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Safety and efficacy of the therapeutic DNA-based vaccine VB10.16 in combination with atezolizumab in persistent, recurrent or metastatic HPV16-positive cervical cancer: a multicenter, single-arm phase 2a study

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SUMMARY

Background Second-line treatment options for persistent, recurrent or metastatic (r/m) cervical cancer are limited. We investigated the safety, efficacy, and immunogenicity of the therapeutic DNA-based vaccine VB10.16 combined with the immune checkpoint inhibitor atezolizumab in patients with human papillomavirus (HPV)16-positive r/m cervical cancer.

Patients and methods This multicenter, single-arm, phase 2a study (NCT04405349, registered 26 May 2020) enrolled adult patients with persistent, r/m HPV16-positive cervical cancer. Patients received 3 mg VB10.16 (every 3 weeks (Q3W) for 12 weeks, hereafter every 6 weeks) combined with 1,200 mg atezolizumab (Q3W) for 48 weeks in total with a 12-month follow-up. The primary endpoints were incidence and severity of adverse events (AEs) and objective response rate (ORR; Response Evaluation Criteria in Solid Tumor V.1.1). ORR was assessed in the efficacy population, being all response-evaluable patients who received any administration of VB10.16 and atezolizumab and had at least one post-baseline imaging assessment.

Results Between June 16, 2020, and January 25, 2022, 52 patients received at least one administration of study treatment. Of these, 47 patients had a minimum of one post-baseline tumor assessment. The median follow-up time for survival was 11.7 months. AEs related to VB10.16 were non-serious and mainly mild injection site reactions (9 of 52 patients). There were no signs of new toxicities other than what was already described with atezolizumab. ORR was 19.1% (95% CI 9.1% to 33.3%). Median duration of response was not reached (n.r.) (95% CI 2.2 to n.r.), median progression-free survival was 4.1 months (95% CI 2.1 to 6.2), and median overall survival 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). HPV16-specific T-cell responses were analyzed in 36 of 47 patients with an increase observed in 22/36 (61%).

Conclusions The therapeutic DNA-based vaccine VB10.16 combined with atezolizumab was safe and well tolerated showing a promising clinically meaningful efficacy with durable responses in patients with persistent, r/m HPV16-positive cervical cancer, especially if PD-L1-positive.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ A number of therapeutic human papillomavirus (HPV) vaccine candidates have been studied in cervical intraepithelial neoplasia (CIN). The first-in-human clinical study VB-C-01 (NCT02529930) evaluated the VB10.16 vaccine in HPV16-positive high-grade CIN 2/3 and demonstrated HPV16-specific T-cell responses in >90% of the subjects.

WHAT THE STUDY ADDS

⇒ The VB C-02 study (NCT04405349) demonstrates the benefit of adding a therapeutic HPV16-specific DNA-based therapeutic vaccine to an immune checkpoint inhibitor (ICI) in late stage patients with previously treated HPV16-positive cervical cancer. VB10.16 was tolerable with a manageable safety profile showing clinically meaningful efficacy with durable responses especially in programmed death-ligand 1 positive patients.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Despite screening programs and prophylactic HPV vaccinations, HPV-associated cancer remains a global public health problem and further investigations of VB10.16 combined with an ICI in HPV16-related malignant lesions are warranted.

INTRODUCTION

Cervical cancer is the fourth most frequent cancer type in women, with more than 600,000 new cases and over 340,000 deaths worldwide annually.[1 2] The leading cause of cervical cancer is persistent human papillomavirus (HPV) infection with a high-risk subtype detected in 99%, of which HPV16 is the most predominant (50–60%).[3] Despite advances in screening programs and prophylactic HPV vaccination, HPV-associated advanced cervical cancer remains a global public health issue with high unmet medical needs.[4]

Platinum-based chemotherapy in combination with the angiogenesis inhibitor bevacizumab has been considered standard of care treatment for patients with persistent, recurrent or metastatic (r/m) cervical cancer for a decade.[5] More recently, immune checkpoint inhibitors (ICIs) targeting the programmed death-(ligand) 1 (PD-1/PD-L1) pathway have gained attention and regulatory approval in this setting. Pembrolizumab (anti-PD-1) has received approval as monotherapy in second-line treatment based on the KEYNOTE-158 study[6] and in combination with platinum-based chemotherapy with or without bevacizumab in first-line setting based on the KEYNOTE-826 study.[7 8] The anti-PD-1 cemiplimab has received European regulatory approval as monotherapy in second-line treatment based on the EMPOWER-Cervical 1 study.[9] Finally, the anti-PD-L1 atezolizumab has demonstrated effect in first-line combination treatment and in the second-line setting as monotherapy as reported in the BEATcc and SKYSCRAPER-04 studies, respectively.[10 11] These data demonstrate that exhausted T cells can be re-activated against viral antigens (such as E6 and E7) and lead to tumor response, but only in a limited proportion of patients with cervical cancer. Thus, there is a rationale for developing therapeutic vaccines with potential synergistic effect on T cells for HPV-driven cancers such as HPV16-positive cervical cancer.[12]

VB10.16 is an investigational, non-integrating DNA-based therapeutic vaccine developed to treat HPV16-associated premalignant and malignant lesions. VB10.16 encodes a recombinant fusion protein consisting of mutation-inactivated HPV16 E6 and E7 oncoprotein antigens linked, via a CH3-hinge dimerization module derived from human immunoglobulin G (IgG3), to the natural human chemokine ligand 3-like 1 (CCL3L1).[13] CCL3L1 attracts and targets professional antigen-presenting cells (APCs) and ensures binding and direct delivery of E6 and E7 antigens to the APCs. The subsequent cross-presentation of E6 and E7 peptides prime and activate cognate T cells, which can then kill cancer cells presenting HPV16 antigens on their cell surface.[14 15] A prior study investigating the safety, immunogenicity, and preliminary efficacy of VB10.16 in patients with HPV16-positive, high-grade cervical intraepithelial neoplasia, showed that VB10.16 was well tolerated, induced a robust and prolonged HPV-specific T-cell response, as well as initial signs of efficacy and upregulation of PD-L1 in the tumorous epithelium.[13] These results, together with previous findings in studies combining HPV vaccines with anti-PD-1 antibodies,[16 17] provide a strong rationale for investigating a potential synergistic effect of VB10.16 with anti-PD-1/PD-L1 checkpoint inhibitors. Here we present data from the VB C-02 phase 2a study (NCT04405349) investigating the DNA-based therapeutic vaccine VB10.16 in combination with atezolizumab in patients with persistent, r/m HPV16-positive cervical cancer.

PATIENTS AND METHODS

Study design and participants

This multicenter, open-label, single-arm, phase 2a study, with a treatment period of approximately 52 weeks (48 weeks of treatment and end-of-treatment visit after 48 weeks+30 days) and a 12-month follow-up period, enrolled patients at 14 sites across 6 European countries. Eligible patients were aged ≥18 years and had non-resectable, confirmed HPV16-positive squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma of the cervix, which was persistent, r/m; had failed or were not eligible for treatment with systemic chemotherapy, radiotherapy, or other standard-of-care anticancer treatments; had a life expectancy of at least 6 months and had the measurable disease as assessed by the investigator as per Response Evaluation Criteria in Solid Tumors (RECIST V.1.1).[18] Patients who had received prior treatment with ICIs were ineligible. The study protocol and its amendments (see online supplemental file 2) were approved by local or national ethics committees for each participating site before study initiation, see online supplemental table S5 for an overview of the ethics approvals for each participating country. The study was done in accordance with the International Council for Harmonisation (ICH) and Good Clinical Practice (GCP) guidelines, Declaration of Helsinki and all applicable laws, and patients were to provide written informed consent prior to any study activity.

Procedures

VB10.16 was supplied as 3.0 mg/mL vials and administered as two 0.5 mL intramuscular injections using the PharmaJet Stratis 0.5 mL Needle-free Injection System (PharmaJet, Colorado, USA). Patients received up to 11 vaccinations of VB10.16 (3 mg) over a period of up to 48 weeks, with five administrations at 3-week intervals during the first 12 weeks (induction period) followed by six administrations at 6-week intervals (maintenance period). Patients also received up to 17 concomitant intravenous administrations of atezolizumab (1,200 mg) every 3 weeks for 48 weeks. Treatment with both study drugs was continued until disease progression or other protocol-specified criteria for treatment discontinuation.

Adverse events (AEs) were monitored throughout treatment and for 30 days after the end of treatment. Serious AEs assessed as related to VB10.16 or atezolizumab were collected during the 12-month follow-up period. AEs were graded for severity in accordance with the National

Cancer Institute Common Terminology Criteria for Adverse Events V.5.0. Treatment-emergent AEs assessed by the investigators as related to either VB10.16, atezolizumab or both are presented in this article.

Safety assessments also included evaluation of physical examination findings and recordings of vital signs for the treatment period, and safety laboratory values for the total duration of the study.

Objective response assessments were performed by imaging every 9 weeks throughout the treatment period, and at the scheduled end-of-treatment visit according to RECIST V.1.1 criteria.

An archival (≤2 years) or fresh (≤28 days before treatment initiation) formalin-fixed paraffin-embedded tumor tissue sample was used to confirm HPV16-positive status and for evaluation of PD-L1 expression status. To confirm HPV16 positivity, HPV genotyping on tumor tissue from study screening was performed in a central laboratory using a PCR-based method (see online supplemental materials, page 3). Tumor tissue samples obtained at study screening were analyzed for PD-L1 expression (Ventana PD-L1 (SP263), Roche Diagnostics, Indiana, USA) in a central laboratory to evaluate PD-L1-positive tumor and immune cells (Tumor Area Positivity (TAP) Score) with ≥5% threshold[19] (see online supplemental materials, page 3).

The immunogenicity of the VB10.16 vaccine was evaluated by analyzing the HPV16 E6/E7-specific T-cell responses using ex vivo interferon (IFN)-γ enzyme-linked immunospot assay (ELISpot; Mabtech AB, Sweden) in triplicates (see online supplemental materials, page 3).

Outcomes

The primary endpoints of the study were: (1) incidence and severity of AEs and (2) antitumor activity assessed by objective response rate (ORR) defined as the proportion of patients, who had a complete response (CR) or partial response (PR) per RECIST V.1.1.,[18] confirmed or unconfirmed, as a best overall response (BOR).

The secondary endpoints were duration of response (DOR), progression-free survival (PFS), overall survival (OS), and evaluation of clonal T-cell immunogenicity of VB10.16 in combination with atezolizumab.

As planned subgroup analysis, ORR was analyzed by PD-L1 status (positive or negative). As post hoc analyses, disease control rate (DCR) was calculated by adding the proportion of patients with stable disease (SD) to ORR, and subgroup analyses of DOR, DCR, PFS and OS were performed by PD-L1 status as well as in PD-L1-positive patients with one prior line of systemic anticancer treatment (SACT) versus two or more lines.

Statistical analysis

To enable discrimination between an ORR of 30% (targeted response rate) and 12% (no clinically relevant effect) (setting alpha at 0.05 and beta at 0.10), a sample size of 45 response-evaluable patients was calculated (with approximately 50 patients to be enrolled to accommodate for a 10% dropout rate). It should be noted that the described power calculation of the study aims to characterize the statistical robustness of the study and is not designed to formally compare against an ORR of 12%.

Safety was assessed in the safety population, defined as patients who received any administration of planned study drugs. All AEs were coded using the Medical Dictionary for Regulatory Activities, V.25.1. AEs were evaluated based on system organ class, frequency, severity, and their potential relationship with study drugs.

Efficacy and immunogenicity were assessed in the efficacy population, being all response-evaluable patients who received any administration of planned study drug and had at least one post-baseline imaging assessment (CT or MR) for objective response evaluation. The ORR and DCR were calculated using descriptive measures including 95% CIs for binomial proportions using the Clopper and Pearson exact method. A predefined subgroup analysis of ORR based on baseline PD-L1 expression status was conducted.

DOR, PFS, and OS were analyzed according to the Kaplan-Meier method and are presented as Kaplan-Meier survival curves and estimates (median with 95% CI). OS until the end of the study, and medians for DOR and PFS were calculated for both the efficacy and safety populations.

As post hoc statistical analyses, an exact two-sided Fisher's exact test has been conducted to compare the ORR in PD-L1-positive versus PD-L1-negative patients. The same two groups have been compared with respect to their survival curves via a log-rank test in the context of the Kaplan-Meier analysis. A log-rank test has also been conducted to compare survival curves for PD-L1-positive patients with one prior line versus PD-L1-positive patients with two or more prior lines of SACT.

Analysis of DOR and PFS included disease progression information based on RECIST V.1.1 and survival status in the treatment period up to the last conducted imaging, planned to be at the end-of-treatment visit (48 weeks+30 days, as done for ORR). An exploratory sensitivity analysis of DOR and PFS included progression and survival status obtained during the 12-month follow-up period via quarterly phone follow-up visits to the end of the study.

As described in the outcomes section, post hoc analyses were performed to further assess the impact of baseline PD-L1 expression status for DOR, PFS, OS and DCR. In a further post hoc subgroup analysis, the impact of a number of previous SACT lines (1 or ≥2) for ORR and DCR was evaluated.

Group comparisons of continuous immunogenicity data were analyzed by Mann-Whitney tests.

Statistical analyses were done with SAS V.9.4 (efficacy) and GraphPad Prism V.9.5.0 (immunogenicity).

This study is registered with ClinicalTrials.gov (NCT04405349) and EudraCT (2019-002328-3).

RESULTS

Study population

Patients were enrolled from June 16, 2020, to January 25, 2022, and the last end-of-treatment visit was November 25, 2022. 52 patients had received a minimum of one administration of VB10.16 and atezolizumab and were included in the safety population. 47 patients with at least one post-baseline assessment of objective response per RECIST V.1.1 were included in the efficacy population. Baseline characteristics for the safety population are shown in table 1.

In this population, 17 (33%) of patients had received prior surgery and 33 (63%) had prior radiotherapy for cervical cancer. 24 (46%) of patients had a history of two or more prior lines of SACT. Most patients (50; 96%) were treated with platinum-based chemotherapy and 40 (77%) had received prior taxanes. All patients were ICI-naïve as per-protocol requirement. 20 (38%) of the patients were previously treated with bevacizumab. The most common locations of metastatic disease at screening were lymph nodes (38 patients; 73%) and lung (17 patients; 33%). Five (10%) patients had lymph node metastases only. Of the 47 response-evaluable patients, 40 (85%) had available tumor material for PD-L1 expression testing at baseline, of these 8 of the biopsies were fresh and 32 were archival. 24 of the 40 patients (60%) were PD-L1-positive, and 16 (40%) patients were PD-L1-negative (table 2).

Of 52 patients, 15 (29%) completed the planned study treatment, whereas 37 (71%) discontinued. 30 patients discontinued due to disease progression, and 2 patients due to death. Three patients discontinued due to AEs, of which one was reported related to study drugs (Consolidated Standards of Reporting Trials (CONSORT) diagram shown in online supplemental figure S1). The median duration of follow-up was 11.7 months (range 0.3–25.0 months). The administered mean total dose was 18.5 mg (6.2 doses) VB10.16 (range 3 mg (1 dose) to the planned full 33 mg (11 doses)). For atezolizumab the administered mean total dose was 9669.2 mg (8.1 doses) ranging from 1,200 mg (1 dose) to the planned full 20,400 mg (17 doses).

Safety

Treatment-related AEs occurred in 35 (67%) patients. AEs assessed by the investigators as at least partly related to VB10.16 occurred in 16 (31%) patients (table 3).

These events were all non-serious and of severity grade ≤2, except for one grade 3 event of arthralgia. The most common AEs assessed as related to VB10.16 were mild (grade 1) injection site reactions (pain, bruising, discomfort) observed in 9 (17%) patients. 13 (25%) patients had potentially immune-related AEs (online supplemental table S3), which were all reported as associated with atezolizumab and of grade 1–2. Most cases were thyroid events. Only one event of grade 2 immune-mediated lung disease led to permanent discontinuation of study drugs.

Table 1 Baseline demographics and clinical characteristics

	Safety population (n=52)
Age, years	47.5 (27–83)
Race	
White	52 (100)
ECOG status	
0	30 (58)
1	22 (42)
Cervical cancer histology	
Squamous cell carcinoma	42 (81)
Adenocarcinoma	8 (15)
Adenosquamous carcinoma	1 (2)
Unknown	1 (2)
PD-L1 status	
PD-L1+	25 (48)
PD-L1–	20 (38)
Unknown	7 (13)
Prior surgery for cervical cancer	17 (33)
Prior radiotherapy for cervical cancer	33 (63)
Prior lines of systemic anticancer therapy	
0	1 (2)
1	26 (50)
≥2	24 (46)
Unknown	1 (2)
Prior systemic anticancer therapies	
Platinum	50 (96)
Taxanes	40 (77)
Bevacizumab	20 (38)
Tisotumab vedotin	7 (13)
Other (gemcitabine, topotecan, ifosfamide, alpelisib)	8 (15)
Tumor burden (metastases) at screening	
Lymph nodes	38 (73)
Lung	17 (33)
Liver	8 (15)
Bone	4 (8)
Skin	1 (2)
Brain	0 (0)
Other ≥10%	
Peritoneum	13 (25)
Uterus	12 (23)
Adrenal gland	6 (12)
Muscle	5 (10)

Data are median or n (%). ECOG=Eastern Cooperative Oncology Group. PD-L1=programmed death-ligand 1 (PD-L1+: Ventana SP263 assay cut-off ≥5% Tumor Area Positivity Score).

Table 2 Summary of response rates

Efficacy population or subgroup	N	Best overall response (BOR) (%)				Response parameter (%)	
		CR (n)	PR (n)	SD (n)	PD (n)	ORR (95% CI)	DCR (95% CI)
All	47	6.4 (3)	12.8 (6)	40.4 (19)	40.4 (19)	19.1 (9.1 to 33.3)	59.6 (44.3 to 73.6)
PD-L1+	24	8.3 (2)	20.8 (5)	45.8 (11)	25.0 (6)	29.2 (12.6 to 51.1)	75.0 (53.3 to 90.2)
PD-L1−	16	6.3 (1)	6.3 (1)	37.5 (6)	50.0 (8)	12.5 (1.6 to 38.3)	50.0 (24.7 to 75.3)
1 prior line of SACT	23	13.0 (3)	17.4 (4)	43.5 (10)	26.1 (6)	30.4 (13.2 to 52.9)	73.9 (51.6 to 89.8)
≥2 prior line of SACT	22	0.0 (0)	9.1 (2)	40.9 (9)	50.0 (11)	9.1 (1.1 to 29.2)	50.0 (28.2 to 71.8)
PD-L1+ and 1 prior line of SACT	15	13.3 (2)	26.7 (4)	40.0 (6)	20.0 (3)	40.0 (16.3 to 67.7)	80.0 (51.9 to 95.7)

PD-L1 expression status is missing for seven patients and SACT status is missing for two patients.
BOR, best overall response; CR, complete response; DCR, disease control rate; n, sample size; ORR, objective response rate; PD, disease progression; PD-L1, programmed death-ligand 1 (PD-L1+=Ventana (SP263) TAP score ≥5%); PR, partial response; SACT, systemic anticancer treatment; SD, stable disease.

Six patients experienced AEs leading to death, none of these were related to study drugs, but were all attributed to disease progression or comorbidity.

The serious treatment-related AEs reported in 5 (10%) patients were all assessed as related to atezolizumab and none to VB10.16 (online supplemental table S1).

Treatment-emergent AEs regardless of causality occurred in 50 (96%) patients (online supplemental table S2).

The observed AEs were generally consistent with the known safety profile of atezolizumab or the underlying disease.

Efficacy

In the protocol-specified efficacy population (n=47), the ORR was 19.1% (95% CI 9.1% to 33.3%). As BOR, 3 (6.4%) patients had CR, 6 (12.8%) had PR, 19 (40.4%) had SD, and 19 (40.4%) had PD (table 2, figure 1).

For PD-L1-positive patients (n=24), the ORR was 29.2% (95% CI 12.6% to 51.1%) versus 12.5% for PD-L1-negative patients (95% CI 1.6 to 38.3) (n=16); p=0.272.

Table 3 Most common treatment-related adverse events (safety population; n=52)

	Related to VB10.16 only		Related to VB10.16 and atezolizumab		Related to atezolizumab only		Total
Grades	G 1–2 n (%)	G 3–4 n (%)	G 1–2 n (%)	G 3–4 n (%)	G 1–2 n (%)	G 3–4 n (%)	All grades (%)
Patients with at least one treatment-related adverse event	10 (19)	0	6 (12)	1 (2)	29 (56)	4 (8)	35 (67)
Treatment-related adverse events, by preferred terms, with an incidence of ≥5%, or any grade 3 or worse event							
Fatigue	0	0	1 (2)	0	6 (12)	1 (2)	7 (13)
Hypothyroidism	0	0	0	0	7 (13)	0	7 (13)
Hyperthyroidism	0	0	0	0	6 (12)	0	6 (12)
Anemia	0	0	0	0	4 (8)	2 (4)	6 (12)
Pruritus	0	0	2 (4)	0	3 (6)	0	5 (10)
Injection site pain*	4 (8)	0	0	0	0	0	4 (8)
Injection site bruising*	3 (6)	0	0	0	0	0	3 (6)
Injection site discomfort	3 (6)	0	0	0	0	0	3 (6)
Arthralgia	0	0	0	1 (2)	2 (4)	0	3 (6)
Myalgia	1 (2)	0	1 (2)	0	1 (2)	0	3 (6)
Dysphagia	0	0	0	0	1 (2)	1 (2)	2 (4)
Renal failure	0	0	0	0	0	1 (2)	1 (2)

G=grade based on CTCAE V.5.0
*The following grouping of events are used in the table: Injection site pain includes adverse event terms “injection site pain” and “administration site pain”. Injection site bruising includes adverse event terms “injection site bruising” and “injection site haematoma”.
CTCAE, Common Terminolgy Criteria for Adverse Events.

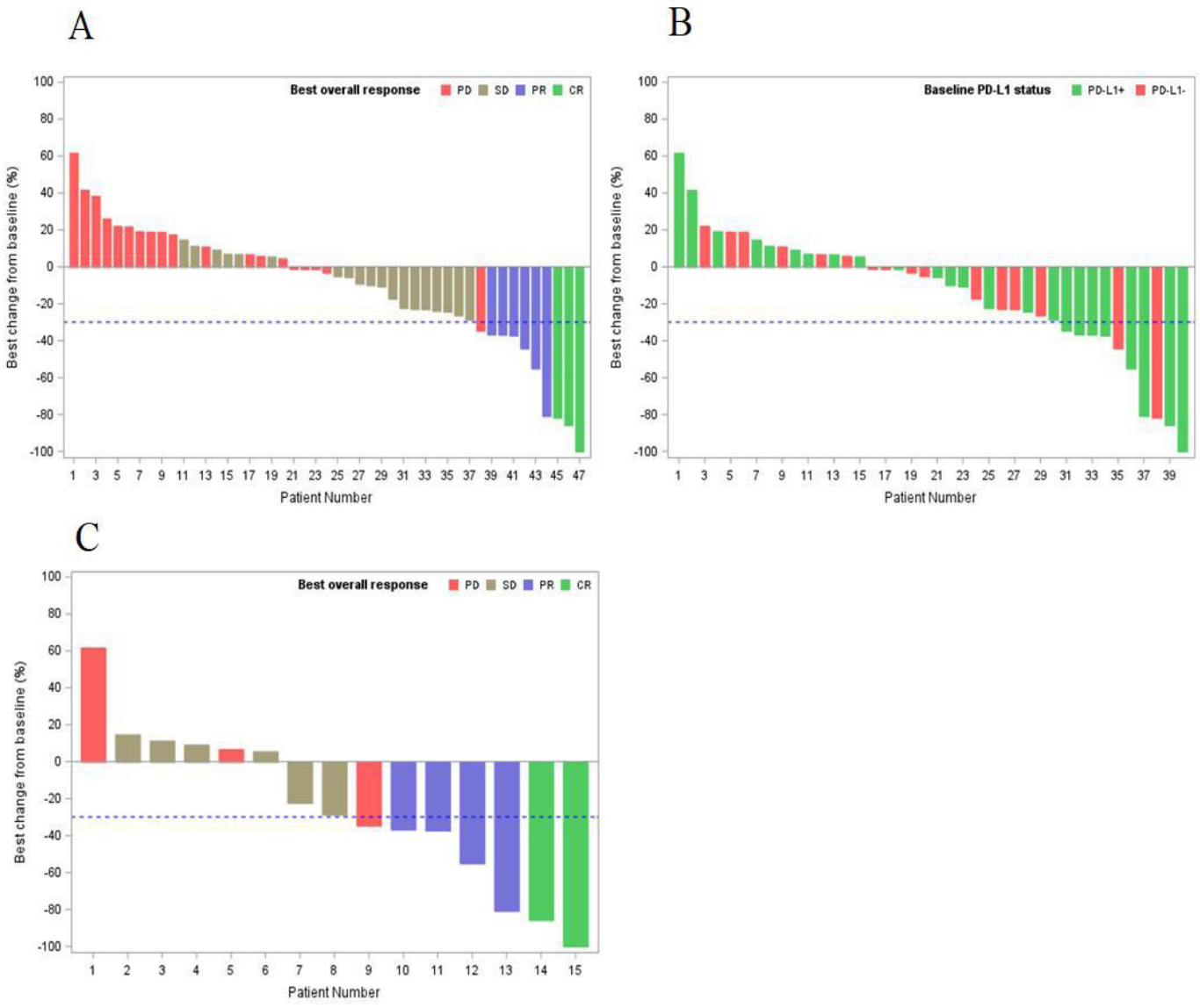

Figure 1 Waterfall plots Waterfall plots illustrate the percentage change between the baseline sum of lesion diameters and the smallest sum of lesion diameter on treatment. (A) All efficacy-evaluable patients (n=47), color-stratified according to best overall response, (B) efficacy-evaluable patients with PD-L1 expression test (n=40) stratified according to known baseline PD-L1 subgroup and (C) for the subgroup of PD-L1+ patients with only one prior line of systemic anticancer treatment (n=15). CR, complete response; PD, progressive disease; PD-L1, programmed death-ligand 1; PR, partial response; SD, stable disease.

Five of 52 patients were excluded from the efficacy population, as they did not fulfill the criterion of having a post-baseline imaging scan (median study participation: 1.2 months; see CONSORT diagram for details on the reason for discontinuation). When including these five patients as non-responders in a post hoc analysis, the ORR was 17.3% (95% CI 7.0% to 27.6%) (9/52).

Up to the end of the treatment period (52 weeks), the median DOR (mDOR) among the 9 (19.1%) responding patients was not reached (n.r.) (two events of progression). DOR for individual patients and individual changes in target lesion size are shown in online supplemental figures S3, S4). Median PFS (mPFS) was 4.1 months (95% CI 2.1 to 6.2) in the treatment period (figure 2, and online supplemental table S4).

As post hoc analyses, the DCR was calculated to be 59.6% for all patients and 75.0% in PD-L1-positive patients. Furthermore, patients with only one prior line of SACT (n=23) had ORR=30.4%, while patients with 2 or more prior lines (n=22) had ORR=9.1%. For the subgroup of PD-L1-positive patients with one prior line of SACT (n=15), the ORR was 40.0% (95% CI 16.3% to 67.7%) and DCR was 80.0% (95% CI 51.9% to 95.7%) (table 2, figure 1).

When including information from the 12-month follow-up period to the end of the study, the mDOR was 16.8 months (95% CI 2.2 to n.r.) in all nine responders (three additional progression events and one death) (online supplemental table S4 and figure S2). For the PD-L1-positive patients, the mDOR was 17.1 months

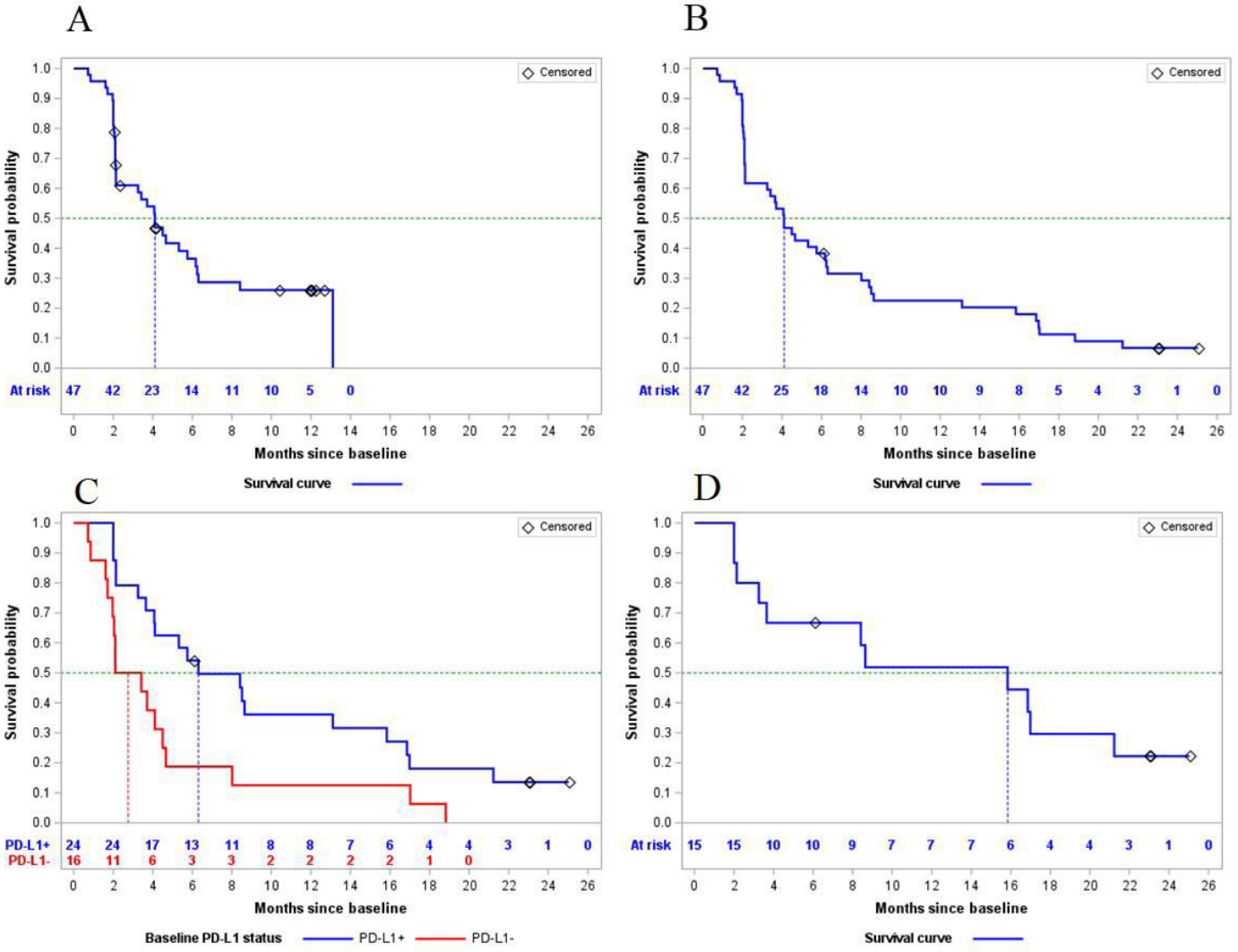

Figure 2 Kaplan-Meier plot of progression-free survival KM curves for progression-free survival. Progression-free survival on treatment was defined from the first study treatment to the first Response Evaluation Criteria in Solid Tumors V.1.1 documented disease progression or death. Duration of response up to the end of the study included progression and survival information reported via phone follow-up visits. (A) On treatment for total efficacy population. (B–D) When including follow-up data up to the end of the study for (B) total efficacy population (C) PD-L1 subgroups and (D) in a subgroup of PD-L1+ patients with one prior line of systemic anticancer treatment. FU, follow up; KM, Kaplan-Meier; PD-L1, programmed death-ligand 1.

(95% CI 2.2 to n.r.) and, for the subgroup of PD-L1-positive patients with one prior line of SACT, it was n.r. (online supplemental table S4 and figure S2).

When including the 12-month follow-up period to the end of study in a post hoc analysis, the mPFS was 6.3 months (95% CI 3.6 to 15.8) for the PD-L1-positive patients versus 2.8 months (95% CI 1.7 to 4.5) in PD-L1-negative patients (p=0.012) and 15.8 months (95% CI 2.1 to 21.2) for the PD-L1-positive patients with one prior line of SACT versus 4.7 months (95% CI 2.0 to 8.5) in PD-L1-positive patients with two or more prior lines of SACT (p=0.029). The median OS (mOS) was 21.3 months (95% CI 8.5 to n.r.) up to the end of the study (n=47) (figure 3, and online supplemental table S4). In the full safety population (n=52), the mOS was 16.9 months (95% CI 8.3 to n.r.), 24.7 months (95% CI 9.1 to n.r.) for the PD-L1-positive patients, and n.r. for the PD-L1-positive patients with one prior line of SACT (figures 2 and 3, and online supplemental table S4).

Immunogenicity

HPV16-specific T-cell responses were analyzed by ex vivo IFN-γ ELISpot in 36 of 47 patients (76.6%) in the efficacy population (non-evaluable samples for 11 patients). Among these, 22/36 (61%) exhibited an increase in HPV16-specific T-cell response and 17/36 (47%) showed a ≥2-fold increase from baseline to peak (post hoc analysis, online supplemental figure S5). Patients with disease control as per RECIST V.1.1 (CR, PR, SD; n=24) showed higher HPV16-specific T-cell responses compared with patients with progressive disease (n=12) both when assessed as peak Spot-forming units (SFU)/10^6 peripheral blood mononuclear cells (p=0.011, post hoc analysis) and fold change from baseline (p=0.035, post hoc

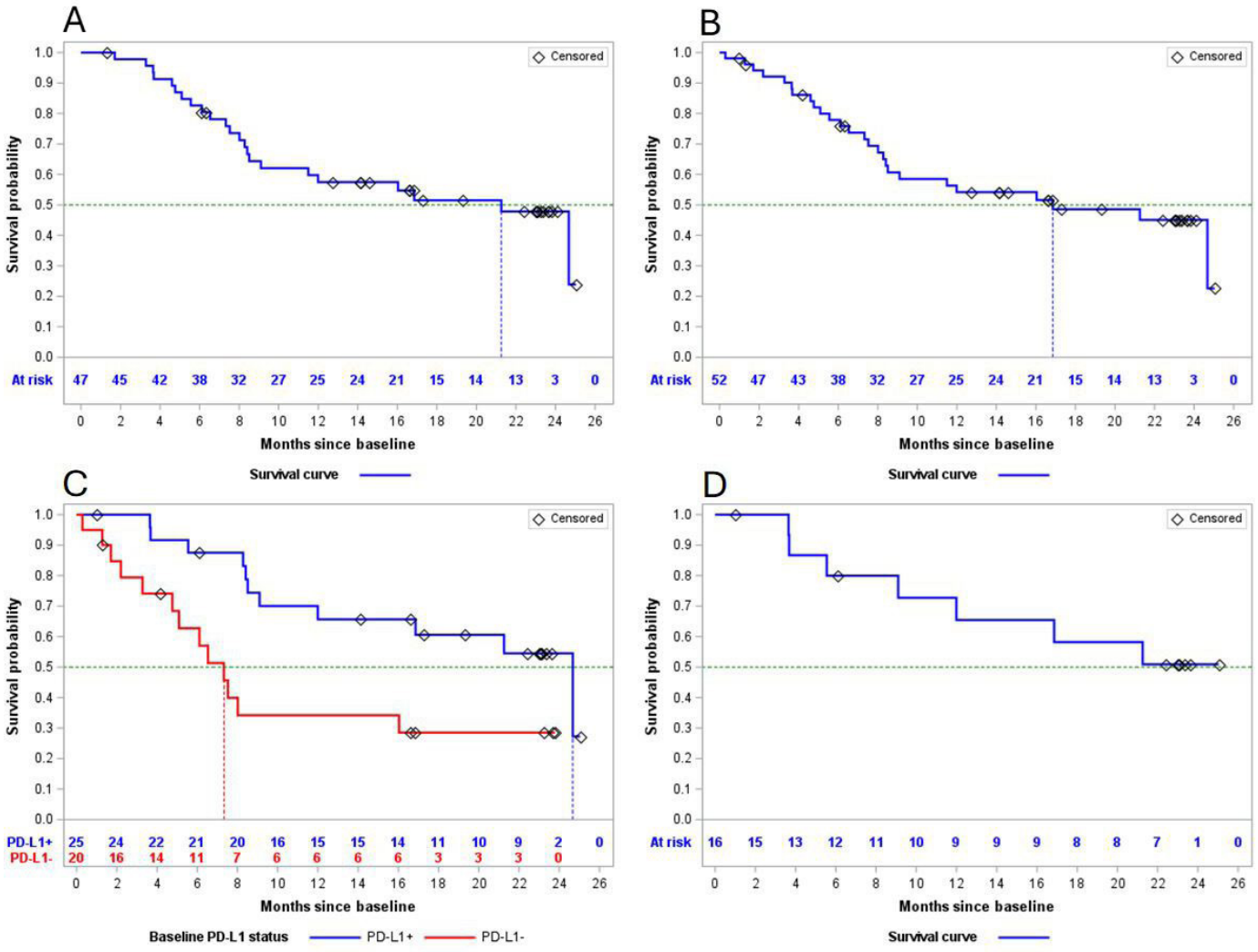

Figure 3 Kaplan-Meier plot of overall survival KM curves for overall survival up to the end of the study in (A) total efficacy population, (B) total safety population, (C) PD-L1 subgroups, and (D) subgroup of PD-L1+ patients with one prior line of systemic anticancer treatment. PD-L1, programmed death-ligand 1.

analysis) (online supplemental figure S6). There was a delay in progression in the 17/36 patients who had a ≥2-fold increase in HPV16-specific T-cell response during treatment (mPFS 8.4 vs 3.7 months (during treatment) and 8.0 versus 3.7 months (to end-of-study)) (post hoc analysis, online supplemental figure S6).

DISCUSSION

In this phase 2a study, the combination of the therapeutic DNA-based HPV16 cancer vaccine VB10.16 with atezolizumab was safe and well-tolerated and showed promising efficacy in patients with persistent, r/m HPV16-positive, ICI-naïve cervical cancer. 46% of the patients were heavily pretreated having received two or more prior lines of SACT. Most AEs reported as associated with VB10.16 were mild-to-moderate (grades 1 and 2), with just one event of arthralgia reported as a grade 3. No serious AEs were ascribed to VB10.16, and no safety concerns were reported for the combination of VB10.16 with atezolizumab. In summary, the tolerability profile appeared to be consistent with the expected tolerability profile of atezolizumab monotherapy.[10 11]

The primary analysis resulted in an ORR of 19.1% in the efficacy population (n=47, table 2). As the ORR in the PD-L1-positive population (TAP≥5%; Ventana PD-L1 (SP263)) was 29.2% (vs 12.5% in PD-L1-negative patients), the overall ORR of 19.1% may partially be explained by the proportional contribution of PD-L1-negative patients enrolled (40% of the 40 patients with evaluable PD-L1 status). Responses were durable, particularly in PD-L1-positive patients, and a clinically meaningful proportion derived clinical benefit from this treatment, which may reduce symptom burden or delay the onset of new symptoms. In other, larger data sets investigating different ICIs and using different tissue requirements, assays, and/or cut-off values for PD-L1 positivity, the prevalence of PD-L1 negativity in r/m cervical cancer has been reported as 11.4% and 36.2%.[7 9] Prevalence of PD-L1 positivity/negativity in the biomarker-unselected BEATcc study investigating atezolizumab remains to be reported.[10] Notably,

the observed ORR in PD-L1-positive, heavily pretreated patients of 29.2% is higher than reported for PD-L1-positive patients exposed to other PD-1/PD-L1 ICIs as monotherapy in a similar second-line advanced disease setting (17–18%).[6 9 20] With regard to PD-L1 ICIs, atezolizumab monotherapy was recently evaluated in PD-L1-positive (TAP≥5%; Ventana PD-L1 (SP263)) patients with r/m cervical cancer in one of two treatment arms in the randomized SKYSCRAPER-04 phase 2 study.[11] For patients, who were on average less pretreated compared with patients in the VB C-02 study, the observed ORR was 15.8%, mPFS was 1.9 months, and mOS 10.9 months (including 33% of the patients crossing over from the atezolizumab monotherapy arm to the combination arm with tiragolumab following disease progression).[11] Despite the single-arm design and a small sample size, the ORR (29.2%) in PD-L1-positive patients is higher than that in historical clinical trials of anti-PD-1/PD-L1 monotherapy in r/m cervical cancer (15.8–17.1%).

A single-country, single-arm phase 2 study (KEYNOTE-567) has investigated the DNA-based vaccine tirvalimogene teraplasmid (GX-188E) in combination with the PD-1 inhibitor pembrolizumab in advanced cervical cancer.[17] In the efficacy-evaluable population (at least 45 days of treatment and at least one post-baseline response assessment; n=60), the ORR was 31.7%, with mDOR 12.3 months, mPFS 3.0 months, and mOS 17.2 months. For the PD-L1-positive population, the ORR was 36.1%, but with shorter mDOR (12.3 months), mPFS (4.4 months), and mOS (23.8 months), comparable with what was seen in the PD-L1-positive population in VB C-02. Notably, in KEYNOTE-567, pembrolizumab was allowed to be given for up to 2 years compared with 1 year of concomitant atezolizumab in VB C-02.

The major limitations of the present phase 2a study include the small sample size and the single-arm study design. Accordingly, the possibility that study population differences affected observed response rates and clinical efficacy in the present study cannot be excluded. Another consideration is that the study was conducted in the setting of evolving first-line treatment for r/m cervical cancer including the addition of pembrolizumab,[7] potentially affecting tumor response in future patients being treated with VB10.16 in combination with ICI rechallenge. VB10.16 appears highly tolerable with an overall safety profile of the VB10.16 plus atezolizumab combination similar to what has been experienced with atezolizumab alone. This can most likely be explained by the non-self-antigen targeting nature of the VB10.16 vaccine together with negligible risk of on-target/off-tumor toxicity. Additionally, biomarker analyses are still ongoing and will be the subject of a future reporting. Translational research data might help to better understand which patients with HPV16-positive advanced cervical cancer would benefit the most from this combination. Based on the data reported here, it seems like the subgroup of ICI naïve patients with PD-L1-positive tumors, who has failed one prior treatment line benefit the most. An improved clinical outcome has also been seen with ICIs in less previously treated patients, for example, when moving from a second-line to a first-line setting.[6 7 10 11] Prospective, randomized clinical trials will be needed to confirm if VB10.16 combined with an ICI can improve clinical outcomes in patients with r/m cervical cancer.

In summary, our data indicate that the DNA-based, HPV16-specific therapeutic cancer vaccine VB10.16 combined with atezolizumab is showing clinically meaningful efficacy with durable responses in HPV16-positive ICI naïve persistent/m cervical cancer which warrants further clinical development. The combination was also safe and well tolerated. The efficacy was further enhanced in PD-L1-positive patients with few prior treatment lines. The clinical observations were further supported by HPV16-specific T-cell responses that were associated with clinical efficacy. VB10.16 in combination with an anti-PD-l/PD-L1 checkpoint inhibitor might have a potential to become an integrated part of the future armamentarium of new innovative therapies for other HPV16-driven cancers in late-stage, as well as earlier, non-metastatic stages of the disease.

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Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Regional Ethic committee Norway Region sør-sør AReference number: 31956, Ethic committee Bulgaria, Reference Number: 0566; Ethic committee Centre Hospital, l'Ardenne, Belgium, Reference number: 2019/20SEP/411; Ethic committee University, Gent, Belgium, Reference Number: 2019/20SEP/411; University Catholique de, Louvain, Belgium; Ethic committee, Reference Number: 2019/20SEP/411; Medizinische Hochschule, Hannover, Germany, Ethic Committee, Reference Number: 8696_AMG_M_2019; Ethic committee of Bulovka University Hospital, Praha, Czech Republic, Reference Number: 7.6.2022/6652; Ethic Committee Masaryk Memorial Cancer institute, Brno, Czech Republic, Reference Number: 2022/1714/MOU; Ethic committee for multicentric clinical trials/the University Hospital Kralovske Vinohrady, Czech Republic, Reference number: KH/40/18/2019; Ethic committee Poland, Reference number: 44/KBL/OIL/202. Participants gave informed consent to participate in the study before taking part.

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