Transcriptomic Profiling Reveals Distinct Immune Signatures in Chronic Hepatitis B and Identifies Blood Transcriptional Modules Associated With Hepatitis B e Antigen Loss Following Therapeutic Vaccination With GS-2829 and GS-6779

WED-578

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Viral Hepatitis B and D: New Therapies, Unapproved Therapies or Strategies

Conclusions

- Therapeutic vaccination with GS-2829 and GS-6779 elicited consistent whole-blood transcriptional immune signatures in healthy participants (HPs) and patients with chronic hepatitis B (CHB)
- Compared with HPs, patients with CHB exhibited a distinct immune signature characterised by a reduced breadth of adaptive signatures and persistence of innate/myeloid programmes
- Among patients with CHB, hepatitis B e antigen (HBeAg) loss is associated with stronger and more durable adaptive immune transcriptional signatures, suggesting that successful antigen control aligns with enhanced T-, B-, and natural killer (NK)-cell transcriptional activity
- Among others, *STAT4, CNBP,* and *KLRC1* were significantly upregulated in patients who achieved HBeAg loss compared with those who did not, with expression levels showing a positive trend with the magnitude of HBeAg decline
- Together, these results support earlier findings that GS-2829 and GS-6779 can stimulate hepatitis B virus (HBV)-specific immune responses in patients with CHB, provide mechanistic insights into immune pathways associated with HBeAg loss, and may inform the design of future functional cure strategies incorporating therapeutic vaccination

Plain Language Summary

- This study tested a prime-boost therapeutic vaccination regimen using GS-2829 and GS-6779 given as alternating injections to healthy participants and patients with chronic hepatitis B
- The goal of this treatment is to help the immune system to better recognise and control the hepatitis B virus, with the aim of achieving a “functional cure,” meaning that the virus remains undetectable in the absence of treatment
- Some patients who received GS-2829 and GS-6779 lost hepatitis B e antigen (a marker of active viral replication) and showed changes in immune markers, suggesting that their immune response better recognized the virus
- These results support earlier findings that GS-2829 and GS-6779 can stimulate immune responses specifically targeting hepatitis B virus

References: **1.** World Health Organization. Global hepatitis report. 2017. Accessed March 9, 2026. https://apps.who.int/iris/rest/bitstreams/1082592/retrieve. **2.** Guidelines for the prevention, diagnosis, care and treatment for people with chronic hepatitis B infection. Geneva: World Health Organization; 2024:53. **3.** Park PG, et al. *Clin Exp Vaccine Res.* 2024;3:21-7. **4.** Gane EJ, et al. Poster at The International Liver Congress; May 7–10, 2025; Amsterdam, the Netherlands. Poster THU-246. **5.** Gane EJ, et al. Poster at AASLD: The Liver Meeting; November 7–11, 2025; Washington, DC, USA. Poster 0197. **6.** Li S, et al. *Nat Immunol.* 2014;15(2):195-204. **7.** Finotello F, et al. *Genome Med.* 2019;11:34. **8.** Chen H, et al. *Clin Gastroenterol Hepatol.* 2020;18:196-204.

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Introduction

- Worldwide, CHB continues to pose a major therapeutic challenge, as existing approved treatments rarely achieve functional cure,[1] characterised as sustained loss of hepatitis B surface antigen (HBsAg) and suppression of HBV DNA below the lower limit of quantitation (LLOQ) at 24 weeks following discontinuation of therapy[2]
- HBV therapeutic vaccination aims to generate or boost preexisting HBV-specific immunity in patients with CHB towards the goal of achieving functional cure[3]
- Arenaviral-vectored vaccines GS-2829 and GS-6779, which encode multiple conserved HBV antigens, induced HBV-specific T-cell responses and HBeAg loss in a subset of Phase 1b study patients with CHB and induced robust T-cell responses, along with high titres of HBsAg antibodies and no anti-vector neutralisation, in HPs[4,5]

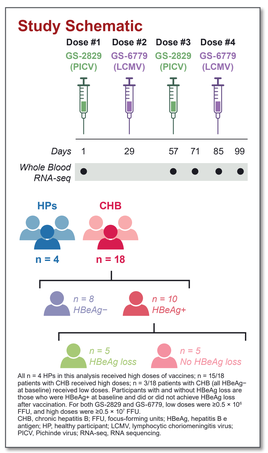
Objective

- To characterise and compare whole-blood transcriptional signatures between HPs and patients with CHB following vaccination, and to elucidate immune mechanisms associated with HBeAg loss

Methods

- Transcriptomic analysis was performed on samples from a subset of HPs and patients with CHB who received GS-2829 and GS-6779 in a Phase 1a/1b study (NCT05770895)
- Patients included in the Phase 1b study had virally suppressed CHB, HBsAg >LLOQ and ≤5000 IU/mL, and alanine aminotransferase <3 × the upper limit of normal, with no cirrhosis
- Whole-blood samples were collected at baseline (day 1), on the day of the third and fourth immunisations (days 57 and 85), and 2 weeks after each (days 71 and 99)
- Whole-blood RNA sequencing was performed to compare transcriptional responses between HPs and patients with CHB
- Differential expression and gene set enrichment analyses (|normalised enrichment score| ≥2; false discovery rate <0.05) using blood transcriptional modules (BTMs) were conducted at postvaccination time points relative to baseline (day 1) as previously described[6]

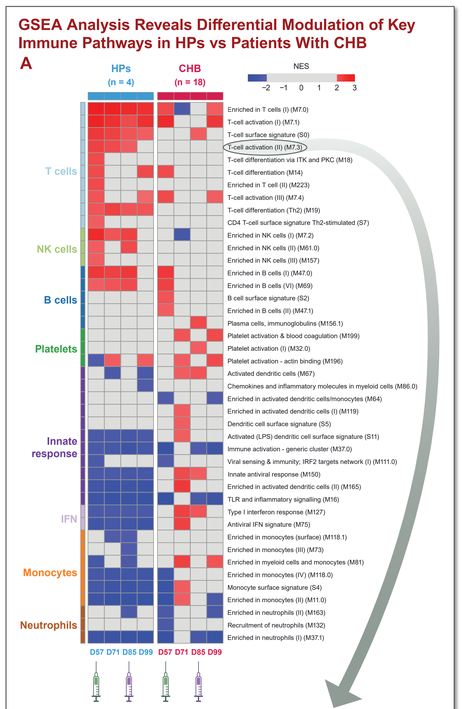
Study Schematic



All n = 4 HPs in this analysis received high doses of vaccines; n = 15/18 patients with CHB received high doses; n = 3/18 patients with CHB (all HBeAg− at baseline) received low doses. Participants with and without HBeAg loss are those who were HBeAg+ at baseline and did or did not achieve HBeAg loss after vaccination. For both GS-2829 and GS-6779, low doses were ≥0.5 × 10^5 FFU, and high doses were ≥0.5 × 10^7 FFU.
CHB, chronic hepatitis B; FFU, focus-forming units; HBeAg, hepatitis B e antigen; HP, healthy participant; LCMV, lymphocytic choriomeningitis virus; PICV, Pichinde virus; RNA-seq, RNA sequencing.

Results

GSEA Analysis Reveals Differential Modulation of Key Immune Pathways in HPs vs Patients With CHB

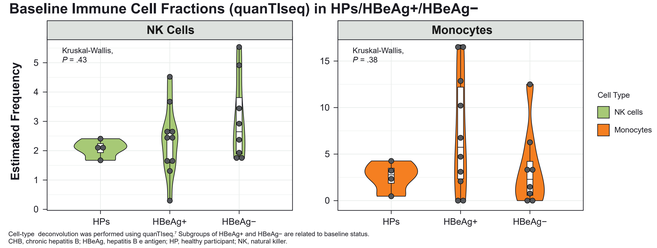


(A) GSEA using a pre-ranked gene list. BTMs with |NES| ≥2 and FDR <0.05 are reported. Different BTMs are functionally grouped and colour-coded by superannotation. Green injections indicate GS-2829 (PICV) and purple injections indicate GS-6779 (LCMV).
(B) Gene networks coloured red (up) and blue (down) indicate log$_2$ FC at D71.
BTM, blood transcriptional module; CD, cluster of differentiation; CHB, chronic hepatitis B; D, day; FC, fold change; FDR, false discovery rate; GSEA, gene set enrichment analysis; HP, healthy participant; IFN, interferon; IRF2, interferon regulatory factor 2; ITK, inducible T-cell kinase; LCMV, lymphocytic choriomeningitis virus; LPS, lipopolysaccharide; NES, normalised enrichment score; NK, natural killer; PICV, Pichinde virus; PKC, protein kinase C; Th2, T helper 2 cell; TLR, toll-like receptor.

- Vaccination with GS-2829 and GS-6779 induces broader and more persistent enrichment of T-cell– and NK-cell–associated transcriptional signatures in HPs compared with patients with CHB
- In HPs, dendritic cell–, interferon-, and monocyte-related BTMs consistently contract at the time of T-cell enrichment following vaccination, whereas in patients with CHB, these signatures do not show a comparable decline and rather appear enriched on day 71, indicating differences in innate-adaptive response patterns between groups

Cell-Type Deconvolution Analysis Suggests Potential Enrichment and Heterogeneity in NK-Cell and Monocyte Subsets Before Vaccination in Patients With CHB vs HPs

Baseline Immune Cell Fractions (quanTIseq) in HPs/HBeAg+/HBeAg−

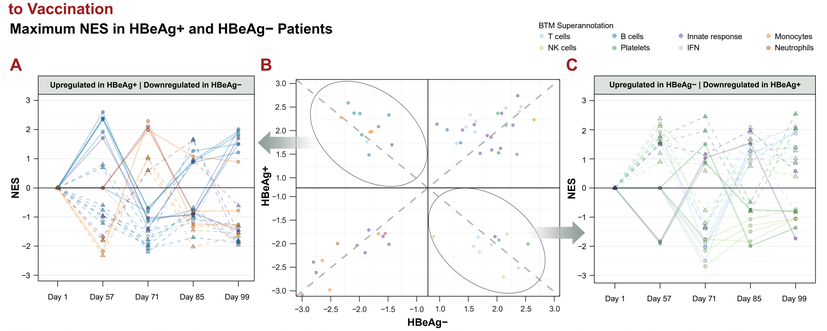


Cell-type deconvolution was performed using quanTIseq.[7] Subgroups of HBeAg+ and HBeAg− are related to baseline status.
CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HP, healthy participant; NK, natural killer.

- Cell-type deconvolution indicates potential enrichment of NK-cell and monocyte populations with greater interindividual variability among patients with CHB compared with HPs at baseline
- Expression patterns of selected NK-cell and monocyte marker genes are consistent with these enrichment trends and suggest potential baseline differences across HPs and CHB subgroups, albeit no statistically significant differences were observed for individual marker genes (data not shown)

HBeAg+ and HBeAg− Patients at Baseline Exhibit Overlapping Yet Distinct Transcriptional Responses to Vaccination

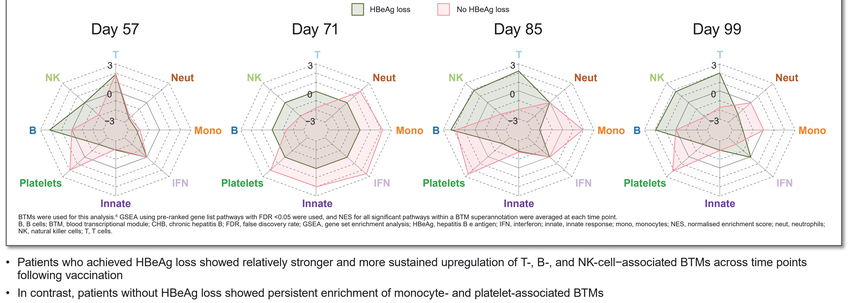
Maximum NES in HBeAg+ and HBeAg− Patients



(B) BTMs were used for this analysis.[8] Maximum NES score is defined by maximum absolute distances from 0 at any postbaseline time point with FDR <0.05 at the time point with the maximum value for one or both of the HBeAg+ and HBeAg− groups. Circles represent individual BTMs.
(A and C) Longitudinal NES score are represented by solid lines with circles for HBeAg+ patients and dashed lines with triangles for HBeAg− patients.
BTM, blood transcriptional module; FDR, false discovery rate; HBeAg, hepatitis B e antigen; IFN, interferon; NES, normalised enrichment score; NK, natural killer.

- Several BTMs related to T, B, and NK cells were enriched in both HBeAg+ and HBeAg− patients
- However, some B-cell–, innate-, and monocyte-related BTMs reached higher peak enrichment (maximum normalised enrichment score [NES]) in HBeAg+ compared to HBeAg− patients (panel **B**, upper left)
- In contrast, a subset of platelet- and NK-cell–related transcriptional responses showed higher peak enrichment (maximum NES) in HBeAg− compared to HBeAg+ patients (panel **B**, lower right)
- Overall, longitudinal NES trajectories suggest that these differences reflect variation in transcriptional response magnitude and timing between HBeAg+ and HBeAg− patients

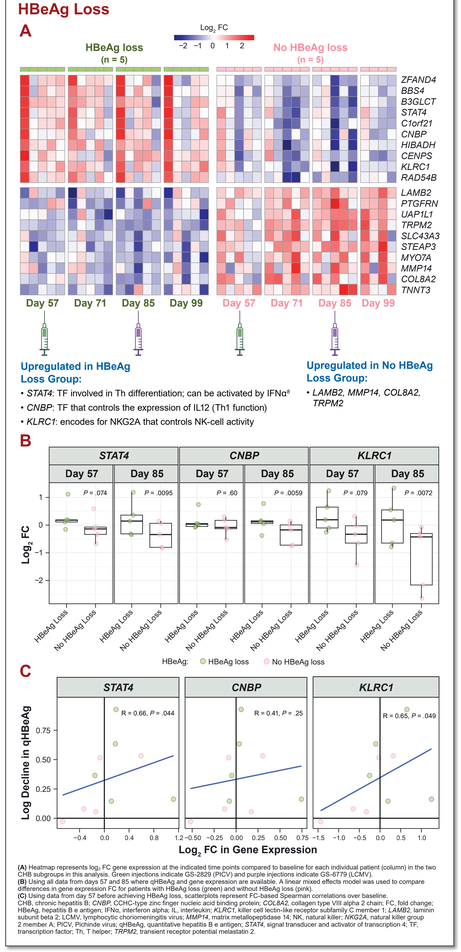
HBeAg+ Patients With CHB Who Achieved HBeAg Loss Showed Enhanced and More Durable Adaptive Immune Responses Following Vaccination



BTMs were used for this analysis.[8] GSEA using pre-ranked gene list pathways with FDR <0.05 were used, and NES for all significant pathways within a BTM superannotation were averaged at each time point.
B, B cells; BTM, blood transcriptional module; CHB, chronic hepatitis B; FDR, false discovery rate; GSEA, gene set enrichment analysis; HBeAg, hepatitis B e antigen; IFN, interferon; innate, innate response; mono, monocytes; NES, normalised enrichment score; neut, neutrophils; NK, natural killer cells; T, T cells.

- Patients who achieved HBeAg loss showed relatively stronger and more sustained upregulation of T-, B-, and NK-cell–associated BTMs across time points following vaccination
- In contrast, patients without HBeAg loss showed persistent enrichment of monocyte- and platelet-associated BTMs

Distinct Transcriptional Programmes Following Vaccination Distinguish Patients With vs Without HBeAg Loss



(A) Heatmap represents log$_2$ FC gene expression at the indicated time points compared to baseline for each individual patient (column) in the two CHB subgroups in this analysis. Green injections indicate GS-2829 (PICV) and purple injections indicate GS-6779 (LCMV).
(B) Using all data from days 57 and 85 where qHBeAg and gene expression are available. A linear mixed effects model was used to compare differences in gene expression FC for patients with HBeAg loss (green) and without HBeAg loss (pink).
(C) Using data from day 57 before achieving HBeAg loss, scatterplots represent FC-based Spearman correlations over baseline.
CHB, chronic hepatitis B; CNBP, CCHC-type zinc finger nucleic acid binding protein; COL8A2, collagen type VIII alpha 2 chain; FC, fold change; HBeAg, hepatitis B e antigen; IFNα, interferon alpha; IL, interleukin; KLRC1, killer cell lectin-like receptor subfamily C member 1; LAMB2, laminin subunit beta 2; LCMV, lymphocytic choriomeningitis virus; MMP14, matrix metallopeptidase 14; NK, natural killer; NKG2A, natural killer group 2 member A; PICV, Pichinde virus; qHBeAg, quantitative hepatitis B e antigen; STAT4, signal transducer and activator of transcription 4; TF, transcription factor; Th, T helper; TRPM2, transient receptor potential melastatin 2.

- HBeAg loss was associated with sustained upregulation of immune genes *STAT4* and *KLRC1*, consistent with activation of interleukin-12 and interferon pathways
- *STAT4*, *CNBP*, and *KLRC1* expression on day 85 differentiated patients with CHB who achieved HBeAg loss from patients who did not achieve HBeAg loss (P <.01) and showed positive trends with the magnitude of HBeAg decline (R = 0.66, R = 0.41, and R = 0.65, respectively, on day 57) before achieving HBeAg loss
- Patients without HBeAg loss showed increased expression of genes linked to extracellular matrix remodelling and cellular stress pathways (eg, *LAMB2*, *MMP14*, *COL8A2*, *TRPM2*), suggesting persistence of tissue remodelling and innate inflammatory signals
- Baseline and on-treatment characteristics of HBeAg-positive patients and correlates of HBeAg loss following GS-2829 + GS-6779 vaccination are presented in poster WED-580