Abstract 3284

HB-201 and HB-202, an Arenavirus-Based Immunotherapy, Induces Tumor T Cell Infiltration in Patients With HNSCC and Other HPV16+ Tumors

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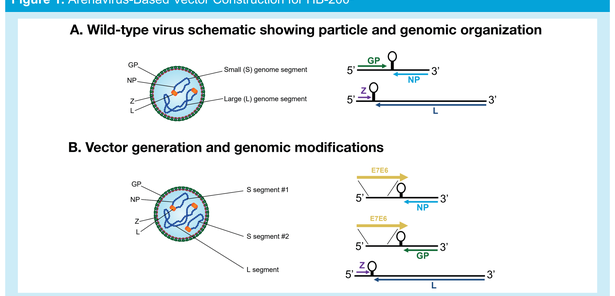
INTRODUCTION

- Human papillomavirus 16 (HPV16) is the most common etiologic agent of HPV-associated cancers.[1] Treatment options are limited for patients with recurrent or metastatic HPV16+ cancers, and the likelihood of long-term survival is low
- Generation and maintenance of HPV16+ cancers requires stable expression of HPV16 E7 and E6 oncoproteins, which are attractive immunotherapeutic targets[2,3]
- HOOKIPA developed HB-201 and HB-202, which are attenuated, replicating arenavirus vectors based on lymphocytic choriomeningitis virus (LCMV) and Pichinde virus (PICV), respectively, expressing the same non-oncogenic HPV16 E7E6 fusion protein (**Figure 1**) (see also **poster #2048**)[2]
- Previously, HB-201 as a single vector and HB-202/201 alternating 2-vector therapy demonstrated to induce potent circulating T cell responses and tumor control in preclinical models[2-5]
- Here, we present data from an ongoing Phase 1 dose-escalation study in patients with treatment-refractory HPV16+ cancers (**Figure 2**; NCT04180215).[6-8] Data from a first-in-human trial demonstrated that patients with HPV16+ head and neck squamous cell carcinoma (HNSCC) treated with single-vector therapy (HB-201) and alternating 2-vector therapy (HB-202/HB-201) rapidly mounted unprecedented levels of E6/E7-specific CD8+ T cells, which exhibited a polyfunctional profile with a clear shift from mono- to polyfunctionality upon repeated administration
- Additionally, characterization of tumor microenvironment (TME) showed a trend of increase in CD8+ T cells post HB200 treatment in conjunction with a decrease of HPV16 DNA

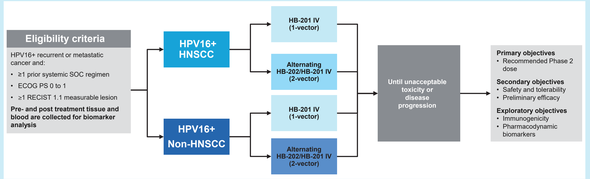
Figure 1. Arenavirus-Based Vector Construction for HB-200[a]



(A) Wild-type virus genomic organization. The large (L) segment encodes for the viral polymerase L and the matrix protein Z, whereas the short (S) segment carries the envelope glycoprotein (GP) and nucleoprotein (NP) genes, separated by intergenic regions. **(B)** HB-200 vector generation. Incorporation of E7E6 transgenic sequences into replicating arenavirus vectors by segregating the NP and GP genes onto artificially duplicated S segments. LCMV, lymphocytic choriomeningitis virus; PICV, Pichinde virus.

[a] HB-200 attenuated-replicating vectors: HB-201 LCMV; HB-202 PICV.

Figure 2. Phase 1 Dose-Escalation Study (NCT04180215)[6-8]



Dosing schedules assessed Q3/6W. Tumor tissue and blood samples (including serum and plasma) were collected during the study unless agreed otherwise between the sponsor and the investigator. Figure only shows IV treatment cohorts. This is the route of administration currently enrolling patients. IT/IV treatment cohorts included in the original study are no longer open for enrollment. ECOG, Eastern Cooperative Oncology Group; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; IT, intratumorally; IV, intravenous; PS, performance status; Q3/6W, every 3/6 weeks; RECIST, Response Evaluation Criteria in Solid Tumors; SOC, standard of care.

METHODS

Study Status

- As of November 1, 2021, 61 patients had been enrolled in the Phase 1/2 study (**Figure 2**)
  - **HB-201 monotherapy:** cohort 1, 5×10^5 replication-competent virus focus-forming units (RCV FFU; n=13); cohort 2, 5×10^6 RCV FFU (n=18); cohort 3, 5×10^7 RCV FFU (n=7). 76.3% were HNSCCs
  - **HB-201 and HB-202 alternating monotherapy:** cohort 1, HB-201 5×10^6 RCV FFU, HB-202 1×10^6 RCV FFU (n=9); cohort 2, HB-201 5×10^6 RCV FFU, HB-202 1×10^7 RCV FFU (n=5); cohort 3, HB-201 5×10^7 RCV FFU, HB-202 1×10^7 RCV FFU (n=5); cohort 4, HB-201 5×10^7 RCV FFU, HB-202 1×10^8 RCV FFU (n=4). 95.7% were HNSCCs
- E6/E7-specific T cells were evaluated in peripheral blood by direct ELISpot and intracellular cytokine staining (ICS) in 32 patients to quantitate and evaluate E6/E7-specific T cell polyfunctionality
- Tumor-infiltrating lymphocytes (TILs) were evaluated in tissue biopsies from 22 patients (6/22 had on-treatment biopsies) using multiplex immunofluorescence (IF) immunohistochemistry (IHC) Vectra Polaris®
- The ImmunoID NeXT® platform was used to conduct molecular profiling of pre- and on-treatment tissue biopsies by whole exome (WES) and RNA sequencing of 18 patients with matched normal blood; 5 had a post treatment biopsy

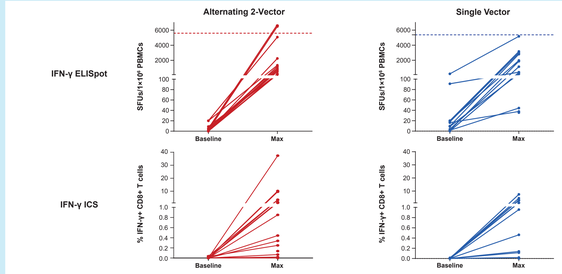
Note: Data are preliminary and include unmonitored data based on current reported electronic data capture, enrollment data, and scan reports as of November 1, 2021.

RESULTS

HB-200 Elicits Unprecedented Levels of Circulating E6/E7-Specific CD8+ T Cells

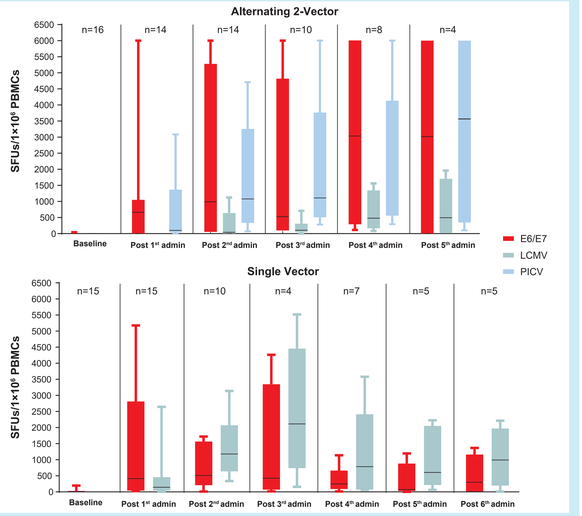
- Peripheral blood mononuclear cells (PBMCs) from patients treated with HB-201 single vector and HB-202/201 alternating vector therapy had a marked increase in antigen-specific T cells as measured by interferon (IFN)-γ ELISpot and ICS (**Figure 3**)
- Highest tumor-specific T cell responses of up to 40% of total CD8+ or >6000 spot-forming units (SFUs)/10^6 PBMCs were observed in patients treated with alternating therapy
- High E6/E7-specific responses in alternating 2-vector therapy are sustained after continual dosing and not overtaken by vector backbone responses (**Figure 4**)
- Proportion of polyfunctional CD8+ T cells, as measured by expression patterns of 4 key markers (tumor necrosis factor [TNF]-α, IFN-γ, interleukin [IL]-2, and CD107a), increased over time for both treatment regimens (**Figure 5**)

Figure 3. PBMCs From Patients Treated With Alternating 2-Vector Therapy Demonstrated Highest Tumor Specific T Cell Responses



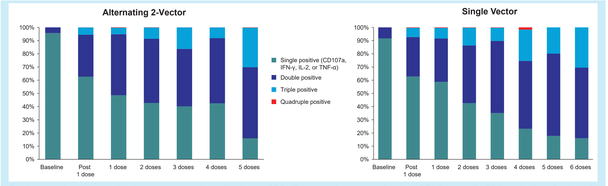
Line graphs represent E6/E7-specific T cells at baseline and maximal response, typically observed 2 to 3 weeks post first administration. **(Top)** Patient max response from baseline as obtained from ELISpot (x-axis: pretreatment and maximal response; y-axis: spot-forming units per 10^6 PBMCs). **(Bottom)** Patient max response from baseline as obtained from ICS (x-axis: pretreatment and maximal response; y-axis: % IFN-γ+ CD8+ T cells). n=16 per group. Dotted line = ULOQ. ICS, intracellular cytokine staining; IFN, interferon; PBMC, peripheral blood mononuclear cell; ULOQ, upper limit of quantification.

Figure 4. High E6/E7-Specific T Cell Responses in Alternating 2-Vector Therapy Are Sustained After Continual Dosing and Not Overtaken by Vector Backbone Responses



PBMCs from patients treated with alternating 2-vector therapy show robust E6/E7-specific responses compared to single vector therapy (**bottom**). Boxplots representing the distribution of ELISpot IFN-γ responses (E6/E7, LCMV-NP, and PICV-NP) over treatment cycles (x-axis: number of administrations; y-axis: spot-forming units [SFUs] per 10^6 PBMCs). **(Top)** HB-202/201 (n=16). **(Bottom)** HB-201 (n=16). Box and whiskers: 25th to 75th percentiles; min, max; median. IFN, interferon; LCMV, lymphocytic choriomeningitis virus; NP, nucleoprotein; PBMC, peripheral blood mononuclear cell; PICV, Pichinde virus.

Figure 5. Temporal Increase in Tumor-Specific Polyfunctional CD8+ T Cells



Stacked bar plots representing the mean proportions of activated CD8+ T cell populations as a fraction of E6/E7-specific CD8+ cells (ie, a fraction of cells positive for ≥1 tested markers TNF-α, IFN-γ, IL-2, and CD107a) in PBMCs of patients undergoing 2-vector and single-vector therapy, respectively. IFN, interferon; IL, interleukin; PBMC, peripheral blood mononuclear cell; TNF, tumor necrosis factor.

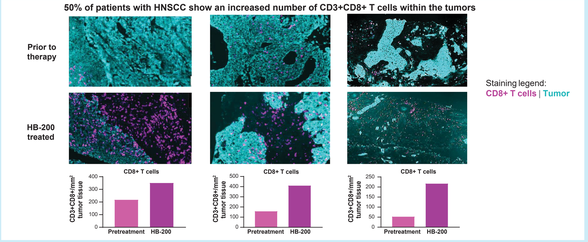
HB-200[a] increases the number of TILs and decreases the levels of HPV16 DNA

- 50% of patients with HNSCC had an increased number of CD8+ T cells in tumor tissue (**Figure 6**)
- 2/6 patients with disease control[b] showed an enrichment of CD8+ T cells in the tumor as obtained from immune cell deconvolution of RNASeq data (**Figure 7, left**)
- A decrease in normalized HPV16 DNA reads was observed in paired biopsy tumor samples post HB-200 per WES (**Figure 7, right**)

[a] HB-200 attenuated-replicating vectors: HB-201 LCMV; HB-202 PICV.

[b] Disease control rate (DCR): stable disease + partial reponse.

Figure 6. Increase in Tumor-Infiltrating Lymphocytes[a]



(Top) Tumor tissues were analyzed by Multiplex IF IHC Vectra Polaris and HALO quantification to determine expression and coexpression of immune markers (CD3, CD4, CD8, Foxp3, PD-L1, PanCK). Hematoxylin-eosin (H&E) staining was performed to assess tissue quality and tumor content for mIF quantification. **(Bottom)** Bar graphs represent number of CD8+ T cells per mm² tumor tissue area. HNSCC, head and neck squamous cell carcinoma; IF, immunofluorescence; IHC, immunohistochemistry; IV, intravenous; mIF, migration inhibition factor; PD-L1, programmed cell death 1 ligand 1; Q3W, every 3 weeks.

[a] Analysis of all available paired (pre- and post treatment) tumor biopsies from patients treated with HB-201 or HB-202/HB-201 IV Q3W intervals (n=6).

Figure 7. Molecular Profiling of Tumor Tissue Suggests an Increase in CD8+ T Cell Signature in Patients With Disease Control and a Decrease of HPV16 DNA

(Left) Boxplots represent the fold-change of CD8+ T cells between pre- and post HB-200 in patients with disease control (red) and progressive disease (blue) as obtained from RNA-seq data. **(Right)** Bar graphs visualize HPV16 normalized DNA reads detected in post treatment tumor (red) as obtained from whole exome sequencing.

[a] Disease control rate (DCR): stable disease + partial reponse.

CONCLUSIONS

- In a first-in-human trial in patients with HPV16+ advanced or metastatic cancers, HB-201 alone or alternating HB-202/201 showed:
  - A strong induction of circulating HPV16 E6/E7-specific polyfunctional CD8+ T cells of up to 40% of total CD8+ T cells
  - The highest tumor-specific T cell responses in patients who received alternating 2-vector therapy
  - High levels of E6/E7-specific T cells are maintained with alternating 2-vector therapy
  - An increase in polyfunctionality of HPV16 E6/E7 specific T cells over time
  - Increased CD8+ TILs in 50% of patients analyzed
  - A decrease in HPV16 DNA in the tumor
- HB-200 represents a powerful new modality in tumor immunotherapy for patients with treatment-refractory HPV16+ cancers

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