Integrative analysis of VB10.16 and atezolizumab in advanced HPV16-positive cervical cancer: Linking biomarker insights to clinical outcomes

Kristina Lindemann[1]*, Michal Zikan[2], Frederic Forget[3], Hannelore Denys[4], Jean-François Baurain[5], Lukas Rob[6], Linn Lena Woelber[7], Frederik Marmé[8], Theresa Link[9], Christian Dannecker[10], Kaja C. G. Berg[11], Roberto S. Oliveri[11], Milena Blaga[11], Anders Rosholm[11], Peter Hillemanns[12], C-02 Investigators

[1]Department of Gynecological Oncology, Oslo University Hospital & Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway; [2]Department of Obstetrics and Gynecology, Bulovka University Hospital, Prague, Czech Republic; [3]Centre Hospitalier de l'Ardenne, Libramont-Chevigny, Belgium; [4]Medical Oncology, University Hospital Ghent, Gent, Belgium; [5]University Clinic Saint-Luc, Bruxelles, Belgium; [6]University Hospital Kralovske Vinohrady, Praha, Czech Republic; [7]Department of Gynecology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [8]Department of Gynecology and Obstetrics, University Hospital Mannheim, Mannheim, Germany; [9]Department of Gynecology and Obstetrics, Medical Faculty and University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany; [10]Gynecology and Obstetrics, Faculty of Medicine, University of Augsburg, Augsburg, Germany; [11]Nykode Therapeutics ASA, Oslo, Norway; [12]Department for Gynecology and Obstetrics, Hannover Medical School, Hannover, Germany

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BACKGROUND

- Therapeutic cancer vaccines offer a promising strategy to enhance anti-tumor responses in combination with immune checkpoint inhibitors.
- VB10.16 is a DNA-based therapeutic HPV16-specific cancer vaccine designed using a unique modular vaccine technology based on linking antigens to a CCL3L1 targeting module to attract and deliver antigens to APCs, for strong T-cell responses (*Figure 1*). Vaccine-induced IFN-γ T-cell responses may sensitize tumors to express PD-L1, which argues a synergistic effect of VB10.16 with immune checkpoint inhibitors such as atezolizumab (anti-PD-L1).
- We recently demonstrated the safety and clinical efficacy of VB10.16, a DNA-based therapeutic cancer vaccine encoding HPV16 E6/E7 oncoproteins fused to CCL3L1 for antigen-presenting cell targeting in HPV16-positive persistent, recurrent or metastatic cervical cancer[1]. In this multicenter open-label phase 2a VB C-02 trial (NCT04405349) the ORR was 19.1%, (95% CI 9.1% to 33.3%). Median DoR was not reached (95% CI 2.2 to n.r.), mPFS was 4.1 months (95% CI 2.1 to 6.2), and mOS was 21.3 months (95% CI 8.5 to n.r.). In programmed death-ligand 1 (PD-L1)-positive patients (n=24), ORR was 29.2% (95% CI 12.6 to 51.1).

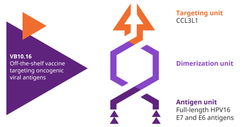


Figure 1: VB10.16; an off-the-shelf HPV16-specific cancer vaccine

- The peripheral and local immune status may be relevant for the clinical benefit of immunotherapies.
- Here we explore the association between HPV16-specific T cell responses, tumor microenvironment (TME) characteristics, and clinical outcomes in the VB C-02 trial.

PATIENTS

BASELINE CHARACTERISTICS (SAFETY POPULATION, N = 52 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 systemic anti-cancer therapy	0	2% (1/52)
	1	50% (26/52)
	≥ 2	46% (24/52)
	Unknown	2% (1/52)
ECOG PS	0	58% (30/52)
	1	42% (22/52)
PD-L1 expression	PD-L1+	48% (25/52)
	PD-L1-	38% (20/52)
	Unknown	13% (7/52)

ECOG PS: Eastern Cooperative Oncology Group Performance Status

STUDY DESIGN AND METHODS

- Between 16 Jun 2020 and 25 Jan 2022, 52 patients with persistent, recurrent or metastatic (r/m) HPV16-positive cervical cancer were enrolled and received VB10.16 (3 mg intramuscularly, Q3W for 12 weeks and then Q6W) combined with atezolizumab (1200 mg intravenously, Q3W) for up to 48 weeks (*Figure 2*).
- Primary endpoints were safety and objective response rate per RECIST v1.1. Secondary endpoints included overall survival (OS) and HPV16-specific T cell responses by IFN-γ ELISpot.
- Predefined exploratory endpoints included systemic immunosuppression and TME inflammatory status in tumors (*Figure 3*).
- Of the 52 patients in the safety population, 47 patients were response-evaluable (efficacy population), and 36 of these patients had pre- and post-baseline PBMC samples.

Figure 2: Trial design

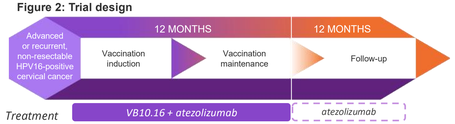
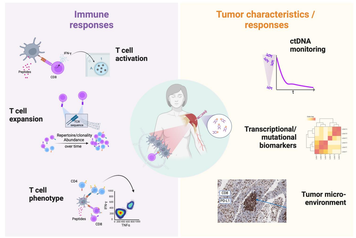


Figure 3: Biomarkers and immunogenicity read-outs



- HPV16-specific peripheral T cell responses were analyzed by *ex vivo* IFN-γ ELISpot (N=36)
- Gene expression in biopsies from baseline tumors were analyzed by the NanoString nCounter® PanCancer IO 360™ Panel (N=29) and 38 cell function/cancer immunity-related gene signatures were analyzed by single-sample GSEA (ssGSEA)
- Longitudinal monitoring of myeloid cells was performed by standard complete blood count with leukocyte differential (N=36)
- T-cell/myeloid-derived suppressor cell (CD3/MDSC) ratio was assessed by flow cytometry in a subset of patients (N=21)

PATIENTS WITH CLINICAL BENEFIT HAD STRONGER T CELL RESPONSES AND MORE FREQUENT ON-TREATMENT REDUCTION OF SYSTEMIC IMMUNOSUPPRESSION

Increased HPV16-specific T cell response in reduced immunosuppressive environment

A higher peak post-baseline HPV16-specific T cell response (IFN-γ ELISpot) was observed in patients with a decrease in frequency of myeloid-derived suppressor cells during treatment, both when analyzed as by hematology analyses (*Figure 5*) and when analyzed as the ratio of T cells to myeloid cells(CD3/MDSC ratio) by flow cytometry (*Figure 6*).

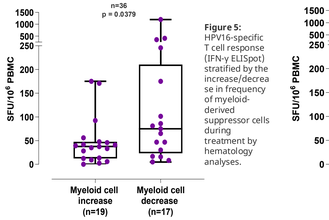


Figure 5: HPV16-specific T cell response (IFN-γ ELISpot) stratified by the increase/decrease in frequency of myeloid-derived suppressor cells during treatment by hematology analyses.

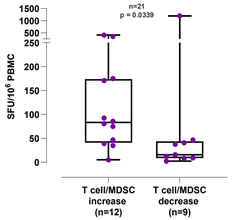
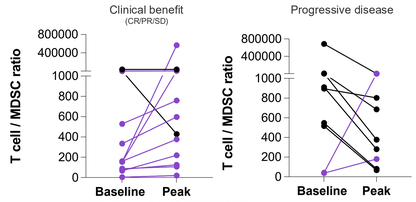


Figure 6: HPV16-specific T cell response (IFN-γ ELISpot) stratified by the increase/decrease in frequency of myeloid-derived suppressor cells during treatment by the ratio of T cells to myeloid cells(CD3/MDSC ratio) from flow cytometry analyses. polymorphonuclear myeloid-derived suppressor cells, PMN-MDSC, defined as: CD3$^-$HLA-DR$^-$CD11b$^+$CD14$^-$/^{dim}CD33^{dim}CD66b$^+$CD15$^{+(2)}$.

Increased T cell / MDSC ratio was associated with clinical benefit

Patients with clinical benefit (CR/PR/SD) more frequently showed an increase in the T cell (CD3)/MDSC ratio during treatment, suggesting a link between successful immunotherapy treatment and reduced systemic immunosuppression (*Figure 7*).



	Increase	Decrease
Clinical benefit	10	2
Progressive disease	2	7

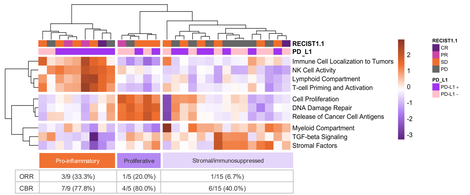
n=21, p=0.0092

Figure 7: The T cell (CD3)/MDSC ratio during treatment in patients with clinical benefit (CR/PR/SD; left figure) and progressive disease (middle figure). Purple color denotes increase in CD3/MDSC ratio. Black color denotes decrease. Data from flow cytometry, n=21 patients. P-values from Fisher's exact test.

GENE EXPRESSION FEATURES OF THE BASELINE TUMOR MICROENVIRONMENT WERE ASSOCIATED WITH ORR AND OS

Gene signatures in the tumor microenvironment associated with clinical benefit

Patients with pro-inflammatory tumor signaling had an ORR of 33.3% and CBR (clinical benefit rate; CR, PR, SD) of 77.8%. A proliferative tumor signature was associated with comparable ORR/CBR, although limited by low sample size. Patients with stromal/immunosuppressed baseline tumors had lower ORR and CBR, but 1 CR and 6 SD was found in this group, totaling to a 40% clinical benefit rate (CR/PR/SD).



	Pro-inflammatory	Proliferative	Stromal/immunosuppressed
ORR	3/9 (33.3%)	1/5 (20.0%)	1/15 (6.7%)
CBR	7/9 (77.8%)	4/5 (80.0%)	6/15 (40.0%)

Figure 8: Hierarchical clustering of gene signatures in baseline tumors produced three clusters: a pro-inflammatory cluster, a proliferative cluster and a stromal/immunosuppressed cluster. Heatmap based top 25% variance ssGSVA gene signatures out of 38 analyzed signatures relevant for cell function and/or cancer immunity, from NanoString nCounter® PanCancer IO 360™ Panel. Clustered with euclidean distance and Ward.D2 linkage.

Prolonged overall survival in patients with pro-inflammatory/proliferative tumors

Patients with pro-inflammatory/proliferative tumors demonstrated significantly prolonged overall survival (mOS n.r) compared with patients with immunosuppressive tumors (mOS 8.3 months).

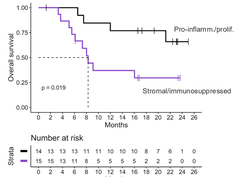


Figure 9: The median survival in patients with pro-inflammatory or proliferative tumors (n=14) was n.r. vs 8.3 months in patients with stromal/immunosuppressed tumors (n=15), p=0.019 Kaplan-Meier, HR=0.27, 95% CI 0.08-0.87).

CONCLUSIONS

- Through integrative analyses of biomarker- and clinical data we demonstrate positive associations between favorable tumor/immune states and patient outcome after treatment with VB10.16 combined with atezolizumab.
- An on-treatment reduction of systemic immunosuppression was associated with improved clinical benefit and indicates that the treatment may mitigate immunosuppression and blood biomarkers could identify responders earlier.
- Insights from TME analyses indicates that the tumor immune environment was predictive of response and survival.
- Together with durable responses, the findings highlights the promise of VB10.16 in combination with atezolizumab and warrants further exploration of this combination therapy.

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Abbreviations:
ORR: objective response rate
DoR: duration of response
n.r.: not reached
mPFS: median progression-free survival
mOS: median overall survival

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* klinde@ous-hf.no

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