Induction of Neoantigen-Specific Immune Responses by VB10.NEO in Combination with Atezolizumab in Heavily Pretreated Patients with Advanced Solid Tumors: Final Analysis of the Phase 1b VB N-02 Trial

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BACKGROUND

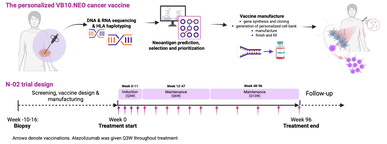
VB10.NEO is a personalized, DNA-based neoantigen vaccine evaluated in combination with atezolizumab in the Phase 1b VB N-02 trial (NCT05018273). Neoantigens are selected using Nykode's NeoSELECT™ platform, which integrates tumor RNA sequencing, whole-exome data, and circulating tumor DNA to prioritize highly immunogenic and clonal neoantigens – including single nucleotide variants and frameshift mutations, adapted to the patient's individual HLA type to optimize presentation. Up to 20 patient-specific neoantigens are encoded into a circular DNA plasmid and delivered intramuscularly using a needle-free jet injection system. The vaccine construct includes a proprietary protein targeting unit that directs antigens to antigen-presenting cells, aiming to elicit robust CD8+ and CD4+ T cell responses. Atezolizumab, an anti-PD-L1 monoclonal antibody, may potentiate this effect by restoring T cell function within the tumor microenvironment. We intended to test this combination treatment in heavily pretreated patients with advanced solid tumors, many of whom have low tumor mutational burden and PD-L1–negative disease. VB N-02 assessed safety, immune activation, and preliminary antitumor activity in this population.

Figure 1: Schematic structure of VB10.NEO

STUDY DESIGN AND METHODS

- VB N-02 is an open-label, dose-escalation, multicenter Phase 1b trial conducted across sites in Germany, Spain, and Norway, as well as the United States (Figure 2).
- Vaccine manufacturing required a median of ~6 weeks from biopsy to first dose. During this period, patients could receive optional bridging therapy per investigator discretion.

Figure 2: Trial Design



The VB N-02 trial used a safety run-in and expansion design, enrolling 3–6 patients per dose level to assess DLTs. Occurrence of ≥2 DLTs in a cohort led to de-escalation or halt of escalation.

Treatment Regimen
VB10.NEO: Three dose levels (3 mg, 6 mg, 9 mg), administered: Q3W for 4 induction doses (C1–C4), then Q6W for 6 doses (C5–C15), then Q12W for up to 5 doses (C17–C33).
Atezolizumab: Fixed IV dose of 1200 mg every 3 weeks (Q3W) for up to 34 cycles.
Imaging Schedule: Tumor assessments were performed every 8 weeks through Week 48, then every 12 weeks thereafter, per RECIST v1.1. Treatment continued until progression, unacceptable toxicity, or investigator-determined lack of clinical benefit.

Key Inclusion Criteria

- Age ≥18 years with ECOG performance status 0–1
- Histologically confirmed locally advanced or metastatic solid tumors
- Disease progression after ≥1 prior line of standard systemic therapy, or no suitable standard options available. Prior immune checkpoint inhibitor therapy permitted
- Measurable disease per RECIST v1.1
- ≥10 predicted tumor neoantigens identified by NeoSELECT™ platform
- Adequate tumor material for DNA/RNA sequencing and vaccine manufacturing
- Adequate hematologic and organ function
- Life expectancy ≥12 weeks
- Availability of fresh or archival tumor tissue (≤4 months old preferred)

Endpoints

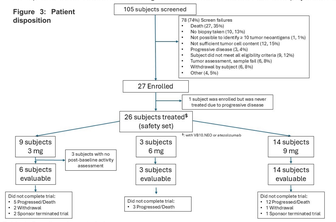
- **Primary**: Safety and neoantigen-specific immune responses
- **Secondary**: ORR, DoR, PFS (per RECIST v1.1), OS
- **Exploratory**: Biomarkers (e.g., ctDNA, immune infiltrates), TCR clonality, PK and ADA for atezolizumab

* Figure made with BioRender.com

PATIENTS

- A total of 26 patients were enrolled between November 2021 and October 2023 at 10 sites in Germany, Spain, Norway, and the United States.
- At the time of the current analysis (October 2024), all 26 patients had discontinued treatment (Figure 3). The primary reason for discontinuation was progressive disease (n=17, 65.4%; n=11 [42.3%] per RECIST v1.1 and n=6 [23.1%] due to clinical progression), followed by death (n=4, 15.4%), subject withdrawal (n=2, 7.7%), and other reasons including non–treatment-related AEs and physician decision (n=3, 11.5%).

Figure 3: Patient disposition



The median age was 61 years (range, 28–72), and 62% were female. Patients had received a median of 5 prior lines of therapy (range, 1–8), and 50% had received prior checkpoint inhibitor therapy. The most common tumor types were head and neck squamous cell carcinoma (15%) and triple-negative breast cancer (15%). Most patients (60%) had tumors with low or negative PD-L1 expression (Table 1).

Table 1: Baseline characteristics

Characteristics	VB10.NEO + Atezolizumab (n=26)
Median age, y (range)	61.0 (28 – 72)
Female, n (%)	16 (61.5)
ECOG PS 0, n (%)	16 (61.5)
Primary tumor diagnosis, n (%)	
• Head and neck squamous cell carcinoma	4 (15.4)
• Triple-negative breast cancer	4 (15.4)
• Non-smell cell lung cancer	3 (11.5)
• Renal cell carcinoma	3 (11.5)
• Adenoid cystic carcinoma of the salivary glands	3 (11.5)
• Melanoma	2 (7.7)
• Colorectal cancer	2 (7.7)
• Other*	5 (19.2)
Stage IV at initial diagnosis, n (%)	10 (38.5)
Sites of metastases, n (%)	
• Lung	17 (65.4)
• Lymph nodes	17 (65.4)
• Liver	10 (38.5)
• Soft tissue	5 (19.2)
• Bone	5 (19.2)
• Brain	2 (7.7)
Prior surgery/radiotherapy, n (%)	19 (73.1) / 17 (64.3)
Prior anti-cancer systemic therapy, n (%)	
• Median number of lines (range)†	5 (1-8)
• ≥3 lines†	19 (79%)
• Prior CPI	13 (50%)
• Prior chemotherapy	20 (76.9)
• Bridging therapy	17 (65.4)
PD-L1 negative or low‡, n (%)	6 (60)
Median time between initial cancer diagnosis and main ICF, months (range)	39.9 (4.2 – 195.2)
Median time between documented advanced disease and main ICF, months (range)	19.0 (-5.7 – 99.2)

* "Other" tumor types were anal cancer, gastric/gastro esophageal junction cancer, intima sarcoma, pancreatic adenocarcinoma and vulvar cancer (one patient each).
† Number of previous lines available for 24 out of 26 patients; relative frequency shown is based on this subgroup.
‡ PD-L1 expression was reported by site per local standards; a formal cutoff for "low" expression was not prespecified. PD-L1 data were available for 10 patients; percentages shown are based on this subgroup.

TREATMENT EXPOSURE
The median duration of exposure to both VB10.NEO and atezolizumab was 10.4 weeks. Patients received a median of 3 cycles of VB10.NEO (range: 1–10) and a median of 4 cycles of atezolizumab (range: 1–12). Out of the 26 patients, only 7 (27%) stayed on the trial long enough to receive more than 4 vaccinations. One patient (3.8%) experienced a dose delay or interruption of VB10.NEO due to an adverse event. Atezolizumab administration was delayed in 4 patients (15.4%) and interrupted in 3 patients (11.5%) due to adverse events.

ACROSS ALL DOSE LEVELS, VB10.NEO INDUCED ROBUST AND DURABLE NEOANTIGEN-SPECIFIC IMMUNE RESPONSES

Neoantigen-specific immune responses were seen in 100% of patients by IVS ELISpot (58% of epitopes) and 22% of patients by *ex vivo* ELISpot (5% of epitopes) using a conservative statistical test for immunogenicity calling (Figure 4). The observed IVS responses constituted both pre-existing stable, amplified and *de novo* responses, and on a patient-level, 85% of patients (11/13) showed *de novo* responses (Figure 5). All vaccine-induced responses by *ex vivo* ELISpot (22% of patients) were *de novo* responses. Literature supports that IVS assays may be particularly suitable to detect memory responses[1,2,3], and the strong responses seen by IVS ELISpot suggest the ability of the vaccine to induce memory responses.

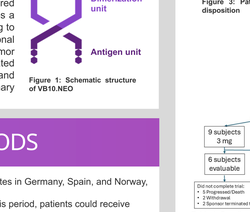


Figure 4: Patient-level and epitope-level summary of immune responses by *ex vivo* and IVS ELISpot. Both *in vitro* stimulated (IVS, n=13 patients) and *ex vivo* (n=18 patients) ELISpot assays were performed on pre- and on-treatment samples (multiple timepoints analyzed, median 2 range 2-3 timepoints, epitopes analyze individually). Positive immunogenicity at each analyzed timepoint was determined by a statistical test with pre-defined thresholds (IVS DFR1.3x, *ex vivo* DFR2x). On a patient-level, positive immunogenicity was called if one or more epitopes were immunogenic.

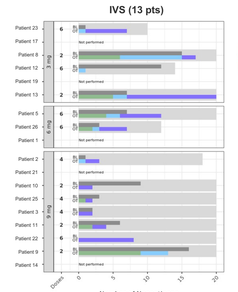


Figure 5: Neoantigen immunogenicity and type of response per patient by IVS ELISpot
The type of response (represented by different colours) was assessed as stable (immunogenic both pre- and on-treatment), amplified (immunogenic pre- and on-treatment with a 1.3-fold increase for IVS), and *de novo* (non-immunogenic pre-treatment and immunogenic on-treatment). Both *de novo* and amplified responses were regarded treatment-induced. The number of immunogenic neoantigens by the different categories are shown on the x-axis. The y-axis represents the patient ID grouped by dose level (3, 6, and 9 mg of VB10.NEO) and the number of received doses are shown (range: 2-6 doses, range 2-3 visits analyzed). Up to 20 epitopes were tested but limited sample availability prevented testing of all neoantigens in a subset of the patients. BL: Baseline response, OT: On-treatment response. Not performed: Samples not available for analysis.

T CELL RECEPTOR SEQUENCING SHOWED PERSISTENT T-CELL CLONE EXPANSION

T cell receptor sequencing of pre- and on-treatment blood samples from 11 patients showed a strong peripheral T cell expansion in all analyzed patients (Figure 6). In 9 of the 11 patients, clones were persistently expanded (≥2 timepoints and first time expanded before week 25; Figure 7). These durably and early-expanded clones constituted both pre-existing expanded as well as *de novo* clones (Figure 8).

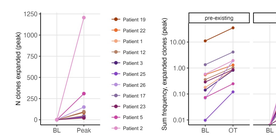


Figure 6: Number of expanded clones at peak and sum frequency of pre-existing expanded and novel clones at baseline and peak. Sequencing of the TCRB locus of the T cell receptor beta chain was performed in pre- and on-treatment blood samples (gDNA, multiple on-treatment timepoints). Significant clonal expansion was determined by differential abundance analyses (binomial test, minimum 5 counts, FDR-adjusted p<0.01). The sum frequency represents the total frequency ("stacked" frequency) of the individual expanded clonotypes. Per definition, *de novo* clones have a frequency of 0 at baseline.

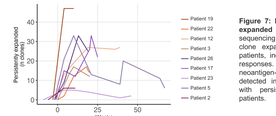


Figure 7: Kinetics of persistently expanded clones. T cell receptor sequencing showed persistent T cell clone expansion in 9 out of 11 patients, indicating durable immune responses. Additionally, putative neoantigen-specific clones were detected in 6/7 analyzed patients, with persistent expansion in 4 patients.

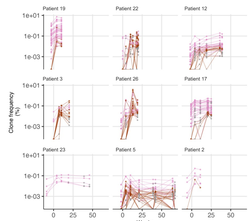


Figure 8: Clone frequencies of persistently and early-expanded clones. Individual clone frequencies of persistently expanded clones in 9 patients displaying such expansion. Y-axis shows the clone frequency in percent on a log scale and x-axis shows the timepoint in weeks. Eight of the 9 patients had persistently expanded *de novo* clones. Pink: pre-existing expanded clones. Dark orange: novel clones. Grey points (NS=non-significant) connected by pink or dark orange edges indicate that the clone in question was significantly expanded on one of the other timepoint but not at that particular timepoint.

SAFETY

Treatment-related adverse events (AEs) of any grade assessed as related to VB10.NEO occurred in 15 patients (58%). The most commonly-reported treatment-related AE was fatigue (3 patients, 12%, Figure 9), followed by increased ASAT, injection site pain, myalgia, asthenia, and pyrexia (each in 2 patients, 8%). Most events were mild to moderate (Grade 1–2). Two patients (8%) experienced Grade 3 AEs: one patient in the 9 mg cohort experienced a Grade 3 transient blood pressure increase (192/81 mmHg), which was considered a dose-limiting toxicity (DLT) potentially related to VB10.NEO, and another patient experienced elevated liver enzymes (ASAT, ALP, and lipase) deemed potentially related to both VB10.NEO and atezolizumab. No VB10.NEO-related AEs were serious or fatal.

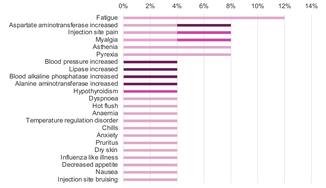


Figure 9: AEs by grade and frequency in overall population

TUMOR RESPONSE AND SURVIVAL

Of 23 subjects with one post-baseline tumor assessment, no subject achieved a CR or PR; the ORR was 0%. Eight subjects (34.8%) had stable disease. The DCR was 34.8% (95% CI: 16.4%, 57.3%). All SD patients exhibited neoantigen-specific immune responses (Table 2).

Table 2: Clinical and biomarker characteristics for 8 patients with stable disease

Patient ID	Dose	Site of primary tumor	PFS (days)	Prior Lines	Prior CPI	PD-L1	IVS ELISpot (treatment-induced response)	Ex Vivo ELISpot (treatment-induced response)	Durable expansion of clonotypes by TCRseq	Confirmed expansion of NeoAg-specific clones by TCRseq
9	9 mg	Anal	67	5	Yes	NA	Positive	Negative (positive baseline)	Not tested	Not tested
22	9 mg	Triple negative breast cancer	127	NA	No	Negative	Positive	Negative	Yes	Yes
1	6 mg	Melanoma	160	2	Yes	NA	Not performed	Negative (positive baseline)	Not tested	Not tested
12	3 mg	Adenoid cystic carcinoma of the salivary glands	241	3	No	Negative	Positive	Negative	Yes	Yes
11	9 mg	Vulvar	36	NA	No	NA	Positive	Negative (positive baseline)	Not tested	Not tested
3	9 mg	Renal cell carcinoma	80	6	Yes	NA	Positive	Negative	Yes	No
17	3 mg	Head and neck squamous cell carcinoma	126	4	Yes	Positive	Not performed	Positive	Yes	Yes
23	3 mg	Adenoid cystic carcinoma of the right gl. submandibularis.	254	1	No	NA	Positive	Negative	Yes	Yes

At data cut-off, 22 of 23 patients (95.7%) had experienced a PFS event. The median PFS was 1.4 months (95% CI: 1.22– 2.20, Figure 10). The 6-month PFS rate was 9.2% (95% CI: 1.6–25.4); no patients remained progression-free at 12 months or beyond. Fourteen patients (60.9%) had died, with 9 (39.1%) censored for OS. The median OS was 6.4 months (95% CI: 4.8–12.9, Figure 10). OS rates at 6, 12, and 18 months were 69.6%, 32.5%, and 26.0%, respectively; 24-month OS was not calculated due to limited follow-up.

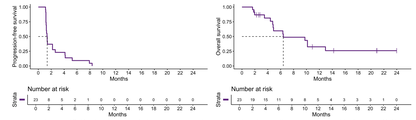


Figure 10: Kaplan-Meier plots for progression-free survival and overall survival (Evaluable Analysis Set)

CONCLUSIONS

- VB10.NEO in combination with atezolizumab was well tolerated and demonstrated a favorable safety profile across all dose levels, with no serious or fatal treatment-related adverse events.
- One DLT—a transient Grade 3 blood pressure increase—was observed at the 9 mg dose level (DL3); as only one DLT occurred, the dose was considered tolerable.
- Despite the absence of objective responses, robust and durable neoantigen-specific immune responses were observed in most evaluable patients.
- The trial enrolled a heavily pre-treated population, with a median of 5 prior therapy lines, frequent prior exposure to checkpoint inhibitors (4/8 SD patients), and predominantly low or negative PD-L1 expression. These characteristics, along with likely low tumor mutational burden in several tumor types and a low fraction of the patients staying on trial beyond the induction period, define a clinically resistant population with limited expected benefit from immunotherapy.
- The median PFS was reached before 2 months, and consequently, a low proportion of the patients stayed in the trial long enough to potentiate a clinically meaningful response.
- Despite these challenges, all patients with stable disease exhibited vaccine-induced T cell responses, as measured by IVS ELISpot, some also showing *ex vivo* immunogenicity signals. TCR sequencing confirmed durable treatment-induced expansion of T cell clones in all evaluable SD cases, including detection of putative neoantigen-specific sequences in 4/5 patients tested.
- These findings demonstrate that VB10.NEO can induce robust and durable peripheral immune responses even in a biologically hard-to-treat setting and support further evaluation in future trials.

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