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Viral Hepatitis

Peginterferon lambda for the treatment of HBeAg-positive chronic hepatitis B: A randomized phase 2b study (LIRA-B)

Henry L.Y. Chan[1], Sang Hoon Ahn[2], Ting-Tsung Chang[3], Cheng-Yuan Peng[4], David Wong[5], Carla S. Coffin[6], Seng Gee Lim[7], Pei-Jer Chen[8], Harry L.A. Janssen[9,10], Patrick Marcellin[11], Lawrence Serfaty[12], Stefan Zeuzem[13], David Cohen[14], Linda Critelli[14], Dong Xu[14], Megan Wind-Rotolo[15], Elizabeth Cooney[14,*], and the LIRA-B Study Team[†]

[1]*The Chinese University of Hong Kong, Hong Kong SAR, China;* [2]*Yonsei University College of Medicine, Seoul, Republic of Korea;* [3]*National Cheng Kung University Hospital, Tainan, Taiwan;* [4]*China Medical University Hospital, Taichung, Taiwan;* [5]*Toronto Western Hospital University Health Network, Toronto, ON, Canada;* [6]*Cumming School of Medicine, University of Calgary, Calgary, AB, Canada;* [7]*National University Hospital, Singapore;* [8]*National Taiwan University Hospital, Taipei, Taiwan;* [9]*Erasmus Medical Center, Rotterdam, Netherlands;* [10]*University Health Network, Toronto, Canada;* [11]*Hôpital Beaujon and INSERM CRI Université Paris Diderot, Clichy, France;* [12]*Hôpital Saint Antoine, Paris, France;* [13]*Johann Wolfgang Goethe University, Frankfurt, Germany;* [14]*Bristol-Myers Squibb Research and Development, Wallingford, CT, USA;* [15]*Bristol-Myers Squibb Research and Development, Lawrenceville, NJ, USA*

Background & Aims: Peginterferon lambda-1a (lambda) is a Type-III interferon, which, like alfa interferons, has antiviral activity *in vitro* against hepatitis B virus (HBV) and hepatitis C virus (HCV); however, lambda has a more limited extra-hepatic receptor distribution. This phase 2b study (LIRA-B) evaluated lambda in patients with chronic HBV infection.

Methods: Adult HBeAg+ interferon-naive patients were randomized (1:1) to weekly lambda (180 μg) or peginterferon alfa-2a (alfa) for 48 weeks. The primary efficacy endpoint was HBeAg seroconversion at week 24 post-treatment; lambda non-inferiority was demonstrated if the 80% confidence interval (80% CI) lower bound was >−15%.

Results: Baseline characteristics were balanced across groups (lambda N = 80; alfa N = 83). Early on-treatment declines in HBV-DNA and qHBsAg through week 24 were greater with lambda. HBeAg seroconversion rates were comparable for lambda and alfa at week 48 (17.5% *vs.* 16.9%, respectively); however lambda non-inferiority was not met at week 24 post-treatment (13.8% *vs.* 30.1%, respectively; lambda *vs.* alfa 80% CI lower bound −24%). Results for other key secondary endpoints (virologic, serologic, biochemical) and post hoc combined endpoints (HBV-DNA <2000 IU/ml plus HBeAg seroconversion or ALT normalization) mostly favored alfa. Overall adverse events (AE), serious AE, and AE-discontinuation rates were comparable between arms but AE-spectra differed (more cytopenias, flu-like, and musculoskeletal symptoms observed with alfa, more ALT flares and bilirubin elevations seen with lambda). Most on-treatment flares occurred early (weeks 4–12), associated with HBV-DNA decline; all post-treatment flares were preceded by HBV-DNA rise.

Conclusions: On-treatment, lambda showed greater early effects on HBV-DNA and qHBsAg, and comparable serologic/virologic responses at end-of-treatment. However, post-treatment, alfa-associated HBeAg seroconversion rates were higher, and key secondary results mostly favored alfa.

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* Corresponding author. Address: Bristol-Myers Squibb, 5 Research Parkway, Wallingford, CT 06492, USA. Tel.: +1 203 677 3651.

E-mail address: elizabeth.cooney@bms.com (E. Cooney).

† Full study team given in appendices.

Abbreviations: 80% CIs, 80% confidence intervals; AE, adverse event; alfa, peginterferon alfa-2a; CHB, chronic hepatitis B; CHC, chronic hepatitis C; HBeAb, hepatitis B e antibody; HBsAb, hepatitis B surface antibody; IFN, interferon; ISG, IFN-stimulated gene; HBeAg+, HBeAg-positive; HBsAg+, HBsAg-positive; Hgb, hemoglobin; lambda, peginterferon lambda-1a; LLOD, lower limit of detection; LLOQ, lower limit of quantification; mITT, modified intent-to-treat; NA, nucleos(t)ide analogue; NK, natural killer; pegIFN-alfa, pegylated interferon alfa; qHBeAg, quantitative hepatitis B e antigen; qHBsAg, quantitative hepatitis B surface antigen; SAE, serious adverse event; SNP, single nucleotide polymorphism; TLR, toll-like receptor; ULN, upper limit of normal.

Introduction

Globally, over 400 million people are chronically infected with hepatitis B virus (HBV), and around 1 million persons die every year from HBV-related complications [1]. Current HBV treatment guidelines recommend the use of a potent nucleos(t)ide analogue (NA) or peginterferon alfa (pegIFN-alfa) for patients with hepatitis B e antigen positive (HBeAg+) or -negative (HBeAg−) chronic hepatitis B (CHB) infection [1–3].

NAs afford long-term virologic suppression correlated with improvements in liver histology and prevention of disease progression, including decompensated cirrhosis, and a lowered risk of hepatocellular carcinoma (HCC) [4,5]. However, NAs are

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limited by the need for long-term treatment in many patients due to limited durability of post-treatment virologic and/or serologic responses [6,7].

PegIFN-alfa administration for 48 weeks is associated with higher off-treatment serologic responses than are observed after 1-year of NA therapy. In HBeAg+ patients, 48–52 weeks of pegIFN-alfa results in HBeAg seroconversion rates at week 24 post-treatment of 29–36% [8–10]. Hepatitis B surface antigen (HBsAg) clearance rates at this time point range from 2–7% [8–10]. However, adverse events (AEs), most commonly constitutional and/or musculoskeletal symptoms, and cytopenias, are frequent and may limit efficacy and adherence [8,11].

Peginterferon lambda-1a (interleukin [IL]-29 or lambda) is a conjugate of the recombinant human Type-III IFN IL-29 and a linear polyethylene glycol chain, with documented activity against HBV and hepatitis C virus (HCV) *in vitro* [12,13]. Type-III IFNs, together with the Type-I/II IFNs and the IL-10 family of cytokines, belong to the Class-II helical cytokine receptor family [14]. The Type-III IFNs are functionally similar to IFN-α/β, however structurally resemble the IL-10 family members, in particular IL-22 [15,16].

Endogenous IFN-λ and IFN-α are produced by host cells following viral infection and toll-like receptor (TLR) stimulation [15,17]. Although induced through similar signaling pathways, IFN-λ and IFN-α induction are differentially regulated and their post-transcriptional and -translational events differ [15]. IFN-λ and IFN-α exhibit distinct antiviral activities, partially determined by differences in cell-type specific receptor expression; IFN-αR is expressed by many cell types, whereas IFN-λR is primarily expressed on epithelial cells, hepatocytes, and plasmacytoid dendritic cells [17–20]. Both IFNs signal via engagement of cell-surface receptors comprising two chains; intracellular signaling occurs in a similar fashion through the JAK-STAT pathway [15]. However, studies in HCV-infected cells show the kinetics and spectrum of IFN-stimulated gene (ISG) induction and resultant antiviral effects for the two IFNs differ *in vitro*; IFN-λ is associated with a more gradual and sustained antiviral effect compared with a more rapid and transient effect seen with IFN-α [21–23]. These findings may relate to differential regulation of IFN-α *vs.* IFN-λ signaling by ISGs induced early following IFN receptor engagement, with UBP43 selectively interacting with IFN-αR creating a refractory IFN-α signaling state. Similar studies using HBV-infected cells have not yet been conducted.

In phase 2 HCV studies, lambda demonstrated comparable efficacy and improved tolerability, with a differentiated safety profile compared with alfa [24]. This phase 2b study (LIRA-B) evaluated the efficacy and safety of lambda *vs.* alfa monotherapy over 48 weeks in IFN-naive patients with HBeAg+ CHB.

Materials and methods

Study design

This was a phase 2b, multicenter, randomized, parallel, double-blind study of 48 weeks of lambda *vs.* alfa monotherapy in IFN-naive patients with HBeAg+ CHB (NCT01204762), conducted at 41 sites in the United States, Canada, France, Germany, Italy, Netherlands, Australia, Hong Kong, Korea, Singapore, and Taiwan between November 2010 and June 2013. Patients were followed for 24 weeks post-treatment to assess off-treatment response rates. Randomization was by designated site personnel via an Interactive Voice Response System. The study was initially designed to be a dose-finding, three-arm study of two lambda doses (240 or 180 μg) based on data from the phase 2 HCV lambda program, *vs.* the standard alfa dose (180 μg). However, the lambda 240 μg dose was discontinued after study initiation, following higher observed rates of bilirubin and/or aminotransferase elevations at this dose in lambda HCV trials. The 13 patients who had been randomized and treated (range, 1–17 weeks) with lambda 240 μg were switched to lambda 180 μg and included in that treatment group; all subsequently enrolled patients were randomized 1:1 to lambda 180 μg or alfa. Data from the lambda 240 μg group did not lend any meaningful insight regarding lambda efficacy or safety, thus are not included. All authors had access to study data, contributed to review and critical revision of the manuscript and approved the final version. The protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review board/independent ethics committee at each site; all patients provided written informed consent.

Study endpoints

The primary study endpoints were the number (%) of patients with serious adverse events (SAEs) and discontinuations due to AEs, and HBeAg seroconversion at post-treatment week 24 (PT24). Patients who discontinued early but had 24 weeks of post-treatment follow-up were included in these analyses. Key additional prespecified efficacy and safety endpoints included mean change from baseline and proportions achieving prespecified thresholds for HBV-DNA; quantitative HBsAg (qHBsAg) and HBeAg (qHBeAg); HBeAg or HBsAg seroconversion or loss (other than PT24 for HBeAg); alanine aminotransferase (ALT) normalization ($\leqslant 1 \times$ upper limit of normal [ULN]); and number (%) with AEs or laboratory abnormalities. Additional efficacy endpoints combining key parameters of interest (i.e. HBV-DNA <2000 IU/ml plus ALT normalization *or* HBeAg seroconversion) were added post hoc.

Patient population

Patients were adults with CHB, defined as HBsAg-positive with another marker of HBV infection (e.g., HBV-DNA, or genotype) on one or more occasions $\geqslant$24 weeks before screening. At screening and at least once $\geqslant$4 weeks before screening, patients had detectable HBeAg and undetectable HBeAg antibodies (HBeAb), plus HBV-DNA $\geqslant 10^5$ copies/ml (17,200 IU/ml). Patients were IFN-naive but prior HBV NA use was allowed if completed >30 days prior. Permitted ALT levels were >ULN (47 U/L) and <10 $\times$ ULN. Cirrhotics (Child-Turcotte-Pugh class A confirmed by liver biopsy/FibroTest) were allowed but capped at 10%.

Key exclusion criteria included human immunodeficiency virus, HCV, or hepatitis delta virus coinfection; other medical conditions contributing to chronic liver disease; history/evidence of hepatic decompensation or HCC, and known alfa intolerance or contraindication to use. Patients with hemoglobin <12 (male) or <11 g/dl (female); platelets <90,000/mm^3; creatinine clearance $\leqslant$50 ml/min (Cockcroft-Gault), or total serum bilirubin >2.5 mg/dl (exception Gilbert's disease); international normalized-ratio >1.2; serum albumin $\leqslant$3.5 g/dl; alpha fetoprotein $\geqslant$100 ng/ml, or partial thromboplastin time >1.5 $\times$ ULN were also excluded.

Assays

HBV-DNA was assessed using the Roche COBAS® TaqMan HPS assay (lower limit of quantitation 29 IU/ml; limit of detection 10 IU/ml). Quantitative HBsAg and qHBeAg were assessed using Abbott Architect assays (linear ranges 0.05–250 IU/ml and 0.22–56.70 PEIU/ml, respectively). Commercially available qualitative assays were used to assess presence of HBeAg, HBsAg, HBeAb, and HBsAb.

Statistical analysis

A two-stage evaluation of the efficacy of lambda *vs.* alfa was planned. In the first stage, non-inferiority was tested and, if established, a second stage would test superiority. A sample size of approximately 85 patients per group provided 80% power to demonstrate non-inferiority of lambda 180 μg to alfa for proportion with HBeAg seroconversion at PT24, assuming a response rate of 32% based on the registrational phase III trial for alfa-2a [8]. Non-inferiority was demonstrated if the lower limit of the 80% CI for the treatment difference between lambda 180 μg and alfa was >−15%.

Efficacy analyses were based on modified intent-to-treat (mITT) analysis and observed methodologies. For both approaches, numerators were patients meeting the response criteria. For mITT analyses, denominators represented all treated subjects and patients with missing data were counted as failures. For observed values analyses, denominators represented those treated subjects with a measurement at the visit week(s) defining the endpoint, and patients with missing data were removed from the analysis.

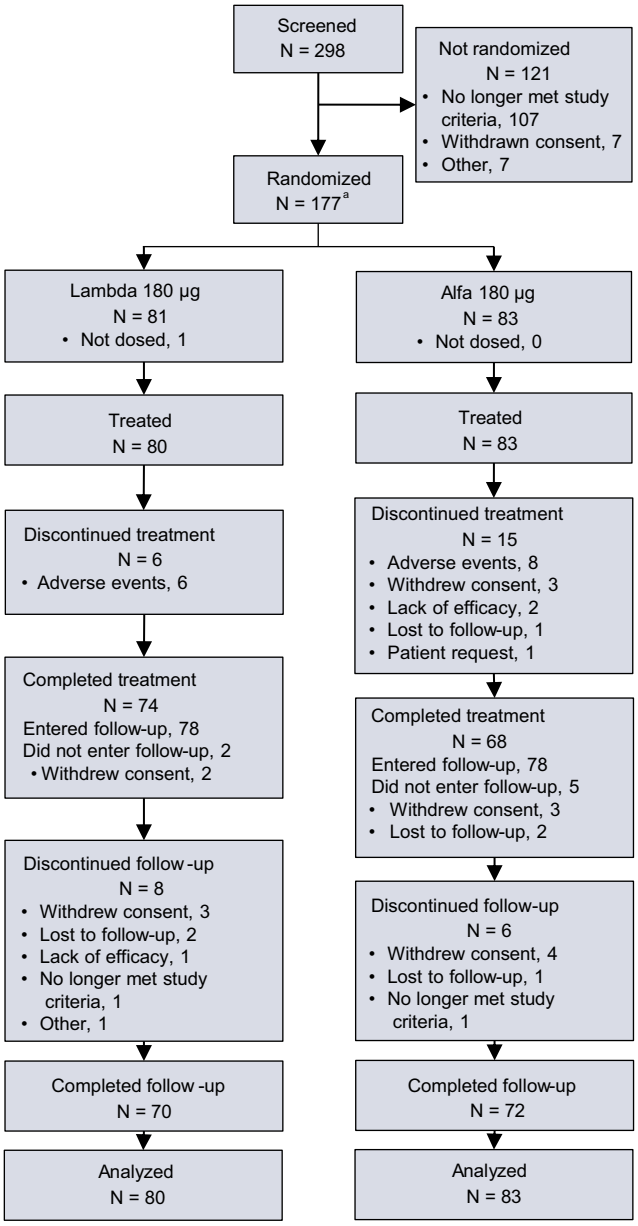

Fig. 1. Patient disposition. [a] Includes 13 patients randomized to a lambda 240 μg arm subsequently discontinued by protocol amendment. See the study design section for details.

Data for those who subsequently started alternative therapy were truncated at time of initiation.

Safety analyses included all patients who received at least one dose of study medication (unless otherwise specified) and included deaths, SAEs, AEs, dose interruptions/reductions, laboratory evaluations, and ALT flares (defined as serum ALT >2 × baseline and >10 × ULN).

Results

Disposition and baseline characteristics

There were 298 patients screened and 176 treated (lambda 240 μg (n = 13); lambda 180 μg (n = 80); alfa 180 μg (n = 83)). The majority (lambda 180 μg (92.5%), alfa (81.9%)) completed the 48-week treatment period. All six patients in the lambda 180 μg and 8/15 in the alfa group who discontinued prematurely did so for AEs (Fig. 1).

Baseline characteristics were balanced between groups (Table 1). Patients were predominantly male (74.8%), Asian (90.2%), non-cirrhotic (95.1%), and NA-naive (95.1%) with median age 34.0 years. Median HBV-DNA was 7.9 $\log_{10}$ IU/ml, ALT 103 U/L (range 26–936), qHBsAg 4.1 $\log_{10}$ IU/ml, and qHBeAg 2.8 $\log_{10}$ PEIU/ml. HBsAg levels were <1500 IU/ml in 8.4% (7/83) alfa and 11.3% (9/80) lambda 180 μg patients, and HBeAg <100 PEIU/ml in 19.3% (16/83) and 23.8% (19/80), respectively. Predominant HBV genotypes were C (55.8%) and B (31.3%), consistent with the Asian majority. Patients with *IL28B* rs12979860 CC host genotype accounted for 73.6%.

Efficacy

Primary efficacy endpoint

HBeAg seroconversion rates were comparable for lambda and alfa during treatment (Table 2) but diverged following treatment discontinuation. Rates at PT24 (primary endpoint) were 30.1% for alfa and 13.8% for lambda, with a lambda *vs.* alfa difference estimate of −0.1635 and 80% CI of −0.2425 to −0.0845. As the lower bound of the 80% CI (−24%) was below the predefined −15% non-inferiority threshold, lambda non-inferiority was not demonstrated.

Secondary efficacy analyses

On-treatment. HBeAg seroconversion at week 48 (end-of-treatment) was observed in 14/80 (17.5%) patients in the lambda group *vs.* 14/83 (16.9%) alfa recipients. The qualitative and quantitative HBeAg responses generally paralleled the HBeAg seroconversion results at week 48: HBeAg loss was 18.8% for lambda *vs.* 18.1% for alfa (Table 2); mean qHBeAg change from baseline was −1.2 *vs.* −1.4 $\log_{10}$ PEIU/ml for lambda *vs.* alfa (Fig. 2A).

A more pronounced early decline in qHBsAg was observed on lambda *vs.* alfa (mean change from baseline through week 24, −0.64 $\log_{10}$ *vs.* −0.33 $\log_{10}$ IU/ml; respectively ($p = 0.011$)); qHBsAg response remained numerically higher on lambda – though not significantly – at week 48 (−0.58 *vs.* −0.34 $\log_{10}$ IU/ml, respectively ($p = 0.149$); Fig. 2B). At week 24, a higher proportion of lambda *vs.* alfa recipients achieved qHBsAg <1500 IU/ml (30.0 *vs.* 19.3%, respectively), whereas responses were comparable at week 48 (26.3% *vs.* 21.7%, respectively). Two lambda recipients *vs.* no alfa recipient achieved HBsAg loss at week 24.

A more pronounced HBV-DNA decline was observed with lambda *vs.* alfa through week 24 (statistically significant through week 16; Fig. 2C). HBV-DNA response subsequently stabilized on lambda *vs.* a continued decline on alfa, with the two groups showing comparable results at week 48 (lambda −2.67, alfa −2.88 $\log_{10}$ IU/ml). The proportion with HBV-DNA <50 IU/ml at end-of-treatment was low in both groups (lambda, 13.8%; alfa, 10.8%).

Median ALT changes from baseline on- and off-treatment are shown in Fig. 3. ALT normalization was lower at week 24 for lambda *vs.* alfa (23.8% *vs.* 33.7%, respectively), likely related to the more frequent early ALT elevations on lambda (see Safety section). At week 48, ALT normalization rates were similar (32.5%, both groups).

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Table 1. Demographics and baseline HBV disease characteristics.

Parameter	Lambda 180 μg N = 80	Alfa 180 μg N = 83
Age, mean [median] years (range)	36.5 [33.0] (21-61)	34.9 [34.0] (19-63)
Male, n (%)	59 (73.8)	63 (75.9)
Asian, n (%)	72 (90.0)	75 (90.4)
Non-cirrhotic, n (%)	75 (93.8)	80 (96.4)
Nucleos(t)ide analog naive, n (%)	76 (95.0)	80 (96.4)
HBV-DNA, mean [median] $\log_{10}$ IU/ml (range)	7.6 [7.9] (4.7-9.3)	7.9 [7.9] (4.9-10.0)
qHBsAg, mean [median] $\log_{10}$ IU/ml (range)	4.0 [4.1] (2.1-5.2)	4.1 [4.1] (1.0-5.4)
qHBeAg, mean [median] $\log_{10}$ PEIU/ml (range)	2.2 [2.7] (-0.4-2.8)	2.3 [2.8] (0.05-2.8)
HBV genotype, n (%)		
A	6 (7.5)	5 (6.0)
B	21 (26.3)	30 (36.1)
C	49 (61.3)	42 (50.6)
D	0	4 (4.8)
E	2 (2.5)	0
Indeterminate/missing	2 (2.5)	2 (2.4)
IL28B rs12979860 CC genotype, n (%)	56 (70.0)	64 (77.1)
ALT, mean [median] U/L (range)	150 [93.5] (30-936)	132 [106] (26-389)

Post-treatment. HBeAg seroconversion rates diverged following treatment discontinuation, favoring alfa *vs.* lambda at PT24, as previously noted. Of the 11/80 lambda-treated patients evidencing HBeAg seroconversion at PT24, 7 showed seroconversion at week 48, and 4 were new responders (the remaining 7/14 responders at week 48 were no longer counted as responders at PT24 due to seroreversion (4), initiation of NA therapy per investigator (2) or loss-to-follow-up (1)); 9/11 responders received 48 weeks and the remaining 2/11 8–9 weeks of lambda. Of the 25/83 alfa recipients with HBeAg seroconversion at PT24, 10 evidenced seroconversion at week 48, and 15 were new events (the remaining 4/14 responders at week 48 were no longer counted as responders at PT24 due to seroreversion (3) or loss to follow-up (1)); 24/25 received 48 weeks and 1/25 36 weeks of alfa. Off-treatment rates of HBeAg loss paralleled seroconversion findings, favoring alfa (32.5%) over lambda (15.0%) at PT24. The post-treatment qHBeAg response was consistent with these findings, showing a mean increase from week 48 in the lambda group *vs.* gradual decline in the alfa group, with the PT24 results favoring alfa (-1.5 $\log_{10}$ PEIU) over lambda (-0.9 $\log_{10}$ PEIU/ml).

Post-treatment, qHBsAg levels plateaued in the alfa group and rose in the lambda group, with similar mean declines (-0.3 $\log_{10}$ IU/ml) observed at PT24. Post-treatment qHBsAg levels <1500 IU/ml remained broadly comparable between the lambda and alfa groups (17.5% *vs.* 10.8%, respectively, at PT24). Through PT24, there remained 2 events of HBsAg loss (without accompanying seroconversion) in the lambda group and one event (with HBsAg seroconversion) in the alfa group.

Post-treatment virologic rebound occurred in both groups, however the loss of virologic effect was greater in the lambda group with PT24 HBV-DNA changes from baseline favoring alfa over lambda (-2.09 *vs.* -1.30 $\log_{10}$ IU/ml). Proportions with HBV-DNA <50 IU/ml declined off-treatment in both groups, but remained numerically higher at PT24 with lambda (6.3%) than alfa (1.2%).

ALT normalization rate at PT24 was numerically lower in the lambda (43.8%) *vs.* alfa group (51.8%), likely related to the greater frequency of post-treatment ALT elevations observed in the lambda group.

Exploratory and subgroup analyses. A post hoc exploratory analysis assessed the proportion of patients who met the combined criteria of HBV-DNA <2000 IU/ml plus ALT normalization during and post-treatment. At week 48, the proportion achieving the combined endpoint (mITT analysis) was numerically higher for lambda *vs.* alfa (17.5% *vs.* 10.8%, respectively). However, at PT24, the proportion achieving this endpoint was numerically lower for lambda (11.3%) *vs.* alfa (16.9%). Similar results for lambda *vs.* alfa were seen in a post hoc analysis of HBV-DNA <2000 IU/ml plus HBeAg seroconversion, where rates were comparable at week 48 (12.5% *vs.* 14.5%, respectively) but numerically lower for lambda than alfa at PT24 (7.5% *vs.* 14.5%, respectively).

HBeAg seroconversion at PT24 was assessed by subgroup analyses utilizing demographic and baseline characteristics

Table 2. Efficacy endpoints summary.

n (%), mITT	Week 48 (end of treatment)		Week 24 post-treatment[a]	
	Lambda 180 μg (N = 80)	Alfa 180 μg (N = 83)	Lambda 180 μg (N = 80)	Alfa 180 μg (N = 83)
HBeAg seroconversion	14 (17.5)	14 (16.9)	11 (13.8)	25 (30.1)
HBeAg loss	15 (18.8)	15 (18.1)	12 (15.0)	27 (32.5)
HBsAg seroconversion	0	0	0	0
HBsAg loss	2 (2.5)	0	2 (2.5)	0[b]
qHBsAg <1500 IU/ml	21 (26.3)	18 (21.7)	14 (17.5)	9 (10.8)
HBV-DNA <50 IU/ml	11 (13.8)	9 (10.8)	5 (6.3)	1 (1.2)
ALT normalization	26 (32.5)	27 (32.5)	35 (43.8)	43 (51.8)
HBV-DNA <2000 IU/ml	22 (27.5)	19 (22.9)	10 (12.5)	14 (16.9)
HBV-DNA <2000 IU/ml and HBeAg seroconversion	10 (12.5)	12 (14.5)	6 (7.5)	12 (14.5)
HBV-DNA <2000 IU/ml and ALT normalization	14 (17.5)	9 (10.8)	9 (11.3)	14 (16.9)

[a]Includes 24-week follow-up from patients who discontinued treatment before week 48.
[b]HBsAg loss evident for one patient at week 12 post-treatment receiving alfa; due to a missed visit, week 24 post-treatment data are not available.

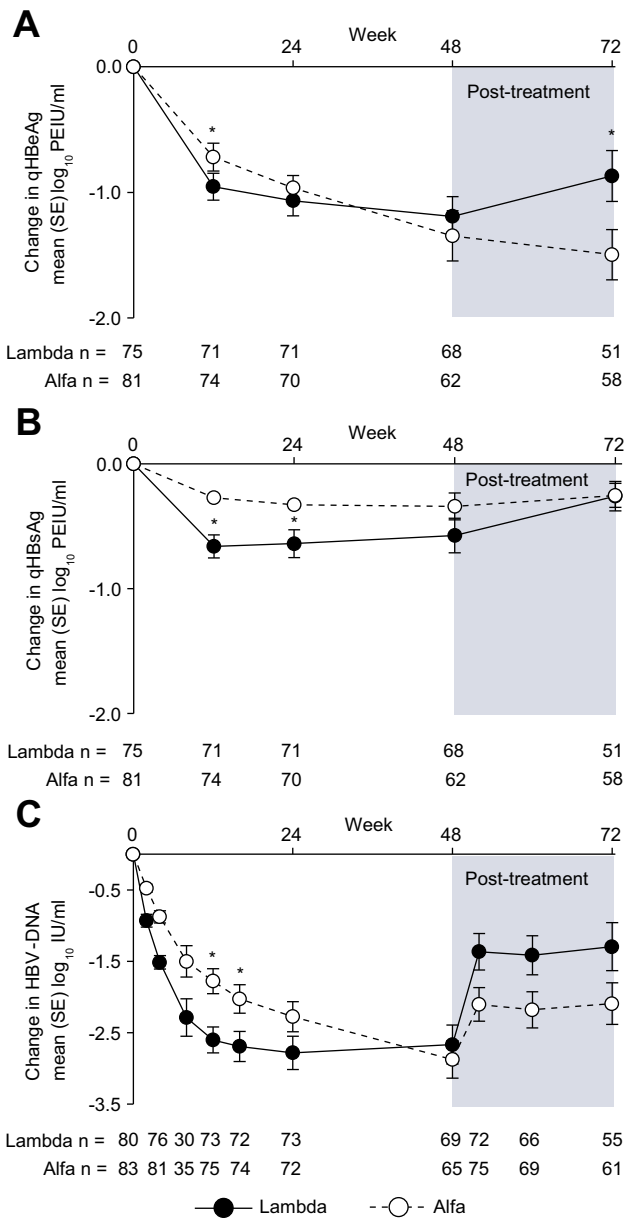

Fig. 2. Quantitative Virologic and Serologic Responses (change from baseline). (A) qHBeAg; (B) qHBsAg; (C) HBV-DNA. Alfa, peginterferon alfa-2a; HBV, hepatitis B virus; Lambda, peginterferon lambda-1a; LLOD, lower limit of detection; LLOQ, lower limit of quantification; qHBeAg, quantitative hepatitis B e antigen; qHBsAg, quantitative hepatitis B surface antigen; SE, standard error. *Significant difference (p <0.05). HBV-DNA: Roche COBAS® TaqMan HPS assay LLOQ 29 IU/ml, LLOD 10 IU/ml; qHBeAg and qHBsAg: Abbott Architect assay, linear ranges 0.22–56.70 PEIU/ml and 0.05–250 IU/ml, respectively.

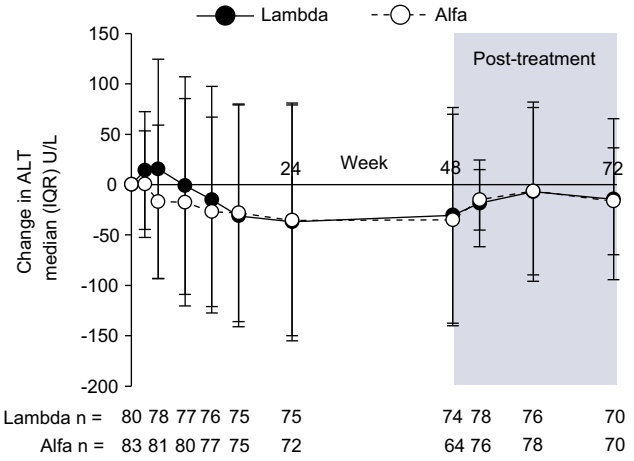

Fig. 3. Changes from baseline in median serum ALT.

previously evaluated for their role in predicting sustained off-treatment response during alfa-2a/2b therapy [8,10,25]. These subgroup data demonstrated consistent findings with the overall analysis, with results mostly favoring alfa (Fig. 4).

Multivariate logistic regression analyses were also conducted to identify predictors of HBeAg seroconversion at PT24 across the two groups, focusing on baseline (same covariates listed in Fig. 4), early on-treatment (qHBeAg, qHBsAg, or HBV-DNA change from baseline at weeks 12 or 24), and end-of-treatment factors (qHBsAg change from baseline, HBeAg seroconversion at week 48) previously identified as predictors for alfa response in CHB. Only gender (odds ratio 3.02 for female *vs.* male; $p = 0.0082$) and HBeAg seroconversion at week 48 (odds ratio 0.068 for no *vs.* yes; p <0.0001) were significantly predictive of outcome, after adjusting for treatment effect.

Safety

On-treatment safety is summarized in Table 3. Lambda was generally well tolerated. There were no deaths. Comparable rates of SAEs (8.8% and 6.0%) and discontinuations due to AEs (7.5% and 9.6%) were observed with lambda *vs.* alfa, respectively.

The majority of SAEs and discontinuations due to AEs for lambda were related to hepatobiliary events, while for alfa the majority of events were related to cytopenias, particularly leukopenia, neutropenia, and thrombocytopenia (grades 1–4) or hepatic enzyme elevations. The frequency of ALT flares (serum ALT >2 × baseline and >10 × ULN) was higher with lambda than alfa. Most ALT flares were asymptomatic; none were associated with clinical or laboratory signs of hepatic impairment. Two lambda events were associated with isolated grade 3 hyperbilirubinemia. The majority of on-treatment ALT flares (lambda 12/13; alfa 3/6) occurred within the first 12 weeks and were associated with an HBV-DNA decline from baseline ($\geqslant$1 $\log_{10}$ IU/ml; see Supplementary Fig. 1 for lambda and alfa patient examples). Four of 13 lambda-treated patients *vs.* none of 6 alfa recipients with an on-treatment ALT flare achieved HBeAg seroconversion through PT24. Most post-treatment ALT flares (lambda 9/12; alfa 3/7) occurred within 12 weeks of treatment discontinuation and were preceded by rebound viremia ($\geqslant$1 $\log_{10}$ IU/ml). There were more dose reductions with alfa (most commonly cytopenias: 15/23, 65%) than with lambda (most commonly hepatic enzyme elevations: 11/12, 92%).

Analysis of AEs of special interest showed numerically higher rates of flu-like symptoms and musculoskeletal symptoms with alfa *vs.* lambda. Rates for other AEs of special interest (constitutional symptoms, neurologic events, and psychiatric events) were generally comparable.

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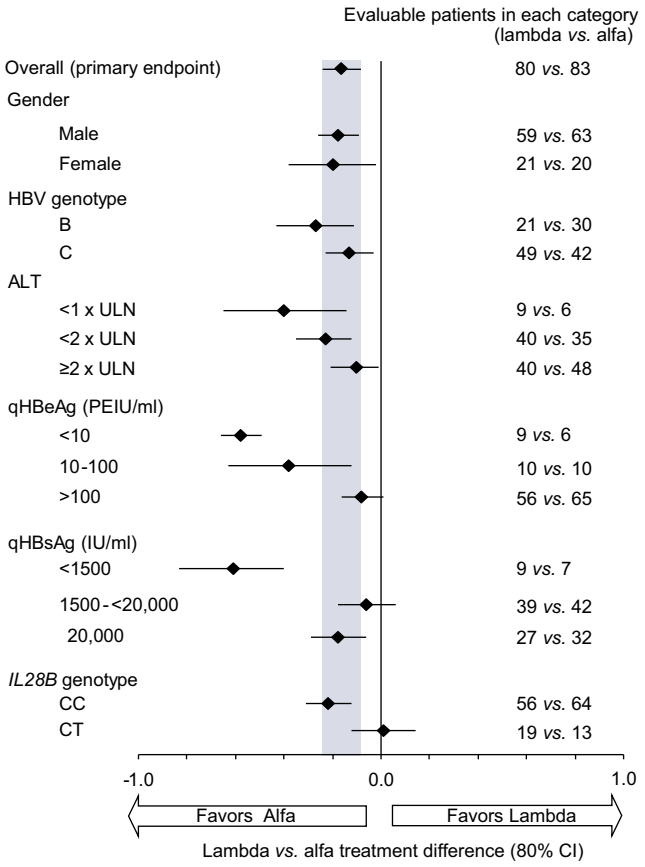

Fig. 4. Treatment differences in proportions with HBeAg seroconversion at week 24 post-treatment by baseline subgroup characteristics. Estimation is stratified by region with Cochran-Mantel-Haenszel weights; 80% confidence interval is based on normal approximation of the binomial distribution. Categories for which treatment difference was not calculated due to low numbers (*IL28B*-TT; HBV genotypes A, D, or E), or subgroups for which only one category could be calculated (age; cirrhosis status), are not shown.

Discussion

Alfa-2a is an approved treatment for patients with CHB, including those with HBeAg+ and HBeAg− disease. The evidence is based on data from a large phase III study conducted in primarily Asian patients, which formed the basis for the drug's approval [8]. These results were substantiated by the subsequent post-marketing Neptune study, which also primarily enrolled Asians [9]. Both studies showed comparable off-treatment HBeAg seroconversion rates to 48 weeks of alfa 180 μg weekly (32–36%, respectively), HBV-DNA suppression (32–42% respectively), and HBsAg seroconversion (2% *vs.* 4%, respectively). Both studies provided post-treatment rates for combined HBV-DNA suppression/ALT normalization; however, only Neptune utilized the currently accepted HBV-DNA criteria of <2000 IU/ml (24%). A number of smaller studies utilizing either alfa-2a or alfa-2b, the majority of which have targeted Asian patients, have demonstrated similar findings. While higher cure rates for CHB are desirable, at the present time alfa remains a recognized and recommended therapy, offering higher off-treatment response rates at early (6 months) and delayed (3–5 years) time points than are achieved on-treatment with HBV NAs [1].

Accordingly, this initial, proof-of-concept study evaluated lambda *vs.* alfa monotherapy in patients with CHB. The rationale for specifically targeting HBeAg+ patients with active viremia and immunoactive disease was based on both the availability of a readily measurable and accepted primary endpoint correlated with clinical benefit (HBeAg seroconversion) and an intent to align with current HBV treatment guidelines recommending interferon use in HBeAg+ CHB be limited to those with immunoactive disease [1–3].

This study was originally intended to be dose-finding for lambda. However, the protocol was subsequently amended to remove the 240 μg dose – due to the increased risk of hepatobiliary laboratory abnormalities observed in lambda HCV studies – and the study continued using only lambda 180 μg or alfa. The decision not to add a second lambda dose was based on the cumulative data from the lambda HCV development program supporting 180 μg as the target dose for treatment of viral hepatitis.

The major finding from this study was that lambda 180 μg did not meet the criteria for non-inferiority to alfa for the primary efficacy endpoint of HBeAg seroconversion at PT24: 11/80 [13.8%] for lambda *vs.* 25/83 [30.1%] for alfa, with a −24% lower bound for the 80% CI for the treatment difference below the prespecified non-inferiority margin of −15%. The off-treatment difference between alfa and lambda was primarily due to more post-treatment HBeAg seroconversions in the alfa group (15 *vs.* 4, respectively) rather than to a difference in HBeAg seroreversion events (3 *vs.* 4, respectively).

Key secondary efficacy endpoint results for lambda were either numerically lower than alfa (HBV-DNA or qHBeAg change from baseline; HBeAg loss; ALT normalization) or comparable (qHBsAg change from baseline; post-treatment HBsAg loss).

The efficacy results cannot be explained by differences in established predictors for interferon treatment response; baseline disease characteristics historically associated with higher alfa response (elevated serum ALT, low HBV-DNA, presence of HBV genotype A or B [25]) were generally well balanced between treatment groups, and consistent with data from major alfa-2a studies [8,9,26]. In addition, the number of patients with baseline qHBsAg or qHBeAg below thresholds which have been shown to be predictive of off-treatment serologic response when attained during treatment, were actually slightly higher in the lambda group. Furthermore, although on-treatment alfa responses were lower in this study compared with historical data, post-treatment alfa responses were similar to published rates [8,9].

During the first 24 weeks on-treatment, lambda demonstrated faster and more pronounced declines in HBV-DNA and qHBsAg, with two events of HBsAg loss *vs.* none with alfa. Results suggest the possibility of an enhanced effect of lambda *vs.* alfa on receptor engagement and signal transduction through the JAK-STAT pathway, increasing ISG production downstream. However, greater stimulation of ISGs in whole blood was seen with alfa than lambda in this study (data not shown), consistent with IFN receptor expression patterns [18–20,27]. The interpretation and clinical relevance of these observations is limited by the fact that intrahepatic ISG responses were not assessed.

The increased occurrence of early ALT flares, the majority of which were associated with a preceding HBV-DNA decline, is also of interest and could relate to differences in the spectrum of ISGs produced by lambda *vs.* alfa, which may differentially engage the host immune system. Data from Kohli's laboratory has

Table 3. On-treatment safety summary.

Patients, n (%)	Lambda 180 μg N = 80	Alfa 180 μg N = 83
Serious adverse events	7 (8.8)	5 (6.0)
Adverse events leading to discontinuation	6 (7.5)[a]	8 (9.6)[b]
Adverse events (any grade) in >15% in any group		
Pyrexia	8 (10.0)	38 (45.8)
Alopecia	9 (11.3)	25 (30.1)
Fatigue	26 (32.5)	24 (28.9)
Headache	11 (13.8)	24 (28.9)
Neutropenia	0	20 (24.1)
Myalgia	3 (3.8)	18 (21.7)
Dizziness	5 (6.3)	13 (15.7)
Pruritus	7 (8.8)	13 (15.7)
ALT increased	15 (18.8)	8 (9.6)
Adverse event categories of special interest[c]		
Constitutional	28 (35.0)	26 (31.3)
Neurologic	18 (22.5)	30 (36.1)
Flu-like	13 (16.3)	45 (54.2)
Musculoskeletal	5 (6.3)	23 (27.7)
Psychiatric	11 (13.8)	15 (18.1)
Grade 3-4 laboratory abnormalities		
ALT increases (>5 × ULN)	33 (41.3)	19 (23.2)
AST increases (>5 × ULN)	27 (33.8)	15 (18.3)
Hyperbilirubinemia (>2.5 × ULN)	3 (3.8)	0
Neutropenia (<750 cells/mm^3)	2 (2.5)	17 (20.7)
Thrombocytopenia (<50,000 cells/mm^3)	0	1 (1.2)
Hemoglobin <9 g/dl or ≥4.5 g/dl ↓ from baseline	0	0
ALT flare[d]	13 (16.3)	6 (7.2)
Dose reductions	12 (15.0)[e]	23 (27.7)[b]
Dose interruptions	8 (10.0)[e]	4 (4.8)[b]

[a]Mostly elevations in hepatobiliary enzymes.
[b]Mostly neutropenia or elevations in hepatobiliary enzymes.
[c]AE categories of special interest are based on preferred terms found in the alfa label reported in at least 5% of patients: Constitutional (fatigue); neurologic (headache and/or dizziness); flu-like (pyrexia and/or chills and/or pain); musculoskeletal (arthralgia and/or myalgia and/or back pain); and psychiatric (depression and/or irritability and/or insomnia).
[d]ALT flare defined as ALT >2 × baseline and >10 × ULN.
[e]In majority of cases, reason was on-treatment ALT flare.

demonstrated that IFN-λ and IFN-α exhibit differential ISG profiles in uninfected *vs.* HCV-infected hepatocyte cell lines, likely due to differences in kinetics of ISG induction [28]. In Kohli's study, IFN-λ was shown to selectively induce certain ISGs, with the differential induction in some cases observed in both infected and uninfected cells, although to a greater degree in infected cells, and in other cases observed only in infected cells. One of these genes is IL-18, a known natural killer (NK) cell stimulatory cytokine. Furthermore, while the IFN-λ receptor has not been found to be expressed directly on NK cells, recently IFN-λ has been shown to activate NK cells and their function through indirect mechanisms mediated via enhanced IL-12 release by macrophages [29].

The explanation for this loss of early lambda advantage requires further investigation but may relate to the differential nature of lambda *vs.* alfa receptor expression and/or signaling within the liver of patients with chronic viral hepatitis, resulting in a down-regulation of lambda receptor expression on infected hepatocytes in response to treatment [30]. Alternatively, lambda may result in a more limited engagement of host immune effectors critical for maintaining virologic control initially achieved through ISG induction. However, without intrahepatic assessments to substantiate either theory, these hypotheses remain speculative.

The accrual of serologic responses post-treatment has been described in prior alfa trials [8], but it is unknown why lower post-treatment rates are seen with lambda. Differences between how lambda and alfa engage the host immune system may play a role, specifically in a disease like CHB where a number of preexisting host defects impact viral suppression and antigen clearance. The lambda receptor is minimally expressed on peripheral blood lymphocytes, in particular T-lymphocytes [15,20]. Conversely, the alfa receptor is widely expressed and alfa can engage both the innate and adaptive arms of the immune system [31], and responses generated during dosing may persist post-treatment. Additionally, alfa has antiproliferative effects on human lymphocytes [31] which may limit detection of alfa's beneficial immune effects until post-treatment follow-up. To this end, we observed a differential effect of alfa *vs.* lambda on host immune cells; during alfa therapy, there was a decrease in absolute numbers of T and B lymphocytes and NK cells, with normalization of cell numbers following discontinuation of therapy, whereas these effects were not observed in the lambda group (data not shown).

Safety data were generally comparable with those previously reported for lambda in chronic HCV infection [24], and consistent with the more restricted lambda receptor distribution [18–20]. The exception was the higher incidence of ALT elevations, which appears largely explained by ALT flares associated with concomitant HBV-DNA decline or rebound viremia; and the lower overall rate of hyperbilirubinemia relative to the HCV experience, likely reflecting absence of concurrent ribavirin use and the associated risk of hemolysis.

In summary, the results from this proof-of-concept study in CHB patients with HBeAg+ disease show greater efficacy for alfa than lambda 180 μg, based on sustained off-treatment serologic, virologic, and biochemical responses. However, the on-treatment findings clearly support a treatment effect for lambda in CHB, with an early virologic response that parallels what has been observed for lambda *vs.* alfa in CHC [24]. All-cause SAEs and AE-related discontinuations were comparable for lambda and alfa. Understanding the mechanism of this early on-treatment effect and subsequent discordance with later treatment effects may lend insight regarding ways of harnessing lambda's beneficial host/virus interactions, and circumventing factors limiting or impairing a treatment effect.

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Conflict of interest

HLC: advisor and speaker for Bristol-Myers Squibb, Gilead, Roche, Novartis and MSD, a speaker for GlaxoSmithKline, Echosens and AbbVie, and has received an unrestricted grant for hepatitis B

research from Roche; SHA: advisor and speaker for Bristol-Myers Squibb, Gilead, Roche, Novartis, MSD, Janssen, and AbbVie; and has received an unrestricted grant for hepatitis B research from Bristol-Myers Squibb, Gilead, and Roche; TTC: nothing to disclose; CYP; nothing to disclose; DW: nothing to disclose; CC: supported by the Canadian institutes of Health Research and has received speaker and advisory board fees and/or research support from Bristol-Myers Squibb, GlaxoSmithKline, Gilead, Janssen Pharmaceuticals, Boehringer Ingelheim, and Merck; SGL: advisory board fees from Gilead, Novartis, Merck Sharpe and Dohme, Bristol-Myers Squibb, Pfizer, Boehringer Ingelheim, AbbVie, Vertex, Tobira, speakers' bureau for Gilead, GlaxoSmithKline, Bristol-Myers Squibb, Novartis, Roche, and educational/research funding from Bristol-Myers Squibb, Novartis, Merck Sharpe and Dohme, and Gilead; PJC: personal fees and other from Bristol-Myers Squibb, grants from Roche and Janssen, and personal fees from Gilead; HLAJ: nothing to disclose; PM: grant, investigator, speaker, and expert for Roche, Gilead, Bristol-Myers Squibb, Novartis, Merck Sharpe and Dohme, and Janssen, investigator and expert for Vertex, and Abbott, grant and investigator for Alios BioPharma, and investigator for Pfizer, and Boehringer Ingelheim; LS: personal fees from Bristol-Myers Squibb, Janssen, Merck Sharpe and Dohme, Gilead, AbbVie, and Roche; SZ: consultancy for AbbVie, Bristol-Myers Squibb, Gilead, Janssen, Merck, speakers' bureau for AbbVie, Bristol-Myers Squibb, Gilead, Janssen, Merck/Merck Sharpe and Dohme; DC, LC, DX, MWR, and EC are employees of Bristol-Myers Squibb.

Author contributions

All authors contributed to review and critical revision of the manuscript and approved the final version of the manuscript. Each author also participated as indicated: HLC: acquisition of data, and interpretation of data; SHA: acquisition of data, and interpretation of data; TTC: acquisition of data, and interpretation of data; CYP; study design/conception, acquisition of data, and interpretation of data; DW: acquisition of data, and interpretation of data; CC: acquisition of data, and interpretation of data; SGL: acquisition of data, and interpretation of data; PJC: acquisition of data, and interpretation of data; HLAJ: acquisition of data, and interpretation of data; PM: acquisition of data, and interpretation of data; LS: acquisition of data, and interpretation of data; SZ: acquisition of data, and interpretation of data; DC: study design/conception, and data analysis; LC: study design/conception; DX: study design/conception, data analysis, and interpretation of data; MWR: study design/conception, and interpretation of data; EC: study design/conception, and interpretation of data.

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Supplementary data

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