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Article type : Original Articles

Editor : Stanislas Pol

ALT flares during nucleotide analogue therapy are associated with HBsAg loss in genotype A HBeAg-positive chronic hepatitis B

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Word Counts

Title: 18

Abstract: 238

Main text: 3623

Key points: 64

Figures: 5

Tables: 3

List of Abbreviations:

LAM	Lamivudine
HBV	Hepatitis B virus
NA	Nucleos(t)ide analogue
ALT	Alanine aminotransferase
CHB	Chronic hepatitis B
ULN	Upper limit of normal
IQR	Interquartile range
TDF	Tenofovir disoproxil fumarate
ADV	Adefovir dipivoxil
DNA	Deoxyribonucleic acid
IU	International unit
PC	Precore
BCP	Basal core promotor
IL	Interleukin
PEIU	Paul Ehrlich international unit
PCR	Polymerase chain reaction

SEM	Standard error of the mean
ANOVA	Analysis of variance
OR	Odds ratio
CI	Confidence interval
SD	Standard deviation
ORF	Open reading frame
BMI	Body mass index

Financial disclosure

Gilead Sciences funded and conducted the original study, including the collection and storage of serum samples. For this study, all clinical data were made available to the investigators, and Gilead were not involved in the analysis or interpretation of data, or the decision to submit for publication.

Statement of Interests

AG, KK, and MS are employees of Gilead Sciences Inc., and own stocks and shares in Gilead Sciences Inc. PM has served as a consultant for Abbot, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead, Janssen/Tibotec, Merck, Novartis, Pfizer, Roche, and Vertex, and has received honoraria from Bristol-Myers Squibb, Gilead, Janssen/Tibotec, Merck, Novartis, and Roche. MB has received honoraria and research grants from Brisol-Myers Squibb, and Gilead. HLJ has received consulting fees and research support from Roche, Merck, Gilead, Bristol-Myers Squibb, Novartis, Santaris, Medtronic, Anadys, Innogenetics, and Kirin. EG has served as a consultant for Achillion, Gilead, Idenix, Janssen, Merck, Novartis, Novira, and Roche. SL has served as a consultant for Gilead. The remaining authors have no personal or financial disclosures.

ABSTRACT

Background

ALT flares during NA therapy are uncommon but occur. Evaluation of ALT flares during nucleos(t)ide analogue (NA) therapy is important as new immunomodulatory therapies for HBV are developed. We evaluated the association between ALT flares and HBsAg loss during long-term therapy for genotype A CHB.

Methods

This analysis included genotype A subjects from a phase III study of tenofovir vs. adefovir in HBeAg-positive HBV. ALT flare was defined as i) a rise in ALT >2x ULN from normal ALT levels; or ii) a rise in ALT >2x baseline ALT level. HBsAg response at week 384 was recorded as one of HBsAg loss vs. HBsAg decline (≥1 $\log_{10}$ IU/ml decline) vs. non-response. The primary analysis evaluated the association between ALT flare and HBsAg response.

Results

54 subjects were included. 23/54 (43%) subjects experienced an on-treatment ALT flare. 45% achieved an HBsAg reduction ≥ 1$\log_{10}$ IU/mL, and of these 67% achieved HBsAg loss at a median of 102 weeks [IQR: 64-156]. Flare was associated with HBsAg decline vs. non-response (67% vs 23%, p=0.002), and were more common in subjects who achieved HBsAg loss vs. non-response (56% vs. 23%), p=0.049). There was a median delay of 56 weeks [IQR: 40-80] between a flare and HBsAg loss.

Conclusion

In genotype A subjects undergoing long term NA therapy, ALT flares predict for HBsAg response. The delay between ALT flare and HBsAg loss has implications for clinical trial design for early phase development of immunomodulatory strategies aiming for HBsAg loss.

Key Words: HBV; Flare; HBsAg loss; Antiviral therapy

Key Points

- This is the largest detailed study of HBsAg loss subjects treated with oral antiviral therapy.
- ALT flares are common in genotype A HBeAg-positive chronic HBV treated with long-term nucleoside analogue therapy.
- ALT flares in this cohort are independently associated with functional cure.
- There is a long delay between ALT flare and HBsAg loss, which has implications for clinical trial design of novel immunotherapeutic strategies.

Introduction

Chronic hepatitis B infection affects more than 248 million people globally and in 2013 was responsible for an estimated 686,000 deaths (1, 2). It is a major contributor to the rising incidence of hepatocellular carcinoma. Despite this significant burden of disease, the immunopathogenesis of chronic HBV infection remains poorly understood. Chronic hepatitis B is very difficult to cure, and the goal of nucleos(t)ide analogue therapy has been long-term suppression of viral replication, rather than viral eradication (3, 4). Functional cure has recently been defined as the loss of hepatitis B surface antigen (HBsAg) with or without anti-HBs production (5), and is now the goal of therapy for new therapeutic candidates.

The host antiviral immune response can be therapeutic, and a number of novel immunotherapies are currently in clinical development to achieve functional cure (6-8). Serum ALT levels are a surrogate marker of hepatic inflammation, indirectly reflecting immune-mediated destruction of infected hepatocytes (9). ALT flares have been associated with achievement of immune control and HBsAg loss in the natural history of chronic hepatitis B, as well as during peginterferon-α therapy (10), leading to the concept of a "therapeutic" flare. ALT flares are reportedly common in the setting of treatment with nucleos(t)ide analogues, but their sequelae have not been investigated (11, 12). The role of host-viral immune interactions in effecting HBsAg clearance during nucleos(t)ide analogue therapy is poorly understood, as is the link with ALT flares. However, a number of novel therapeutic strategies, including the siRNA technologies, are being developed to induce a potentially therapeutic flare.

Study GS-US-174-0103 was a randomised, double-blind phase III study of 266 HBeAg-positive subjects with immune clearance disease comparing the antiviral efficacy of tenofovir disoproxil fumarate (TDF) to adefovir dipivoxil (ADV) (ratio 2:1) monotherapy for 48 weeks in chronic hepatitis B subjects, followed by open-label TDF for a further 336 weeks (12). The primary clinical endpoints included plasma HBV DNA <80 IU/mL and histological improvement. HBeAg loss or seroconversion and HBsAg loss or seroconversion were secondary endpoints. At the conclusion of the study, a total of 26 subjects, or 9.8% of the study cohort, had achieved HBsAg loss. The majority of these functional cures were infected with genotype A virus (n = 16, 6.0%), with the others being either genotype D (n = 9, 3.4%) or genotype B (n = 1, 0.4%).

The aim of the current study was to i) evaluate the frequency of ALT flares during nucleos(t)ide analogue therapy; ii) evaluate the association between on-treatment ALT flare and HBsAg responses; and iii) compare these to the presence of PC-BCP variants, among genotype A patients treated with long-term nucleos(t)ide analogue therapy in the GS-US-174-0103 study.

Methods

Subjects

All genotype A-infected subjects in study GS-US-174-0103 who received at least 48 weeks of treatment were included in the analysis (Table S1). Given that the number of patients achieving HBsAg loss when infected with other genotypes was low, the analysis was restricted to genotype A subjects (Table S2). Quantitative HBsAg response was monitored to week 384 (end of study), or for 24 weeks after HBsAg loss, whichever endpoint was first.

Definitions and Assays

The upper limit of normal for ALT in this study was gender-based as per, being 30 IU/L for males and 19 IU/L for females (3). An ALT flare was defined as an ALT 2x the upper limit of normal after achieving a normal ALT and/or a rise in the ALT level on-treatment to ≥2x baseline ALT level (13). These cut-offs were chosen as a presumptive marker of immunological activity, rather than strict clinical significance. Flares >5x ULN were investigated to exclude other causes of elevated ALT. HBsAg loss was confirmed on two samples 6 months apart, whereas a significant HBsAg decline was defined as a ≥1 $\log_{10}$ IU/ml drop in HBsAg level from baseline but persistent HBsAg positivity at the end of follow-up. HBsAg non-response occurred if HBsAg decline was <1 $\log_{10}$ IU/ml at the end of follow-up. Quantitative HBsAg was measured on the Abbott Architect platform (Abbott Park, IL) using serum samples taken every 12 weeks for the duration of the study (lower limit of detection = 0.05 IU/ml). Qualitative anti-HBs analysis was performed on the same platform, with a lower limit of detection of 10 IU/L. Quantitative HBeAg was measured on the Roche Elecsys platform (Roche Diagnostics, Mannheim, Germany), with a lower limit of detection of 0.3 PEIU/ml.

PCR Amplification and Population-based Sequencing

A) Precore / basal core promotor region (PC-BCP)

The PC-BCP sequencing protocol has been published in detail previously (14). In brief, the PC/core region was amplified from baseline samples by PCR. Primers were designed to amplify the negative regulatory element, core upstream regulatory sequence, BCP regulatory regions, and the complete PC/core coding region over two rounds of nested PCR. The amplicon was purified then sequenced using the PCR primers. Gel electrophoresis of the sequencing reactions was carried out by MicroMon (Department of Microbiology, Monash University, Clayton, Australia). Cut-off for variant detection is approximately 20-25%.

B) Small HBsAg region

Sequencing of the polymerase region was performed by Gilead Sciences using Sanger sequencing as part of the parent study to screen for baseline antiviral resistance mutations within the overlapping polymerase gene. These data were used in this study to determine the small HBsAg sequence at baseline for the purpose of clustal analysis.

Statistical Analysis

All statistical analyses were performed using Stata v14.2 (StataCorp, TX). Normally distributed data is reported as mean ± standard error (SEM). Non-parametric data was analysed as median and interquartile range (IQR). Categorical data was analysed as number and percentage. Bivariate analyses of outcome variables were conducted using the Student's t-test or Mann-Whitney as appropriate. One-way ANOVA or the Kruskal-Wallis test were used when there was more than one category. Categorical variables were analysed using chi-square or Fisher's exact test. Multivariate regression was used to determine independent factors associated with development of an ALT flare. A two-sided p value of 0.05 was considered statistically significant.

Ethics

The parent study was conducted following approval from independent ethics committees or institutional review boards at all participating study sites (NCT00116805). Informed consent was obtained for the parent study but was not required for the current data analysis.

Results

There were 54 genotype A subjects who received a minimum of 48 weeks of treatment (median = 384 weeks [IQR: 264-384]). The characteristics of the cohort are described in

198 Table 1 and Table S1. All subjects were HBeAg-positive. Patients were classified according to
199 their HBsAg response - twenty-four (45%) subjects achieved a significant HBsAg decline and
200 of these, 16 (30% of the overall cohort) went on to achieve HBsAg loss. HBsAg loss was
201 observed at a median of 102 weeks of treatment [IQR: 64-156]. Thirty (55%) subjects were
202 HBsAg non-responders at the end of follow up (Table 1). Age, gender, ethnicity, liver
203 histology (fibrosis stage / necro-inflammatory grade), treatment allocation, and median
204 baseline ALT level were comparable across the three HBsAg response groups. Baseline
205 median levels of HBV DNA, HBeAg, and HBsAg were lower in the HBsAg non-responder
206 group, and these subjects were more likely to be infected with PC-BCP variant virus, as
207 previously described (15).
208
209 ***On-treatment ALT flares were common in patients with genotype A HBV infection treated***
210 ***with nucleos(t)ide analogues***
211 An on-treatment ALT flare was experienced by 23 (43%) subjects (Table 2, Figure S1, and
212 Table S3), with the median peak ALT in this group being 203 [IQR: 97-457] IU/L. Six of the 23
213 had a flare defined by an ALT >2x baseline vs. 17 of the 23 defined by an ALT 2x the upper
214 limit of normal (Table S4). The median time to ALT flare was 40 weeks [IQR: 8-44]. ALT flares
215 occurred earlier in patients treated with TDF (median 16 weeks [IQR: 8-40] compared to
216 ADV treated patients (median 42 weeks [IQR: 40-56], p-value vs. TDF = 0.002). All patients
217 who flared were viraemic at the time of the flare; of note, the later flares in ADV treated
218 subjects were associated with a mean HBV DNA of 4.89 $\log_{10}$ IU/ml [sd: 0.82] despite more
219 than 24 weeks treatment duration. HBV DNA levels were <2,000 IU/mL by week 24 in all
220 TDF-treated subjects, and at the time of flare were at a mean of 3.07 $\log_{10}$ IU/ml [sd:1.53].
221
222 ***Higher baseline viraemia was associated with an increased likelihood of an on-treatment***
223 ***ALT flare***
224 Univariate logistic regression was used to determine clinical factors associated with ALT
225 flare (Table S5). Variables included were age, gender, body mass index, histologic fibrosis
226 and inflammatory scores, ethnicity, treatment arm, detectable baseline PC-BCP variants,
227 and baseline levels of HBV DNA, HBeAg, and HBsAg. Virologic factors including infection
228 with wildtype virus (p=0.03), and levels of HBV DNA (p=0.005), HBeAg (p=0.009), and HBsAg
229 (p=0.008) were associated with ALT flare. These were entered into a multivariate logistic

regression model. Following stepwise elimination of non-significant variables, only baseline HBV DNA level remained significantly associated with an on-treatment ALT flare (p=0.005), with higher baseline HBV DNA level associated with increased likelihood of flare (OR 3.75 per $\log_{10}$ IU/ml increase [95% CI: 1.48-9.53]).

On-treatment ALT flares were associated with HBsAg response

The pre-specified composite end-point for the primary analysis was the association between on-treatment ALT flare and HBsAg response (HBsAg loss or significant HBsAg decline) vs. HBsAg non-response. ALT flares were more common in subjects who achieved HBsAg loss (9/16 [56%]) or significant HBsAg decline (7/8 [88%]) vs. HBsAg non-response (7/30 [23%]), p=0.049 and 0.002, respectively (Table 3). The median time to ALT flare among subjects who achieved HBsAg loss was 14 weeks [IQR: 8-42]. The median time to HBsAg loss was 102 weeks [IQR: 64-156]. There were 7 HBsAg loss subjects who were not observed to have an ALT flare. Three subjects had no observable fluctuations in ALT and four subjects had elevations in ALT that coincided with control of viraemia but did not meet study criteria for an ALT flare. One of these four subjects, who was TDF-treated, experienced a sustained elevation in ALT between 1-2x the upper limit of normal for 40 weeks of treatment (Subject 5703, Figure S3).

Seven (88%) of the eight HBsAg decline subjects experienced a study defined ALT flare. Five of these subjects were in the TDF only arm, and two were in the ADV-TDF arm. The median time of flare was at 22 weeks [IQR: 8-44]. The mean baseline HBsAg level in the HBsAg decline subjects was 5.10 [SD: 0.33] $\log_{10}$ IU/ml (Table 1), and levels declined to their nadir by a mean of 2.09 [SD: 0.73] $\log_{10}$ IU/ml.

HBeAg seroconversion was common, both in subjects who achieved an HBsAg response and non-response (HBsAg loss / HBsAg decline: 21/24 (88%) vs. HBsAg non-response: 23/30 (77%)). However, there was no association between the occurrence of an on-treatment ALT flare and the attainment of HBeAg seroconversion (50% vs 41%, p = 0.73). Additionally, the HBsAg response group experienced a significantly greater median decline in HBsAg levels 24 weeks post-flare (1.34 $\log_{10}$ IU/ml [IQR: 0.82-2.31] vs 0.12 $\log_{10}$ IU/ml [IQR: 0.03-0.31] amongst non-responders, p=0.002). The magnitude of the week 24 post-flare decline

correlated strongly with the peak flare ALT (Pearson's r=0.75, p<0.0001). Accelerated HBV DNA decline was not observed post flare. An ALT flare appeared to result in a more rapid reduction in HBsAg when compared to those who did not flare (Figure 1).

Peak ALT level was higher in subjects who achieve HBsAg loss

Among subjects who experienced an ALT flare, the peak ALT level was significantly higher in those who achieved HBsAg loss. Median ALT in this group was 457 IU/L (IQR: 254 – 627) vs. HBsAg decline (median ALT 203 IU/L [IQR: 97 – 278]) and non-responders (median ALT 104 IU/L [IQR: 68 – 149]) (Figure 2).

ALT flares were independently associated with HBsAg response

Bivariate regression was performed on all baseline clinical factors, as well as the occurrence of an on-treatment ALT flare, to determine which variables were associated with an HBsAg response. ALT flare, HBV DNA level (p=0.002), HBeAg level (p<0.0001), HBsAg level (p=0.002), and infection with PC-BCP wildtype virus (p=0.001) were associated with HBsAg loss. After multivariate regression with stepwise elimination of non-significant predictors, only baseline HBeAg level (p=0.003) and the occurrence of an on-treatment ALT flare (p=0.02) were associated with an HBsAg response.

ALT flares associated with HBsAg loss occurred later in ADV vs TDF treated patients

Of the 9 patients who achieved HBsAg loss, 5 were TDF treated, and 4 were ADV-TDF switch treated. HBsAg seroconversion (ie, development of anti-HBs) was seen in 12 (75%) of the 16 HBsAg loss subjects; of the four who did not, one had an ALT flare, whereas three did not. The median time to ALT flare was 14 weeks [IQR: 8-42] in subjects who achieved HBsAg loss. TDF treated subjects had early flares at ≤16 weeks with rapid control of viraemia, observed in four subjects (Figure 3). The fifth TDF treated patient (6204) had a lower degree of necroinflammation at baseline, and a less acute decline in viraemia, and reached criteria for an ALT flare later at week 40 (Figure 4). This flare was lower in magnitude. In contrast, the four ADV-TDF treated subjects had persistent viraemia greater than 5 $\log_{10}$ IU/ml of DNA at the last level measured prior to a flare, in keeping with the reduced potency of ADV. Flares were seen between 40 and 56 weeks of treatment (Figure 5) in those who had a slow ADV response. HBV DNA was higher at both week 4 (6.93 $\log_{10}$ IU/ml [sd: 0.72] vs 5.32 $\log_{10}$

294 IU/ml [sd: 0.84], p=0.002) and at week 12 (6.59 $\log_{10}$ IU/ml [sd:0.77] vs 4.46 $\log_{10}$ IU/ml
295 [sd:1.25], p=0.003) in these subjects.
296
297 ***There was a significant lag period between ALT flare and HBsAg loss***
298 ALT flare preceded HBsAg decline in all study subjects. In those who achieved HBsAg loss,
299 this response was seen at a median of 102 weeks of treatment [IQR: 64-156]. There was a
300 significant time delay of a median of 56 weeks [IQR: 40-80 weeks] between ALT flare and
301 HBsAg loss (Figure S2). Reduction in levels by at least 1 $\log_{10}$IU/ml in the HBsAg decline
302 group, however, occurred soon after a flare (median 12 weeks [IQR:8-26], Figure S2).
303
304 ***ALT flares in non-responder subjects preceded HBV DNA suppression***
305 Seven (23%) of the non-responder subjects experienced an ALT flare during treatment
306 (Figure S4 and Table S1). ALT flare occurred at a median of 24 weeks [IQR: 4-48] of
307 treatment in this group. As noted, the peak ALT level in this population was significantly
308 lower than in HBsAg responders (104 IU/L vs 457 IU/L / 203 IU/L, respectively, Figure 2).
309 Although HBsAg decline was not observed, in 6/7 of these subjects, the ALT flare occurred
310 just prior to HBV DNA suppression below the limit of detection (Figure S4).
311
312 ***Infection with HBV variants at the PC and/or BCP region is associated with a reduced***
313 ***likelihood of an ALT flare and HBsAg response***
314 We have previously observed a negative relationship between PC-BCP variants and the
315 achievement of HBsAg loss in this cohort (15). We were therefore interested in whether
316 HBV variants might be associated with resistance to the occurrence of an ALT flare, or with
317 resistance to HBsAg decline in the event of an ALT flare. Population sequencing of the PC-
318 BCP region, as well as the S-ORF (open reading frame) was successful for 52 of the 54
319 subjects. Thirty (58%) subjects were found to be infected with wildtype HBV, whereas 22
320 (42%) harboured PC-BCP variants. ALT flares occurred less often in subjects with PC-BCP
321 variants vs. wildtype HBV infection (56% vs. 76%, p=0.04, Table 3). When an ALT flare
322 occurred, there was a trend for PC-BCP-infected subjects to be less likely to achieve an
323 HBsAg response (2/5 vs. 13/16, p=0.12). We also tested for an association between variants
324 in the S ORF and HBsAg response. However, when S ORF sequence data for all these
325 genotype A subjects were aligned, no variants were detected.

Discussion

This is the first study to examine in detail the occurrence of ALT flares during nucleos(t)ide analogue therapy and their association with HBsAg kinetics in genotype A patients. We have demonstrated that ALT flares occur frequently in this cohort and predict for subsequent HBsAg decline and HBsAg loss.

On-treatment ALT flare was observed in 43% of genotype A infected HBV subjects. ALT flare was more common among subjects who achieved HBsAg loss or HBsAg decline (67%) vs. HBsAg non-response (23%). Peak ALT flare was higher in subjects who achieved an HBsAg response than in HBsAg non-response, and the post-flare viral kinetics were more pronounced, indicating that the magnitude of the flare may correlate with the strength of the underlying antiviral immune response. Those who experienced a flare had a less pronounced suppression of HBV DNA in response to treatment, particularly amongst the ADV group who were generally slow responders to treatment. This is consistent with a necessary role for persistent virion stimulation as a trigger for ALT flare. ALT flares occurred in the first year of treatment and were seen earlier in patients randomized to TDF (median 16 weeks) than ADV (median 44 weeks). In ADV treated subjects, flares were associated with a mean HBV DNA of 4.89 $\log_{10}$ IU/ml [sd: 0.82] despite more than 24 weeks treatment duration.

A prolonged lag period was observed between ALT flare and HBsAg loss (median 56 weeks). We hypothesize that ALT flare achieves HBV control, with eventual HBsAg loss occurring through hepatocyte turnover. This concept should be considered in future prospective studies aiming for HBV cure. The lag period has practical implications for clinical trial design of immunomodulatory cure strategies aiming to enhance the anti-HBV immune response to achieve HBsAg loss – prolonged follow-up periods will be required to capture HBsAg loss itself.

It has previously been recognised that HBsAg loss is more common in genotype A infection in patients treated with nucleos(t)ide analogues, similar to treatment with pegylated interferon (16). In association with this, we have shown that on-treatment ALT flares are also common. Additionally, ALT flares are more common in HBsAg loss subjects than in non-

responders (17, 18). This may indicate that the strength of the immune response and / or the degree of immune recovery is greater in these patients and this is what is necessary to achieve immunologic control of chronic hepatitis B. Unfortunately, immunological studies were not possible in this cohort. Future translational studies will be required to interrogate the antiviral mechanisms driven by ALT flare and should also compare the differences between genotype A vs. non-genotype A-infected patients.

HBsAg loss was also independently associated with higher HBeAg levels in this study. This was independent of the presence of PC-BCP variants. HBeAg has been shown to have an immunomodulatory effect in chronically infected subjects, attenuating innate immune antiviral responses (19). It could, therefore, be hypothesised that a higher level of HBeAg results in greater immune paresis, and that the subsequent reduction of HBeAg during treatment allows an effective immune response to occur. This raises the possibility of therapeutic approaches designed to rapidly reduce circulating HBeAg and could be used as an adjunctive therapy to new treatments (20).

A subset of subjects who achieved functional cure did so without an ALT flare. This may have simply been due to a missed flare. Alternatively, this could be explained by the non-cytolytic model of cytokine-mediated viral clearance, which would not manifest with an ALT flare (21-23). This mechanism is in contrast to the cytolytic model of adaptive CD8+ T cell-mediated HBV clearance via hepatocyte killing, which has been well described (24, 25). It is also likely that both mechanisms are at play concurrently, but perhaps the dominance of a given pathway results in the observed clinical presentation in an individual patient.

Not all subjects who experienced an ALT flare achieved an HBsAg response. This is likely to be multi-factorial. In general, the ALT flare in non-responders was lower level, perhaps associated with weaker antiviral effect. It may be that these HBV have developed as yet undefined mechanisms for 'immune escape' (26). Alternatively, HBsAg levels in these patients include virions, subviral particles, as well as HBsAg particles derived from integrated HBV. Current HBsAg assays cannot differentiate between these particles; integrated HBsAg is unlikely to be cleared by immune-mediated antiviral effect.

There are limitations to this study. Firstly, ALT is a crude marker of an anti-HBV immune response, and as such, these data do not allow speculation about mechanisms underlying a flare. Secondly, the overall number of subjects was small, and limited to HBeAg-positive, genotype A-infected subjects. It should be noted, however, that this study has shown one of the highest reported rates of functional cure, and therefore provides a unique opportunity to examine and explore in detail factors that may be responsible for the development of cure, to inform future therapies and trial design. Importantly, however, factors leading to functional cure in HBeAg-negative disease, and in those infected with other genotypes, requires further study, as functional cure associated with nucleos(t)ide analogue therapy in these patients is rare.

In conclusion, we have demonstrated a strong association between on-treatment ALT flare and HBsAg decline and HBsAg loss in genotype A, HBeAg-positive HBV-infected subjects treated with long-term nucleos(t)ide analogues. The data suggest that ALT flares may be important for achieving HBsAg loss and support the evaluation of immunotherapeutic strategies to induce a therapeutic hepatitis flare.

Table 1 Baseline characteristics of the study cohort, stratified according to end-of-study HBsAg response.

	Overall	Non-Response	HBsAg Decline (≥1 $\log_{10}$ IU/mL)	HBsAg Loss	*p*-value
n (%)	54 (100)	30 (55.6)	8 (14.8)	16 (29.6)	-
Weeks of Follow Up, med (IQR)	384 (264-384)	384 (300 – 384)	384 (294 – 384)	350 (228 – 384)	0.38
Age, med (IQR)	39 (25-50)	37 (24 – 47)	37 (26 – 43)	44 (36 – 52)	0.19
Males, n (%)	46 (85)	24 (80)	8 (100)	14 (88)	0.35
Ethnicity, n (%)					
White	43 (80)	21 (70)	7 (88)	15 (94)	0.14
Black	9 (17)	7 (23)	1 (13)	1 (6)	0.32
Asian	1 (2)	1 (3)	0 (0)	0 (0)	
BMI, med (IQR)	25 (22-29)	25 (21 – 29)	23 (22 – 24)	27 (23 – 29)	0.13
ADV-TDF Arm, n (%)	17 (31)	8 (27)	3 (38)	6 (38)	0.70
Ishak Fibrosis Score, med (IQR)	4 (2-6)	4 (2 – 6)	5 (3 – 6)	4 (3 – 6)	0.88
Cirrhosis, n (%)	17 (31)	10 (33)	3 (38)	4 (25)	0.49
Knodell Inflammatory Score, med (IQR)	9 (9-10)	9 (9 – 9)	9 (9 – 9)	9 (9 – 10)	0.53
Baseline ALT (IU/L), med (IQR)	109 (85-198)	106 (81 – 196)	134 (86 – 215)	117 (100 – 190)	0.69
Baseline HBV DNA (Log_{10} IU/mL), mean (sd)	7.98 (0.94)	7.64 (0.99)	8.73 (0.60)	8.23 (0.66)	**0.004**

Baseline HBeAg (Log_{10} PEIU/mL), mean (sd)	2.96 (0.89)	2.57 (1.00)	3.53 (0.12)	3.39 (0.42)	**0.0008**
Baseline HBsAg (Log_{10} IU/mL), mean (sd)	4.62 (0.61)	4.40 (0.56)	5.10 (0.33)	4.80 (0.62)	**0.004**
Wildtype HBV by Population Sequencing, n (%)	30 (58)	11 (38%)	7 (88%)	12 (80%)	**0.005**

Table 2 There were statistically significant differences between the HBsAg responses when comparing the group who had an ALT flare vs. those who did not. The differences were significant for both the composite outcome of any HBsAg response, as well as the individual components of HBsAg decline or HBsAg loss, as compared to non-responders.

	Overall N=54	ALT Flare N=23	No ALT Flare N = 31	p-value (vs. non-response)
HBsAg response, n (%) (HBsAg loss + HBsAg decline ≥1 log_{10})	24 (45%)	16 (69%)	8 (26%)	0.0019
HBsAg loss, n (%)	16 (30%)	9 (39%)	7 (23%)	0.049
HBsAg decline, n (%) (HBsAg decline ≥1 log_{10})	8 (15%)	7 (30%)	1 (3%)	0.002
HBsAg non-response, n (%) (HBsAg decline <1 log_{10})	30 (56%)	7 (30%)	23 (71%)	--

Table 3 Wildtype HBV infection is associated with an increased likelihood of an on-treatment ALT flare (p=0.04)

	No Flare	Flare	Total
PC and/or BCP Variant, n (%)	17 (55)	5 (24)	22 (42)
Wildtype, n (%)	14 (45)	16 (76)	30 (58)
Total	31	21	52

Figure Legends

Figure 1 An ALT flare is likely to result in a rapid decline in HBsAg, as compared to those who did not flare. Each bar represents the decline in HBsAg for an individual subject. In subjects who had a flare, decline is between HBsAg at time of flare and 24 weeks post-flare. In subjects without an ALT flare, change in HBsAg is the average decline between baseline to week 72 separated into 3 intervals (week 0-24, week 24-48, and week 48-72) (13)

Figure 2 Box plot of peak ALT in those experiencing an on-treatment flare (n=23) by HBsAg response. There was no statistically significant difference between the HBsAg decline and HBsAg loss groups.

Figure 3 Graph depicting mean ALT changes over time for the first 48 weeks of treatment, separated by treatment group, and comparing complete HBsAg responders to the rest of the cohort. Crosses indicate a statistically significant difference compared to all other groups. Error bars indicate SEM.

Figure 4 Individual subject plots over the first 144 weeks of treatment for those in the TDF treatment cohort who achieved HBsAg loss following an ALT flare. Subject 3058 lost HBsAg at week 180 but did not achieve on-study HBsAg seroconversion. Subject 4318 achieved HBsAg seroconversion at week 152.

Figure 5 Individual subject plots over the first 144 weeks of treatment for those in the ADV-TDF switch treatment cohort who achieved HBsAg loss following an ALT flare.

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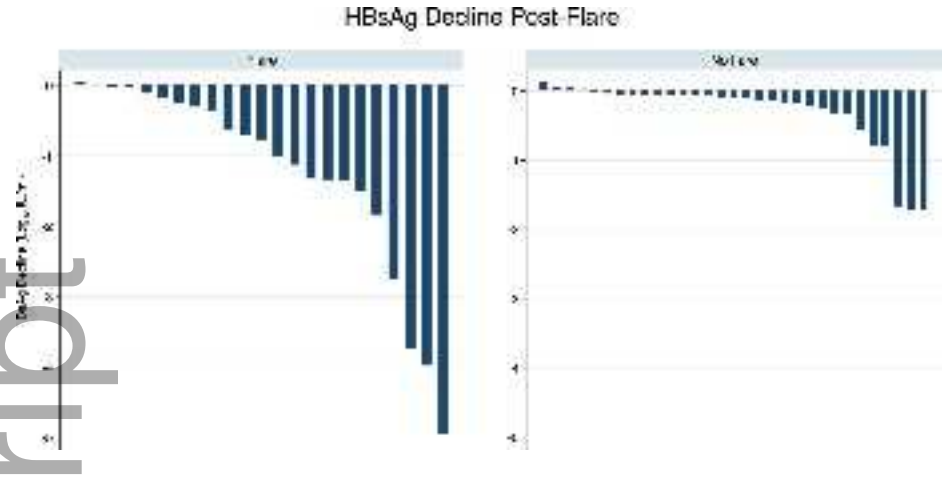

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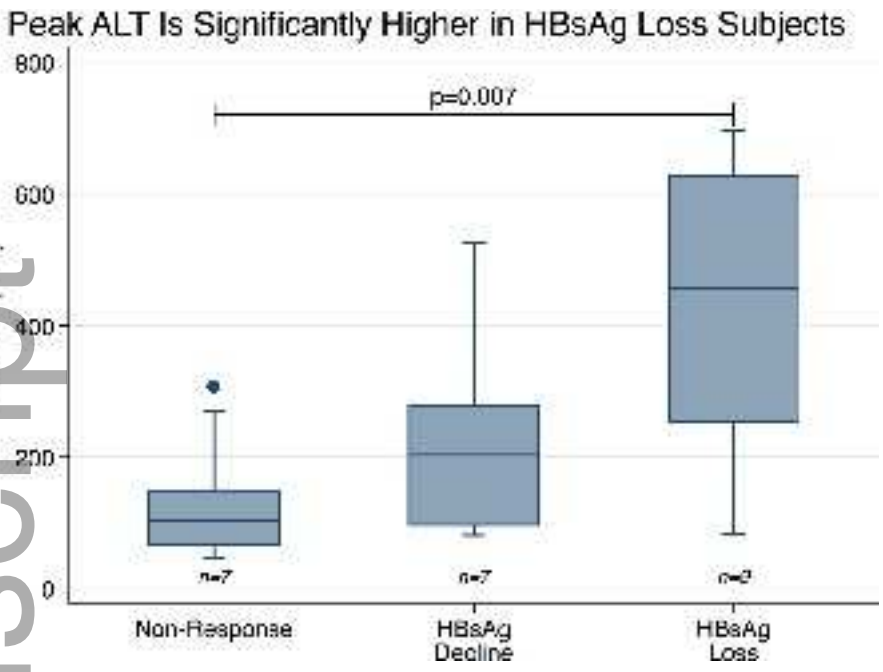

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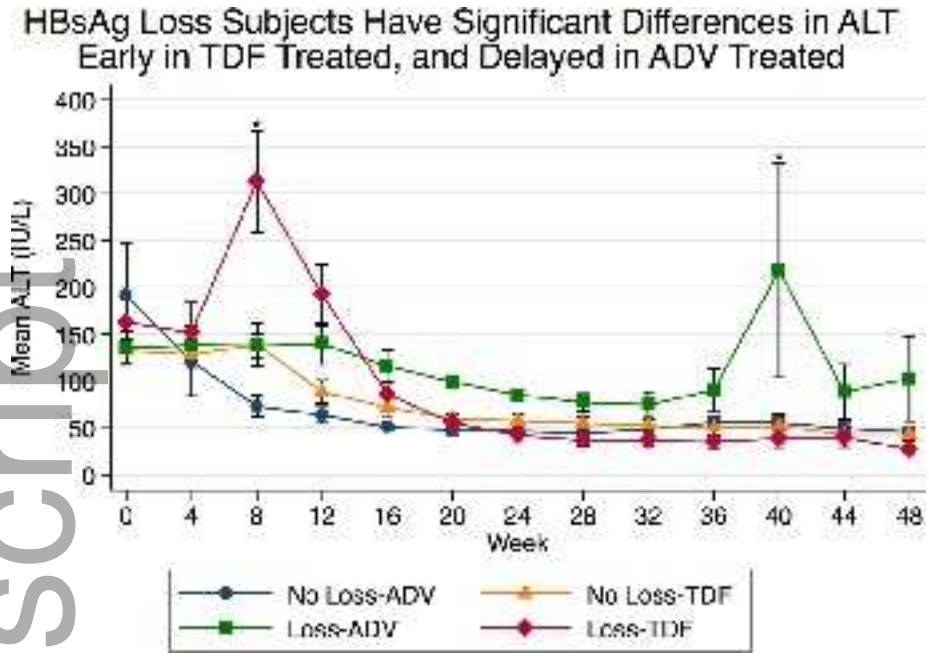

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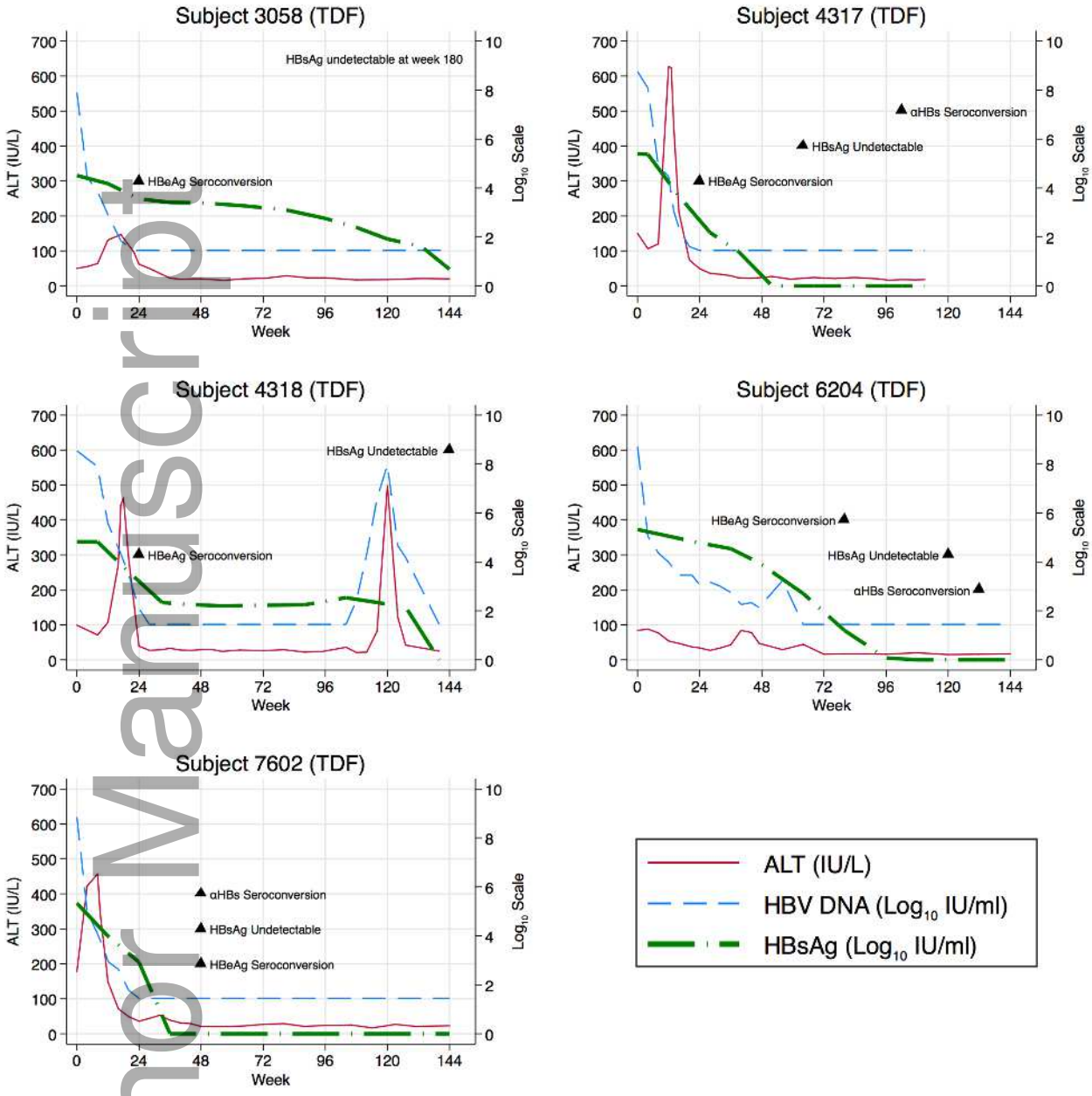

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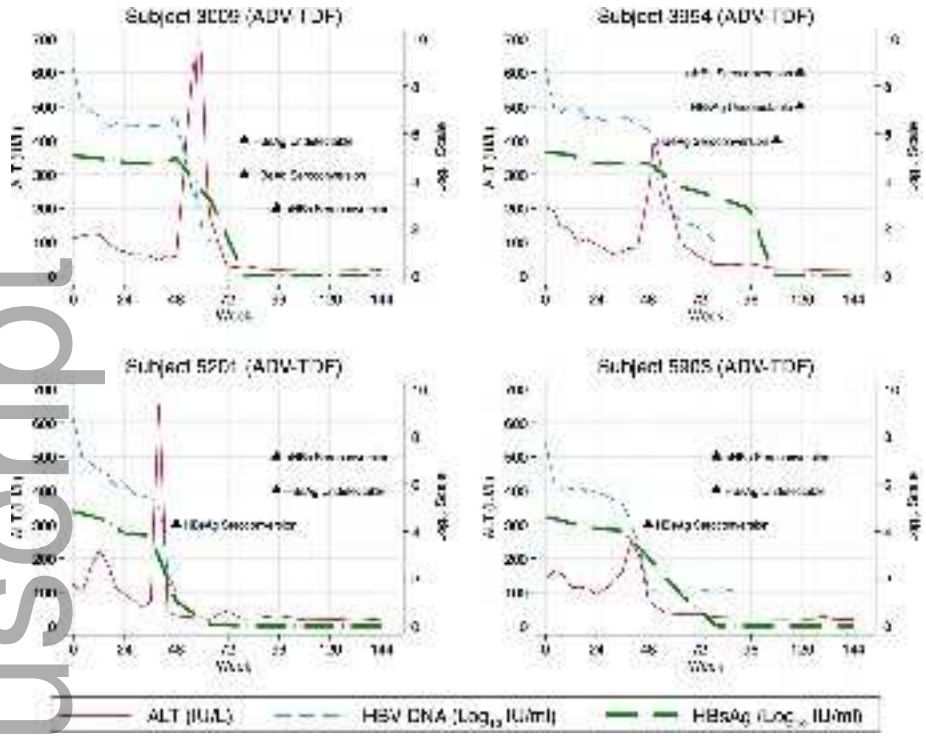

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