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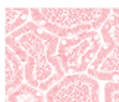
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RESEARCH PAPER



Safety and immunogenicity of the therapeutic vaccine TG1050 in chronic hepatitis B patients: a phase 1b placebo-controlled trial

Fabien Zoulim^a, Claire Fournier^b, François Habersetzer^c, Martin Sprinzl^d, Stanislas Pol^e, Carla S Coffin^f, Vincent Leroy^g, Mang Ma^h, Heiner Wedemeyerⁱ, Ansgar W Lohse^j, Robert Thimme^k, Karine Lugardon^l, Perrine Martin^m, Bérangère Bastien^l, Benoit Sansas^l, Nathalie Adda^l, Celine Halluard^l, Kaïdre Bendjama^l, Maud Brandely^l, and Geneviève Inchauspé^m

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ABSTRACT

Treatment of chronic hepatitis B (CHB) typically requires life-long administration of drugs. Cohort and pre-clinical studies have established the link between a functional T-cell-mounted immunity and resolution of infection. TG1050 is an adenovirus 5-based vaccine that expresses HBV polymerase and domains of core and surface antigen and has shown immunogenicity and antiviral effects in mice. We performed a phase 1 clinical trial to assess safety and explore immunogenicity and early efficacy of TG1050 in CHB patients. This randomized, double blind, placebo-controlled study included two sequential phases: one single dose cohort (SD, $n = 12$) and one multiple (3) doses cohort (MD, $n = 36$). Patients, virally suppressed under nucleoside(tide) analog NUC therapy, were randomized 1:1:1 across 3 dose levels (DL) and assigned to receive 10^9 , 10^{10} , 10^{11} virus particles (vp) of TG1050 and then randomized within each DL to placebo (3:1 and 9:3 vaccines/placebo in each DL, respectively, for the SD and MD cohorts). Cellular (ELISPOT) and antibody responses (anti-Adenovirus), as well as evolution of circulating HBsAg and HBcrAg, were monitored. All doses were well tolerated in both cohorts, without severe adverse event. TG1050 was capable to induce IFN- γ producing T-cells targeting 1 to 3 encoded antigens, in particular at the 10^{10} vp dose. Overall, minor decreases of HBsAg were observed while a number of vaccinees reached unquantifiable HBcrAg by end of the study. In CHB patients under NUC, TG1050 exhibited a good safety profile and was capable to induce HBV-specific cellular immune response. These data support further clinical evaluation, especially in combination studies.

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Introduction

Over two billion people have been infected by HBV worldwide and approximately 257 million are currently chronically infected and at risk of developing cirrhosis and hepatocellular carcinoma.¹ Current therapies include nucleos(t)ide analogs (NUC) inhibiting viral replication and nonspecific immunomodulatory pegylated-IFN α . Despite controlling HBV replication and improving liver histology in most patients, a functional cure of infection defined by HBsAg seroclearance is seldom achieved (globally 3–5% of the treated patients) leading to costly and lifelong NUC therapy. Novel therapies increasing cure rate are urgently needed.²

Development of immune-modulators, targeting either the adaptive or innate arm of the host's immune system, represents a growing class of HBV therapeutics.³ Rationale supporting this development stems from numerous cohort studies of patients chronically infected as well as who resolved infection,

both spontaneously or after treatment, together with earlier studies performed in non-human primates. Taken together these studies have established the key role of a strong, multi-specific, sustained HBV-specific T cell response in viral control and resolution of infection.^{4,5} In patients resolving infection, development of broad repertoire of HBV-specific CD8+ and CD4+ T cell-producing cytokines and displaying cytolytic properties has been correlated with virus control and/or elimination.^{6–8} In contrast, chronic hepatitis B (CHB) patients display weak, narrowed and dysfunctional HBV immune T cell responses.^{9–11} Interestingly, long-term treatment with NUC has been described to restore, at least partially, functionality of HBV-specific CD8+ T-cells (proliferation and effector functions).¹² In contrast, conflicting data have been reported on the existence and/or role of innate immunity. The long described stealth nature of HBV to innate sensors has been recently challenged

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by data suggesting that liver macrophages and hepatocytes may be involved in early recognition of HBV while the IFN mediated response is inhibited by some ill-defined viral components.¹³⁻¹⁵

Three T-cell based immunotherapeutic approaches for treatment of CHB are currently pursued:³ one aiming at inducing novel functional HBV-specific immune responses similar to those described in natural resolution of infection (e.g. *de novo* priming of functional T-cells); one aiming at rescuing dysfunctional HBV-specific T-cell responses (e.g. blocking inhibitory pathways); one based on engineered HBV-specific TCRs. TG1050 is an immunotherapeutic based on a non-replicative human adenovirus and encodes for a large fusion protein comprising truncated HBV Core, modified polymerase (POL) deleted of its catalytic sites and two HBsAg/Envelope (ENV) domains rich in T-cell epitopes.¹⁶ It was shown to induce functional HBV-specific T cells (including cytolytic activity) in HBV-free mice and/or in HBV-persistent mouse models and to exert antiviral effects (i.e. both on HBV viremia and circulating levels of HBsAg).^{16,17} These results prompted the clinical development of this therapeutic vaccine. We report here results of the first-in-man study with TG1050 administered to CHB patients under NUC. Safety (primary end-point) together with analyses of immunogenicity and antiviral efficacy (secondary-end-points) was assessed in a phase 1b, dose-finding placebo-controlled trial.

Patients and methods

Patients

Eligible patients were chronic hepatitis B (CHB) infected, male or female, ages 18–65 years receiving nucleo(s)ides treatment (entecavir (ETV) or tenofovir (TDF) for at least 2 years (duration of NUC administration). Mean duration of NUC treatment before vaccine administration ranged from 3.8–6.1 and 5.1–6.1 years, respectively, for SD and MD patients while mean duration of disease ranged from 14–24.8 and 14.9–19 years, respectively. All the 48 patients enrolled had undetectable levels of circulating HBV DNA for at least 6 months and three of them were HBeAg positive. Additional enrollment criteria included serum alanine aminotransferase (ALT) \leq the upper limit of normal (defined as <25 for females and <35 for males); the absence of cirrhosis determined using FibroScan® or Fibrosure®/FibroTest® score together with APRI score. Patients enrolled had a transient elastography score <10.5 kPa or Fibrosure®/FibroTest® score <0.48 and APRI score <1 . Patients were excluded if they had coinfection with human immunodeficiency virus (HIV), hepatitis C virus (HCV) or hepatitis D virus (HDV), immunosuppressive disorders or evidence of hepatocellular carcinoma or any other liver cancer. Visits comprised clinical evaluation, full laboratory evaluation, ECG (baseline), FibroScan® or Fibrosure®/FibroTest® (baseline and week 48). Intensity of adverse events (AEs) was graded according to NCI Common Toxicity Criteria for Adverse Events version 4.03.

Study design

The study was designed as a two parts study: in the first part, patients received a single dose of TG1050 while in the second part, patients received 3 weekly doses (Figure 1(a), SD and

MD cohorts, respectively, by the subcutaneous route (SC)). Patients enrolled in SD and MD cohorts underwent 13 and 15 visits, respectively, including screening, baseline, and end-of-study visit at week 52/54. In SD and MD cohorts, 12 and 36 patients were randomized 1:1:1 across 3 dose levels (DLs) of 10^9 , 10^{10} , 10^{11} virus particles (vp) and then randomized 3:1 within each DL to placebo (four patients in each dose group included one placebo in SD cohort; nine patients in each dose group included three placebo in MD cohort (Figure 1(a)). Consort flow diagram is shown in Figure 1(b). All patients except 1 completed the study and received all injections as planned. The study was conducted in 12 investigational centers in France, Germany, and Canada in accordance with the Declaration of Helsinki and Good Clinical Practice standards and with approvals from the appropriate ethical review committees/institutional review boards. All patients provided informed consent before participating in any study-related procedure (trial is referenced in clinicalTrials.gov under the identifier NCT02428400).

Therapeutic vaccine

TG1050 has been previously described.⁶ It is based on an E1 and E3 deleted non-replicative human adenovirus serotype 5 (Ad5) which encodes, under a CMV promoter, a large fusion protein composed of a truncated form of the HBV Core, the entire Polymerase (POL) and two small domains derived from the Envelope (ENV). TG1050 was produced using HER96 cells and supplied as a frozen suspension formulated in the Adenovirus Reference Material buffer, available in clear glass vials stored at or below -60°C .

Neutralizing anti-Ad5 antibodies (anti-Ad5-NA)

Serum levels of neutralizing anti-Ad5 NA antibodies were evaluated using a chemiluminescence-based assay developed, optimized and validated internally as previously described.¹⁸ The assay is based on the capacity of patient serum to inhibit the infection of A549 cells by Ad5 encoding for the firefly luciferase (Ad5-luc). Briefly, 7 three-fold dilutions of patient serum starting from a 1/10 dilution were incubated during 1 h at room temperature with 54.5×10^7 vp/ml (MOI 25) of Ad5-luc before inoculation to 2.5×10^4 A549 cells. After 17 h of incubation at 37°C , 5% CO_2 , the luciferase activity was measured after addition of One-Glo luciferase substrate on cell layers. Luminescence of samples was plotted versus log of serum dilution and titer was defined as the dilution factor inhibiting 90% of the maximum luminescence signal (ND90) using the Spearman–Kärber method. Patients with titers at 45 or below were classified as patients without significant Ad5 pre-immunity. Patients below a titer of 105 (median titer of the study population) were arbitrarily adjudicated as low pre-immunity patients, those above 105 were considered high pre-immunity patients.

ELISPOT analysis

Up to 50 mL of whole blood was drawn on 10 mL Sodium heparin tubes at five longitudinal visits along the course of the clinical trial at Baseline, week 2,4 and 12 for the SD cohort

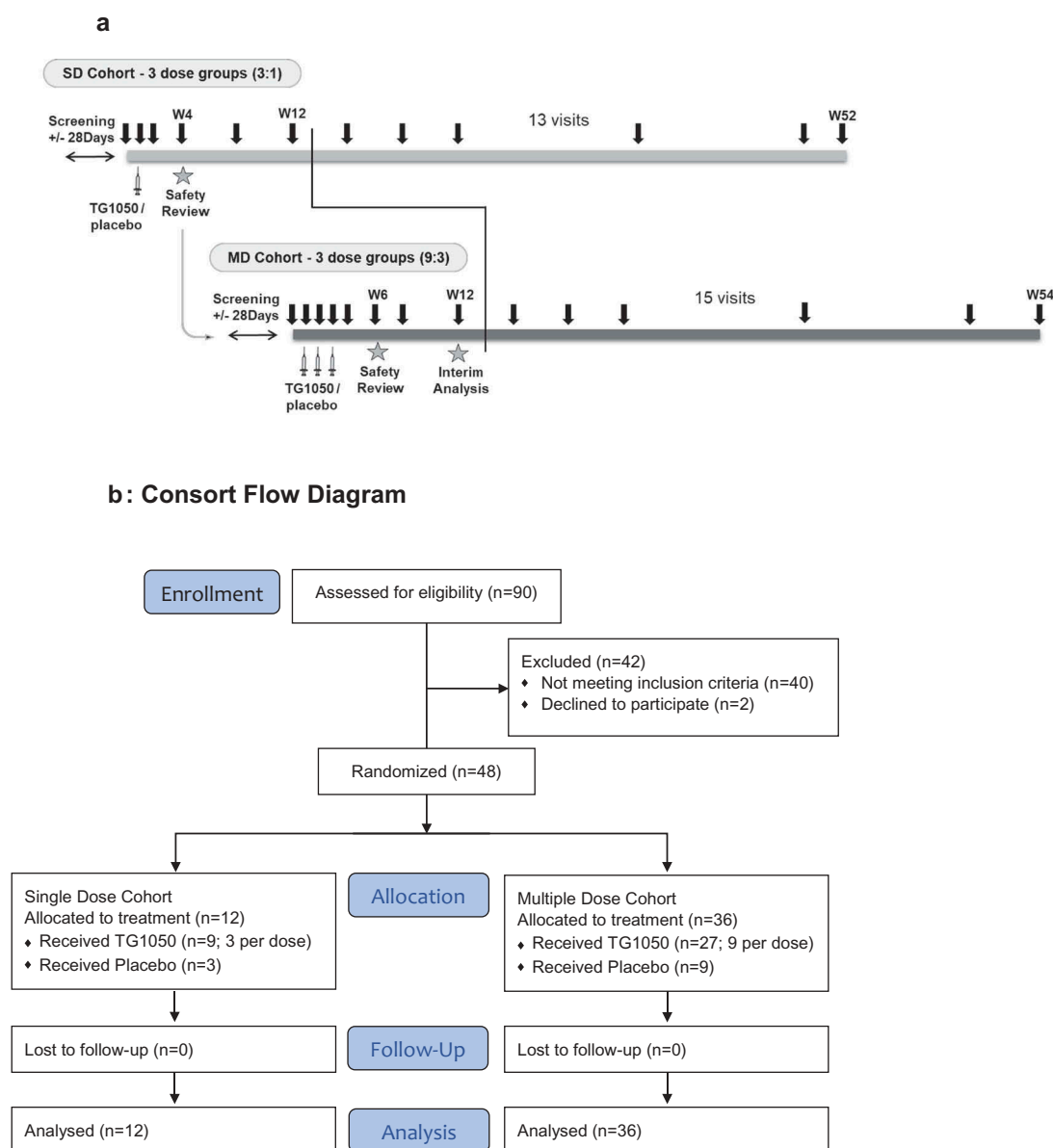


Figure 1. (a) Schematic representation of study design and conduct. Duration (52–54 weeks), vaccine administration (1 or 3, syringes) and blood samplings (black arrows) are indicated together with the number of patient's visits (13 and 15 for SD and MD cohorts, respectively). Week 12 (W12) represents time of interim analysis. (b) Consort flow diagram.

and at Baseline, week 4, 6 and 12 for the MD cohort. Peripheral blood mononuclear cells (PBMC) were extracted from patient blood in trained laboratories within 12 h after collection by ficoll gradient separation. Cells were stored in nitrogen vapors until analysis. Analysis of T cell responses was performed using IFN γ Enzyme-Linked immunospot assay (ELISPOT) after 10 days of *in vitro* expansion. Briefly, after thawing, cells were seeded at 2×10^6 cells per mL per well of 24-well culture plates in RPMI+10%FCS medium and in the presence or not of stimulating antigens (1 μ g/mL of HBV antigen peptide or CMV peptide pool (PepMix HCMVA, JPT Peptide Technology, Germany) at 0.1 μ g/mL or an anti CD3 antibody used as the maximum signal of the assay). Antigens used for PBMC stimulation were pools of 80 overlapping 15-mer peptides covering the sequences for Core, POL, ENV domains as well as a non-vaccine antigen, the

HBV X protein. Because of its large size, peptides covering the POL were spread across three different peptide pools (P1, P2, P3). At day 4 and day 7, half of the medium from each well was discarded and replaced by RPMI+10%FCS medium supplemented media with 100 IU/mL of recombinant human IL-2 (R&D System, USA). At day 10, cells were harvested, counted, and plated at 2×10^5 cells per well of ELISPOT IFN- γ plates (Mabtech, Sweden) in quadruplicates. After 24 h incubation, ELISPOT plates were revealed according to the manufacturer's instructions, then dried before spot counting by an Immunospot reader (Cellular Technology Limited Europe GmbH, Germany). Longitudinal assessment from a given patient was conducted over the same assay run in quadruplicates. To be considered evaluable patients must had a baseline level lower than the assay maximum signal (anti-CD3). Antigenic response was normalized using medium

value. Positive response was defined as an elevation of the number of spots at least equal to a doubling from baseline and superior to 100 spots (representing 3 times the assay inter-variability).

Circulating viral parameters: HBV DNA, HBsAg, HBcrAg

Serum HBV-DNA quantification was performed by quantitative PCR using Cobas Ampliprep/Cobas Taqman HBV test (Roche Diagnostics, Germany). Serum HBsAg was quantified with Elecsys HBsAg II kit/Cobas e411 (Roche Diagnostics, Germany) according to manufacturer's instructions. Quantitative levels of HBcrAg were determined using the Lumipulse G HBcrAg assay on the LUMIPULSE G1200 Analyzer (Fujirebio Europe, Belgium) after denaturation of proteins by incubation at 60°C for 30 min according to manufacturer's instructions. Due to the denaturation step, the assay measures simultaneously denatured HBeAg, HBcAg and the pre-Core protein p22cr (aa -28 to aa 150). The assay measurement linear range spans from 3 to 7 logU/ml, hence adequate quantification is achieved at a concentration of 3 log U/ml (defined as Limit of Quantification or LoQ) or above, and patients below were considered negative.

Statistical analysis

Statistical analyses were performed using SAS® software version 9.4. Descriptive statistics were used for this phase I/Ib study, and descriptive statistics were done by cohort (SD, MD), treatment arm (TG1050 or placebo) and TG1050 dose. Continuous variables were summarized using number of observations (N), arithmetic mean (Mean) and standard deviation (SD). Categorical variables were presented using the number of observations (N) and percentages (%).

All the safety analyses were performed on the study population. Adverse Events (AE) were collected from the first treatment administration and up to 28 days after the last TG1050 administration or after the safety follow-up visit. AEs were graded using CTCAE version 4.03. The number of events, number, and percentage of patients were summarized by preferred terms (PT) as per MedDRA dictionary version 21.1 by descending order based on the number of events.

For HBsAg and HBcrAg, graphical representations of evolution over time were done for each cohort and each dose

level. Subgroup analyses were performed in patients with values at baseline higher than the LoQ for HBcrAg.

For ELISpot data, T-cells immune responses were evaluated at baseline, week 2, 4 and 12 for the SD cohort and at baseline, week 4, 6 and 12 for the MD cohort.

Results

This was a double-blind, randomized, placebo-controlled and dose-finding study. Objectives were to assess dose-related safety of TG1050 administered as single and multiple doses (SD and MD, respectively), early antiviral activity and induced immune responses (cellular-based HBV antigen-specific immunity and anti-Ad5 NA). TG1050 was administered at three different dose levels (DL) of 10^9 , 10^{10} , 10^{11} virus particles (vp). Patients received the allocated randomized product as one or three weekly subcutaneous injections, respectively in SD and MD cohorts. Baseline characteristics of the patients are presented in Table 1 (SD) and 2 (MD). An important difference in the selection of patients was the detection (or lack) of pre-existing anti-Ad5 neutralizing antibody (NA): Patients in SD cohort were all anti-Ad5 NA-undetectable while no selection on pre-existing anti-Ad5 NA was set in the recruitment of patients in MD cohort. Some reports have described that pre-existing anti-Ad5 NA may blunt adenovirus-based vaccines induced immunogenicity.^{19,20} Hence, it was important to first evaluate the safety of TG1050 in absence of NA such as in the SD cohort, a scenario in which TG1050 mechanism of action (induction of T-cells) should be optimal. Based on available information, no patients enrolled in the trial were vertically infected.

TG1050 is safe in patients with CHB

No severe adverse events (SAE), no sign of immune-related AE and sign of hepatotoxicity were reported in SD and MD cohorts (Tables 3 and 4). Overall 24 patients (60 events) exhibited IMP-related AEs whereas 26 patients (52 events) exhibited IMP-not related AEs. Most of IMP-related AEs were in SOC (System Organ Class) "General disorders and administration site conditions" (57%; 10/12 events in SD cohort and 24/48 events in MD cohort), usually injection site reactions. Among the 60 IMP-related AEs, all were of grade 1 except six AEs that were grade 2 (see Tables 3 and 4). There was no ALT flare. ALT fluctuations remained minor and never exceeded 1.5 ULN. Overall, no AE leads to discontinuation of treatment.

Table 1. Demographic characteristics at baseline for SD cohort.

	Placebo N = 3	10^9 vp N = 3	10^{10} N = 3	10^{11} vp N = 3	Overall N = 12
Gender (female/male)	0/3	2/1	1/2	2/1	5/7
Mean age (years) (SD)	52.3 (4.04)	44.0 (8.00)	50.0 (7.21)	39.0 (11.14)	46.3 (8.73)
Mean duration of HBV disease (years) (SD)	20.4 (3.71)	24.8 (10.50)	17.1 (11.15)	14.0 (9.93)	19.1 (8.97)
Nucleo(s)tidies treatment (ENT/TDF)	1/2	1/2	2/1	1/2	5/7
Mean duration of nucleo(s)tidies treatment before vaccine administration in years (SD)	5.9 (3.46)	4.1 (2.28)	6.1 (3.76)	3.8 (0.96)	5.0 (2.65)
HBeAg status (negative/positive)	3/0	3/0	2/1	3/0	11/1
HBV genotype D	1 (33.33%)	1 (33.33%)	0 (0.00%)	0 (0.00%)	2 (16.67%)
HBV genotype Unknown ¹	2 (66.67%)	2 (66.67%)	3 (100.00%)	3 (100.00%)	10 (83.33%)
Mean Alanine Aminotransferase (U/L) (SD)	23.7 (5.51)	21.0 (13.89)	26.3 (7.57)	16.0 (1.73)	21.8 (8.21)

Numerical values are shown as mean (SD) and frequency variables as n (%), BMI, body mass index; ENT, entecavir; TDF, tenofovir.

¹ Unknown genotype because all patients were virally suppressed.

Table 2. Demographic characteristics at baseline for MD cohort.

	Placebo N = 9	10 ⁹ vp N = 9	10 ¹⁰ vp N = 9	10 ¹¹ vp N = 9	Overall N = 36
Gender (female/male)	0/9	2/7	1/8	3/6	6/30
Mean age (years) (SD)	50.6 (8.68)	45.8 (8.24)	47.9 (12.91)	49.4 (9.74)	48.4 (9.79)
Mean duration of HBV disease (years) (SD)	14.9 (7.65)	19.0 (9.08)	15.1 (10.41)	17.2 (9.71)	16.5 (9.03)
Nucleo(s)tides treatment (ENT/TDF)	3/6	4/5	2/7	5/4	14/22
Mean duration of nucleo(s)tides treatment before vaccine administration in years (SD)	5.1 (2.01)	5.7 (2.55)	6.1 (3.14)	5.6 (2.86)	5.6 (2.58)
HBeAg status (negative/positive)	8/1	9/0	8/1	9/0	34/2
HBV genotype A	0 (0.00%)	1 (11.11%)	0 (0.00%)	0 (0.00%)	1 (2.78%)
HBV genotype B	0 (0.00%)	0 (0.00%)	1 (11.11%)	0 (0.00%)	1 (2.78%)
HBV genotype D	0 (0.00%)	0 (0.00%)	1 (11.11%)	1 (11.11%)	2 (5.56%)
HBV genotype E	0 (0.00%)	1 (11.11%)	1 (11.11%)	1 (11.11%)	3 (8.33%)
HBV genotype Unknown	9 (100.00%)	7 (77.78%)	6 (66.67%)	7 (77.78%)	29 (80.56%)
Mean Alanine Aminotransferase (U/L) (SD)	25.0 (9.21)	23.4 (13.27)	21.6 (7.02)	24.4 (9.25)	23.6 (9.60)

Numerical values are shown as mean (SD) and frequency variables as n (%), BMI, body mass index; ENT, entecavir; TDF, tenofovir.

Table 3. Number (%) of patients with TG1050-related adverse events (AE) in SD cohort.

MedDRA Preferred Term	Place N=3		10 ⁹ vp N=3		10 ¹⁰ vp N=3		10 ¹¹ vp N=3		Overall N=12	
	N (%)	Ev	N (%)	Ev	N (%)	Ev	N (%)	Ev	N (%)	Ev
OVERALL	1 (33.3%)	2	0 (0.0%)	0	1 (33.3%)	2	3 (100.0%)	8	5 (41.7%)	12
Injection site reactions	0 (0.0%)	0	0 (0.0%)	0	1 (33.3%)	2	3* (100.0%)	5	4 (33.3%)	7
Influenza like illness	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (33.3%)	1	1 (8.3%)	1
Pain	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (33.3%)	1	1 (8.3%)	1
Pyrexia	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (33.3%)	1	1 (8.3%)	1
Dizziness	1 (33.3%)	1	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (8.3%)	1
Somnolence	1 (33.3%)	1	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (8.3%)	1

All AE were grade 1 except 1 of grade 2 (indicated by an Asterix)

Table 4. Number (%) of patients with TG1050-related adverse events (AE) in MD cohort.

MedDRA Preferred Term	Placebo N=9		10 ⁹ vp N=9		10 ¹⁰ vp N=9		10 ¹¹ vp N=9		Overall N=36	
	N (%)	Ev	N (%)	Ev	N (%)	Ev	N (%)	Ev	N (%)	Ev
OVERALL	2 (22.2%)	3	5 (55.6%)	10	6 (66.7%)	19	6 (66.7%)	16	19 (52.8%)	48
Injection site reactions	0 (0.0%)	0	2 (22.2%)	2	3 (33.3%)	6	4 (44.4%)	7	9 (25.0%)	15
Fatigue	1* (11.1%)	1	0 (0.0%)	0	2 (22.2%)	2	1 (11.1%)	1	4 (11.1%)	4
Body temperature increase	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	3	0 (0.0%)	0	1 (2.8%)	3
Feeling hot	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	2	0 (0.0%)	0	1 (2.8%)	2
Cough	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	2	1 (2.8%)	2
Vertigo	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	2 (22.2%)	2	2 (5.6%)	2
Nausea	0 (0.0%)	0	1 (11.1%)	1	1 (11.1%)	1	0 (0.0%)	0	2 (5.6%)	2
Upper respiratory tract	0 (0.0%)	0	1 (11.1%)	1	1 (11.1%)	1	0 (0.0%)	0	2 (5.6%)	2
Headache	0 (0.0%)	0	2* (22.2%)	2	0 (0.0%)	0	0 (0.0%)	0	2 (5.6%)	2
Lymph node pain	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	1	0 (0.0%)	0	1 (2.8%)	1
Diarrhea	1* (11.1%)	1	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (2.8%)	1
Chills	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	1	1 (2.8%)	1
Pain	0 (0.0%)	0	1* (11.1%)	1	0 (0.0%)	0	0 (0.0%)	0	1 (2.8%)	1
Pyrexia	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	1	0 (0.0%)	0	1 (2.8%)	1
Genital herpes	0 (0.0%)	0	1 (11.1%)	1	0 (0.0%)	0	0 (0.0%)	0	1 (2.8%)	1
Influenza	1 (11.1%)	1	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (2.8%)	1
White blood cells urine	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	1	1 (2.8%)	1
Myalgia	0 (0.0%)	0	1* (11.1%)	1	0 (0.0%)	0	0 (0.0%)	0	1 (2.8%)	1
Dysgeusia	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	1	1 (2.8%)	1
Sleep disorder	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	1	0 (0.0%)	0	1 (2.8%)	1
Night sweats	0 (0.0%)	0	1 (11.1%)	1	0 (0.0%)	0	0 (0.0%)	0	1 (2.8%)	1
Pruritus	0 (0.0%)	0	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	1	1 (2.8%)	1
Hot flush	0 (0.0%)	0	0 (0.0%)	0	1 (11.1%)	1	0 (0.0%)	0	1 (2.8%)	1

All AE were grade 1 except 5 of grade 2 (indicated by an Asterix)

TG1050 antiviral activity

Evolution of two viral markers was measured in the study following administration of TG1050, circulating HBsAg and HBcrAg.

HBsAg levels

Expectedly, enrolled patients displayed a heterogeneous level of HBsAg at baseline ranging from 2.24 to 4.58 log U/ml (Supplementary Figure 1 and Supplementary Table 1). For

patients of SD cohort, observed decrease of HBsAg over the time study (week 52) was quite limited (Figure 2(a)). Decreases approximated or reached at most 0.2 log from baseline values (Figure 2(a) and Supplementary Table 1). For patients in MD cohort, similar observations were made, i.e., for most patients decrease of HBsAg remains minor (<0.2 log) except for six patients: one Placebo (MD02), one (MD11), three (MD20, MD27, MD23) and one (MD29) patients, respectively, in 10⁹, 10¹⁰ and 10¹¹ vp groups who reached

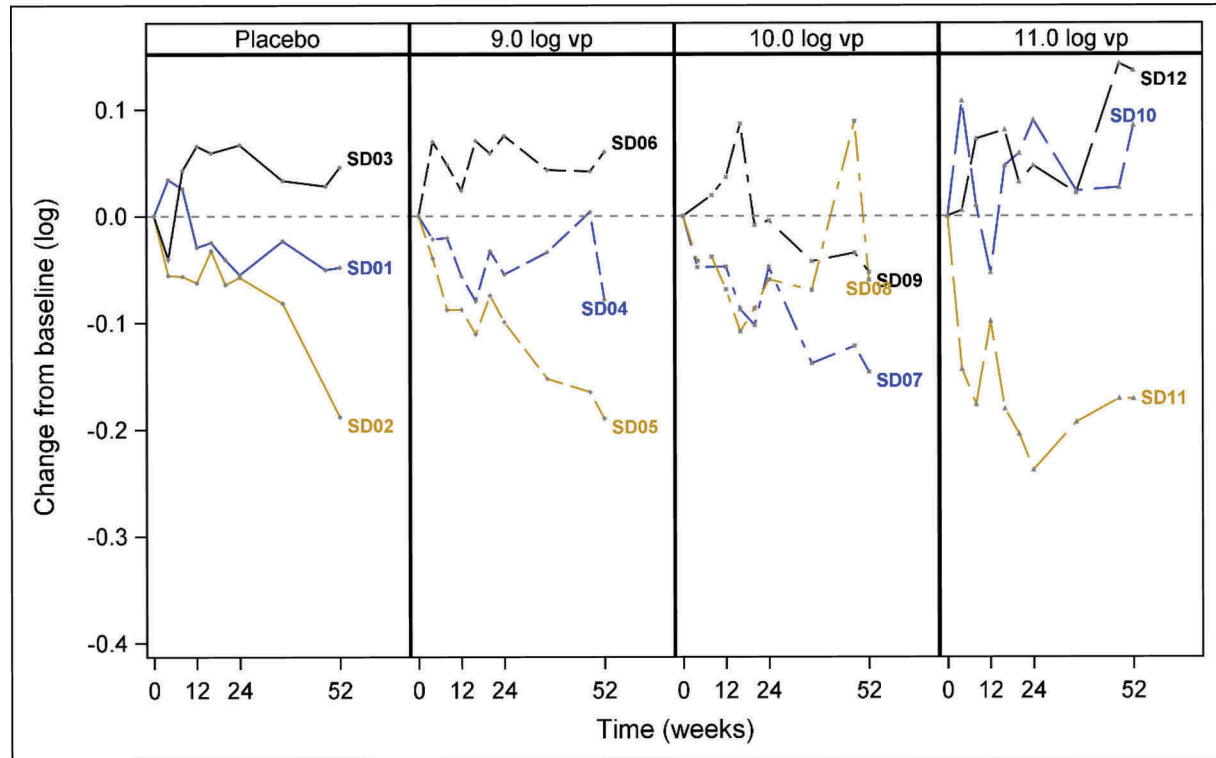
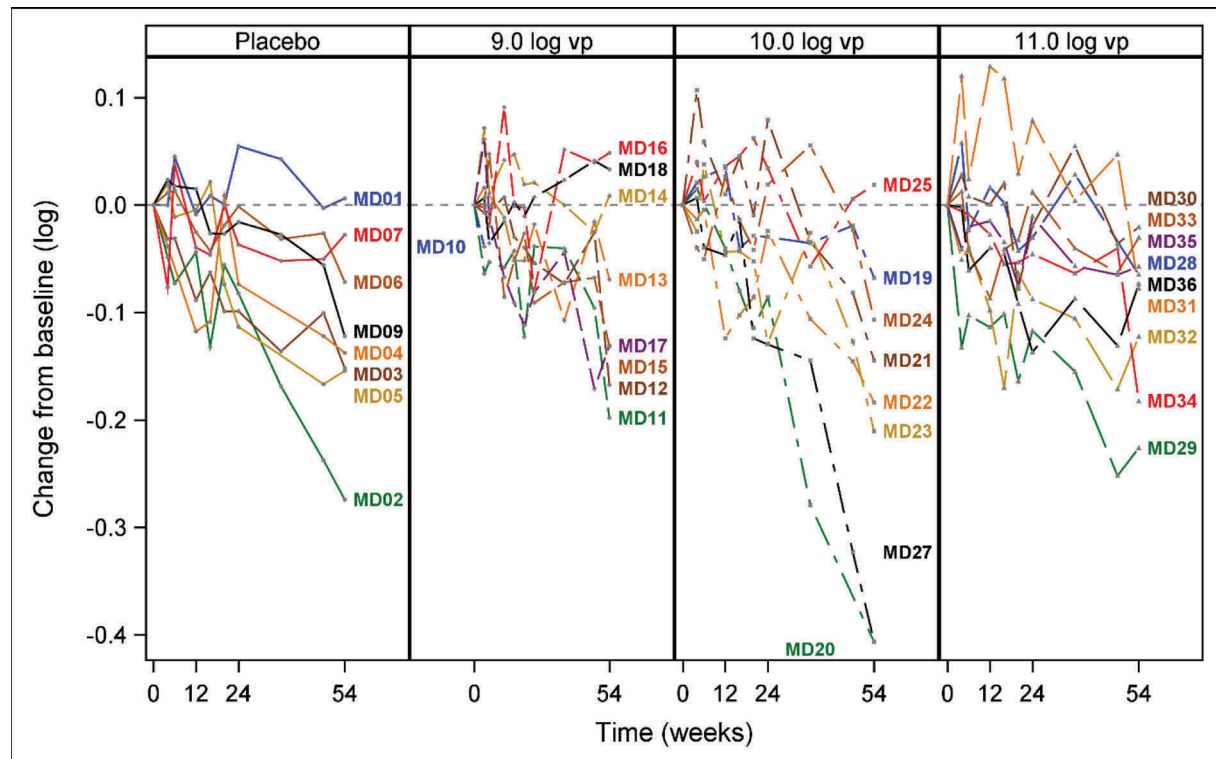
a**b**

Figure 2. Longitudinal titration of serum HBsAg in SD and MD cohorts. Data are represented as changes from baseline. Each colored line represents an individual patient. Dots represent HBsAg delta from baseline measured for a given time point (from time of vaccine administration (0) till week 52/54 (last time point)). The four groups of patients from SD (Figure 2(a)) and MD (Figure 2(b)) cohorts are indicated (Placebo and 10^9 , 10^{10} , 10^{11} vp – injected groups). For MD cohort, the two patients displaying 0.4 logs decrease are outlined.

>0.2 logs decrease, including two patients (MD20 and MD27) reaching 0.4 logs (10^{10} vp group). Maximal decreases were typically observed late post-vaccination (W54). No particular common baseline clinical characteristics could be found for these patients.

HBcrAg levels

Overall, a significant number of SD patients (75%) had no detectable levels of HBcrAg or levels below the LoQ of the assay, while this number was lower in MD cohort (17%) (Supplementary Table 1), consistent with the fact that most patients were HBeAg negative.²¹ Similar to HBsAg, baseline levels of quantifiable HBcrAg were quite heterogeneous, ranging from 3 to 6.7 log AU/ml (Supplementary Table 1). Hence, the number of SD patients evaluated was too small and analysis not relevant (data not shown). For MD cohort specifically, the number of patients displaying baseline values above the LoQ was 6, 8, 8 and 7 in the Placebo, 10^9 , 10^{10} and 10^{11} vp cohort, respectively. Overall, majority of patients did not display a significant change in HBcrAg levels with the following exceptions: one patient in the Placebo group (MD02) went from a detectable level at baseline to level below LoQ at end of study (Figure 3 and Supplementary Table 1); Among the vaccinees, we did observe a significant decrease of HBcrAg (>1 log) for one patient in the 10^9 vp group (MD17). Furthermore, two patients in the 10^9 vp cohort (MD15, MD18), three patients in the 10^{10} vp cohort (MD19, MD21, MD25) and one patient in the 10^{11} vp cohort (MD35) showed undetectable or unquantifiable level of HBcrAg during the course of and up-to end of study (Figure 3 and Supplementary Table 1). A common observation for those patients is that they all displayed low baseline levels (ranging from 3 to 3.5 log AU/ml). Larger cohort studies are required to determine whether loss of HBcrAg detectability is directly linked to vaccine administration in these patients or may have been due to the natural evolution of infection.

Adenovirus-specific immune responses

Anti-Ad5 NA was measured through the study in all patients. Per design, all SD patients displayed undetectable antibodies while MD patients displayed titers ranging from undetectable ($n = 10$ or 27.7%) up to 1116 (one patient in the 10^9 vp group) (Supplementary Table1).

Overall, injection of TG1050 resulted in an increase of anti-Ad5 NA in 17% and 47% of patients, respectively, from SD and MD cohorts (Supplementary Figure 2A and 2B). Peak was typically observed at week 4 or 12. For MD cohort, peaks were typically observed at week 4, plateauing thereafter for many weeks and/or starting to decrease in some cases by week 8. The highest levels of detectable antibodies were observed in two patients from the 10^{11} vp MD dose group (MD28 and MD36). Except for these two patients, elevations in antibody titer observed were comparable across the 3 DL.

TG1050 induces HBV-specific cellular immune responses

In order to analyze HBV-specific T-cell responses during the course of the study, a 10-day *in vitro* stimulation IFN- γ ELISPOT assay was performed. Such assay characterized by a rather long stimulation time is a standard in HBV immunology as the high tolerogenic environment found in CHB typically precludes (unless cell enrichment is performed) detection of responses.²¹ Assay was successfully performed for patients of SD cohort. In contrast, a strong background signal was observed in several MD patients exhibiting higher levels of pre-existing anti-Ad5 NA, precluding interpretation of analysis (data not shown). High baseline levels of anti-Ad5 NA have been in some studies shown to be associated with a reduction of vaccine immunogenicity;^{19,20} yet no consensus exists on a putative inhibiting antibody threshold. We hence

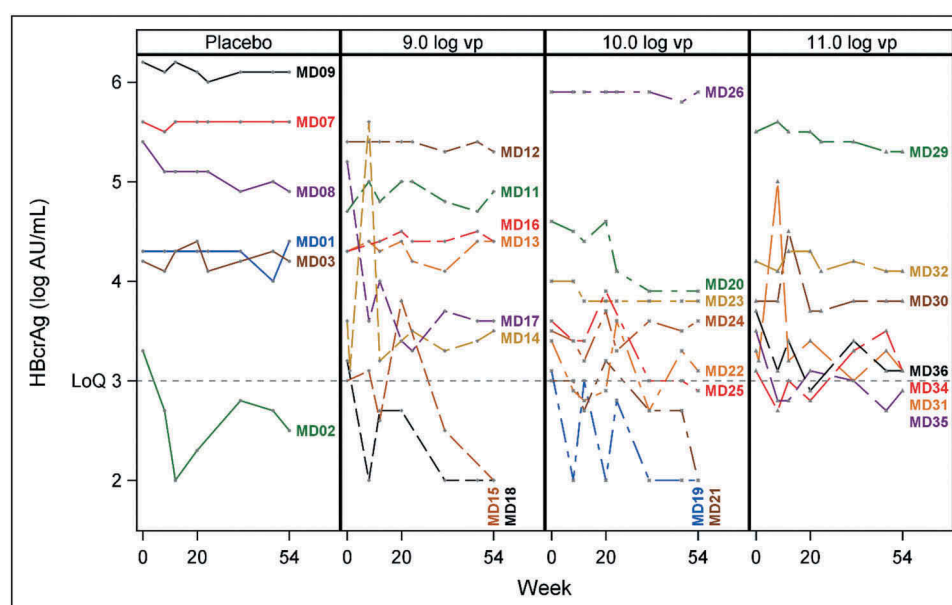


Figure 3. Individual HBcrAg evolution over time in MD cohort. Only patients displaying a baseline HBcrAg level > LoQ are represented. Data are presented as changes from baseline. Each colored line represents an individual patient. Dots represent HBcrAg values measured for a given time point (from time of vaccine administration (0) till week 52/54 (last time point)). The four groups of patients are indicated (Placebo and 10^9 , 10^{10} , 10^{11} vp – injected groups).

arbitrarily allocated MD patients into two groups based on their baseline neutralizing titer: those harboring baseline titers $>$ to the median anti-Ad5 NA (value of 105) and those with levels $<$ to the median. As background ELISPOT signals were typically too high in the first group (whether placebos or vaccines), we report only here data obtained for the second group with lower anti-Ad5 NA titer levels.

To report changes induced by the vaccine, we plotted for each patient, increases of spots from baseline (Figure 4) but also indicated the raw number of spots obtained for each patient (after background correction) (Supplementary Figure 3). This latter representation reflects well the high heterogeneity in the level of responses detected as well as the potentially important intra-subject variability. Based on these observations, we have

defined positivity with relatively stringent criteria (see Materials and Methods section). Regarding placebos in both SD and MD cohorts, minimal ELISPOT responses were observed that did not reach the criteria for positivity for respectively Pol, Core and Env (Figure 4(a-c)). Similar observations were made for patients receiving the lower vaccine dose 10^9 vp, whether in SD or MD cohort (Figure 4(a-c)). In contrast, greater numbers of spots were clearly detected for several patients in the two higher vaccine doses (10^{10} and 10^{11} vp). For these doses, strongest responses targeted Core, followed by Pol and Env, were seen at week 2 for SD patients and between week 4 and 6, or even at week 12 for MD patients (Figure 4(a-c)). Of note, week 2 was not tested in the MD patients (sample not collected at that time point). A maximum number of responders were observed in the

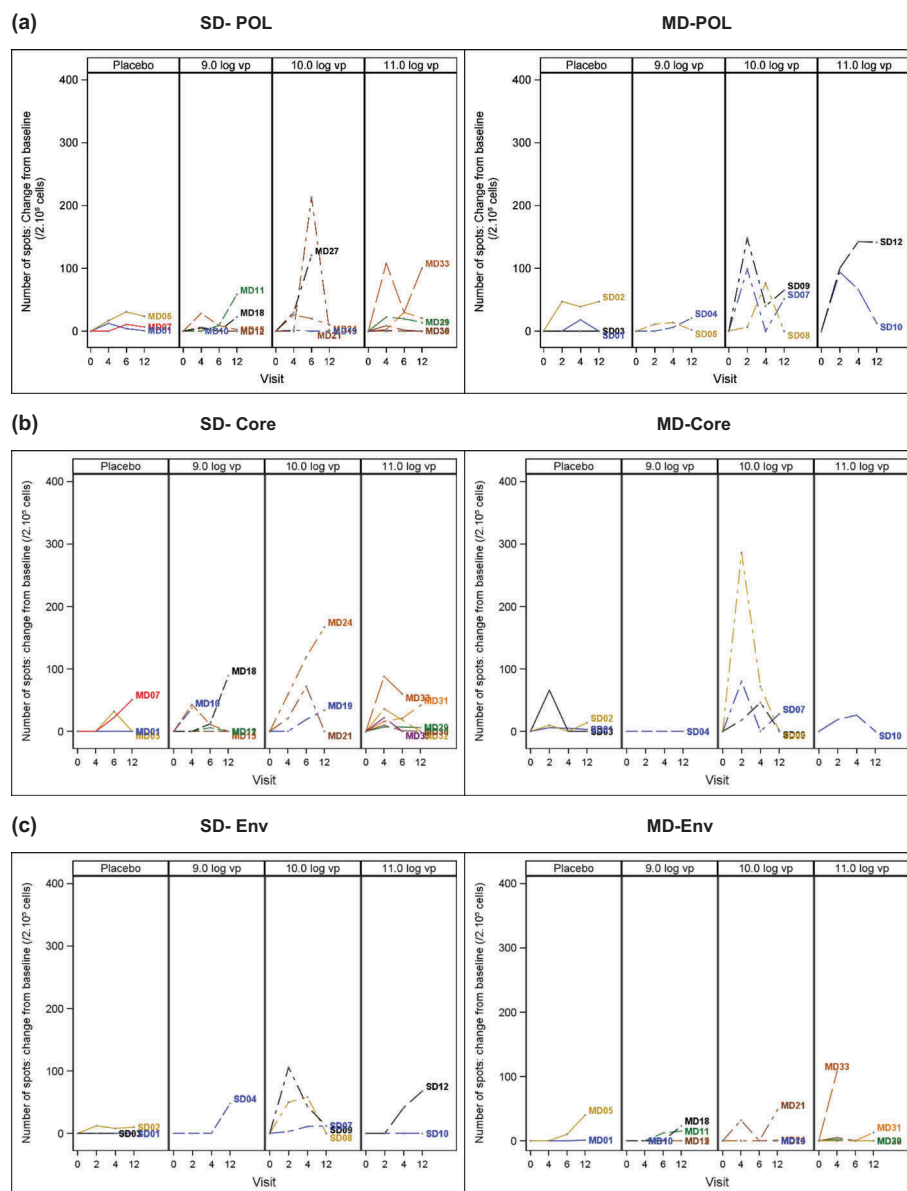


Figure 4. TG1050 induced – HBV-specific T-cell responses. Blood samples were taken at 4 time points (at baseline, week 2, week 4 and week 12 for SD cohort and at baseline, week 4, week 6 and week 12 for MD cohort) post-initial vaccine administration and ELISPOT assays performed using purified PBMCs as described in Materials and Methods. Data are reported as number of spots changes from baseline for 2×10^5 cells/ml. For each cohort, the four groups of patients are indicated (Placebo and 10^9 , 10^{10} , 10^{11} vp – injected groups). Stimulation with the various peptide pools covering whole polymerase (3 pools, Figure 4(a)), the Core (1 pool, Figure 4(b)) and Env (1 pool, Figure 4(c)) were added for each antigen; change from baseline was normalized by the number of peptide pools tested for each patient (i.e., for some patients, only 1 or 2 Pol peptide pools were tested). Dotted line indicates the threshold of positivity (100 spots) as defined in Materials and Methods.

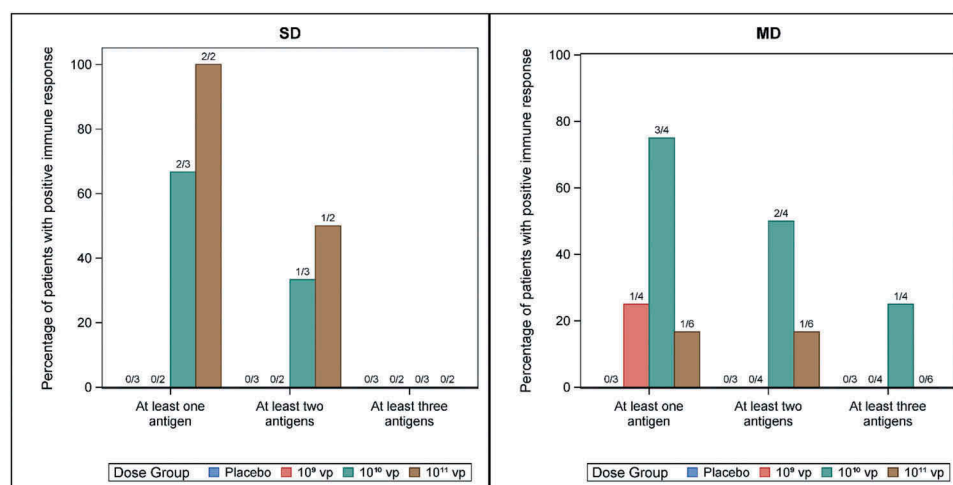


Figure 5. Number of antigens targeted following TG1050 administration. Percentage of patients displaying Pol and/or Core and/or Env- ELISPOT-specific responses following TG1050 administration is shown (at least one, two or three antigens targeted) over the course of analysis. Data are shown for SD and MD cohorts and for all four groups tested (Placebo and 10⁹, 10¹⁰, 10¹¹ vp – injected groups).

10¹⁰ vp dose. Figure 5 summarizes the percentage of patients with positive immune responses, for each cohort and vaccine doses, specific to 1 or 2 or 3 antigens targeted by the vaccine. Overall, 4/7 patients in SD cohort had a response against at least one antigen including two patients developing response against multiple antigens. Similarly, 5/10 patients vaccinated in MD cohort had responses against at least one antigen and four of them had a poly-specific response. As mentioned above, most responses are observed with the 10¹⁰ and 10¹¹ vp doses, although it is not clear whether the highest dose of 10¹¹ vp is linked to a higher immunogenicity.

To determine whether immune responses observed were vaccine specific, we also assessed response against the HBV X protein, a protein not contained in TG1050. We did not observe major increase of reactive T cell targeting this antigen except for one patient in SD cohort showing an elevation at week 12 (data not shown).

Overall, these data indicate that TG1050 is capable to induce functional, IFN- γ producing cells in chronically infected patients. Detection of POL-specific cells was prominent likely reflecting the large size of this protein. TG1050 was also, albeit to low levels, able to induce detectable cells specific of the highly tolerogenic HBsAg. Of note, we could not observe any association between detection of circulating IFN- γ positive cells, whether at baseline or following vaccination, and decrease of HBsAg or HBcrAg in serum.

Discussion

In this study, we report for the first time the clinical evaluation, in patients receiving NUC therapy, of a multi-antigenic adenovirus-based T-cell-inducing vaccine for the treatment of CHB. The primary end-point of the study was reached, TG1050 was shown to be safe at all doses tested, following either a single or multiple administration. Most reported AEs were typical of adenovirus-based vaccination, i.e., inflammation at injection sites that waned with time. We did not observe any flares of the disease nor significant ALT elevations in this first-in-man (FIM) study. Importantly, no SAE was reported during the study.

A number of secondary end points were assessed. We were able to document immunogenicity of TG1050 in the highly tolerant context of CHB infection, albeit not in all patients. In order to gain insights into HBV-specific T-cell responsiveness, we used a conventional, well described, *in vitro* expansion IFN- γ ELISPOT assay.^{22–24} This assay, although only semi-quantitative, has allowed to demonstrate in various cohort studies the particularly low frequencies of HBV-specific CD8 + T-cells during CHB infection, these cells being mainly documented in low viral load patients.^{24,25} Following vaccination with TG1050 in the immune-reactive group of patients, majority of IFN- γ producing cells were observed in patients receiving the two highest doses of vaccine: up to 100% and 75% of the patients of SD and MD cohort, respectively, developed a response specific to at least one of the vaccine antigens with the most immunogenic dose (Figure 5). Overall, the intermediate dose in MD cohort (10¹⁰ vp) appears to be most immunogenic in terms of both the number of reactive patients and the number of recognized antigens although small number of patients precluded the reaching of statistical significance. Interestingly, it has been published in various pre-clinical studies that an intermediate dose of Ad5-based vaccines is typically associated with better quality of T-cells (higher polyfunctionality, strongest proliferative capacity and lower expression of exhaustion markers) compared with higher doses.^{26–28} Unfortunately, we could not perform additional immune assays such as recently reported in the field (e.g., tetramer analysis, *ex vivo* ELISPOT, phenotypic analysis^{25,29–31}) in order to better characterize the detected cells due to limited sample quantities. Such analyses will need to be conducted in future clinical trials. It is difficult to speculate on the possible cytolytic function of the induced T-cells in the absence of further functional studies. No ALT elevations were observed in the study except for very minor and transient flares (ranging from 42 to 60 IU/L) seen in six patients from the MD cohort (data not shown). Those did not appear to correlate with antiviral activity (see below). Peak of ELISPOT responses were typically observed at week 2 (SD cohort) or week 4–6 (MD cohort) although week 2 time point not being assessed for MD earlier peaks may have

been missed. With a few exceptions, those waned with time, at least in the periphery. Educated T-cells may have migrated to the liver to exert their effector function and hence were possibly no longer detectable in blood. Validation of this hypothesis is beyond the scope of our study and use of semi-invasive procedures based on fine-needle aspirates of livers is recommended.³² As HBV genotype for the majority of patients was not known, due to suppressed HBV DNA, level of cross-reactivity of the induced T-cells by our genotype D-derived vaccine could not be appreciated and encouraging pre-clinical data are clearly in support TG1050 use in a wide range of HBV patients.³³

In the majority of immune-reactive MD patients from the 10^{10} vp dose, we could detect T-cells targeting both POL and Core. In a recent study, Rivino et al.,³⁴ followed patients prior to and after NUC therapy discontinuation and performed biomarker analysis to identify immunological biomarkers predictive of safe discontinuation. The presence of T cells specific of POL and Core was proposed as a potential marker as these cells were prevalent in patients who did not experience a disease flare after therapy arrest. Hence, priming *de novo* and/or boosting existing functional POL and Core T cells during NUC therapy with TG1050 may be beneficial and provide optimal safety in therapy arrest settings. In particular, inducing POL-educated T-cells would be important as POL-specific CD8 + T cells appear to be more severely exhausted during CHB than Core ones.³¹ POL is a very large antigen, notoriously difficult to purify and engineer in genetically stable vectors for vaccine development. To our knowledge, only one other vaccine beside TG1050, i.e. HB-100, contains the full-length POL and has been tested in the clinic.^{4,35} This DNA vaccine consists of a pool of 5 plasmid DNAs expressing in addition to POL, Core, S/S1/S2 Ags, X, and IL12. In a therapy cessation setting in patients receiving Lamudivine, following arrest, virological responders displayed predominant Core and POL IFN- γ producing cells validating the interest of including these two antigens in a therapeutic vaccine. Despite an attempt to simplify this complex vaccine (development of HB110 based on only 3 plasmids in place of 5), the complexity of this product appears to have led to discontinuation of its clinical development.

We did not measure T-cell responses specific of our vector and performed only limited analysis of anti-adenovirus NAs pre-existing before administration of TG1050 or induced following vaccination (3 time points total). NAs were only poorly induced and/or boosted by TG1050. Full interpretation of these data would require analysis of additional time-points, booster – effects may have taken place very early post-vaccination and been missed, or blunted by a robust pre-existing Adeno-specific T cells. This lack of priming and/or boosting-effect may also reflect a yet non-optimal vaccine administration conditions (schedule, route of injection – see below), parameters that could be studied in further trials.

While immunogenicity of TG1050 in a CHB situation is encouraging, we were not able in this trial to document it for all patients. ELISPOT analysis from patients who displayed baseline titers of anti-Ad5 NA > median value (105) was not exploitable due to high background signals, whether PBMCs were from placebo or vaccine groups. Presence of high levels of anti-Ad5 NA has been described in a number of studies as blunting Ad5-vaccine T-cell immunogenicity. HIV- and Ebola- vaccine trials have reported that very high levels of neutralizing antibodies

(typically the top 20 percentiles of subjects) abrogate detection of specific T-cells, while other levels (majority of subjects) either have no impact or only reduce the number of vaccine antigens targeted and/or the intensity of induced T-cell responses.^{19,20,36} Other clinical trials (Tuberculosis and Malaria) have failed to document any impact of preexisting anti-Ad5 NA on induction vaccine antigen-specific T – cells.^{37,38} It would be important in future trials to use immune-assays not requiring prolonged *in vitro* stimulation step to avoid generating background signals in order to precisely evaluate if and which pre-existing anti-Ad5 NA titers may impact TG1050 immunogenicity. In general, intra-dermal (ID) immunization generates greater immune responses than intramuscular (IM) or SC injection³⁹ due to the presence of more and different dendritic cells in the dermis, which facilitate antigen capture.⁴⁰ A recent study by Enama et al.,⁴¹ has compared in the clinic the three routes of administration, i.e., ID or IM or SC, of an Ad5-HIV vaccine. Although number of subjects was small, the Ad5 vaccine injected ID performed the best, both at the level of T- and B-cell-based assays (ELISPOT and ELISA). Changing to ID route constitutes a very attractive option for future development of TG1050.

While overall serum HBsAg did not decrease significantly following TG1050 administration (0.4 log maximal decrease), a few patients in the vaccinated groups displayed robust decreases of circulating HBcrAg: for one patient reaching >1 log and for six patients, reaching undetectable or unquantifiable levels by end of study (observed in one placebo patient). Serum hepatitis B core-related antigen (HBcrAg) represents a very interesting biomarker shown to correlate with intrahepatic covalently closed circular DNA (cccDNA) transcriptional activity in chronic hepatitis B patients.⁴² Development of therapeutics capable to impact on the functionality of cccDNA is actively pursued by the field. Elimination or blocking of cccDNA activity would be synonymous of definitive cure, the ultimate clinical goal. In that respect, our data are intriguing, they clearly require consolidation before proposing a solid interpretation. For TG1050, it was not possible at this early clinical development stage to validate whether the changes observed are linked to the vaccination and/or define (predictive) markers associated with the observed loss of quantifiable core antigen. Patients reaching undetectable or unquantifiable HBcrAg levels did display low HBcrAg level at baseline. In all cases, maximal decreases, whether for HBsAg or HBcrAg, were observed at longest follow-up time points (week 48–54) suggesting that time could be important to achieve efficacy post-immuno-intervention in CHB, similar to what we have observed in pre-clinical models^{16,17} and also in vaccine-based clinical therapy in oncology.⁴³ These elements are in favor of longer-term follow-up analysis and/or more aggressive vaccination schedules in order to reach maximal vaccine efficacy in virally suppressed patients. In this trial, we could not observe any association between detected peripheral-blood HBV-specific immune responses and reduction of circulating HBcrAg (or HBsAg) levels. As discussed earlier, such association should be studied at the liver level as vaccine primed-T-cells is expected to have migrated to the liver to exert their effector function, a situation that we have described in our pre-clinical models (¹⁶ and unpublished data).

The field of therapeutic vaccination in CHB has had several failures in the past, there is a renewed interest in the field with the development of novel vaccine formulations. The great majority of past vaccines displayed a poor antigenic complexity and were mainly focused on HBsAg but also on recombinant protein-based platforms or non-electroporated DNA vaccinology neither optimal for induction of a strong, large T-cell-based immunity (see Lobaina Y and Michel ML for review⁴⁴⁻⁴⁵). TG1050 includes for the first time a potent T-cell inducing platform (adenovirus) and a complex antigenic design (including the entire POL).³ While original, our study has some limitations which would be of interest to address in future trials e.g., poor representativity of HBeAg + patients, lack of analysis of HBV-specific B-cell responses and adeno-specific – T cell ones in order to address potential antigenic competition issues, lack of knowledge on infecting genotypes. The concept of combining vaccines and NUC with newly developed antivirals capable to temporally lower circulating antigen load or with agents blocking inhibitory pathways is generating strong interest and represents innovative avenues for the treatment of CHB.^{42,43} Data collected in this clinical study as well as pre-clinical combination packages with TG1050 (P Martin, personal communication) support further clinical development of TG1050 in triple combination settings.

Authors' contribution

ZF, FC, HF, SM, PS, CC, LV, MM, WH, LA, TR, were in charge of clinical trial conduct at clinical sites; BB, SB performed statistical analysis; PM, KB, IG were in charged of biomarker analysis; NA, HC, BM of clinical trial supervision, KL supervised the overall project. May add the contributions of authors in discussing the results and reviewing the manuscript if relevant.

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Disclosure of potential conflicts of interest

These authors disclose the following: Karine Lugardon, Perrine Martin, Bérangère Bastien, Sansas Benoit, Halluard Celine, Bendjama Kaïdre, Brandely Maud, Inchauspé Geneviève are employees of Transgene. Fabien Zoulim is consultant for Transgene.

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