Therapeutic vaccine BRII-179 restores HBV-specific immune responses in patients with chronic HBV in a phase Ib/IIa study

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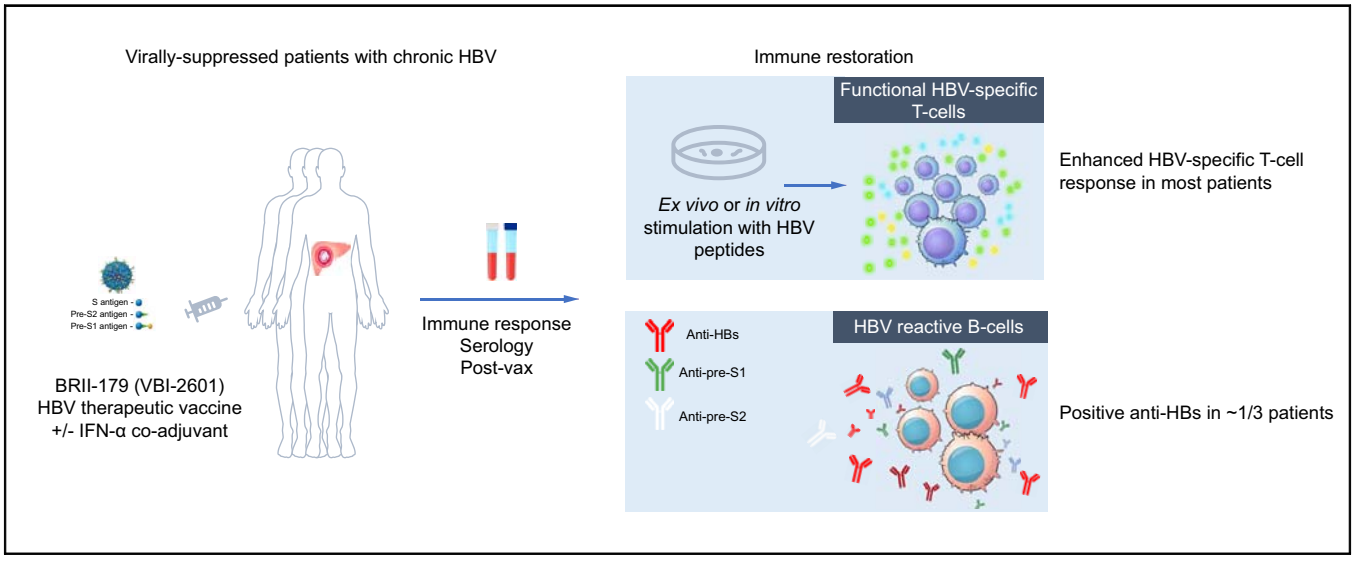
Graphical abstract



Highlights

- BRII-179 admixed with or without IFN-α is safe and well tolerated.
- BRII-179 can induce antigen-specific humoral and T-cell responses in patients with CHB.
- BRII-179-induced immune responses were insufficient to reduce serum HBsAg in virally suppressed patients.
- These data support the further clinical evaluation of BRII-179, especially in combination with other therapies.

Lay summary

BRII-179 is a therapeutic vaccine designed to improve the immune response in patients with chronic hepatitis B. In this study, BRII-179 alone or with a low dose of interferon-α was safe, well tolerated, and induced enhanced HBV-specific antibody and T-cell responses in patients with chronic hepatitis B. However, BRII-179 treatment alone had minimal effect on patient's virological status. The potential of BRII-179 to achieve a functional cure in conjunction with other agents is being evaluated in the clinic.

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Research article

JHEP Reports

Therapeutic vaccine BRII-179 restores HBV-specific immune responses in patients with chronic HBV in a phase Ib/IIa study

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Background & Aims: Functional cure of chronic HBV infection (CHB) without life-long treatment requires the restoration of defective HBV-specific humoral and cellular immunity. Therapeutic vaccines based on the major structural and non-structural proteins have been tested in patients with CHB but have shown scarce immunogenicity. BRII-179, also known as VBI-2601, is a novel formulation comprised of all 3 HBV surface envelope proteins (Pre-S1, Pre-S2, and S). Safety, antiviral activity, and immunogenicity of BRII-179 admixed with co-adjuvant interferon (IFN)-α were assessed in patients with CHB.
Method: This randomized, open-label, controlled phase Ib/IIa study included 2 dose levels, 20 μg BRII-179 (Part 1, n = 25) and 40 μg BRII-179 (Part 2, n = 24). Patients, virally suppressed under nucleos(t)ide analogue (NA) therapy were randomized 1:2:2 into 3 cohorts in Part 1 and 1:1 into 2 cohorts in Part 2 to receive 4 monthly intramuscular injections of BRII-179 admixed with/without 3 MIU IFN-α. Antibody and cellular responses to HBsAg, as well as evolution of circulating HBsAg were monitored.
Results: Both 20 μg and 40 μg BRII-179 with/without IFN-α were well tolerated with no severe adverse events. BRII-179 induced anti-HBs responses in >30% patients in all treatment cohorts, however, moderate anti-Pre-S1 or anti-Pre-S2 antibody responses were only observed in patients receiving BRII-179 with IFN-α. BRII-179 also restored S-, Pre-S1-, Pre-S2-specific IFN-γ-producing T-cells in the majority of treated patients. Overall, no notable reduction of HBsAg was observed after BRII-179 treatment.
Conclusion: In patients with CHB under NA therapy, BRII-179 with/without IFN-α exhibited a good safety profile and induced HBV-specific B- and T-cell immune responses. These data support further clinical evaluation of BRII-179 in combination with other therapies.
Clinical Trial Number: ACTRN12619001210167
Lay summary: BRII-179 is a therapeutic vaccine designed to improve the immune response in patients with chronic hepatitis B. In this study, BRII-179 alone or with a low dose of interferon-α was safe, well tolerated, and induced enhanced HBV-specific antibody and T-cell responses in patients with chronic hepatitis B. However, BRII-179 treatment alone had minimal effect on patient's virological status. The potential of BRII-179 to achieve a functional cure in conjunction with other agents is being evaluated in the clinic.

Keywords: CHB; BRII-179; immunotherapy; IFN-alpha; HBV-specific immune response.
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Introduction

Chronic HBV infection (CHB) remains an important global public health problem that leads to serious sequelae over time, including cirrhosis, liver failure, and hepatocellular carcinoma.[1] An estimated 290 million people are living with CHB worldwide, and almost 800,000 people die annually due to associated complications.[2]

Current treatment options for CHB are limited.[3] The main option is long-term nucleos(t)ide analogue (NA) therapy. Despite

its ability to control HBV replication and improve liver histology in most patients,[3] a functional cure of infection (defined as a sustained HBsAg loss in blood with or without seroconversion to anti-HBs) that avoids the need for costly and life-long treatment is rarely achieved. An alternative treatment option is to employ a finite course of immunomodulatory therapy with pegylated interferon-α (PEG-IFN-α), which can induce a functional cure in a small percentage of patients (<10%) but has poor tolerability.[4] The failure of NA therapy to produce a functional cure and the limitations of PEG-IFN-α therapy highlight the clinical need for novel therapies to increase cure rates.[3]

The main obstacle to HBV clearance is the profound state of immune exhaustion which is thought to be driven by the combination of decades of high-dose antigenic stimulation and the tolerogenic environment in the liver.[5] Early studies in chimpanzees demonstrated that T-cells represent a major factor associated with HBV immune control and clearance of HBV.[6,7] Immune control is further exemplified by the spontaneous resolution of infection, as indicated by a loss of detectable circulating HBsAg (±anti-HBs seroconversion) that can occur in a small subset of patients even after the establishment of CHB.[8] These studies established the key role of the robust, multispecific and sustained HBV-specific T-cell response in immune control of infection. Additionally, humoral immunity might also play an important role.[9,10] Therefore, immunotherapeutic approaches are being developed that target innate or adaptive arms of the host's immune system based on the concept of redirecting the failed immune response to better mimic the features that characterize spontaneous resolution.

BRII-179, also known as VBI-2601, is a virus-like particle (VLP)-based immunotherapeutic derived from the prophylactic Sci-B-Vac® vaccine containing the large (L), middle (M), and small (S) envelope proteins. Sci-B-Vac® was used previously in a therapeutic setting in patients with CHB and showed signs of efficacy, *i.e.*, restoration of anti-HBs to >10 mIU/ml in the vaccinated groups, a higher HBeAg seroconversion frequency, and suppression of HBV DNA to <4 log copies/ml in the anti-HBs responders compared to non-responders.[11] BRII-179 is comprised of a new adjuvant formulation of higher doses of the antigens contained in Sci-B-Vac®. In preclinical studies, BRII-179 induced HBV-specific T helper type 1 (Th1) cellular and antibody responses in both HBV-free and HBV-persistent mouse models, and the addition of low dose IFN-α further enhanced its activity.[12] These results prompted the clinical development of BRII-179 for the treatment of patients with CHB, either alone or in combination with other modalities.

Herein, we report the results of the first-in-human study of BRII-179, administered with or without co-adjuvant IFN-α, to patients with CHB who were virally suppressed with NA therapy. Safety, tolerability, immunogenicity, and antiviral activity were assessed in an open-label, randomized, phase Ib/IIa study.

Patients and methods

Patients

Eligible patients were male or female, aged 18–60 years, with a BMI of 18–32 kg/m^2, who were receiving NA treatment for CHB at least 6 months prior to screening. All patients enrolled had detectable levels of circulating HBsAg (50–5,000 IU/ml) at screening. Additional enrollment criteria included serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤2x the upper limit of normal; the absence of cirrhosis determined using a liver stiffness value <8.5 kPa by Fibroscan. Patients were excluded if they had co-infection with human immunodeficiency virus, hepatitis C virus, or hepatitis D virus, immunosuppressive disorders, or evidence of hepatic decompensation, or previous vaccination with HBV vaccine or previous treatment with IFN-α. Visits comprised clinical evaluation, full laboratory evaluation, electrocardiogram (baseline) and Fibroscan (baseline).

Study design

In this study, BRII-179 admixed with/without IFN-α was administered intramuscularly to non-cirrhotic patients with virologically suppressed CHB every 4 weeks until week 12 (4 doses). All patients continued with the NA regimen that was ongoing at the time of screening until the end of the study at week 24. This study comprised 2 parts, Part 1 and Part 2, which were used to investigate 2 dose levels of BRII-179. Twenty-five eligible patients were randomized by Block method (1:2:2) into Part 1 to receive NA only (Cohort A), NA plus 20 μg BRII-179 (Cohort B), NA plus 20 μg BRII-179 + 3 million international unit (MIU) IFN-α (Cohort C), respectively. Patients in Cohort A may be eligible to participate in Part 2 of the study following completion of the week 16 visit of Cohort A participation. Otherwise, Cohort A participants are to complete the study follow-up period to week 24. Twenty-four eligible patients were randomized (1:1) into Part 2 to receive NA plus 40 μg BRII-179 (Cohort D), NA plus 40 μg BRII-179 + 3 MIU IFN-α (Cohort E). Consort flow diagram is shown in Fig. 1A. All except 1 patient completed the study and received all injections as planned. The study was conducted in 12 investigational centers including Hong Kong, New Zealand, Australia, Thailand, South Korea and mainland China in accordance with the International Conference on Harmonisation Guidance for Good Clinical Practice guidelines and all applicable local regulatory requirements and laws (registered at http://www.anzctr.org.au/Default.aspx under registration number ACTRN12619001210167). The study protocol and informed consent forms were reviewed and approved by Institutional Review Board/Independent Ethics Committee at the site. Participants provided written informed consent before any study-related procedures were performed.

Antiviral and antibody response to HBs, Pre-S1 and Pre-S2 antigens

Serum HBsAg and anti-HBs were quantified with Elecsys HBsAg II quant II kit and Elecsys Anti-HBs II kit/Cobas e411 (Roche Diagnostics, Germany) according to manufacturer's instructions. HBcrAg was measured using Lumipulse® G HBcrAg assay from Fujirebio according to manufacturer's instructions. HBV RNA was determined by quantitative reverse-transcription PCR as described in the supplementary information.

Positive anti-HBs response was defined at any post-baseline visit with either (i) post-baseline anti-HBs ≥2 IU/L if anti-HBs was undetectable at baseline or (ii) post-baseline anti-HBs ≥5 times the baseline anti-HBs if baseline anti-HBs was ≥2 IU/L.

Antibodies to Pre-S1 (anti-Pre-S1) and Pre-S2 (anti-Pre-S2) were measured at baseline and all subsequent visits using a customized ELISA protocol as described in the supplementary information.

Human PBMC isolation and HBV-specific T-cell response evaluation

Peripheral blood mononuclear cells (PBMCs) isolated by density-gradient centrifugation using Ficoll-Paque were

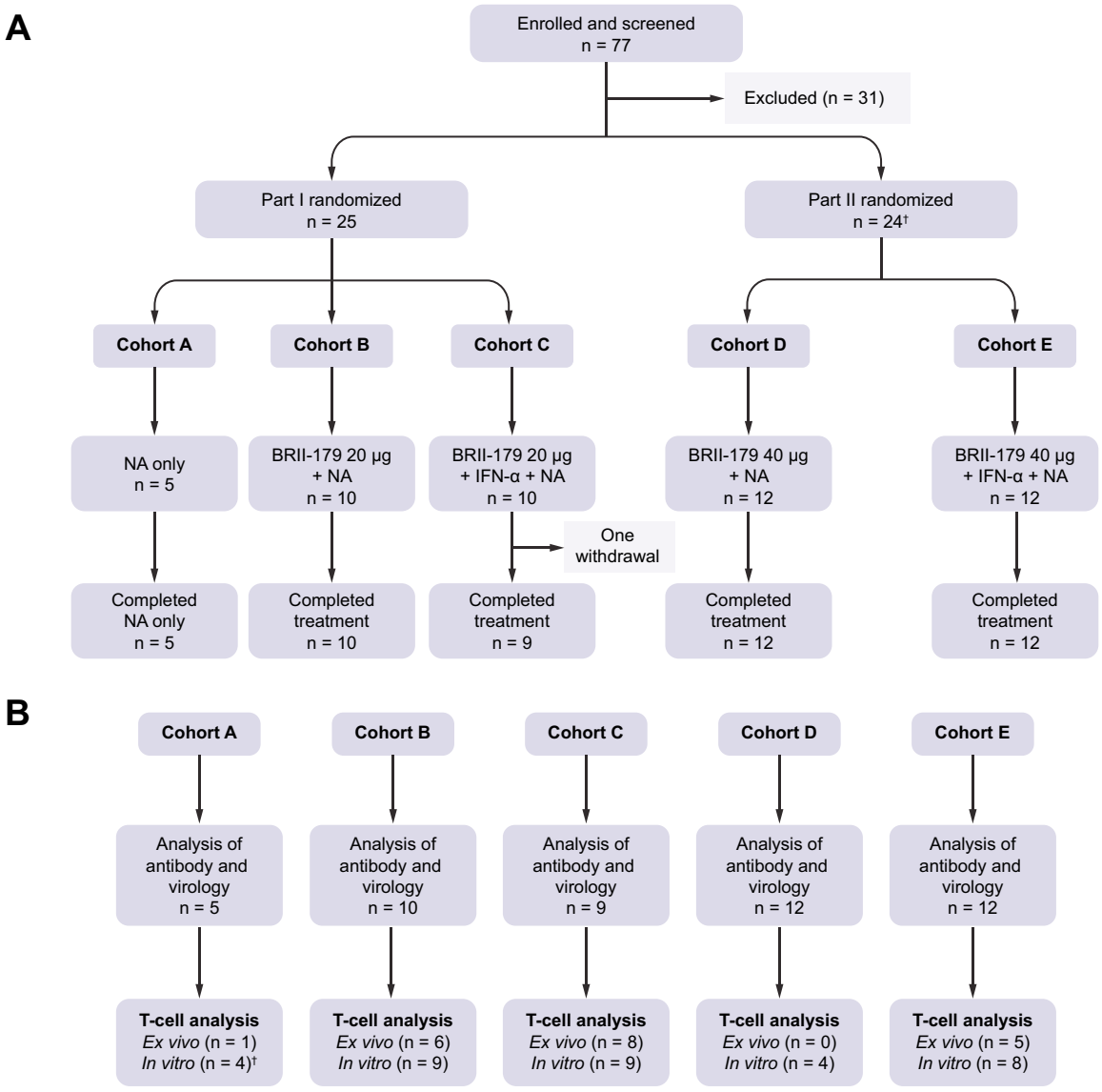

Fig. 1. Patient disposition by treatment. (A) Patient disposition (B) Flow chart of immunogenicity and virology analysis in participants throughout the trial. †Including 3 patients from Cohort A randomized to Cohort E after Part 1 week 16 visit. IFN-α, interferon-α; NA, nucleos(t)ide analogue.

cryopreserved and stored in liquid nitrogen until use.[13] Details regarding the evaluation of HBV-specific T-cell responses are provided in the supplementary information.

Statistical analysis

No formal sample size calculation was performed for this exploratory study. All the safety analyses were performed on the study participants who received at least 1 dose of vaccine or were randomized to Cohort A. Adverse events (AEs) were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 23.0. Antiviral activity and immunogenicity were summarized based on observed data, *i.e.*, missing data were excluded from the summaries. The graphs and statistical analyses were performed in GraphPad Prism 9.0. Statistical significance was determined by paired *t* test. The statistical analysis was performed using SAS software version 9.4 (SAS Institute, Cary, NC).

Results

Patients

This was a phase Ib/IIa, randomized, open-label, controlled study. Objectives were to assess the safety and tolerability of 4 doses of BRII-179 administered monthly, as well as early antiviral activity and HBV-specific immune responses. BRII-179 admixed with and without IFN-α was administered intramuscularly at 2 dose levels (Part 1 and Part 2). Patients enrolled into Cohort A (n = 5) continued background NA therapy without study treatment injections. Of the 5 patients in Cohort A, 3 were randomized and rolled over to Part 2 after finishing the week 16 visit. In addition, 1 patient in Cohort C was prematurely withdrawn from the study (Fig. 1A). A total of 45 patients (3 rolled over from Cohort A to Cohort E after week 16 visit) completed the study and were included in the final safety analysis (Fig. 1A). Immunogenicity and virology readouts were analyzed based on observed data (Fig. 1B). Baseline demographics and disease characteristics were

generally balanced across the 5 treatment cohorts with more males than females, and similar medians and ranges of age (45-49 years) and BMI (22.1-26.8) across treatment cohorts (Table 1).

BRII-179 safety in patients with CHB

No severe AEs, deaths, or signs of hepatotoxicity were reported (Table 2). A total of 38 patients (267 events) exhibited treatment-emergent AEs, which were mostly transient and of mild to moderate severity. The most frequently occurring AEs (>10%) reported in the treatment cohorts included fatigue, injection site pain, influenza-like illness, pyrexia, chills, nausea, diarrhea, headache, dizziness, myalgia, and nasopharyngitis. Pyrexia, influenza-like illness, chill, headache, nausea, diarrhea, and myalgia (known AEs associated with interferon) occurred more frequently in those receiving BRII-179 co-administered with 3 MIU IFN-α than those receiving BRII-179 alone (Table 2). No ALT flares were recorded. Overall, there were no safety concerns related to BRII-179.

BRII-179 antiviral activity in HBsAg

Enrolled patients displayed a heterogeneous level of HBsAg at baseline, with a median level of ~3.0 log IU/ml (ranging from 1.83 to 3.71 log IU/ml) (Table 1). Decreases in HBsAg were limited after 4 doses of vaccine treatment (Fig. 2). In most patients, the decrease of HBsAg was minor (<0.2 log) except in 1 HBeAg-positive patient in the 40 μg BRII-179 Cohort D who reached >1 log reduction accompanied by HBeAg seroconversion (Fig. 2).

BRII-179 induces antibody responses to HBV surface antigens

Antibody responses to surface antigens were measured in the serum throughout the study in all patients except one who was withdrawn due to an AE (flu-like illness during COVID-19 outbreak). Boosting and/or restoration of anti-HBs antibody response was observed in 19/43 (44.2%) BRII-179 recipients with titers of 2-10 mIU/ml in 5 patients, 10–100 mIU/ml in 9 patients, and >100 mIU/ml in 5 patients (Fig. 3A). Overall, 4 monthly injections of BRII-179 with or without IFN-α resulted in an increase of anti-HBs titers in 60%, 33%, 50% and 33% of patients, respectively, from treatment Cohorts B to E, with no detectable anti-HBs responses in the untreated Cohort A (Fig. 3B and Table 3). The peak anti-HBs level was typically observed at week 12 or 16, at or 1 month after the last dose of injection. The peak titers declined gradually at the end of study. Except for 1 patient who received 40 μg BRII-179 admixed with IFN-α, who achieved the highest antibody titer (9,020 mIU/ml), the elevations in antibody titer were similar across all 4 treatment groups, ranging from 2.1–998.8 mIU/ml. Using a qualitative ELISA-based assay, anti-Pre-S1 and anti-Pre-S2 antibody responses were also measured and were only detected in patients who received BRII-179 admixed with IFN-α in Cohort C (56% and 44%, respectively) and Cohort E (8.3% and 8.3%, respectively) (Fig. 3C, 3D and Table 3).

Restoration of HBsAg-specific T-cells in patients with CHB after administration of BRII-179

To determine whether HBsAg-specific T-cells were boosted and/or restored after administration of BRII-179, PBMCs from patients with CHB were tested *ex vivo* (20/46 patients) and after *in vitro* expansion (34/46 patients) for responses to the HBV Pre-S1, Pre-S2 and S regions using IFN-γ ELISpot assays (Fig. 1B).

Among a total of 19 evaluable patients from the treatment cohorts, a clear increase of antigen-specific T-cells was

Table 1. Characteristics of the participants at baseline.[*]

Characteristic	Cohort A NA (n = 5)	Cohort B BRII-179 20 μg + NA (n = 10)	Cohort C BRII-179 20 μg + IFN-α + NA (n = 10)	Cohort D BRII-179 40 μg + NA (n = 12)	Cohort E BRII-179 40 μg + IFN-α + NA (n = 12)
Age (year)					
Median	49	49	47	45.5	45
Range	31-54	33-59	24-60	31-56	28-57
Male n (%)	4 (80.0)	8 (80.0)	8 (80.0)	10 (83.3)	8 (66.7)
Race n (%)					
Asian	4 (80.0)	8 (80.0)	8 (80.0)	12 (100.0)	12 (100.0)
BMI (kg/m^2)					
Median	26.7	25.2	26.8	24.3	22.1
Range	26.1-31.5	18.4-32	21.6-30.5	19.8-30.8	18.1-29.6
HBsAg (IU/ml)					
Median	215	935	651	1,447	1,511
Range	77-1,871	78-5,092	169-1,259[#]	188-4,929	68-4,650
HBeAg-positive, n (%)	2 (40.0)	3 (30.0)	1 (10.0)	4 (33.3)	6 (50.0)
Anti-HBe-reactive n (%)	2(40.0)	8 (80.0)	9 (90.0)	7 (58.3)	5 (41.7)
Anti-HBs n (%)					
≤2 mIU/ml	5 (100)	7 (70)	9 (90)	10 (83.3)	11 (91.7)
ALT (IU/L)					
Median	17	22	25	23.5	15.5
Range	13-54	11-33	11-35	13-45	8-26
Duration of HBsAg positivity					
Median (month)	82.3	143.3	159.8	72.5	145.2
Range (month)	35.7-294.9	14.0-318.9	67.4-325.4	21.0-211.3	30.9-310.6
Duration of NA therapy					
Median (month)	77.5	52.8	77.8	36.4	40.8
Range (month)	31.8-113.6	14.0-139.7	25.9-294.0	13.4-160.2	13.1-169.6

ALT, alanine aminotransferase; IFN-α, interferon-α; NA, nucleos(t)ide analogue.
[*] Baseline: the last available non-missing value collected prior to the first administration of investigational product or randomization date for the NA-only cohort.
[#] 1 participant with baseline HBsAg 8,695 IU/ml was excluded due to a protocol deviation.

Table 2. Frequency and severity of treatment-emergent adverse effect.

Patients, n (%)	Cohort A NA (n = 5)	Cohort B BRII-179 20 μg + NA (n = 10)	Cohort C BRII-179 20 μg + IFN-α + NA (n = 10)	Cohort D BRII-179 40 μg + NA (n = 12)	Cohort E BRII-179 40 μg + IFN-α + NA (n = 12)
Any TEAEs	2 (40.0)	7 (70.0)	10 (100.0)	10 (83.3)	11 (91.7)
Severe AEs	0	0	0	0	0
Drug-related AEs	0	6 (60.0)	9 (90.0)	8 (66.7)	11 (91.7)
TEAEs (any grade) in ≥10% of patients in any treatment					
Fatigue	0	4 (40.0)	4 (40.0)	5 (41.7)	8 (66.7)
Headache	0	2 (20.0)	5 (50.0)	0	8 (66.7)
Injection site reaction	0	4 (40.0)	4 (40.0)	5 (41.7)	7 (58.3)
Myalgia	0	1 (10.0)	4 (40.0)	1 (8.3)	7 (58.3)
Pyrexia	0	0	3 (30.0)	0	6 (50.0)
Nasopharyngitis	0	0	0	3 (25.0)	0
Influenza-like illness	1 (20.0)	1 (10.0)	2 (20.0)	0	1 (8.3)
Chills	0	0	2 (20.0)	0	1 (8.3)
Nausea	0	1 (10.0)	2 (20.0)	0	2 (16.7)
Diarrhea	0	0	2 (20.0)	0	2 (16.7)
Cystoid macular oedema	1 (20.0)	0	0	0	0
Dizziness	0	1 (10.0)	0	0	2 (16.7)
Abdominal pain upper	0	0	1 (10.0)	0	1 (8.3)
Dyspepsia	0	0	1 (10.0)	0	1 (8.3)
Lethargy	0	0	1 (10.0)	0	0
Joint stiffness	0	0	1 (10.0)	0	0
Upper respiratory tract infection	0	0	1 (10.0)	0	0
Viral pharyngitis	0	0	1 (10.0)	0	0
Blood creatine phosphokinase increased	0	0	1 (10.0)	0	0
Anxiety	0	0	1 (10.0)	0	0
Depressed mood	0	0	1 (10.0)	0	0
Depression	0	0	1 (10.0)	0	0
Insomnia	0	0	1 (10.0)	0	0
Pruritus	0	1 (10.0)	0	1 (8.3)	1 (8.3)
Rash	0	1 (10.0)	0	0	0
Nasal congestion	0	1 (10.0)	0	0	0
Skin laceration	0	0	1 (10.0)	0	0
Decreased appetite	0	0	1 (10.0)	0	0
Hypertension	0	0	1 (10.0)	0	0

AEs, adverse events; IFN-α, interferon-α; NA, nucleos(t)ide analogue; TEAEs, treatment-emergent adverse events.

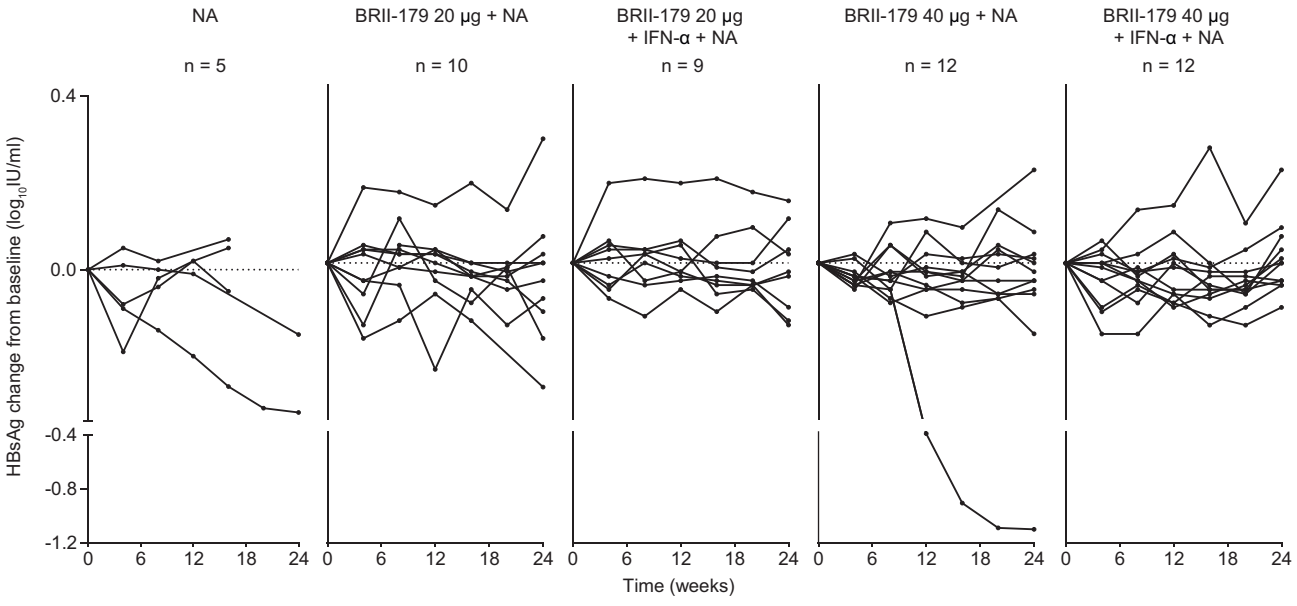

Fig. 2. Longitudinal titration of serum HBsAg. Data are represented as HBsAg change in log scale from baseline (day 1) up to week 24 in 5 cohorts of patients with chronic HBV infection. IFN-α, interferon-α; NA, nucleos(t)ide analogue.

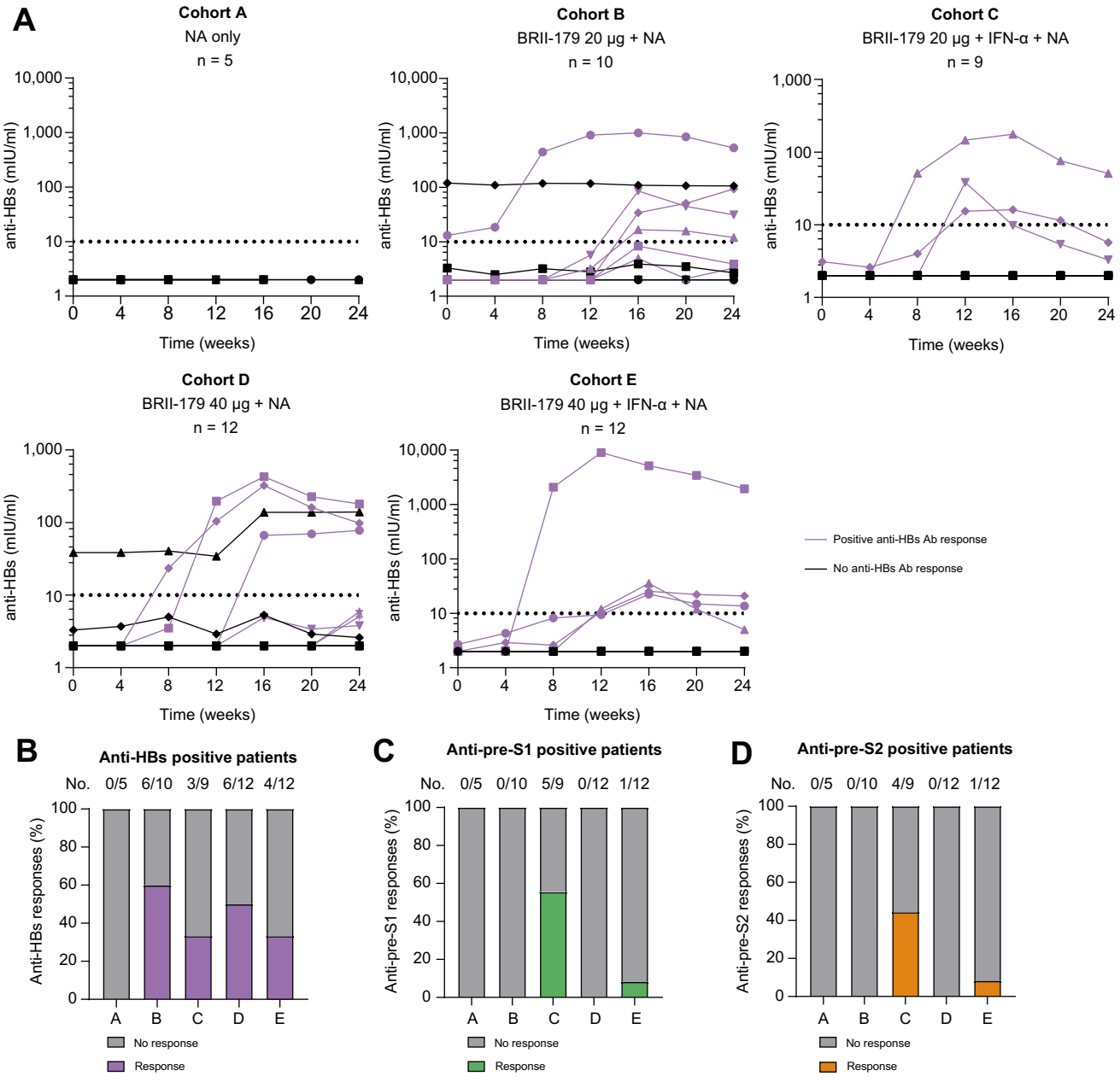

Fig. 3. Antibody responses to surface antigens in chronic HBV patients post BRII-179 treatment. (A) Individual anti-HBs titration over time in 5 cohorts. (B) The percentage of individuals with vaccine-induced positive anti-HBs responses by cohorts. (C, D) The percentage of individuals with positive anti-Pre-S1 (C) or Pre-S2 (D) antibody responses by cohorts. IFN-α, interferon-α; NA, nucleos(t)ide analogue.

detectable *ex vivo* in 3 out of 8 (37.5%) patients (C01, C03, C05; Fig. 4A and Table 3) at both timepoints post final vaccination in Cohort C, who received 20 μg BRII-179 admixed with IFN-α. A modest increase was also present in another 2 patients from this Cohort (C04, C07). In addition, 1 patient (E06) in 5 tested patients from Cohort E (20%) had a response to the Pre-S1 peptide pool after vaccination (Fig. 4A and Table 3). There was no detectable *ex vivo* response among 6 evaluable patients in Cohort B and no patient samples were available from Cohort D for testing. In order to analyze the proliferative potential of HBV-specific T-cell responses induced by BRII-179, T-cells were characterized with 2 different *in vitro* expansion methods: PBMCs were either stimulated with a mixture of HBV Pre-S1, Pre-S2 and S peptide pools (Fig. 4B and Fig. S1) or with the recombinant vaccine proteins (Fig. 4C and Fig. S2) for 9–10 days before performing IFN-γ ELISpot assays. We detected an increase of IFN-γ-producing Pre-S1-, Pre-S2- and S-specific T-cells in the majority of BRII-179 recipients 1 and 2 months after the last dose of vaccine (at weeks 16 and 20; Fig. 4B and 4C). In peptide-expanded PBMCs, increased T-cell responses were detected in 67% (6/9), 78% (7/9), 75% (3/4), and 50% (4/8) of patients in Cohort B, C, D, and E, respectively (Fig. 4B and Table 3). Similarly, in vaccine protein-expanded PBMCs, increased T-cell responses were detected in 56% (5/9), 44% (4/

Table 3. Immunogenicity of BRII-179 in patients with CHB.

Treatment	Antibody response*				T-cell response**			
	Anti-HBs	Anti-Pre-S1	Anti-Pre-S2	Overall response	*Ex vivo*	Peptides (*In vitro*)	Vaccine (*In vitro*)	Overall response
NA therapy	0/5 (0%)	0/5 (0%)	0/5 (0%)	**0/5 (0%)**	0/1 (0%)	0/4 (0%)	0/4 (0%)	**0/4 (0%)**
BRII-179 20 μg + NA	6/10 (60%)	0/10 (0%)	0/10 (0%)	**6/10 (60%)**	0/6 (0%)	6/9 (66.7%)	5/9 (55.56%)	**6/9 (66.7%)**
BRII-179 20 μg + IFN-α + NA	3/9 (33.3%)	5/9 (55.6%)	4/9 (44.4%)	**6/9 (66.7%)**	3/8 (37.5%)	7/9 (77.8%)	4/9 (44.4%)	**7/9 (77.8%)**
BRII-179 40 μg + NA	6/12 (50%)	0/12 (0%)	0/12 (0%)	**6/12 (50%)**	n.a.	3/4 (75%)	3/4 (75%)	**4/4 (100%)**
BRII-179 40 μg + IFN-α + NA	4/12 (33.3%)	1/12 (8.3%)	1/12 (8.3%)	**4/12 (33.3%)**	1/5 (20%)	4/8 (50%)	4/8 (50%)	**5/8 (62.5%)**

CHB, chronic HBV infection; IFN-α, interferon-α; NA, nucleos(t)ide analogue.
* Positive anti-HBs response was defined at any post-baseline visit with either (i) post-baseline anti-HBs ≥2 IU/L if anti-HBs undetectable at baseline or (ii) post-baseline anti-HBs ≥5 times the baseline anti-HBs if baseline anti-HBs was ≥2 IU/L. Positive anti-PreS1 or anti-PreS2 response was determined for each cohort using the criteria of post-baseline ELISA optical density ratio (relative to baseline) value ≥ the population mean + 3 population standard deviations from patients treated with NA therapy only.
** Positive T-cell response was defined as a quantity of spot-forming units post-vaccination of at least 3 times the pre-vaccination maximum.

9), 75% (3/4) and 50% (4/8) of patients in Cohort B, C, D, and E, respectively (Fig. 4C and Table 3).

Magnitude and breadth of restored HBsAg-specific T-cells after BRII-179 vaccination

The magnitude and breath of HBV Pre-S1, Pre-S2 and S-specific T-cells present in the T-cell lines obtained by stimulation of PBMCs with HBV Pre-S1, Pre-S2 and S peptide pools (Fig. 5A) or with the vaccine protein (Fig. S2A) were analyzed. Significantly higher magnitudes of HBsAg-specific T-cell responses were detected against all 3 peptide pools at week 16 post-vaccination compared to pre-vaccination. There was no significant difference between week 16 and 20. Prior to BRII-179 vaccination, a small proportion of patients with CHB were primarily reactive to the S peptide pool only – both at screening (27%) and randomization day 1 (20%) (Fig. 5B). By contrast, the majority of patients responded to at least 1 of the Pre-S1, Pre-S2 and S peptide pools after vaccination (overall 77% at week 16 and 76% at week 20) (Fig. 5B). Similar results were obtained in vaccine-expanded cell lines (Fig. S2B).

Overall, these data indicate that BRII-179 admixed with or without IFN-α is capable of boosting HBV-specific T-cells in

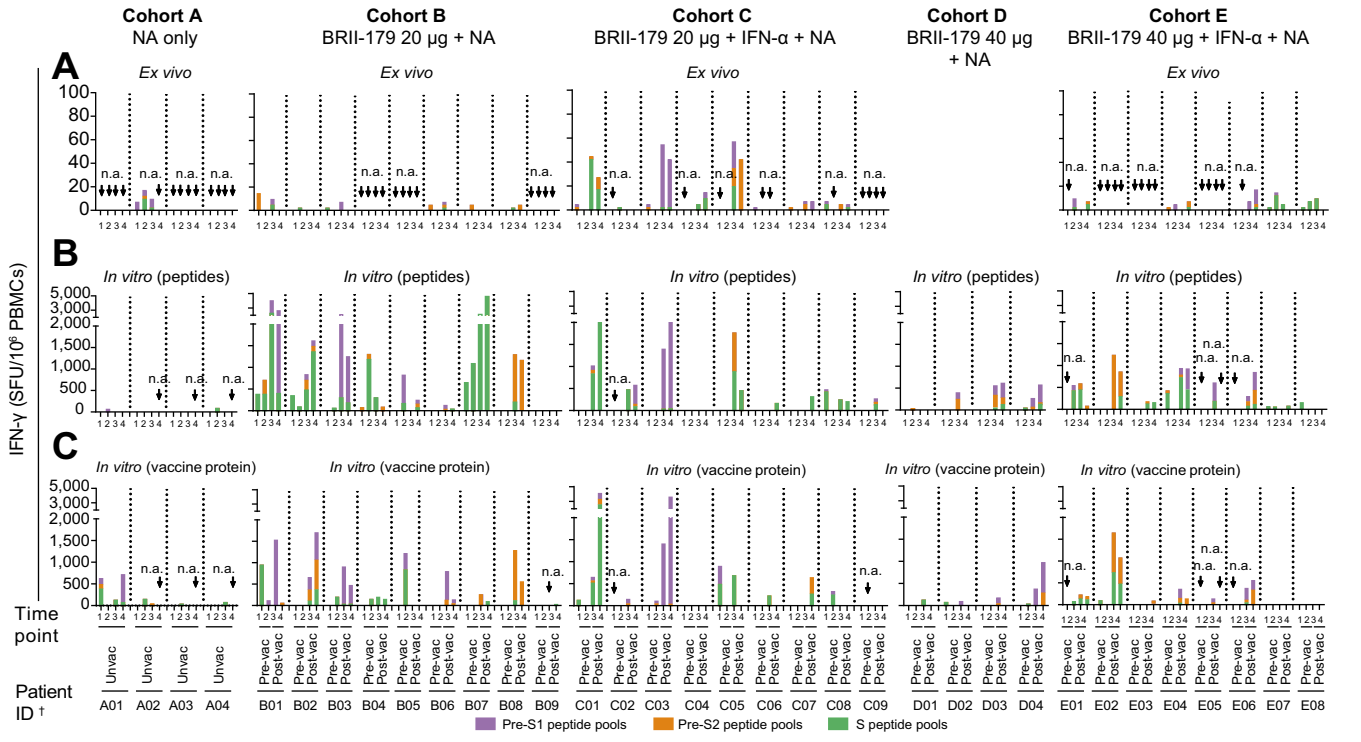

Fig. 4. HBsAg-specific T-cells in patients with CHB after administration of BRII-179. (A) Frequency of HBsAg-specific IFN-γ-secreting T-cells by *ex vivo* ELISpot in PBMCs from BRII-179-treated patients with CHB at 2 timepoints pre-vaccination and 2 timepoints post-vaccination. (B and C) PBMCs from treated patients with CHB were expanded *in vitro* for 9-10 days in the presence of a mixture of Pre-S1, Pre-S2 and S peptide pools (B) or BRII-179 vaccine protein (C) prior to ELISpot assays. The quantification of SFUs of IFN-γ-secreting T-cells is shown. Each 4-bar set shows the total T-cell responses of each individual specific to Pre-S1, Pre-S2, and S peptide pools at time point 1 (screening), time point 2 (randomization day 1), time point 3 (week 16), and time point 4 (week 20). †A02, A03 and A04 subjects in Cohort A were assigned into E01, E05 and E06 in Cohort E, respectively, after week 16 visit. CHB, chronic HBV infection; IFN-α/γ, interferon-α/γ; NA, nucleos(t)ide analogue; n. a., not applicable; PBMCs, peripheral blood mononuclear cells; SFUs, spot-forming units.

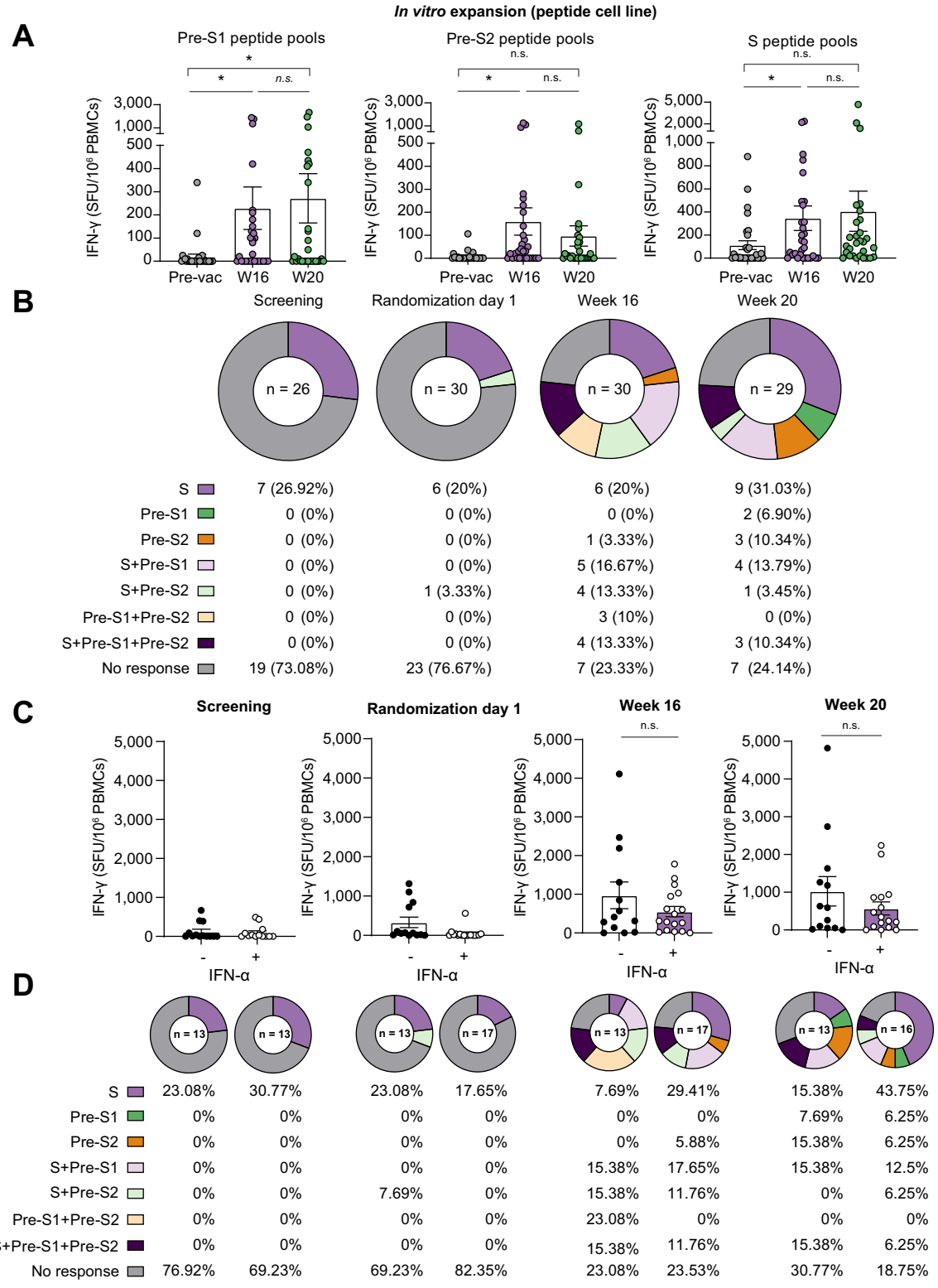

Fig. 5. Magnitude and breadth of vaccine-induced HBsAg-specific T-cells. (A, C) Magnitude of the total T-cell responses specific to Pre-S1, Pre-S2 and S in peptide-expanded PBMCs from BRII-179-treated patients with CHB at the time points of pre-vaccination (screening and randomization day 1) and post-vaccination (week 16 and week 20). (C) Total HBsAg-specific T-cell responses in BRII-179-treated patients with and without IFN-α. Pre-vac is the average of SFUs at the time points of screening and randomization day 1. *p <0.05; n.s., not significant. (B, D) Breadth of T-cell response against Pre-S1, Pre-S2 and S antigen, and the percentage of individuals with Pre-S1-, Pre-S2- and S-specific T-cell responses by *in vitro* expansion ELISpot in patients with CHB before and after BRII-179 vaccination (B) or with and without IFN-α co-administration. (D) T-cell responses to HBV Pre-S1, Pre-S2, or S peptide pools were considered positive if the results were at least 2 times the maximum of the negative controls and >30 SFUs per 10^6 PBMCs for *in vitro* expansion ELISpot. CHB, chronic HBV infection; IFN-α/γ, interferon-α/γ; PBMCs, peripheral blood mononuclear cells; SFUs, spot-forming units.

chronically infected patients. Although based on a limited number of patients, BRII-179 was able to induce a detectable *ex vivo* response to HBsAg in 4 out of 13 (31%) patients who received 20 or 40 μg of BRII-179 co-administrated with IFN-α; we did not observe significant differences between HBsAg-specific T-cell responses in patients who did or did not receive co-adjuvant IFN-α by *in vitro* expansion ELISpot analysis (Fig. 5C,D).

Discussion

This is a first-in-human clinical study to evaluate a multiple-dose therapeutic vaccine BRII-179 for the treatment of patients with CHB receiving NA therapy. The primary endpoint of the study was reached, as treatment with 20 or 40 μg BRII-179 admixed with or without 3 MIU IFN-α every 4 weeks for 12 weeks was safe and well tolerated. All AEs observed during the study appeared to be dose independent.

A number of secondary end points were assessed. Although not in all patients, HBV-specific humoral and cellular immune responses induced by BRII-179 were detected in a significant number of patients with CHB. BRII-179 is a new vaccine formulation composed of a higher dose of the same antigens contained in the prophylactic vaccine Sci-B-Vac® with an altered adjuvant formulation. Sci-B-Vac® has been shown to elicit protective antibody titers correlated with cellular immunity in low- or non-responders to alternate HBV vaccines comprised of the small HBsAg only.[14] Moreover, the co-administration of a 20 μg dose of Sci-B-Vac® and lamivudine to patients with CHB was shown to restore anti-HBs responses.[11] The anti-HBs responders had a significantly higher HBeAg seroconversion rate and more frequent HBV DNA suppression than anti-HBs non-responders, demonstrating the functional relevance of anti-HBs seroconversion.[11] By contrast, a yeast-derived recombinant small HBs vaccine (100 μg), with a strong AS02B adjuvant, was used in combination with lamivudine in 195 HBeAg+ chronic HBV patients.[15] Most of the vaccinated patients developed anti-HBs responses and there was evidence for HBsAg-specific lymphoproliferative responses in the limited (n = 6) number of patients evaluated, but there was no improvement in HBeAg seroconversion or in kinetics of HBV DNA decline when compared to the lamivudine only control group.[15] Collectively, these data suggest that the presence of the Pre-S1 and Pre-S2 domains may be associated with an enhanced immunogenic response in the therapeutic setting. The impact of T-cell epitopes within the Pre-S1 region on cellular immunity has already been demonstrated in prior studies in the CHB setting.[14,16] With this in mind, BRII-179 is based on the same protein components of Sci-B-Vac®, including the Pre-S1, Pre-S2, and HBsAgs, but was optimized with a new adjuvant formulation to improve Th1-skewed antibody and T-cell immunity, as demonstrated in mouse models.[12] BRII-179-induced immune responses could be further enhanced by admixing with a low dose of IFN-α in these preclinical studies.[12]

In this study, therapeutic vaccination with BRII-179 (admixed with or without IFN-α) led to a notable elevation in humoral responses in patients with CHB receiving NA therapy. There were no significant differences in antibody responses observed across all treatment cohorts, suggesting that BRII-179 can induce anti-HBs responses in a significant number of patients at dose levels of 20 μg and 40 μg regardless of the addition of IFN-α. However, the peak antibody titers in most antibody responders were observed after 3 or 4 doses of vaccination (at weeks 12 or 16), indicating that the antibody response was attenuated in the highly tolerant patients compared to healthy individuals in whom the protective titer (≥10 mIU/ml) was generally reached after the second dose.[17] These data also suggest that a longer duration of treatment may be required for optimal and sustained responses. It is noteworthy that anti-Pre-S1 and anti-Pre-S2 antibodies were also detected qualitatively but only in patients who received BRII-179 co-administered with 3 MIU IFN-α, suggesting that IFN-α may enhance antibody responses to the less tolerized Pre-S1 and Pre-S2 antigens, perhaps by promoting dendritic cell maturation and subsequently more efficient antigen presentation at local injection sites. This is also supported by increased *ex vivo* T-cell responses to HBsAg, which were only observed in the cohorts that received vaccine admixed with IFN-α. The impact of IFN-α as a co-adjuvant needs to be further evaluated in future clinical trials.

HBV-specific T-cell responses were also elicited by therapeutic vaccination. Here we found that potent HBsAg-specific T-cell responses were elicited by BRII-179 with or without IFN-α co-adjuvant. At least 62.5% of patients displayed robust restoration of antigen-specific T-cells in all vaccine treatment cohorts. Although enhanced antigen-specific T-cell responses by *ex vivo* ELISpot were only observed in patients who received BRII-179 with IFN-α treatment (3 in Cohort C and 1 in Cohort E), the majority of BRII-179-treated patients with or without IFN-α co-adjuvant showed T-cell responses to vaccination, by *in vitro* expansion ELISpot, with similar response rates. Intracellular cytokine staining of a limited number (n = 7) of IFN-γ ELISpot-positive cell lines showed that antigen-specific TNF-α- and/or IFN-γ-producing CD4 and CD8 T-cells were induced and/or restored (Fig. S3), which is consistent with the findings of Th1-skewed immune responses induced by BRII-179 in the mouse model.[12] To further enhance T-cell responses, future adjuvant selection should be focused on the candidates that can elicit strong antigen cross-presentation to induce an augmented CD8 T-cell response.

Although BRII-179 displayed promising immunogenicity in patients with CHB in our study, we did not observe a notable association between detected HBV-specific immune responses and reductions of circulating HBsAg levels. There was also no significant change in circulating HBV RNA and HBcrAg levels across all treatment groups throughout the study (data not shown). In the present study, only 1 patient receiving NA plus 40 μg BRII-179 showed a decline of >1 $\log_{10}$ IU/ml in HBsAg serum levels between baseline and week 24, which was probably driven by the HBeAg seroconversion. Anti-HBs responses were detected in >30% patients after 4 doses of vaccine; however, most of the peak antibody titers observed 1 month after the last dose were <100 mIU/ml, with a median of 35.6 mIU/ml, and the titers declined over time. If 1 mIU of anti-HBs can bind and/or neutralize 2 IU HBsAg,[18,19] the induced anti-HBs levels in the majority of antibody responders would have minimal ability to neutralize and lower circulating HBsAg, considering the baseline HBsAg levels. In addition, T-cell responses were only evaluated at 2 time points, 1 or 2 months after the last dose of BRII-179 (week 16 and week 20), with the responses to S and Pre-S2 trending lower after week 16. These observations suggest that 4 doses of BRII-179 used in the present study are not sufficient to establish

the sustained immune response required for successful long-term viral control. Likewise, the short-term viral suppression induced by the combination therapy of Sci-B-Vac® and lamivudine in patients with CHB was not sustained after the discontinuation of the vaccine.[11] The lack of sustained responses may reflect suboptimal vaccine administration conditions *e.g.*, schedule, co-adjuvant parameters that could be studied in future trials. Moreover, it is difficult to speculate on the potency and breadth of coverage for the detected polyclonal anti-HBs in serum. As the HBV genotype for the majority of patients is unknown, due to the suppressed HBV DNA at study entry, the levels of cross-reactivity of the induced antibodies and T-cells by our genotype A-derived vaccine could not be determined. The potential cross reactive antibody response induced by the vaccine could also be affected by mutations in the antibody epitope regions in HBs proteins in patients with CHB.

Additionally, we reasoned that the immunity induced by the current treatment regimen is not sufficiently potent to suppress HBV replication in the studied patients, based on their levels of baseline viremia and circulating HBsAg. Preclinical study results demonstrated that low HBsAg levels at the beginning of treatment with a therapeutic vaccine are critical for the induction of HBV-specific $CD8^+$ T-cell responses, an essential compartment for effective viral control.[20] This is corroborated by the clinical observation that a combination of Sci-B-Vac® and NAs induced anti-HBs production and maintenance of low HBsAg levels in 3 patients with CHB and low baseline HBsAg levels, whereas this combination was ineffective in a patient with CHB and a high antigen load.[21] Other baseline characteristics such as age, duration of infection *etc.* could also contribute to the lack of therapeutic effect.[9,13,22] Therefore, selection of a subgroup of patients who are younger or have lower HBsAg levels, or employment of therapeutic modalities to reduce the antigen load before BRII-179 vaccination, may be necessary to achieve functional cure.

There were several limitations in the present study. First, only a limited number of patients were recruited in each cohort. However, the consistent lack of severe side effects and the positive immunogenic responses observed only in the BRII-179-treated groups adequately demonstrated its safety profile and potential efficacy. Second, the observed efficacies may not be generalizable across different HBV genotypes due to the lack of genotype information. Third, the initial promising results of positive anti-Pre-S1 and anti-Pre-S2 responses seen in this study were observed using "conventional" IFN-α. Further studies using PEG-IFN-α are required.

In conclusion, in this randomized phase Ib/IIa study, NA plus BRII-179 with or without co-adjuvant IFN-α was safe, well tolerated and immunogenic, but of limited efficacy in virally suppressed non-cirrhotic patients with CHB. Considering the magnitude and breadth of HBV-specific humoral and cellular immune responses induced by BRII-179, the lack of significant antiviral efficacy may be related to high baseline HBsAg levels and insufficient BRII-179 doses in this study. A recently initiated clinical study (NCT04749368) is evaluating the combination of HBV-specific small-interfering RNA and BRII-179, with the aim of reducing immunosuppressive viral antigen levels via gene silencing before stimulating HBV-specific immunity with multi-doses of BRII-179.

Abbreviations

AE, adverse event; ALT, alanine aminotransferase; Anti-HBs, hepatitis B surface antibody; BMI, body mass index; CHB, chronic hepatitis B; ELISpot, enzyme-linked immune absorbent spot; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IFN-α, interferon-α; IM, intramuscular; IU, international units; NA, nucleos(t)ide analogue; PEG-IFN-α, pegylated interferon-α; PBMCs, peripheral blood mononuclear cells; SAE, serious adverse events; Th1, T helper type 1.

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Conflict of interest

Ji Y, Li D, Zhang Y, Hamatake R, Li C, Li Y, and Zhu Q: Employees of and hold stock and options in Brii Biosciences. Ahmed T, Anderson D: Employees of and hold stock in VBI Vaccines, which own an interest in BRII-179. Lim TH: Data Safety Monitoring Board or Advisory Board: Brii Biosciences (BRII-179-001 study). Yoon JH: Grants: AstraZeneca, Daewoong Pharmaceuticals, Hanmi Pharmaceuticals. Lim Y: Grants/Consultant/Payments: Gilead Sciences; Data Safety Monitoring Board or Advisory Board: Gilead Sciences, Vaccitech. Strasser S: Payment or honoraria: AbbVie, Gilead, MSD, Eisai, Ipsen, AstraZeneca, BMS, Roche, Guebert; Data Safety Monitoring Board or Advisory Board: AbbVie, CSL Behring, MSD, AstraZeneca, Roche, Eisai, Bayer, Ipsen, Norgine, Novartis. Yuen M: Grants: Assembly Biosciences, Arrowhead Pharmaceuticals, Bristol Myers Squibb, Fujirebio Incorporation, Gilead Sciences, Merck Sharp and Dohme, Springbank Pharmaceuticals, Roche; Consultant: AbbVie, Aligos Therapeutics, Arbutus Biopharma, Bristol Myer Squibb, Dicerna Pharmaceuticals, Finch Therapeutics, GlaxoSmithKline, Gilead Sciences, Janssen, Merck Sharp and Dohme, Clear B Therapeutics, Springbank Pharmaceuticals, Roche; Payment or honoraria: Arbutus Biopharma, Bristol Myer Squibb, Discerna Pharmaceuticals, Fujirebio Incorporation, Gilead Sciences.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

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Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supplementary data

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Author names in bold designate shared co-first authorship

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