LB049

Preliminary Analysis of Immunogenicity of HB-201 and HB-202, an Arenavirus-Based Cancer Immunotherapy, in Patients With Advanced HPV16-Positive Cancers

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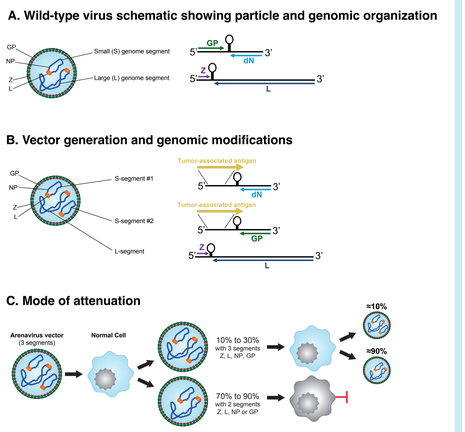
INTRODUCTION

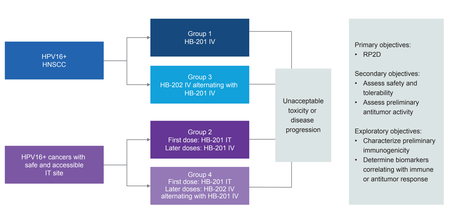
- The human papillomavirus (HPV), especially HPV type 16, is the cause of numerous solid tumors, such as cervical, head and neck, vaginal, and anal cancers[1]
- For patients with HPV16+ recurrent or metastatic cancers, treatment options are limited, and the likelihood of long-term survival is low
- HPV16-specific E7 and E6 oncogenes are attractive targets for immunotherapy because they are expressed in all HPV-infected cells and drive the cells' transformation into cancer cells[2,3]
- Therefore, HOOKIPA Pharma Inc. developed HB-201 and HB-202, which are attenuated, replicating arenavirus vectors based on lymphocytic choriomeningitis virus (LCMV) and Pichinde virus (PICV), respectively, expressing a nononcogenic HPV16 E7E6 fusion protein for induction of a HPV16+ tumor–specific immune response (**Figure 1**)[2]
  - In preclinical models, both HB-201 alone and sequential administration of HB-202 followed by HB-201, were safe and efficacious. Alternating 2-vector therapy induced E7- and E6-specific CD8$^+$ T cell responses that accounted for up to 50% of circulating T cells[2,4]
- Here, we present the first immunogenicity results from the Phase 1 portion of an ongoing first-in-human, open-label, Phase 1/2 clinical trial in heavily pretreated patients with prior failure of an anti–PD-1/PD-L1 and/or platinum-based chemotherapy for HPV16+ cancers. This trial is evaluating different dose levels and schedules of monotherapy injections of HB-201 alone or HB-202 alternating with HB-201 (**Figure 2**; NCT04180215)[5,6]

Figure 1. HOOKIPA'S Proprietary Arenavirus Vector Construction



MONOTHERAPY STUDY METHODS

Figure 2. Phase 1 Dose-Escalation Study (NCT04180215)[a,5,a,b]



[a]3+3 dose escalation with additional biomarker- and schedule-finding cohorts
Dosing schedules assessed include (1) q3w for cycles 1-5 then q9w ± 6 and (2) q2w.
[b]All injections consist of specified vector monotherapy.

HNSCC, head and neck squamous cell carcinoma; IT, intratumoral; IV, intravenous; q2w, every 2 weeks; q9w, every 6 weeks; RP2D, recommended Phase 2 dose.

- Serum cytokine and chemokine patterns from 66 samples (12 patients at up to 13 time points) were evaluated by the 30-plex Meso Scale Discovery (MSD) panel
- Direct IFN-γ ELISpot and intracellular cytokine staining (ICS) were conducted on 5 and 3 patients, respectively, with samples from baseline and day 15
  - Cryopreserved and thawed peripheral blood mononuclear cells (PBMCs) from 7 patients were stimulated with overlapping HPV16 E6/E7 peptides for 24 h (± 2 h) for direct *ex vivo* IFN-γ ELISpot measurement. Enough cells were available from 5 of 7 patients to be evaluated by ELISpot at the time of data cutoff
  - Three patients were evaluated by ICS at baseline and day 15. PBMCs were stimulated for 6 hours, washed for subsequent immunostaining, and analyzed by polychromatic flow cytometry. The 3 patients evaluated by ICS were among the 7 patients whose cells were tested by ELISpot

RESULTS

Study Status

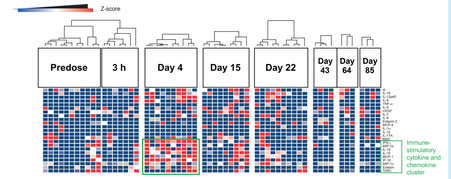
- As of February 17, 2021, 32 patients had been enrolled in the Phase 1/2 study. Cohort doses and patients enrolled are:
  - HB-201 monotherapy: cohort 1, 5×10^5 replication-competent virus focus-forming units (RCV FFU; n=13); cohort 2, 5×10^6 RCV FFU (n=13)
  - HB-201 and HB-202 alternating monotherapy: cohort 1, HB-201 5×10^6 RCV FFU, HB-202 1×10^6 RCV FFU (n=5); cohort 2, HB-201 5×10^6 RCV FFU, HB-202 1×10^7 RCV FFU (n=1)
    - Patients: 78.2% had head and neck squamous cell carcinoma, 75% were male, median age was 62 y (range, 30-86 y), 59.4% had ECOG performance status 1, and the median prior lines of therapy was 3 (range, 1-8)
    - Note: data are preliminary and include unmonitored data based on current reported electronic data capture, enrollment data, and scan reports as of February 17, 2021. Data are subject to change

Distinct Cytokine and Chemokine Signature After Treatment With HB-201 Monotherapy

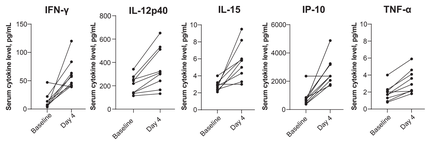
- Hierarchical clustering of serum 30-plex analysis showed that IFN-γ levels increased in 90% of patients after a single administration of HB-201 (**Figure 3a**)
- An IFN-γ signature in serum posttreatment is an early sign of immune activation. On day 4 after treatment with a single dose of HB-201, levels of IFN-γ, IL-12p40, IL-15, IFN-inducible protein (IP)-10, and TNF-α increased in nearly all 9 patients in this analysis (**Figure 3b**)
- It is hypothesized that these changes in immune-stimulatory cytokine and chemokine levels are an early sign of natural killer (NK) and T cell activation
- This balanced and physiological increase in systemic cytokine levels also suggests virus-induced immune activation
- Changes in cytokine levels were generally not associated with adverse events

Figure 3. Distinct Serum Cytokine/Chemokine Signatures on Day 4 Posttreatment

A. **Results of 30-plex cytokine and chemokine analyses for 12 patients over 8 time points are presented. Day 4 data were available for 10 of 12 patients. Analytes (pg/mL) were converted to *z* scores. Hierarchical clustering was performed by visit day and each analyte level.**



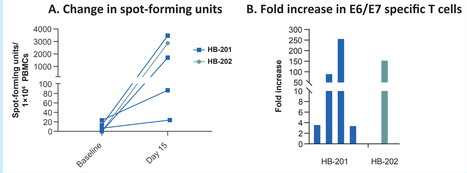
B. **Effects of HB-201 on expression of select key cytokines 4 days posttreatment. Nine of 12 patients had both baseline and day 4 samples**



Strong HPV16 E6/E7-Specific T Cell Responses

- The number of circulating functional E6/E7-specific T cells in HB-201– and HB-202–treated patients reached levels that allowed detection in an *ex vivo* direct ELISpot (ie, without *in vitro* expansion of T cells)
- All patients tested to date (n=5) had a strong induction of antigen-specific T cell responses to HPV16 E6/E7 overlapping peptides from baseline to day 15 (**Figure 4A**)
- Up to a 250-fold increase in antigen-specific IFN-γ–secreting T cells from baseline to day 15 was observed in 4 patients who received 1 dose of HB-201 monotherapy systemically (IV). A 150-fold increase was observed in 1 patient after a single dose of HB-202 IV monotherapy (**Figure 4B**)

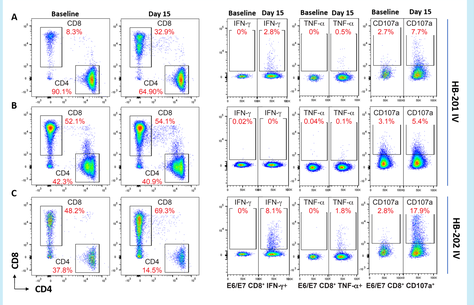
Figure 4. Direct IFN-γ ELISpot Analysis Shows a Strong E6/E7-Specific T Cell Response After a Single IV Dose of Vector Monotherapy
Assessment of PBMCs for E6/E7-specific T cells at baseline and day 15 for individual patients.



High Single-Digit Percentages of Circulating HPV16-Specific CD8$^+$ T Cells

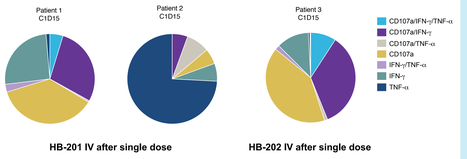
- Cryopreserved PBMCs from 3 patients (2 treated with HB-201 and 1 treated with HB-202) were stimulated with HPV16 E6/E7 overlapping peptides for 6 hours. The frequency of IFN-γ+, TNF-α+, IL-2+, and CD107a$^+$ CD4$^+$ and CD8$^+$ T cells was determined by ICS followed by multicolor flow cytometry
- **Figure 5A-C** shows representative pseudocolor plots with the frequencies of CD4$^+$ and CD8$^+$ T cells and frequencies of IFN-γ+, TNF-α+, and CD107a$^+$ cells gated on CD8$^+$ T cells at baseline and day 15 for 3 patients
  - Two patients had an increase in T cells, predominantly CD8$^+$ T cells, within the total peripheral T cell population after 1 dose of HB-201 (8.3% vs 32.9%; **Figure 5A**) and HB-202 (48.2% vs 69.3%; **Figure 5C**) at baseline vs day 15, respectively. One patient treated with HB-201 had no change in the fraction of CD8$^+$ T cells (**Figure 5B**)
  - E6/E7-specific IFN-γ+ CD8$^+$ T cells increased substantially following single doses of HB-201 or HB-202. Antigen-specific CD8$^+$ T cells increased from 0% at baseline to 2.8% on day 15 following a single dose of HB-201 (**Figure 5A**). Following a single dose of HB-202, antigen-specific CD8$^+$ T cells increased from 0% at baseline to 8.1% on day 15 (**Figure 5C**) Similarly, E6/E7 specific CD8$^+$ T cells had a higher expression of CD107a at day 15. One patient had a slight increase in TNF-α+ and CD107a$^+$, but not IFN-γ+, CD8$^+$ T cells (**Figure 5B**)

Figure 5. Single Dose of HB-201 and HB-202 Elicited a Substantial Increase in Circulating HPV16-Specific IFN-γ+ CD8$^+$ T Cells



- Relative frequencies of circulating E6/E7-specific CD8$^+$ T cells that stained positive for the degranulation marker CD107a and/or the cytokines IFN-γ and TNF-α are shown for 3 patients in **Figure 6**
- Each pie chart represents the relative frequency of HPV16 E6/E7-specific CD8$^+$ T cells with each combination of the 3 functional responses after a single administration of HB-201 or HB-202

Figure 6. Multifunctionality of Circulating HPV16-Specific CD8$^+$ T cells



C1D15, cycle 1 day 15.

CONCLUSIONS

- These preliminary data from a first-in-human trial with arenavirus vectors demonstrated for the first time that patients with HPV16+ cancer who were injected systemically with E7E6-expressing HB-201 or HB-202 as monotherapy had:
  - An increase in key systemic proinflammatory cytokine and chemokine levels, which suggests a virus-induced immune activation
  - A strong induction of circulating HPV16 E6/E7-specific polyfunctional CD8$^+$ T cells up to 8% after the first dose
- Data presented here are from the second dose level for HB-201 and the first dose level for HB-202. Additional doses are being explored in this study
  - Various doses and treatment schedules (ie, alternating 2-vector therapy with HB-201/HB-202 and combination with anti–PD-1 monoclonal antibodies) are being explored in additional cohorts
- Arenavirus vectors expressing E7E6 may constitute a new potential therapy for patients with immunotherapy- and/or chemotherapy-refractory HPV16+ cancers
- In addition to multiple ongoing and planned translational analyses, clinical data are being collected from this ongoing study and will be presented at another upcoming scientific meeting

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AUTHOR DISCLOSURES

Kia Katchar, Michael Schwendinger, Xioaping Qing, Daniel Pinschewer, Klaus Orlinger, Henning Lauterbach, Igor Matushansky: HOOKIPA Pharma Inc. Inc. Diane DaSilva: None.

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