A003

# High-throughput TCR sequencing demonstrates induction of long-lasting HPV16-specific T-cell responses in VB10.16 vaccinated advanced cervical cancer patients

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## BACKGROUND

- Vaccine-induced T-cell responses is a primary pharmacodynamic readout in cancer vaccine clinical trials. High-throughput sequencing of T cell receptors (TCRs) is emerging as a rapid and scalable method, suitable for late-stage clinical trials, and offers sensitive and accurate quantification of the T-cell repertoire.
- VB10.16 is a therapeutic HPV16-specific cancer vaccine designed using a unique modular vaccine technology based on linking antigens to a CCL3L1 targeting module. CCL3L1 attracts and delivers antigens to APCs, to induce strong T-cell responses.
- Vaccine-induced IFNɣ T-cell responses may sensitize tumors to express PD-L1 which argues a synergistic effect with immune checkpoint inhibitor atezolizumab (anti-PDL1)


**Figure 1:** VB10.16; an off-the-shelf HPV vaccine

## PATIENTS

| BASELINE CHARACTERISTICS (N = 52 ENROLLED PATIENTS) | | |
|---|---|---|
| **Median age, years (range)** | | 47.5 (27-83) |
| **Histology** | ◆ Squamous cell carcinoma | 81% (42/52) |
| | ◆ Adenocarcinoma | 15% (8/52) |
| | ◆ Adenosquamous carcinoma | 2% (1/52) |
| | ◆ Unknown | 2% (1/52) |
| **Prior lines of SACT (range 0-5)** | ◆ 0 | 4% (2/52) |
| | ◆ 1 | 50% (26/52) |
| | ◆ ≥ 2 | 46% (24/52) |
| **ECOG PS** | ◆ 0 | 56% (29/52) |
| | ◆ 1 | 44% (23/52) |
| **PD-L1 expression** | ◆ PD-L1+ | 48% (25/52) |
| | ◆ PD-L1- | 39% (20/52) |
| | ◆ Unknown | 14% (7/52) |

*SACT: Systemic Anti-Cancer Therapy*
*ECOG PS: Eastern Cooperative Oncology Group Performance Status*

## STUDY

- At the cut-off date of 22 December 2022, 52 patients with recurrent or metastatic HPV16-positive cervical cancer were enrolled. Of these, 47 patients were included in the efficacy analysis.
- Patients received up to 11 doses of 3 mg VB10.16 intramuscularly in combination with intravenous atezolizumab 1200 mg for up to 48 weeks, until disease progression or unacceptable toxicity. Anti-tumor activity was evaluated using RECIST 1.1 criteria.

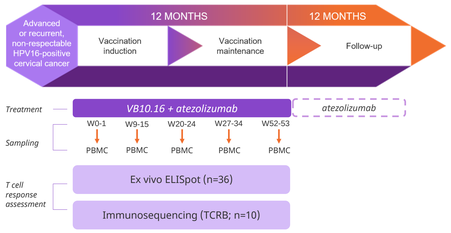


**Figure 2: Trial design and sampling overview**

- T cell responses were assessed by *ex vivo* IFNɣ ELISpot (n=36).
- Sequencing of the T-cell receptor beta chain (TCRB) locus was performed using Immunosequencing (Adaptive Biotechnologies) on up to five timepoints per patient in 10 patients selected based on i) availability of PBMCs and ii) representing a range of clinical response groups (response, stable-, and progressive disease).
- *De novo* and pre-existing expanded clones (baseline vs on-treatment) were determined by a differential abundance framework, using binomial models in pair-wise comparisons (FDR<0.01). Clones with significantly higher on-treatment frequency and a baseline frequency of 0 were defined as *de novo*, remaining significant clones were defined as pre-existing expanded. Longitudinal sampling enabled tracking of expanded TCRs in 7 patients, 4 of which had samples from >2 on-treatment timepoints.
- TCRB specificity was assessed by matching sequences to a proprietary database of confirmed HLA class I HPV16-specific TCRs (Adaptive Biotechnologies). The database was constructed from querying the TCRB repertoires of 92 healthy donors with 146 epitopes from HPV16 E6 and E7 and high-predicted affinity to common Class I alleles. Epitopes were encoded as transgenes within the MIRA assay to query T cells.

## IMMUNOGENICITY

### Stronger HPV16-specific T cell-responses in patients with disease control

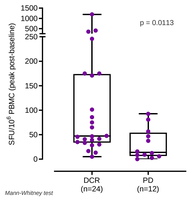


**Figure 3:** Patients with disease control (DCR) had a higher peak post-baseline HPV-specific T-cell response as assessed by ex vivo IFN-g ELISpot (N=36).

### Delayed progression in patients with >2-fold increase in T cell response

| | Median progression-free survival |
|---|---|
| >2-fold T cell response | 8 months |
| <2-fold T cell response | 3.7 months |

**Figure 4:** Patients with >2-fold increase in HPV16-specific T cell response from baseline to peak (*ex vivo* IFN-g ELISpot) had a numerical improvement in the median progression-free survival with 8 versus 3.7 months.

### TCR sequencing supports treatment-induced expansion of T cells

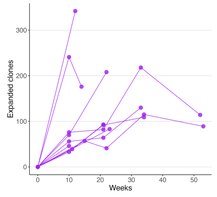


**Figure 5:** Immunosequencing of the TCRB locus demonstrated a rapid and persistent on-treatment T-cell expansion with a peak of 46-342 significantly expanded clones (on-treatment versus baseline, N=10), in support of functional assays showing treatment-induced T-cell responses.

### Expansion of pre-existing and *de novo* clones during treatment

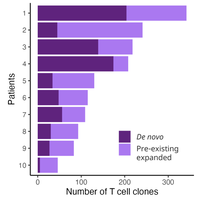


**Figure 6:** Among expanded clones there was a significant contribution of *de novo* clones (range 5-204, on-treatment versus baseline, peak expansion shown, N=10 patients).

### Sum frequency of expanded clones

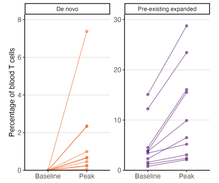


**Figure 7:** The sum frequency of *de novo* clones (left) constituted a median of 0.66% of the peripheral T-cell pool at peak (range 0.04-7.37%). At baseline, pre-existing expanded clones (right) had a median sum frequency of 3.59% (range 0.77-15.08) and increased to a median of 8.20% at peak (range 2.11-28.75%)

### Increased breadth of HPV16-specific CD8 T cell clones in patients with disease control

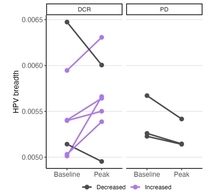


**Figure 8:** The breadth of verified HPV16-specific HLA Class I-restricted TCRs increased in 5/7 patients with disease control. HPV16-specific TCRs were analyzed as the overlap with a proprietary database of confirmed HLA class I HPV16-specific TCRs.

## LONGITUDINAL TCR EXPANSION

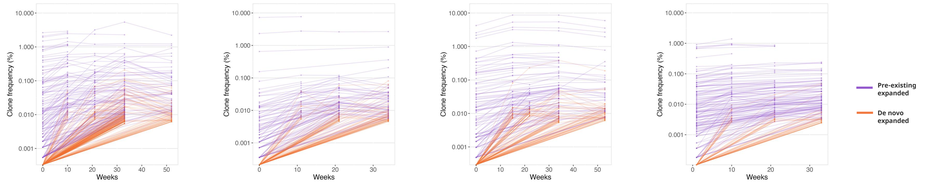


**Figure 9:** Four patients had >2 post-baseline samples and were analyzed for longitudinal TCR expansion kinetics. All 4 patients displayed clones that were persistently expanded from baseline to the on-treatment timepoints as well as continued expansion at each on-treatment timepoint (both *de novo* and pre-existing expanded), indicating on-going clonal expansion throughout treatment (up to one-year). Y-axis: clone frequencies of *de novo* and pre-existing expanded clones (individual dots) at available on-treatment timepoints, x-axis, from pairwise analyses versus baseline. Purple: expanded pre-existing clones, orange: *de novo* clones. Clones that were expanded at any on-treatment timepoint are shown at baseline. Lines connect clones from their baseline frequencies to the on-treatment frequencies, and across visits if expanded at multiple timepoints (non-expanded clones at each timepoint not shown).

## CONCLUSION

- We demonstrate induction of strong and long-lasting HPV16-specific T-cell responses after treatment with VB10.16 and atezolizumab in advanced cervical cancer patients.
- Induction of HPV16-specific T-cell responses (*ex vivo* ELISpot) was correlated with improved clinical outcome.
- Immunosequencing allowed longitudinal tracking of expanded TCRBs.
- Although the sample size was low, HPV16-specific annotation demonstrated a potential relevance of increased HVP16-specific T cell diversity for disease control.

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