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Albinterferon alfa-2b

Prop INN; USAN

Immunomodulator
Treatment of Hepatitis C virus

ABF-656
Albumin interferon α
Albuferon®

Recombinant fusion protein composed of recombinant human albumin genetically fused at its *C*-terminus to the *N*-terminus of recombinant human interferon alfa-2b

1-585-Serum albumin (human) fusion protein with interferon α-2b (human)

Human serum albumin (585 residues) fusion protein with human interferon α-2b (165 residues)

CAS: 472960-22-8

EN: 295108

Abstract

Interferon α (IFN-α) was the standard treatment for chronic hepatitis C virus (HCV) for many years. However, unmodified IFN-α has a short elimination half-life ($t_{1/2}$) of about 2-3 h and requires frequent and prolonged injections. Researchers therefore initiated a search for modified IFNs with prolonged half-lives. Pegylated IFN-α (PEG-IFN) emerged and combination therapy including PEG-IFN and ribavirin has become the mainstay of treatment. However, the combination is associated with a high discontinuation rate, frequent dose modifications, low tolerability and a high nonresponder rate. In the continued search for novel therapies for HCV, albinterferon alfa-2b (ABF-656, albumin interferon α, Albuferon®) was designed using albumin fusion technology to fuse the gene expressing human serum albumin to therapeutically active IFN-α. The novel recombinant molecule exhibited improved efficacy, tolerability and pharmacokinetics in IFN-experienced and -naïve patients with HCV as compared to standard therapy including PEG-IFN-α. Albinterferon alfa-2b is currently undergoing phase III development in combination with ribavirin in IFN-naïve and nonresponding patients with HCV. Phase I trials in patients co-infected with HCV and human immunodeficiency virus (HIV) are also under way.

Background

Hepatitis C virus (HCV) infection initially causes acute hepatitis and the World Health Organization (WHO) estimates that 3-4 million people worldwide are infected with the virus each year. However, only 25-30% of the cases are symptomatic, since manifestations such as jaundice, fatigue, abdominal pain, dark urine, loss of appetite, intermittent nausea and vomiting may not appear until the chronic stage (*i.e.*, infection lasting for longer than 6 months). The WHO has reported that 180 million people worldwide have chronic HCV and the Centers for Disease Control and Prevention (CDC) estimates that about 3.9 million individuals in the U.S. alone are infected with HCV, 2.7 million of whom are chronically infected. Chronic HCV impairs quality of life and can result in complications such as cirrhosis, end-stage liver disease and hepatocellular carcinoma. About 20% of patients with liver disease and cirrhosis will develop end-stage liver disease, making chronic HCV the leading cause of liver transplantation in the U.S. (1-4).

The principal goal in the treatment of chronic HCV is the prevention of progressive hepatic fibrosis leading to advanced liver disease through eradication of viral HCV in the blood. If HCV is cleared rapidly, the risk of complications is eliminated in the majority of patients. Interferons (IFNs) represent the standard of treatment for HCV patients. IFNs are a family of cytokines that are involved in the regulation of cell growth and differentiation and exert antiviral, antiproliferative and immunomodulatory activity. There are two classes of IFNs: type I (IFN-α, IFN-β, IFN-ω and IFN-κ) and type II (IFN-γ). The majority of cell types produce type I IFNs within hours of antiviral infection and IFN-α has been shown to exert the most potent antiviral activity against HCV. In the clinic, the standard therapy for chronic HCV for decades has been IFN-α. However, unmodified IFN-α has a short elimination half-life ($t_{1/2}$) of about 2-3 h in humans and therefore requires frequent (*e.g.*, once daily or 3 times/week) and

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prolonged (6-12 months or more) injections when used as a treatment for chronic HCV. This led researchers to search for modified IFNs with prolonged half-lives. Pegylation of IFN-α (PEG-IFN-α) significantly prolongs the plasma clearance of IFN-α. Moreover, combination therapy with PEG-IFN-α plus ribavirin has become the mainstay of treatment. Pegylation of IFN results in a prolonged $t_{1/2}$ of about 40-80 h and increases the sustained virological response (SVR) rates. Studies have reported that combination of PEG-IFN-α plus ribavirin therapy resulted in an SVR of approximately 55% in patients with chronic HCV (1, 5-10).

However, there is a need for improved therapies for chronic HCV. The number of nonresponders is growing and subpopulations such as individuals infected with HCV genotype 1, African Americans and patients with advanced liver disease exhibit much lower SVR rates and a greater number of nonresponders. In addition, PEG-IFN plus ribavirin combination therapy is associated with a discontinuation rate of 13-22% and about one-third of the patients require dose modifications due to adverse events. The low tolerability of combination therapy results in decreased compliance, which significantly reduces virological outcomes (9-11).

Researchers therefore continue to search for novel therapies for chronic HCV that have improved tolerability and would therefore ameliorate compliance and virological outcomes. The result to date is the discovery of several IFNs which are currently under active development for the treatment of HCV infection (Table I). In an attempt to reduce dosing frequency and improve efficacy and tolerability profiles as compared to conventional IFN-α therapies, albinterferon alfa-2b (ABF-656, albumin interferon α, Albuferon®) was designed using albumin fusion technology, which involves fusing the gene expressing human serum albumin to a therapeutically active protein. Human serum albumin is the most prevalent naturally occurring human blood protein, exhibits a $t_{1/2}$ of 19 days and is devoid of enzymatic or immunological activity. The novel recombinant molecule albinterferon alfa-2b resulting from this technology is a single-polypeptide (85.7 kDa) fusion protein that has potentially improved pharmacokinetics, tolerability, efficacy, dosing options and compliance compared to PEG-IFN. Albinterferon alfa-2b was therefore chosen for further development for the treatment of HCV (12, 13).

Preclinical Pharmacology

The antiviral activity of albinterferon alfa-2b was examined *in vitro* against human (WISH), bovine (MDBK) and simian (COS-1) cell lines infected with encephalomyocarditis virus (EMCV) or vesicular stomatitis virus (VSV). The agent exhibited marked activity against all cell lines (EC_{50} = 1.6, 0.12 and 1.85 ng/ml, respectively), with activity similar to that reported for other forms of recombinant IFN-α. Albinterferon alfa-2b exhibited potent antiproliferative activity *in vitro* against a human Burkitt's B-cell lymphoma-derived cell line (Daudi) that is highly sensitive to type I IFNs (EC_{50} = 2.72 ± 0.72 ng/ml) and induced mRNA expression of IFN-inducible genes in human peripheral blood mononuclear cells (PBMCs) in a manner not significantly different from IFN-α (12).

The *in vitro* antiproliferative and antiviral activity of albinterferon alfa-2b was also compared to that of IFN-β. Albinterferon alfa-2b was less potent than IFN-β in inhibiting the proliferation of Daudi cells (EC_{50} = 28 pM *vs.* 5.4 pM) and exhibited concentration-dependent antiviral activity against A549 lung epithelial cells infected with VSV that was about 9-fold lower than that of IFN-β (EC_{50} = 14 pM *vs.* 1.5 pM). IFN-β was approximately 8-fold more effective than albinterferon alfa-2b in activating the IFN-stimulated responsive element (ISRE) pathway in an epithelial cell line (EC_{50} = 58 pM *vs.* 7.2 pM). However, both IFNs induced IFN target genes in Daudi cells in a comparable manner (13).

The anti-HCV activity of albinterferon alfa-2b was compared to that of interferon alfa-2b, pegylated interferon alfa-2a and pegylated interferon alfa-2b using three HCV replicon cell lines: GSB (Huh7 HCV replicon), H801 (IFN-resistant Huh7 HCV replicon) and SL1 (HeLa HCV replicon). When GSB cells were incubated with IFNs at equivalent patient serum C_{max} concentrations, albinterferon alfa-2b exhibited greater antiviral activity than the PEG-IFNs. The activity observed for the three modified IFNs against IFN-resistant cells was comparable, suggesting that modification does not overcome IFN resistance. Similar anti-HCV activity was observed for albinterferon alfa-2b in both human liver and nonliver cells. The expression of IFN target genes in human liver cells was similarly induced by all modified IFNs (14).

Two studies using cytopathic effect (CPE) assays and infected Vero cells demonstrated the potent antiviral

Table I: Interferons under development for the treatment of hepatitis C (from Prous Science Integrity®).

Interferon	Source	Phase
Albinterferon alfa-2b	Human Genome Sciences/Novartis	III
Human leukocyte interferon alfa*	HemispheRx	III
Interferon omega	Intarcia	II
Interferon alfa-2b XL	Flamel Technologies	I/II
HCV-Interferon Enhancing Therapy (HCV-IET)	Transition Therapeutics	I/II
Interferon alfa (R-7025)	Roche/Maxygen	I (on hold)
Belerofon® (interferon alfa)	Nautilus Biotech	I
PEG-interferon lambda (IL-29)	ZymoGenetics	I
Natural human multisubtype interferon alfa	GeneTrol Biotherapeutics	Preclinical
Interferon alfa-8	RioTech Pharma	Preclinical

*Launched in 1989 for the treatment of genital warts.

activity of albinterferon alfa-2b against Ebola virus, Pichinde virus, Venezuelan equine encephalitis virus (VEE), Punta Toro virus (PTV), yellow fever virus, West Nile virus and severe acute respiratory syndrome (SARS) virus (Toronto-2 strain). IC_{50} values ranged from < 0.1 ng/ml for PTV to 19 ng/ml for VEE; the IC_{50} values obtained in Vero cells infected with the Ebola or SARS virus were 0.4 and 2 ng/ml, respectively. Both albinterferon alfa-2b and IFN-β induced similar IFN target genes and the ISRE signal transduction pathway (15, 16).

Treatment of EMCV-infected WISH, MDBK or COS-1 cells with sera from cynomolgus monkeys administered i.v. or s.c. albinterferon alfa-2b (30 or 300 μg/kg) resulted in dose-dependent antiviral activity for 8 days or more postdosing. In contrast, sera from monkeys administered IFN-α on days 0, 2 and 4 produced only slightly elevated antiviral activity on day 1 and no significant activity on subsequent days. Further examination of sera from albinterferon alfa-2b-treated animals revealed significant increases in 2',5'-oligoadenylate synthetase (OAS) mRNA for up to 240 and 192 h, respectively, after a single s.c. dose of 30 or 300 μg/kg. Significant increases for up to 48 h in OAS genes were also observed in monkeys administered the agent i.v. (30 μg/kg) (12).

Another study using sera from rhesus monkeys administered albinterferon alfa-2b (50 μg/kg i.v. or 50 or 300 μg/kg s.c.) or IFN-β (25 μg/kg s.c.) compared neopterin (a marker of type I IFN activity) levels and OAS(p69) mRNA expression and showed that albinterferon alfa-2b exhibited enhanced activity. Dose-dependent increases in serum neopterin were observed in albinterferon alfa-2b-treated monkeys, which were sustained for 2-3 days and 1 day following s.c. and i.v. dosing, respectively. While the 300 μg/kg s.c. dose of albinterferon alfa-2b resulted in elevated neopterin levels for more than 10 days, increases in neopterin seen with IFN-β were sustained for about 5-7 days. The maximal neopterin response and the AUC for neopterin were significantly higher for the 300 μg/kg albinterferon alfa-2b group compared to other treatment groups. At 24 h postadministration, both albinterferon alfa-2b and IFN-β induced a > 100-fold increase in OAS(p69) mRNA expression, although significantly higher levels were observed at 14 days postdosing in sera from animals receiving albinterferon alfa-2b as compared to IFN-β. Similar increases in OAS(p69) mRNA were observed with both doses of albinterferon alfa-2b and both routes of administration (13).

Pharmacokinetics and Metabolism

The biodistribution and excretion of [^{125}I]-albinterferon alfa-2b (200 μg/kg s.c.; 0.74 MBq/rat) were examined in rats. Radioactivity was highest in blood and slow elimination was observed. There was no tissue accumulation. The excretion rate in urine was 80.1%, with additional excretion observed in bile following liver metabolism (17).

The pharmacokinetics of single-dose albinterferon alfa-2b (30 μg/kg i.v. or s.c.) were examined in cynomolgus monkeys. Clearance and terminal $t_{1/2}$ values were 0.9 ml/h/kg and 68 h, respectively, following i.v. administration and apparent clearance (*i.e.*, clearance/bioavailability), terminal $t_{1/2}$ and bioavailability were 1.4 l/h/kg, 93 h and 64%, respectively, following s.c. administration. When compared to s.c. IFN-α, the clearance rate and $t_{1/2}$ value for albinterferon alfa-2b were 140-fold slower and 18-fold longer, respectively (12).

The pharmacokinetics of albinterferon alfa-2b (50 μg/kg i.v. or 50 or 300 μg/kg s.c.) were also compared to IFN-β (25 μg/kg s.c.) in rhesus monkeys. The terminal $t_{1/2}$ values for i.v. and s.c. albinterferon alfa-2b were 24 and 36-40 h, respectively, which was approximately 4-5 times greater than that for s.c. IFN-β ($t_{1/2}$ = 8 h). The clearance rate for albinterferon alfa-2b was about 140-fold slower than that for IFN-β (4.7-5.7 ml/h/kg *vs.* 791 ml/h/kg). The volume of distribution for i.v. albinterferon alfa-2b was 172.4 ml/kg, which approximated the extracellular water volume, and the s.c. bioavailability was 87%. Antiviral $t_{1/2}$ values for both s.c. doses of albinterferon alfa-2b were 32 and 37 h, respectively, as compared to about 9 h for s.c. IFN-β (13, 16).

A multicenter, open-label, escalating-dose phase I/II study in 119 IFN-α-experienced patients with chronic HCV examined the pharmacokinetics of albinterferon alfa-2b (7-900 μg as a single or two s.c. injections 14 days apart). C_{max} increased dose-proportionally following a single dose of 20-900 μg, with mean C_{max} values of 1.6 and 34 ng/ml obtained following a single dose of 20 and 900 μg, respectively. Monophasic elimination of the agent was observed, with a mean elimination $t_{1/2}$ of 159 h obtained after a single dose and some accumulation observed in patients receiving two injections. In patients administered two injections of 400-900 μg, mean trough levels of the agent were 50% greater on day 28 as compared to day 14. Gender had no significant effect on C_{max} or AUC values. However, a significant correlation was found between greater body weight and lower AUC values. Safety and efficacy data are discussed below (18).

The pharmacokinetics of two s.c. injections of albinterferon alfa-2b (200, 450, 670, 900 and 1200 μg) given 14 days apart were determined in a phase II study conducted in 56 IFN-α-naïve patients with genotype 1 chronic HCV. The pharmacokinetics of the agent were linear across the dose ranged tested. Gender did not appear to significantly affect the pharmacokinetics, although greater body weight was associated with significantly decreased exposure. The mean absorption $t_{1/2}$ and t_{max} values were 23-31 h and 65-87 h, respectively. A linear increase in the mean C_{max} was observed (11.3 ng/ml at 200 μg and 74.8 ng/ml at 1200 μg). Serum drug accumulation of 17-30% was observed following the second dose and mean clearance values were similar for all doses (49-60 ml/h). The agent appeared to be distributed to tissue since the mean volume of distribution ranged from 10.1 to 12.1 l. The mean elimination $t_{1/2}$ values ranged from 134 to 153 h which, were similar to values obtained in the above phase I/II trial. These results suggest that the pharmacokinetics are similar in both IFN-

naïve and IFN-experienced patients. Safety and efficacy data from this trial are discussed below (19).

Data from over 600 patients with chronic HCV enrolled in 4 clinical trials were used to assess the pharmacokinetics of albinterferon alfa-2b (900-1800 μg s.c. for up to 48 weeks) and pegylated interferon alfa-2a (s.c.). The pharmacokinetics of both IFNs were consistent with a one-compartment model with first-order absorption. The pharmacokinetics of albinterferon alfa-2b were linear and the protein detected in the systemic circulation was found to be intact and levels were maintained at day 4 and 14 postinjection. The elimination $t_{1/2}$ for albinterferon alfa-2b was about 2-fold slower than that of PEG-IFN-α (about 6 days *vs.* 3 days), thus supporting dosing at 2-4-week intervals. It was estimated that more than 95% of steady state would be achieved by the time the third every-2-weeks dose was administered. Similar accumulation was observed for both agents. It was suggested that albinterferon alfa-2b was more extensively distributed, since its apparent volume of distribution was greater than that of pegylated interferon alfa-2a (approximately 11 l *vs.* 5 l). The pharmacokinetics of albinterferon alfa-2b were not affected by previous IFN exposure, age, gender, race or fibrosis stage (20).

Clinical Studies

A multicenter, open-label, dose-escalating phase I/II study in 119 patients with chronic HCV who failed previous IFN-α-based therapy examined the safety, tolerability and pharmacodynamics of albinterferon alfa-2b (7-900 μg s.c.) given as single or double (14 days apart) injections. The agent exhibited a favorable safety profile at all doses. There were no discontinuations due to adverse events, the most common of which were headache (56%), fatigue (52%), erythema at the injection site (38%), arthralgias (32%) and pyrexia (27%). Following a single injection of 40 μg or more, *OAS1* was induced and maintained for at least 28 days. Moreover, antiviral activity, reflected as dose-related decreases in HCV RNA of 1 log IU/ml or more, was seen in 47% and 59% of the patients in the 120-900-μg single-injection cohorts and 400-900-μg double-injection cohorts, respectively. Further analysis of 21 subjects enrolled in the 400-900-μg cohorts revealed sustained induction of up to 1,000-fold of IFN-specific genes over a 28-day period. In addition, temporal regulation of granulysin and downregulation of DcR2, a regulator of apoptosis, were observed with treatment. A significant positive correlation was detected between induction of *IFI44* and *OAS1* gene expression and antiviral response on day 28 (18, 21).

An open-label, dose-ranging phase II study in 56 IFN-α-naïve patients with genotype 1 chronic HCV examined the safety and efficacy of two s.c. injections (14 days apart) of albinterferon alfa-2b (200, 450, 670, 900 and 1200 μg s.c.). Treatment was well tolerated. The majority of adverse events were mild to moderate, with the most common side effects including headache (73%), chills (63%), fatigue (31%) and arthralgia (55%). Reductions in HCV RNA at week 4 of 2 $\log_{10}$ IU/ml or greater were achieved in 69% of the patients in the 900- and 1200-μg cohorts (mean decrease = 3.2 $\log_{10}$ IU/ml) (19).

The efficacy and safety of albinterferon alfa-2b (1500 μg s.c. every 2 or 4 weeks) in combination with ribavirin (800 mg/day) were examined in a multicenter, randomized, open-label phase II trial conducted in 43 patients with genotype 2 or 3 chronic HCV. Treatment was well tolerated, with similar safety profiles obtained for dosing every 2 or 4 weeks. While 10% of the patients receiving albinterferon alfa-2b every 2 weeks required dose reductions, none were reported in the every-4-weeks cohort. Antiviral response rates were similar for albinterferon alfa-2b dosed every 2 or 4 weeks in patients with genotypes 2 and 3. The overall end-of-treatment response rates at week 24 were 71% and 82% for the respective dosing schedules. The study also reported that baseline insulin resistance determined using the Homeostasis Assessment Model Insulin Resistance Index (HOMA-IR) was significantly correlated with antiviral response at week 4, such that patients with a significantly lower baseline HOMA-IR (1.7) had a rapid virological response while those with higher HOMA-IR (3.3) did not (22).

Preliminary safety and efficacy results were reported from a randomized, dose-ranging phase II study conducted in 115 patients with chronic HCV who failed previous IFN-α-based therapy. Subjects received s.c. albinterferon alfa-2b at doses of 900, 1200, 1500 or 1800 μg every 2 weeks or 1200 μg every 4 weeks combined with oral ribavirin (1000-1200 mg/day). Data were available at week 8 for the highest doses and after 24 weeks for the other dose groups. Treatment was well tolerated. Neutropenia developed in 24% of the patients, but was stabilized by week 8 and could be managed with dose reductions. Overall safety profiles including the type, incidence and severity of adverse events were similar in all cohorts. A significantly greater antiviral response was observed in the 1800-μg cohort at week 8 as compared to other treatment groups; the percentage of patients achieving a decrease in HCV RNA of 2 log or more at 8 weeks was 64% in the group receiving 1800 μg every 2 weeks, compared to 48%, 42%, 32% and 29%, respectively, in those receiving 900, 1200 and 1500 μg every 2 weeks and 1200 μg every 4 weeks. The antiviral response rates at week 24 were similar across the 900-1500-μg cohorts (25-35%) (23).

An ongoing randomized phase IIb trial in 458 IFN-α-naïve patients with genotype 1 chronic HCV compared the efficacy of s.c. albinterferon alfa-2b (900 or 1200 μg every 2 weeks or 1200 μg every 4 weeks) and pegylated interferon alfa-2a (180 μg weekly) for 48 weeks, with a 24-week follow-up. Both IFN therapies were combined with ribavirin (1000-1200 mg/day). All treatment and dosing schedules were well tolerated. No significant differences were observed across the treatment groups in the incidence of grade 3-4 adverse events or in discontinuation rates due to adverse events. The lowest incidence of hematological reductions was noted in the group receiving 1200 μg every 4 weeks, while the incidence was sim-

ilar for all other treatment groups. The rates of antibody development to IFN were significantly lower on albinterferon alfa-2b compared to PEG-IFN-α. Quality of life as measured by SF-36 was found to be the best in patients on 900 μg every 2 weeks. The greatest antiviral activity (HCV RNA-negative) at week 12 was observed in the group receiving 1200 μg every 2 weeks (74% *vs.* 66.1%, 52.3% and 62.5% for 900 μg every 2 weeks, 1200 μg every 4 weeks and PEG-IFN-α, respectively). A rapid viral response at week 12 was significantly and positively correlated with end-of-treatment virological responses in all treatment groups. The antiviral response rates (*i.e.*, HCV RNA reduction of 2 log IU/ml or more) in the subgroup of patients who achieved a rapid viral response were 97%, 100% and 97% for the respective albinterferon alfa-2b groups and 100% for the PEG-IFN-α group. At week 2, significantly more patients achieving a rapid antiviral response were observed in both 1200-μg dosing groups compared to PEG-IFN-α. SVR rates at 12 weeks were similar for all treatment groups (53-59%). Analysis of subgroups stratified by body mass index (BMI) indicated that those patients in the PEG-IFN-α cohort with a high BMI (25 kg/m^2 or greater) had a lower antiviral response at week 12 compared to those in the cohort receiving 1200 μg every 2 weeks. It was concluded that maximal early antiviral activity was seen in the patients treated with albinterferon alfa-2b every 2 weeks and that this improved dosing schedule could result in comparable or even superior sustained antiviral efficacy as compared to weekly dosing with PEG-IFN-α (24-27).

Albinterferon alfa-2b is presently undergoing phase III clinical development for the treatment of chronic HCV (28, 29). Phase I trials are also under way in patients co-infected with HCV and HIV (30).

Sources

Human Genome Sciences, Inc. (US); co-developed worldwide with Novartis AG (CH).

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