Title:

A therapeutic antigen-presenting cell-targeting DNA vaccine VB10.16 in HPV16-positive high-grade cervical intraepithelial neoplasia: results from a phase 1/2a trial

Authors and Affiliations:

- Peter HILLEMANNS, 30625 Hannover, Hannover Medical School, Department of Gynecology and Obstetrics, Germany
- Agnieszka DENECKE, 30625 Hannover, Hannover Medical School, Department of Gynecology and Obstetrics, Germany
- Linn WOELBER, 20246 Hamburg, University Medical Center Hamburg-Eppendorf, Department of Gynecologic Oncology, Germany
- Gerd BÖHMER, 30159 Hannover, IZD Institut für Zytologie und Dysplasie, Germany
- Matthias JENTSCHKE, 30625 Hannover, Hannover Medical School, Department of Gynecology and Obstetrics, Germany
- Karoline W SCHJETNE, 0349 Oslo, Nykode Therapeutics ASA, Norway
- Karsten MH BRUINS SLOT, 0349 Oslo, Nykode Therapeutics ASA, Norway
- Agnete B FREDRIKSEN, 0349 Oslo, Nykode Therapeutics ASA, Norway

Running title:

VB10.16 DNA vaccine in HPV16-positive cervical intraepithelial neoplasia

Key words:

VB10.16, Human Papilloma Virus 16, cervical intraepithelial neoplasia, first-in-human clinical trial, therapeutic vaccine

Financial Support:

This study was funded by Nykode Therapeutics ASA (formerly Vaccibody AS) and co-funded by the Research Council of Norway (grant no. 219596 and 214866)

Corresponding author:

Professor Peter HILLEMANNS

Hannover Medical School

Department of Gynecology and Obstetrics

Carl-Neuberg-Straße 1, 30625 Hannover, Germany

Tel. +49 511 532 6144

Fax +49 511 532 6145

Email: Hillemanns.Peter@MH-Hannover.de

Disclosure of Potential Conflicts of Interest

P. Hillemanns has received honoria for lectures from Roche, AstraZeneca and MSD.

A.B. Fredriksen, K. W. Schjetne and K.M.H. Bruins Slot are all employees of Nykode Therapeutics and hold ownership interest (including patents).

L. Woelber has received Honoria from Roche, TESARO/GSK, medac oncology, Pfizer, AstraZeneca, TEVA, Omniamed, promedicis, MSD_Eisai, Seagene and research support from medac oncology, Roche diagnostics, Greiner BioOne outside the submitted work.

No potential conflicts of interest were disclosed by the other authors.

Manuscript word count:

Translational relevance: 149 words

Abstract: 250 words

Main text: 4106 words

Number of Figures and Tables:

Main section: 2 tables and 3 figures

Supplement: 4 tables and 4 figures

ABSTRACT

Purpose: To evaluate the safety, immunogenicity and efficacy of a therapeutic DNA vaccine VB10.16, using a unique modular vaccine technology that is based on linking antigens to CCL3L1 targeting module, in women with HPV16-positive high-grade cervical intraepithelial neoplasia (CIN).

Patients and Methods: We conducted a first-in-human, open-label, phase I/IIa clinical trial of VB10.16 in subjects with confirmed HPV16-positive CIN 2/3. The primary endpoint was the proportion of participants with adverse events, including dose-limiting toxicities. Secondary outcome measures included measuring the E6/E7-specific cellular immune response. In the Expansion cohort HPV16 clearance, regression of CIN lesion size and grading were assessed during a 12-month follow-up period.

Results: A total of 34 women were enrolled: 16 in two Dose cohorts and 18 in the Expansion cohort. No serious adverse events or dose-limiting toxicities were observed, and none of the subjects discontinued treatment with VB10.16 due to an adverse event. Mild to moderate injection site reactions were the most commonly reported adverse event (79%).

HPV16-specific T-cell responses were observed after vaccination in the majority of the subjects. In the Expansion cohort, HPV16 clearance was seen in 8 of 17 evaluable subjects (47%). Reductions in lesion size were seen in 16 subjects (94%) and 10 subjects (59%) had regression to CIN 0/1. Correlation between strong IFN-γ T cell responses and lesion size reduction was statistically significant ($p < 0.001$)

Conclusions: The novel therapeutic DNA vaccine VB10.16 was well tolerated and showed promising evidence of efficacy and strong HPV16-specific T-cell responses in subjects with high-grade CIN.

Translational Relevance

High-grade cervical intraepithelial neoplasia (CIN) caused by infection with human papillomavirus (HPV) most often precedes the development of cervical carcinoma. HPV E6 and E7 viral antigens are only expressed by HPV-infected cells and thus act as tumor-specific antigens that are attractive targets for therapeutic cancer vaccines. VB10.16 is a novel vaccine designed using a unique modular vaccine technology based on linking antigens to a CCL3L1 targeting module and developed to treat HPV16-associated premalignant and malignant lesions. We conducted a first-in-human trial of VB10.16 monotherapy in subjects with CIN 2 or 3 and demonstrated that VB10.16 is well tolerated and generated robust HPV16-specific E6 and E7 T-cell responses. We observed regression of lesion size and CIN grading in a majority of treated subjects. Vaccine-induced T-cell responses were shown to be correlated to reduction of lesion size and grading indicating that VB10.16 was able to elicit a clinically relevant immune response.

Introduction

Cervical carcinoma is often preceded by high-grade cervical intraepithelial neoplasia (CIN) and remains one of the most common cancers in women worldwide, with GLOBOCAN statistics from 2018 reporting over 560,000 new cases and over 300,000 deaths (1). This makes it the fourth most common cancer in women worldwide (2). Almost all carcinomas of the cervix are associated with HPV infections (2,3). Among more than 35 HPV types found in the genital tract, HPV16 accounts for 50-60% of cervical cancer cases, followed by HPV18 (10-20%) (4). These distributions are generally consistent worldwide (5–7). HPV16 is associated with a greater risk of progression from infection to CIN (8,9). CIN grade 2 and grade 3 are considered high-grade squamous intraepithelial lesions and, if left untreated, around 30% of CIN 3 lesions will progress to carcinoma (10). Standard treatment for high-grade CIN is cervical excisional surgery (conization) that is associated with some important long-term risks (e.g., pre-term delivery), especially in younger women (11).

Current prophylactic HPV vaccines have been available for over 10 years, with vaccination in approximately 40% of the targeted population worldwide (12,13). However, prophylactic vaccines are not able to treat pre-established infections or eradicate existing cancerous lesions and CIN (14). HPV infections and HPV-related malignancies will continue to be a public health issue in the coming decades. The development of effective non-surgical treatment options such as therapeutic HPV vaccines and other anti-cancer therapies is therefore still relevant (15).

VB10.16 is an antigen-presenting cell (APC) targeting, DNA-based therapeutic vaccine that has been developed to treat HPV16-associated premalignant and malignant lesions. VB10.16 includes the E6 and E7 tumor-specific antigens that are expressed by HPV16-infected cells. The vaccine encodes a recombinant protein consisting of mutation-inactivated E6 and E7 proteins, linked to the natural human chemokine (C-C motif) ligand 3-like 1 (CCL3L1 or LD78β) in a dimeric format. The chemokine CCL3L1 attracts APC and when binding to its receptor CCR5 expressed on APC delivers the E6 and E7 antigens directly to the APCs, thereby increasing antigen loading and cross presentation through direct delivery of the antigen by receptor ligation and internalization. (16,17). The mature APCs can migrate to the lymph nodes where they activate antigen-specific T cells. These activated T cells are then able to kill cancer cells that express the relevant antigen (18,19). This unique mechanism of action, targeting the antigens to chemokine-receptors on APCs, induces a powerful cellular immune response against the antigens compared to conventional therapeutic vaccines, which only deliver the antigens (16,17). The VB10.16 vaccine holds antigens from HPV16 and will thus induce an immune response specifically to the virus strain infecting the transduced cells.

We conducted a first-in-human, open-label, multicenter, phase I/IIa trial to assess the safety and immunogenicity of two different dosing schedules of 3 mg VB10.16 in women with HPV16-positive CIN 2 and examined the safety, immunogenicity and preliminary efficacy of VB10.16 in an Expansion cohort including subjects with HPV16-positive CIN 2 or CIN 3.

Subjects and Methods

Study Design and Subjects

This single-arm, open-label study was conducted at 4 study sites in Germany between September 2015 and January 2019. An initial dosing phase was performed in two cohorts of 8 participants each, to evaluate safety and immunogenicity of 3 mg VB10.16 using different dosing schedules. Results from this phase were subject to an interim analysis after 6 participants in each dose cohort had completed immunological assessments 16 weeks after receiving the first dose of VB10.16. Results were reviewed by a Cohort Review Committee that advised on the selection of the VB10.16 regimen to be further evaluated in a subsequent Expansion cohort of 18 subjects based on safety and immunological results (**Supplementary Figure S1**).

Eligible women were aged at least 18 years, had pathology-confirmed HPV16-positive high-grade cervical intraepithelial neoplasia (CIN 2 for the initial Dosing cohorts, or CIN 2 or 3 for the Expansion cohort), and agreed to the protocol-mandated biological sampling. All participants were required to have adequate bone marrow and liver function. Participants were considered ineligible if colposcopy showed more than 2 cervical quadrants of CIN 3, or evidence of severe pelvic inflammatory disease or cervicitis, or other severe gynecological infection. Participants with atypical glandular cells, adenocarcinoma in situ, malignant cells, or suspected micro-invasive or invasive disease were excluded. Participants were also excluded if they had clinically significant autoimmune disease or known immunodeficiency, previous vaccination against HPV, or administration of any live vaccination within the preceding 90 days. An extensive list of inclusion and exclusion criteria is listed in the Supplement section (**Supplementary Table S1**). The protocol allowed for conization of subjects during the study period and the decision to perform a conization was at the discretion of the investigator.

The study was conducted in accordance with the principles of the Declaration of Helsinki, and of Good Clinical Practice, and was approved by the Paul Ehrlich Institute and Ethics Committees of participating sites in Germany before screening subjects. Eligible subjects were identified by participating investigators and all subjects provided written informed consent before undergoing any study procedures. The trial is registered at ClinicalTrials.gov (NCT02529930).

Plasmid design

VB10.16 is a non-replicative, non-integrating, DNA plasmid of 5994 base pairs. It encodes a single recombinant homodimer protein consisting of three modules: mutation-inactivated E6 and E7 protein from HPV16 linked to the natural human chemokine CCL3L1 via a Dimerization Module derived from human Immunoglobulin G (IgG3) as shown in **Figure 1.** The described coding region was inserted in high-expression vector, pUMVC4a, to generate VB10.16 which was produced in *E. coli* DH1 in compliance with cGMP at Cobra Biologics Ltd., Keele, UK.

Study procedures

VB10.16 was administered as two 0.5 mL intramuscular injections into the lateral deltoid muscles using the PharmaJet® Stratis 0.5 mL Needle-free Injection System (Golden, Colorado, USA). Participants in the initial dosing phase received 3 vaccinations of 3 mg VB10.16 and two

dosing regimens were evaluated: in Cohort 1 participants received vaccinations at Weeks 0, 3 and 6; in Cohort 2 vaccinations were administered at Weeks 0, 4 and 12. Participants in the Expansion cohort received 4 vaccinations of 3 mg VB10.16 (Weeks 0, 3, 6 and 16) (**Supplementary Figure S1**).

HPV16 positivity of all subjects was verified by a Cobas® HPV Test performed at the study site and obtained within four weeks prior to start of study treatment.

Safety was evaluated by recording adverse events (AEs, Common Terminology Criteria for Adverse Events, version 4.0) and through regular scheduled evaluations of safety laboratory parameters, vital signs, physical examinations, and electrocardiograms (ECGs). Injection site related adverse events were solicited through the use of a diary in each subject.

A DLT was defined as a clinically significant toxicity or abnormal value assessed as unrelated to the underlying disease, or concomitant medication and considered related to the study treatment.

Regression of CIN lesions and lesion size was evaluated at the study sites by colposcopic examination and by histological assessment of representative cervical biopsies (at Screening and after 2 months, 4 months, 6 months 9 month and 12 months of the first administration of VB10.16). More than one lesion could be followed by the investigator for this purpose.

Clearance of HPV was evaluated at the study sites using a Cobas® HPV Test (Roche Molecular Diagnostics, Pleasanton, California) and/or p16 immunohistochemistry assessment of cervical biopsies (at Screening and 2 months, 4 months, 6 months 9 month and 12 months of the first administration of VB10.16).

Biopsies of cervical lesions were obtained at screening, after 4 months, and after 6 months to analyze PD-L1 expression (clone 22C3) by immunohistochemistry.

IFNγ ELISpot assay

Blood samples were obtained at pre-specified time points to monitor cellular immune responses (Supplement Figure 2). Immunogenicity of the vaccine was evaluated in terms of the cellular immune response against the E6/E7 viral antigens, using enzyme-linked immunospot assay (ELISpot) to assess systemic T-cell responses. Cryopreserved and thawed peripheral blood mononuclear cells (PBMCs) were cultured in RPMI-1640 overnight at 37 °C, 5% CO_2. After resting, PBMCs were cultured with HPV16 E6 or E7 peptides pools peptide pools in RPMI supplemented with 10% FCS for 5 days at 37 °C 5% CO_2 (2×10^6 cells/wells in 24 well plate). At day 5, each condition was harvested and seeded in ELISpot plates at 2×10^5 cells/well. PBMCs were then re-stimulated with HPV16 E6 or E7 peptide pools or anti-CD3 (positive control). Unstimulated PBMCs served as negative controls. After 24 hours incubation, spots were developed according to manufacturer's instructions and counted using CTL reader. HPV-specific responses were calculated by subtracting the mean number of spots in the unstimulated cells from the mean number of spots in experimental wells and shown as spot-forming units (SFU) per 10^6 PBMCs. The assay was performed in quadruplicates.

Outcome Measures

The primary endpoint, the proportion of subjects with AEs, including any DLTs, laboratory assessments, and physical findings, was analyzed in the Safety Evaluable Population, comprising all subjects who received any amount of VB10.16.

Immunogenicity endpoints were analyzed in the Immunogenicity Evaluable Population, comprising all subjects who underwent an immunologic assessment during the study.

Efficacy endpoints (CIN lesion size, CIN regression and HPV-clearance) were analyzed in the Efficacy Evaluable Population in the Expansion cohort comprising all subjects with at least 1 post-baseline colposcopic assessment and Cobas® HPV Test. These outcomes were all assessed locally by the investigators at prespecified timepoints.

Statistical Analysis

The sample size for this exploratory, first-in-human trial was based on clinical and practical considerations, not on a formal statistical power calculation. An interim analysis was planned after completion of the initial dosing phase. Statistical analyses were generally descriptive, using counts and percentages for categorical measures, and mean, median, standard deviation, minimum, maximum for continuous measures. A Mann–Whitney test was used to analyze differences in immune responses in subjects with and without reductions in lesion size. A generalized linear model with a Gamma distributed dependent variable and inverse link function was fitted to the data. An ANOVA analysis on the resulting single term model resulted in a p-value for SFU. Detailed description of the generalized linear model is available in the Supplementary information. P values less than 0.05 were considered significant. All statistical analyses were performed using SAS® (version 9.4; SAS Institute, Cary, NC, USA).

Data availability

The data generated in this study are available within the article and its supplementary data files and at Clinicaltrials.gov (NCT02529930). Please contact the corresponding author for requests for additional data.

Results

Subjects disposition and baseline characteristics

A total of 38 women were screened for the study; 4 women failed to meet all the eligibility criteria and 34 women were enrolled in the study and received treatment with VB10.16 (**Supplementary Figure S3**). Demographics and baseline characteristics were comparable between cohorts (**Table 1**). A table outlining the representativeness of study participants is included in the Supplement section (**Supplementary Table S2**).

One subject enrolled in the Expansion cohort was subsequently found to be HPV16 negative after having received 2 vaccinations, and treatment was thereafter discontinued. This subject was followed for safety until Week 24 and was included in the safety analyses but was excluded from immunogenicity and efficacy analyses, since VB10.16 can only be effective in subjects with HPV16. The remaining 33 enrolled subjects received all scheduled vaccinations. Conization was permitted under the protocol and 6 enrolled subjects underwent this procedure after having received all scheduled vaccinations with VB10.16. One subject in the Expansion cohort discontinued before the scheduled 6 months follow-up visit.

Safety

No serious adverse events and DLTs were reported in the safety evaluable population (n=34), and none of the subjects discontinued treatment due to an adverse event. Adverse events were reported in all subjects except one and were typically mild to moderate in severity. The most common solicited and unsolicited treatment-related AEs (≥10%) reported during the period from administration of the first VB10.16 dose to 30 days post last dose are listed in **Table 2**. Most treatment-related AEs were "General disorders and administration site conditions", mainly injection site reactions. The majority of such injection site reactions (81%) resolved within 4 days and were mild in nature, with 99% of events of Grade 1 or 2 severity. Other commonly reported treatment-related AEs (≥10%) were headache, hyperesthesia and erythema, all of Grade 1-2. Grade 3 AEs were reported in 3 subjects (9%): 1 participant with emotional distress and 1 participant with arthritis that were both not considered related to treatment with VB10.16 by the treating physicians, and 1 participant with injection site pain and hyperesthesia that were both considered to be treatment related. No Grade 4 or 5 AEs were reported.

Treatment-related late emerging AEs (occurring during Week 24 to 12 months) were reported in 1 participant in Cohort 2 (alopecia) and 2 subjects in the Expansion cohort (influenza-like illness and injection site pruritus).

A comparison of results between Cohort 1, Cohort 2 and Expansion cohort showed similar overall treatment-related AEs by System Organ Class with few category exemptions and few differences (**Supplementary Tables S3 A-C**).

No noticeable changes in vital signs, ECG, or performance status were observed during the study period. A few patients experienced Grade 2, 3 and 4 lab value events, but none of these were considered as related to VB10.16 (**Supplementary Table S4**).

Clinical efficacy and HPV clearance in Expansion cohort

Preliminary evidence of efficacy was assessed in 17 evaluable subjects with CIN 2/3 that were enrolled in the Expansion cohort and received vaccinations with VB10.16 at week 0, 3, 6 and 16. Three subjects were not followed up for the complete 12 months period: two subjects had a conization performed after 5 and 10 months, respectively, and one subject withdrew from study after 9 months.

A reduction in lesion size was observed in 16 of the 17 evaluable subjects (94%), who were followed for up to 12 months. Twelve subjects (71%) had lesions size reductions of more than 50% compared with their baseline lesion size. Regression of lesions to CIN 0 or CIN 1 was observed in 10 subjects (59%). A complete regression of CIN (CIN 0) was seen in 8 subjects (47%). (**Figure 2**).

HPV16 clearance was observed in 8 evaluable subjects (47%) as assessed by at least one test (Cobas® HPV Test or p16 immunohistochemistry assessment of cervical biopsies) during the 12 months follow-up period.

Clinical efficacy and HPV clearance in initial Dosing cohorts

Preliminary evidence of efficacy was also assessed in 16 evaluable subjects with CIN 2 at baseline that were enrolled in the two initial Dosing cohorts and received vaccinations with VB10.16 at week 0, 3, 6 in Cohort 1, and at week 0, 4 and 12 in Cohort 2. Four subjects (two in each cohort) were not followed up for the complete 12 months period: these subjects had a conization performed after 4, 6, 6 and 7 months, respectively

A reduction in lesion size was observed in 6 of the 8 evaluable subjects (75%) in Cohort 1 and in 4 of the 8 evaluable subjects (50%) in Cohort 2. Regression of lesions to CIN 0 or CIN 1 was observed in 3 subjects (38%) in Cohort 1 and 3 subjects (38%) in Cohort 2. A complete regression of CIN (CIN 0) was seen in 2 subjects (25%) in Cohort 1 and 2 subjects (25%) in Cohort 2.

HPV16 clearance was observed in 3 evaluable subjects (38%%) in Cohort 1 and 3 subjects (38%) in Cohort 2, as assessed by at least one test (Cobas® HPV Test or p16 immunohistochemistry assessment of cervical biopsies) during the 12 months follow-up period.

Induction of HPV16-specific IFN-γ responses

Systemic T cell responses against HPV16 E6 and E7 viral antigens were assayed by IFN-γ ELISpot individually in isolated PBMCs. PBMCs were collected at baseline and post vaccination visits, and functional T cell responses are reported for 31 of 33 evaluable subjects.

HPV16-specific T cell responses were increased from baseline at least at one timepoint after vaccination in 6 of the 7 (85%) evaluable subjects in Cohort 1 (**Figure 3A**), with the peak response observed at Week 7 one week after the third vaccination. Increased IFN-γ T cell response post baseline was observed in all 7 (100%) evaluable subjects in Cohort 2 (**Figure 3B**).

Both dosing regimens demonstrated that a homologous boost vaccination with VB10.16 was well tolerated, and the T cell response was increased after multiple vaccinations.

IFN-γ ELISpot in Cohort 1 (Week 0, 3, and 6) showed faster, stronger, and longer lasting T-cell responses compared with Cohort 2 (Week 0, 4 and 12), and based on both immunogenicity and safety findings, this dosing regimen was selected for the Expansion cohort. In addition to the induction vaccinations, an additional vaccination at Week 16 was included in the Expansion cohort to study whether T-cell immune responses could be further amplified and maintained by multiple vaccinations.

In the Expansion phase, strong T-cell responses were observed for all subjects (N=17) with an average 7.9-fold increase (range 0-63-fold) indicating that an increase in the number of vaccinations elicited a more robust and longer lasting T cell responses. T-cell responses were increased from baseline in 16 of 17 subjects (94%) after vaccination, and in 13 subjects (76.5%) more than 2-fold (**Figure 3C**). The additional dose at Week 16 demonstrated amplified and prolonged immune responses compared to the dosing cohort 1(**Figure 3D**).

The majority of the subjects (29 of 31 evaluable subjects) demonstrated a vaccine-induced T cell response, and a response was seen against both E6 and E7 antigens (**Supplementary Figure S4**).

HPV16-specific immune responses correlated with lesion size regression

A total of 26 (79%) of the 33 subjects enrolled into Cohort 1, 2 and Expansion Cohort showed a lesion size reduction, and an exploratory analysis demonstrated a clear statistically significant correlation ($p < 0.001$) between strength of T cell response and reduction in lesion size. Most patients with strong T cell responses and lesion size reduction also presented with regression to no CIN or CIN 1, indicating that VB10.16 induced a clinically relevant immune response (**Figures 3E and F**).

PD-L1 upregulation in CIN lesions

Expression of PD-L1 in cervical biopsies was assessed by immunohistochemistry at baseline and at week 16 and week 24 in subjects enrolled in the Expansion cohort. The data shown in **Figure 4**, indicate a trend towards an increased level of PD-L1 after VB10.16 vaccination which may delay or inhibit T-cell mediated elimination of affected cells. Strong IFNγ responses were observed and lead to the expectation that PD-L1 was upregulated in the tumoral epithelium as a response to the strong immune response elicited by the VB10.16 vaccine. An upregulation of PD-L1 (>1%) was observed in all 6 patients, who did not achieve a regression to no CIN or CIN 1 during the follow-up period.

DISCUSSION

In this first-in-human study the APC targeted, therapeutic DNA vaccine VB10.16 was generally safe and well tolerated in women with HPV16-positive high-grade CIN. The most common treatment-related adverse events were injection site reactions that were predominantly mild to moderate in severity and of limited duration. Furthermore, immunogenicity of VB10.16 was demonstrated, with a robust and prolonged HPV16-specific T-cell response after vaccination in the majority of the subjects. The two Initial dosing cohorts demonstrated that the HPV16-specific T cell response is increased by more frequent vaccinations, and the 3-week vaccination regimen in combination with an additional vaccination demonstrated induction of the most rapid, strong, and long-lasting T cell responses.

Clearance of HPV16 and evidence of partial and complete regression of CIN lesions was observed in a majority of subjects in the Expansion cohort, indicating promising signs of efficacy of VB10.16. A regression of lesions to no CIN or CIN 1 was observed in 10 (59%) subjects. This seems to be in line, or better, when compared with findings from other studies investigating therapeutic vaccines targeting E6 and E7 that reported regression rates to no CIN or CIN 1 in women with high-grade CIN (20–22). The observed HPV clearance rate of 47% in subjects treated with VB10.16 is also supportive for the HPV-specific mechanism of action of VB10.16. Caution should, however, be exercised when performing cross-trial comparisons as the included study populations, number of treated subjects and study follow-up periods vary between studies.

Interestingly, the induction of strong HPV16-specific T-cell responses was correlated with lesion size reduction in most treated subjects, indicating that T-cells induced by the VB10.16 vaccine were clinically active. A robust IFN-γ T-cell response was observed in all subjects who received four VB10.16 injections. A strong T-cell response was generated against both E6 and E7 antigens in all subjects and a significant correlation to lesion size reductions was evident for both E6 and E7-specific T-cells. The unique modular vaccine technology of VB10.16 that is based on linking antigens to the chemokine CCL3L1 targeting module might contribute to cross presentation enabling a strong T-cell response. In trials performed in similar settings as ours investigating vaccines that are not directly targeting antigen presentation to APCs for uptake of HPV antigens, T-cell responses were only elicited in a limited number of subjects (21,23,24). Furthermore, in contrast to other therapeutic HPV vaccines holding both HPV16 and HPV18 antigens, the immune response elicited by VB10.16, and demonstrated in IFNγ ELISpot, is specific against the HPV strain in the infected lesion. Homologous vaccination of the VB10.16 vaccine with initial priming doses to activate the immune system, followed by an additional dose of the same vaccine also offers a simple and easy vaccination regime compared to heterologous prime-boost vaccines that use different types of vaccine technologies.

The promising, though preliminary, signs of efficacy and the upregulation of PD-L1 observed in this study provide a strong rationale for combining VB10.16 with an anti-PD-1/PD-L1 checkpoint inhibitor. Combination therapy with a checkpoint inhibitor blocking PD-1/PD-L1 interaction between the activated T-cells and tumor cells might have resulted in improved clinical responses in our study. Such a combinatorial approach is supported by a recent study of nivolumab in combination with ISA101b, a synthetic long-peptide therapeutic HPV16 vaccine,

in patients with HPV16-positive head and neck cancer. This study showed promising results in terms of overall response rate and overall survival compared to historical data in patients receiving PD-1 inhibition alone (25). Another study that combined treatment with a therapeutic DNA vaccine targeting E6 and E7 (GX-188E) and pembrolizumab in patients with HPV16/18-positive advanced cervical cancer also showed improved response rates compared with historical data from patients who received treatment with pembrolizumab alone (26). A phase 2 study of VB10.16 in combination with the PD-L1 inhibitor atezolizumab is currently ongoing in women with HPV16-positive advanced cervical cancer (NCT04405349). This trial uses a schedule of VB10.16 with a similar 3-week dose interval in an induction phase.

The use of a 2-phase approach is typical in early phase studies with an exploratory focus and was of particular benefit in the present study, where a clear difference in immune responses between the initially studied dose regimens was observed, and results from the interim analysis prompting the addition of a fourth vaccination.

Most subjects were followed up for an extended period (up to 12 months) after having received 3 or 4 VB10.16 vaccinations allowing for an adequate characterization of its safety profile. Our study was, however, both limited in size and had extensive exclusion criteria, which were necessary to protect the safety of participating individuals given that this was a first-in-human study with VB10.16. This resulted in the population under examination being more homogenous compared to a real-world situation. Further, we excluded women who had received prior prophylactic HPV vaccination from our study.

Importantly, the Expansion cohort included both subjects with CIN 2 lesions and more severe CIN 3 lesions. As our trial was phase 1 and did not have a placebo or control arm, the observed regressions of lesion size that were seen in most subjects will have to be interpreted with some caution. Biopsies that were taken from CIN lesions during the study period might have resulted in decreased lesion sizes. CIN lesions are also known to have relatively high spontaneous regression rates, although such rates are generally lower (<30%) in subjects with CIN 2 or CIN 3 lesions that were enrolled in our study (21,27,28). Spontaneous regression of CIN 3 lesions caused by HPV16 that were included in the expansion cohort are reported to be even more rare (27). In conclusion, vaccination of women with HPV16-positive high-grade CIN using the unique modular vaccine technology of VB10.16 that is based on linking antigens to a CCL3L1 targeting module, was generally well tolerated, and induced rapid, strong and long-lasting immune responses specific for E6 and E7 antigens. Promising signs of efficacy were observed in subjects who received VB10.16 using a homologous vaccination regimen. A strong T cell response was demonstrated in subjects with lesion size reduction indicating that VB10.16 induced a clinically relevant immune response.

Acknowledgements

The authors thank the subjects and their families for participating in the clinical trial. We would also like to thank the staff at the study sites for their contribution to the clinical trial and members of VB10.16 vaccine program at Nykode Therapeutics ASA for supporting the clinical trial and manufacturing VB10.16. This study was funded by Nykode Therapeutics ASA (formerly Vaccibody AS) and co-funded by the Research Council of Norway (grant no. 219596 and 214866).

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca Cancer J Clin. 2018;68:394–424.

2. Arbyn M, Weiderpass E, Bruni L, Sanjosé S de, Saraiya M, Ferlay J, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Global Heal. 2020;8:e191–203.

3. Plummer M, Martel C de, Vignat J, Ferlay J, Bray F, Franceschi S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Global Heal. 2016;4:e609–16.

4. Crow JM. HPV: The global burden. Nature. 2012;488:S2–3.

5. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of Human Papillomavirus in Cervical Cancer: a Worldwide Perspective. Jnci J National Cancer Inst. 1995;87:796–802.

6. Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Brit J Cancer. 2003;88:63–73.

7. Ronco G, Ghisetti V, Segnan N, Snijders PJF, Gillio-Tos A, Meijer CJLM, et al. Prevalence of human papillomavirus infection in women in Turin, Italy. Eur J Cancer. 2005;41:297–305.

8. Jaisamrarn U, Castellsagué X, Garland SM, Naud P, Palmroth J, Rosario-Raymundo MRD, et al. Natural History of Progression of HPV Infection to Cervical Lesion or Clearance: Analysis of the Control Arm of the Large, Randomised PATRICIA Study. Plos One. 2013;8:e79260.

9. Stoler MH, Wright TC, Sharma A, Apple R, Gutekunst K, Wright TL, et al. High-risk human papillomavirus testing in women with ASC-US cytology: results from the ATHENA HPV study. Am J Clin Pathol. 2011;135:468–75.

10. McCredie MR, Sharples KJ, Paul C, Baranyai J, Medley G, Jones RW, et al. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. Lancet Oncol. 2008;9:425–34.

11. Kyrgiou M, Athanasiou A, Kalliala IEJ, Paraskevaidi M, Mitra A, Martin-Hirsch PP, et al. Obstetric outcomes after conservative treatment for cervical intraepithelial lesions and early invasive disease. Cochrane Db Syst Rev. 2017;11:CD012847.

12. Cheng L, Wang Y, Du J. Human Papillomavirus Vaccines: An Updated Review. Nato Adv Sci Inst Se. 2020;8:391.

13. Bruni L, Diaz M, Barrionuevo-Rosas L, Herrero R, Bray F, Bosch FX, et al. Global estimates of human papillomavirus vaccination coverage by region and income level: a pooled analysis. Lancet Global Heal. 2016;4:e453–63.

14. Chabeda A, Yanez RJR, Lamprecht R, Meyers AE, Rybicki EP, Hitzeroth II. Therapeutic vaccines for high-risk HPV-associated diseases. Papillomavirus Res. 2017;5:46–58.

15. Rumfield CS, Roller N, Pellom ST, Schlom J, Jochems C. Therapeutic Vaccines for HPV-Associated Malignancies. Immunotargets Ther. 2020;9:167–200.

16. Fredriksen AB, Sandlie I, Bogen B. DNA Vaccines Increase Immunogenicity of Idiotypic Tumor Antigen by Targeting Novel Fusion Proteins to Antigen-Presenting Cells. Mol Ther. 2006;13:776–85.

17. Fredriksen AB, Bogen B. Chemokine-idiotype fusion DNA vaccines are potentiated by bivalency and xenogeneic sequences. Blood. 2007;110:1797–805.

18. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;392:245–52.

19. Mellman I, Steinman RM. Dendritic Cells Specialized and Regulated Antigen Processing Machines. Cell. 2001;106:255–8.

20. Choi YJ, Hur SY, Kim T-J, Hong SR, Lee JK, Cho C-H, et al. A Phase II, Prospective, Randomized, Multicenter, Open-Label Study of GX-188E, an HPV DNA Vaccine, in Patients with Cervical Intraepithelial Neoplasia 3. Clin Cancer Res. 2020;26:1616–23.

21. Trimble CL, Morrow MP, Kraynyak KA, Shen X, Dallas M, Yan J, et al. Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. Lancet. 2015;386:2078–88.

22. Harper DM, Nieminen P, Donders G, Einstein MH, Garcia F, Huh WK, et al. The efficacy and safety of Tipapkinogen Sovacivec therapeutic HPV vaccine in cervical intraepithelial neoplasia grades 2 and 3: Randomized controlled phase II trial with 2.5 years of follow-up. Gynecol Oncol. 2019;153:521–9.

23. Kim TJ, Jin H-T, Hur S-Y, Yang HG, Seo YB, Hong SR, et al. Clearance of persistent HPV infection and cervical lesion by therapeutic DNA vaccine in CIN3 patients. Nat Commun. 2014;5:5317.

24. Steenwijk PJ de V van, Ramwadhdoebe TH, Löwik MJG, Minne CE van der, Meer DMAB der, Fathers LM, et al. A placebo-controlled randomized HPV16 synthetic long-peptide vaccination study in women with high-grade cervical squamous intraepithelial lesions. Cancer Immunol Immunother. 2012;61:1485–92.

515 25. Massarelli E, William W, Johnson F, Kies M, Ferrarotto R, Guo M, et al. Combining
516 Immune Checkpoint Blockade and Tumor-Specific Vaccine for Patients With Incurable Human
517 Papillomavirus 16–Related Cancer: A Phase 2 Clinical Trial. Jama Oncol. 2019;5:67.

518 26. Youn JW, Hur S-Y, Woo JW, Kim Y-M, Lim MC, Park SY, et al. Pembrolizumab plus GX-
519 188E therapeutic DNA vaccine in patients with HPV-16-positive or HPV-18-positive advanced
520 cervical cancer: interim results of a single-arm, phase 2 trial. Lancet Oncol. 2020;21:1653–60.

521 27. Motamedi M, Böhmer G, Neumann HH, Wasielewski R von. CIN III lesions and regression:
522 retrospective analysis of 635 cases. Bmc Infect Dis. 2015;15:541.

523 28. Zhang J, Lu C. Spontaneous Regression of Cervical Intraepithelial Neoplasia 2: A Meta-
524 analysis. Gynecol Obstet Inves. 2019;84:562–7.

525

526

527

528
529
530

Tables and Figures

Table 1: Baseline characteristics

Baseline characteristics	VB10.16 Dose Cohort (3 mg/mL)			
	Cohort 1	Cohort 2	Expansion	Overall
Number of Subjects	8	8	18	34
Age (years)				
N	8	8	18	34
Mean	31.4	27.4	29.1	29.2
18 to 64	8 (100.0%)	8 (100.0%)	18 (100.0%)	34 (100.0%)
Cervical Dysplasia Categorization				
CIN 2	8 (100.0%)	8 (100.0%)	8 (44.4%)	24 (70.6%)
CIN 3	0	0	10 (55.6%)	10 (29.4%)
HPV16 Present	8 (100.0%)	8 (100.0%)	17 (94.4%)	33 (97.1%)
Other High-risk HPV Present	3 (37.5%)	5 (62.5%)	7 (38.9%)	15 (44.1%)
ECOG Performance Status				
0	8 (100.0%)	8 (100.0%)	18 (100.0%)	34 (100.0%)

CIN, cervical intraepithelial neoplasia; ECOG, Eastern Co-operative Oncology Group; HPV, Human Papilloma Virus. All enrolled subjects were Caucasian.

538 **Table 2: Common solicited and unsolicited treatment-related AEs (≥10%) reported during**
539 **the period from administration of the first VB10.16 dose to 30 days post last dose in all**
540 **cohorts combined**

MedDRA System Organ Class MedDRA preferred term	**Overall (%)**
Number of Subjects	34
General disorders and administration site conditions	**32 (94%)**
Injection site pain	27 (79%)
Injection site erythema	17 (50%)
Injection site hypersensitivity	14 (41%)
Injection site hyperaesthesia	13 (38%)
Injection site swelling	11 (32%)
Swelling	6 (18%)
Fatigue	5 (15%)
Pain	5 (15%)
Nervous system disorders	**22 (65%)**
Headache	13 (38%)
Hyperaesthesia	13 (38%)
Skin and subcutaneous tissue disorders	**14 (41%)**
Erythema	11 (32%)

AE, adverse event; MedDRA, Medical Dictionary for Regulatory Activities

541

Figure 1: Diagram of the therapeutic DNA vaccine VB10.16 designed by the unique modular vaccine technology linking antigens to a CCL3L1 targeting module. (A) The VB10.16 DNA vaccine was constructed through insertion of a coding sequence (CDS) encoding inactivated E7 and E6 HPV16 proteins linked to the chemokine CCL3L1 including its native signal peptide, through a human immunoglobulin G (IgG3) based dimerization unit consisting of hinge region 1 of human IgG3, hinge region 4 of human IgG3 and CH3 domain of human IgG3 into pUMVC4a expression vector. (B) The translated Vaccibody protein consists of inactivated E6 and E7 HPV16 proteins linked to the human chemokine CCL3L1 through a human immunoglobulin G (IgG3) based homodimerization unit.

Figure 2: Best overall change from baseline in CIN lesions. Each bar in the waterfall plot represents one subject indicating maximum change in lesion size and CIN staging during the 12 months follow-up period in all evaluable subjects enrolled in the Expansion cohort (n=17) with CIN 2 or CIN 3 at baseline. Changes from baseline in lesion size and grading were assessed locally. Grey scaling indicates the CIN grading where 10 subjects showed no CIN or CIN 1 as best response. One subject had a conization performed before the 24 weeks follow up visit (first bar).

CIN, cervical intraepithelial neoplasia.

Figure 3: VB10.16 induced strong and long-lasting HPV16-specific T cell response after homologous boost vaccination significantly correlated with lesion size regression. Patients' PBMCs were analysed before (V1), during (1 weeks post each vaccination) and 8 weeks after (week 24) vaccination with VB10.16. The number of HPV16 E6- and E7-specific IFN-γ secreting cells was determined individually by IFN-γ ELISPOT assays after 5-day *in vitro* stimulation with HPV16 E6 or E7 peptide pools. Shown are the spot-forming units (SFU) per 10^6 PBMCs (average of triplicates) after subtracting the background number of spots (37.1±6.8) at pre-vaccination and peak response post-vaccination. Bars represent stacked E6 and E7 peptide-specific baseline (grey) and post-vaccination (black) response in the dosing Cohort 1 (A), dosing Cohort 2 (B), and Expansion Cohort (C). The kinetic of immune response is illustrated for Cohort 1 and Expansion Cohort (D). Error bars represent SEM. IFNγ HPV16-specific T cell responses were significantly correlated with lesion size regression (E, F). A comparison between lesion size regression as best response against peak IFN-γ response post vaccination of participants in cohort 1, 2 and expansion cohort are visualized by floating bars. A Mann–Whitney test was used to compare groups, indicated by the p-value ($p < 0.001$). Floating bars show min, median and max values. Open, grey and closed dots represent cohort 1, 2 and expansion cohort. A generalized linear model with gamma distribution and inverse model link function was fitted to the data in figure 3F. An ANOVA analysis was used to generate the p-value for SFU (details in supplementary information).

The HPV16 type was confirmed for all patients by COBAS HPV Test prior to vaccination.

582 *PBMC samples at baseline were lost in 2 subjects. SFU, spot forming units; PBMC, peripheral*
583 *blood mononuclear cell*

584

585 **Figure 4: PD-L1 Expression increased in lesions after VB10.16 vaccination.** PD-L1
586 expression was assessed by immunohistochemistry in cervical biopsies collected at screening,
587 and at week 16 and week 24 after first vaccination. PD-L1 is reported at screening and maximum
588 response at post vaccination visit in subjects enrolled into the Expansion Cohort.

589 *PD-L1, programmed death ligand 1; pre-vac, before vaccination; post-vac, after vaccination*

Figure 1

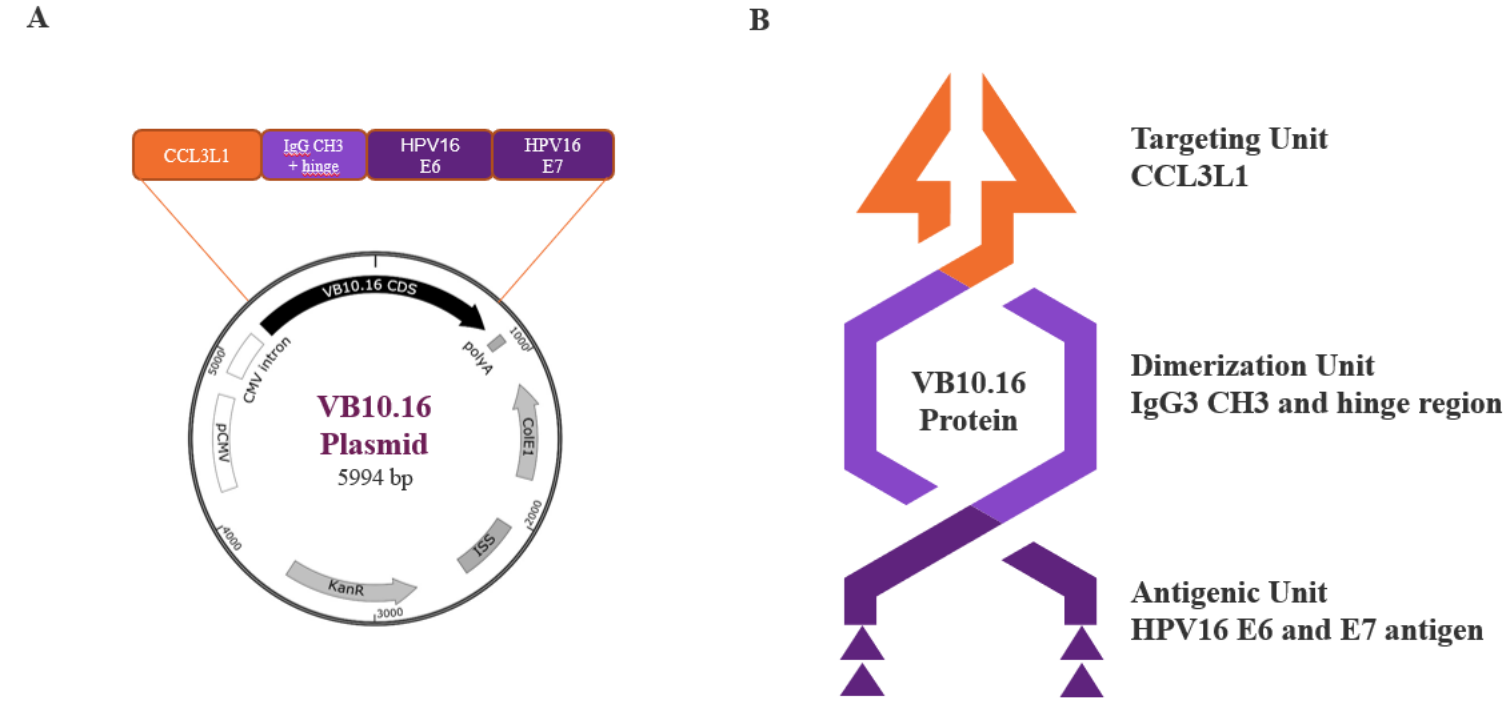
A
CCL3L1
IgG CH3 + hinge
HPV16 E6
HPV16 E7
VB10.16 CDS
polyA
ColE1
ISS
KanR
pCMV
CMV intron
VB10.16 Plasmid
5994 bp
1000
2000
3000
4000
5000
B
VB10.16 Protein
Targeting Unit
CCL3L1
Dimerization Unit
IgG3 CH3 and hinge region
Antigenic Unit
HPV16 E6 and E7 antigen

Figure 2

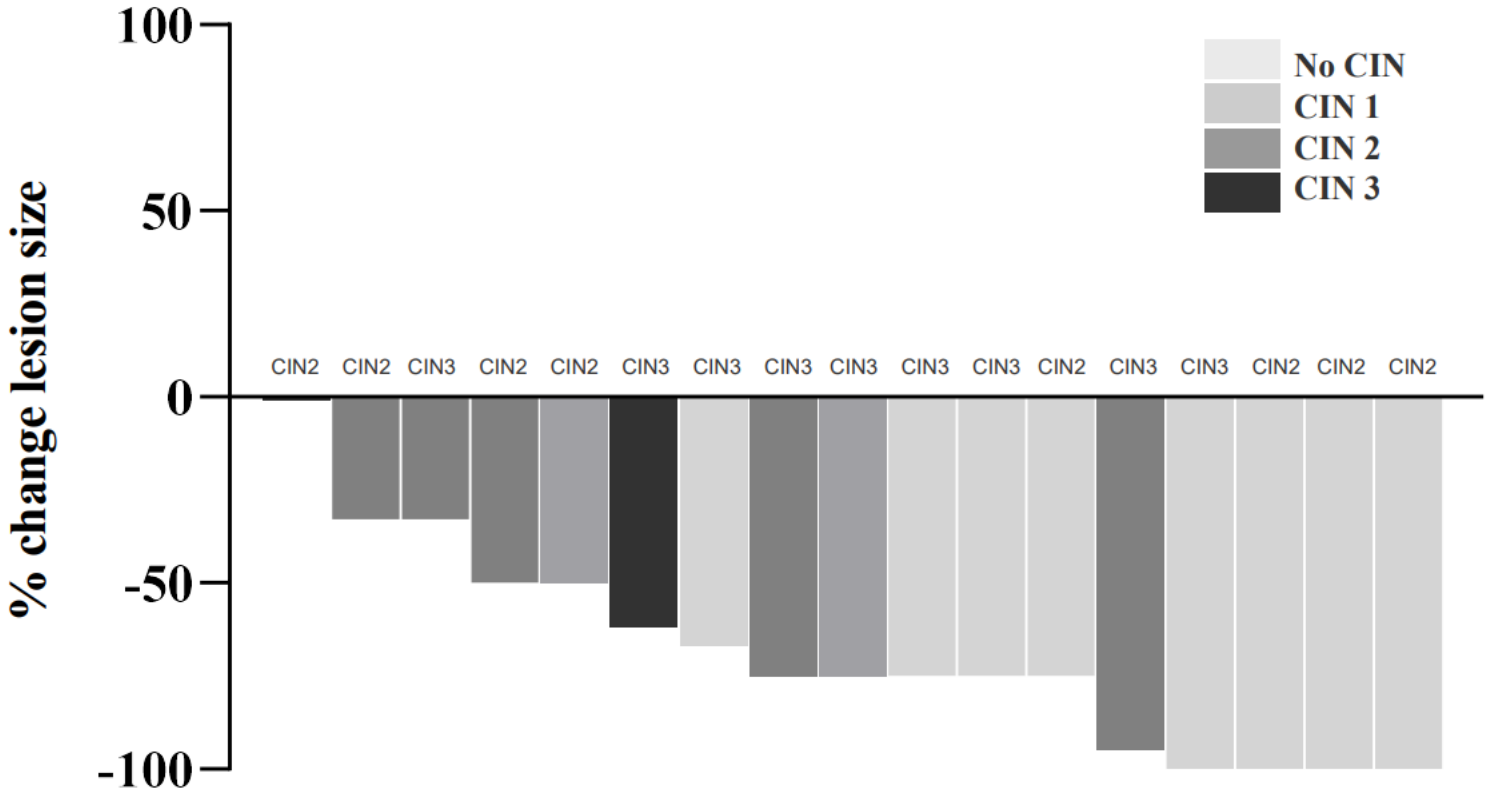
100
50
0
-50
-100
% change lesion size
No CIN
CIN 1
CIN 2
CIN 3
CIN2
CIN2
CIN3
CIN2
CIN2
CIN3
CIN3
CIN3
CIN3
CIN3
CIN3
CIN2
CIN3
CIN3
CIN2
CIN2
CIN2

Figure 3

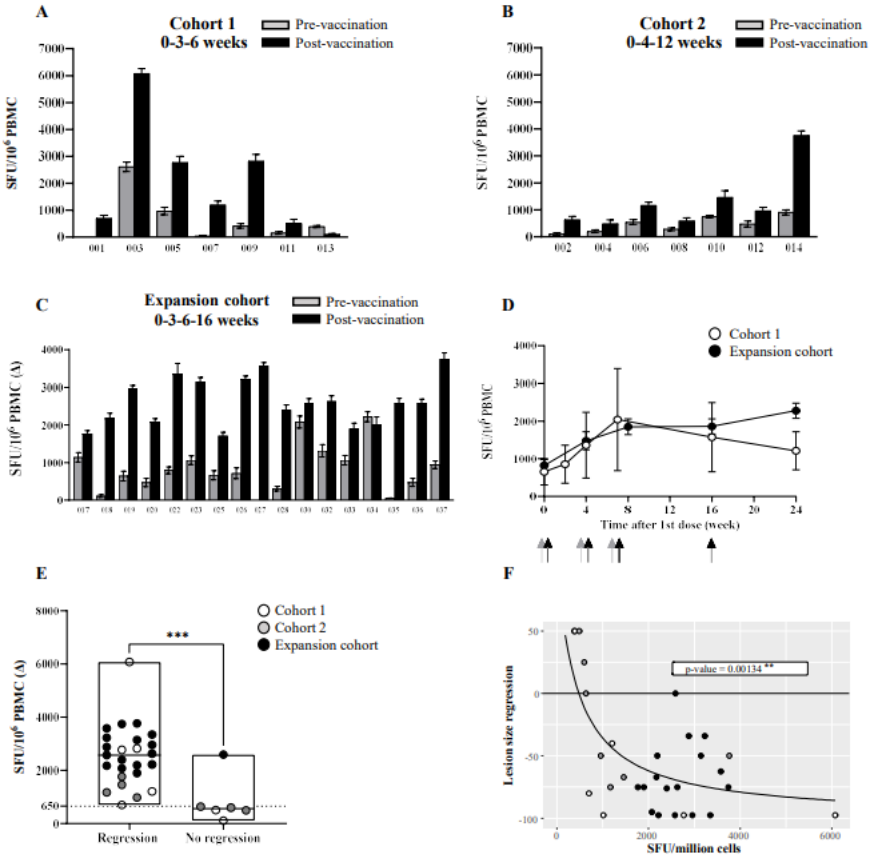
A
Cohort 1
0-3-6 weeks
Pre-vaccination
Post-vaccination
SFU/10^6 PBMC
7000
6000
5000
4000
3000
2000
1000
0
001
003
005
007
009
011
013
B
Cohort 2
0-4-12 weeks
Pre-vaccination
Post-vaccination
SFU/10^6 PBMC
7000
6000
5000
4000
3000
2000
1000
0
002
004
006
008
010
012
014
C
Expansion cohort
0-3-6-16 weeks
Pre-vaccination
Post-vaccination
SFU/10^6 PBMC (A)
4000
3000
2000
1000
0
017
018
019
020
022
023
025
026
027
028
030
032
033
034
035
036
037
D
Cohort 1
Expansion cohort
SFU/10^6 PBMC
4000
3000
2000
1000
0
0
4
8
12
16
20
24
Time after 1st dose (week)
E
Cohort 1
Cohort 2
Expansion cohort
***
SFU/10^6 PBMC (A)
8000
6000
4000
2000
650
0
Regression
No regression
F
p-value = 0.00134 **
Lesion size regression
50
0
-50
-100
0
2000
4000
6000
SFU/million cells

Figure 4