitis during pregnancy was started in 2013.<sup>44</sup>

According to the Ministry of Health of Indonesia, the therapeutic agents that is used for hepatitis B and C consist of nucleoside analog and recombinant protein. For hepatitis B, pegylated hIFN $\alpha$ 2a is widely used whereas the recommended nucleoside analogs are lamivudine, adefovir, entecavir, telbivudine and tenofovir. For hepatitis C, the standardized therapy is a combination of modified or unmodified hIFN $\alpha$ 2 with ribavirin. In 2016, sofosbuvir is also used as therapeutic agent on hepatitis C treatment. This new drug has already been approved by US FDA since 2013, but the first generic version was launched in 2015.<sup>47</sup>

As the second highest hepatitis endemy, the need of rhIFN $\alpha$ 2a as anti hepatitis in Indonesia is very high. Just until recently, Indonesia still imported the rhIFN $\alpha$ 2a. To stimulate domestic production on biosimilar, National Agency for Drug and Food Control (NADFC) Republic of Indonesia has assigned Regulation of Biosimilar with id number PK BPOM nomor 17 tahun 2015. The strategic plan on development of biopharmaceutical by Ministry of Health of Indonesia, states that interferon alpha is going to produce in

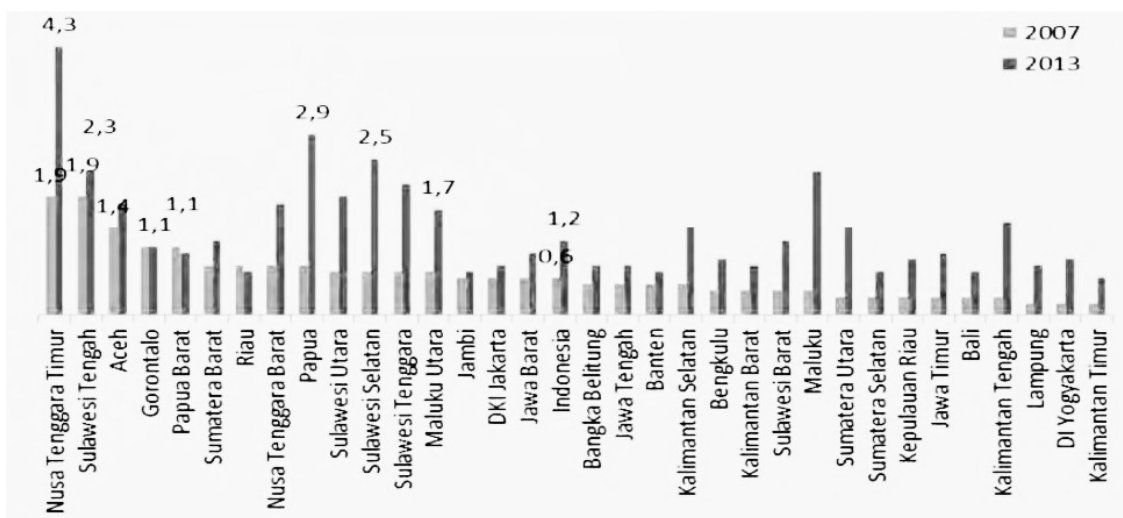


Figure 7 The Prevalance of Hepatitis in Indonesia<sup>44</sup>

2019–2021.<sup>48,49</sup> The production is expected to involve the academia, businessman and government.

Indonesian Institute of Sciences as biggest research institution in Indonesia has developed rhIFN $\alpha$ 2a since 2014. The recombinant protein is produced in methylotropic *Pichia pastoris*.<sup>50</sup> The development of rhIFN $\alpha$ 2a is not only produced as biosimilar but also as its biobetter form through albumin fusion technology. The protein has been purified and characterized. In preliminary biological activity determination, the fusion form has ability to inhibit human breast cancer MCF-7 cell line with 20% lower activity comparing to the unmodified form. The activity of rhIFN $\alpha$ 2a was determined by combination with other cancer drug, that is tamoxifen. Another report was development of rhIFN $\alpha$ 2b mutein with better antiproliferative activity comparing to the wildtype. The mutation was applied to substitute cysteine at position 1 and 98. It has been known that the disulfide bridge formed by the two cysteines is not important in protein biological activity.

## Conclusions

hIFN $\alpha$ 2a is a wide biological activity therapeutic protein that is used in hepatitis B and C treatments. The use of hIFN $\alpha$ 2a has a great challenge due to protein pharmacokinetic profiles which related to viral relapse. Protein modification is important to improve protein properties so it can increase the elimination half-life, decrease the therapy frequency and improve the quality of patient.

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## Conflict of Interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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