Journal for ImmunoTherapy of Cancer

Phase I study of intratumoral administration of CV8102 in patients with advanced melanoma, squamous cell carcinoma of the skin, squamous cell carcinoma of the head and neck, or adenoid cystic carcinoma

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To cite: Eigentler T, Thomas I, Samoylenko I, *et al*. Phase I study of intratumoral administration of CV8102 in patients with advanced melanoma, squamous cell carcinoma of the skin, squamous cell carcinoma of the head and neck, or adenoid cystic carcinoma. *Journal for ImmunoTherapy of Cancer* 2025;**13**:e009352. doi:10.1136/jitc-2024-009352

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/jitc-2024-009352).

Accepted 24 November 2024

Check for updates

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ABSTRACT

Background CV8102, a toll-like receptor 7/8 and RIG I agonist, has demonstrated antitumor immune responses in preclinical studies. We investigated intratumoral (IT) administration of CV8102 in patients with anti-programmed cell death protein-1 (PD-1) therapy-naïve or anti-PD-1 therapy-refractory cutaneous melanoma (cMEL) and in patients with advanced cutaneous squamous cell carcinoma, head and neck squamous cell carcinoma and adenoid cystic carcinoma.

Methods This open-label, cohort-based, phase I dose escalation study aimed to establish the maximum tolerated dose (MTD), recommended dose (RD), safety and preliminary efficacy of CV8102 as monotherapy or in combination with a PD-1 inhibitor. The preliminary efficacy of the RD was assessed in patients with cMEL in the expansion cohorts.

Results Between September 2017 and October 2022, 98 patients were enrolled in monotherapy and combination therapy dose escalation and dose expansion cohorts. Two patients in the CV8102 monotherapy dose escalation cohort experienced relevant toxicities at the 900 µg dose level. One patient had Grade 3 aspartate transaminase/alanine aminotransferase elevation which met dose-limiting toxicity (DLT) criteria. Another patient experienced Grade 3 immune-mediated pneumonitis. No DLTs occurred in the combination therapy dose escalation cohort. The MTD was not formally reached and the RD for expansion was 600 µg. Common treatment-emergent adverse events were fever (57%), chills (37%) and fatigue (25%). In the dose escalation part, objective responses occurred in 3/33 patients treated with CV8102 as monotherapy and in 2/25 patients treated with CV8102 plus a PD-1 inhibitor. In the expansion cohorts in patients with anti-PD-1 therapy-refractory melanoma, 0/10 patients treated with CV8102 as monotherapy and 5/30 patients (17%) treated in combination with a PD-1 inhibitor experienced objective responses.

Conclusions IT CV8102 was generally well tolerated with preliminary signs of efficacy as monotherapy and in combination with a PD-1 inhibitor.

Trial registration number NCT03291002.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Preclinical studies have demonstrated that intratumoral injection of CV8102, a single-stranded, non-coding, non-capped RNA with poly-U repeats, complexed with a cationic peptide induces antitumor immune responses with local and systemic antitumor effects.

⇒ CV8102 mediates its immunostimulatory properties by simultaneously triggering toll-like receptor 7/8 and RIG-I signaling.

BACKGROUND

Skin cancers are one of the most common groups of cancers worldwide, with an estimated 1.5 million new cases in 2020.[1] About 20% are melanomas[1] and 20% are squamous cell carcinomas.[2] There were an estimated 324,635 new diagnoses of melanoma and

WHAT THIS STUDY ADDS

⇒ Repeated intratumoral administration of CV8102 appears safe and feasible as monotherapy and in combination with programmed cell death protein-1 (PD-1) antibodies.

⇒ CV8102 demonstrated preliminary signs of efficacy as monotherapy and in combination with a PD-1 inhibitor; in the expansion part of the study, the objective response rate was 17% in patients with anti-PD-1 therapy-refractory cutaneous melanoma treated at the RP2D of 600 µg in combination with a PD-1 inhibitor; overall, 8/98 patients experienced shrinkage of non-injected lesions of more than 30%.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study demonstrates the safety and first evidence of biological and clinical activity of intratumoral CV8102 including patients with anti-PD-1 therapy-refractory melanoma treated in combination with anti-PD-1 antibodies.

57,043 deaths globally in 2020,[1 3] and 2,402,221 new cases of cutaneous squamous cell carcinoma (cSCC) and 56,100 deaths globally in 2019.[4] For head and neck cancer, there were an estimated 931,931 new cases and 467,125 deaths globally in 2020.[5] In contrast, adenoid cystic carcinoma (ACC) is a rare cancer, representing 1% of all head and neck cancers, with an estimated 200,000 cases having ACC worldwide, annually.[1 6 7]

Programmed cell death protein-1 (PD-1) inhibitors have become a main pillar of treatment for advanced melanoma, and SCCs of the skin or head and neck. However, not all patients respond with some having primary resistance, while others develop secondary resistance.[8 9] Additionally, some patients experience serious treatment-related toxicities, particularly after treatment with systemic combinations.[8 9] In addition, ACC is a non-inflamed (cold) tumor of the head and neck that has shown to be largely resistant to PD-1 inhibitors alone.[10–13] Hence, there is an unmet need for new well-tolerated treatment modalities to enhance the clinical efficacy of PD-1 inhibitors in these tumor types.

Since skin and head and neck cancers are generally easily accessible, they are ideal targets for intratumoral (IT) administration. IT administration of immunomodulating therapies can induce local and systemic immunological responses with the aim of promoting tumor cell death and, thus, the release of tumor-derived antigens which activate tumor-specific effector T cells.[14] IT administration offers the advantage of higher bioavailability in the tumor microenvironment (TME) of injected lesions and limited systemic exposure, reducing systemic toxicity while maximizing the potential to activate IT immune cells. IT treatment can thus enhance treatment response when co-administered with systemic therapies.[8] Several IT treatments have demonstrated clinical benefit as monotherapy and in combination with systemic immune checkpoint inhibitors (CPIs) therapies in patients with skin and head and neck cancers.[15 16]

The immuno-stimulatory agent, CV8102, is a toll-like receptor (TLR) 7/8 and RIG I agonist[17] comprising a synthetic RNA complexed with a polymeric carrier.[17 18] The RNA component contains an uncapped U-rich RNA sequence containing several poly-U-repeats (described in patent WO2009/095226). The polymeric carrier is formed by a disulfide crosslinked cationic peptide (WO2009/095226).[17 18] In preclinical models (BALBC/c mice challenged with CT26 cells, or the A20 B-cell lymphoma line), IT administration of CV8102 showed dose-dependent antitumor effects. In the CT26 model, activation of innate immune responses in the TME and draining lymph nodes was also demonstrated.[18] CV8102 in combination with a systemic PD-1 inhibitor further enhanced antitumoral responses inducing tumor infiltration and activation of $CD8^+$ T cells. When mice that had cleared the tumor were rechallenged 4 months after the primary challenge with the same tumor and left untreated, no tumor growth was observed. This showed that initial treatment had provided sufficient immunological memory to eradicate the tumor cells on rechallenge. Clinical safety of intramuscular administration of CV8102 alone and in combination with a licensed rabies vaccine has been demonstrated in healthy volunteers.[19] In addition, a phase I/II trial of a multipeptide antigen vaccine (IMA970A) with CV8102 administered as an adjuvant demonstrated safety and immunogenicity after intradermal administration in patients with early-to-intermediate hepatocellular carcinoma.[20] In light of these findings, we investigated IT CV8102 as a new local treatment for patients with advanced cutaneous melanoma (cMEL), cSCC, head and neck squamous cell carcinoma (hnSCC) or ACC.

PATIENTS AND METHODS

Study design

This was a multinational, open-label, cohort-based, phase I dose escalation and expansion study of CV8102 monotherapy or in combination with a PD-1 inhibitor in patients with cMEL, cSCC, hnSCC or ACC (figure 1). The primary objectives were to establish the maximum tolerated dose (MTD) and recommended dose (RD) for IT CV8102 alone or in combination with a standard dose of a PD-1 inhibitor (recommended combination dose; RCD). Safety primary outcomes were to characterize the tolerability and safety profile of IT CV8102 administered alone and in combination with a PD-1 inhibitor. Secondary outcomes were to evaluate the antitumor activity of IT CV8102 by Response Evaluation Criteria in Solid Tumors (RECIST V.1.1)[21] and immune-related Response Evaluation Criteria in Solid Tumors (irRECIST),[22] to evaluate median duration of response (DOR) and median progression-free survival (PFS) per irRECIST and RECIST V.1.1 criteria and to evaluate the extent of tumor response at injected and non-injected lesions. Exploratory objectives were to evaluate the effects of CV8102 on systemic immune parameters and other peripheral

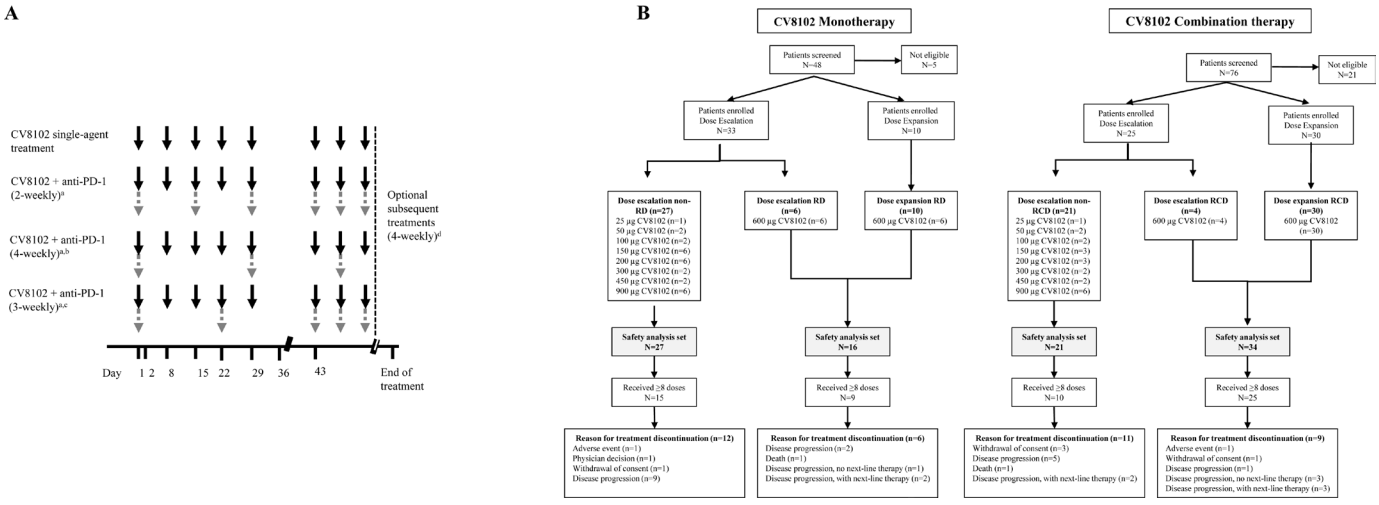

Figure 1 (A) Treatment schedule. (B) Trial profile for the monotherapy and combination therapy dose escalation and dose expansion parts. PD-1, programmed cell death protein-1. [a]Patients in the monotherapy dose expansion and dose escalation cohort and the combination dose escalation cohort, and anti-PD-1 refractory patients in the combination dose escalation cohort. with evidence of clinical benefit received further injections until progression. [b]Nivolumab (2-weekly CV8102 administration both for 2- and 4-weekly nivolumab schedule). [c]Pembrolizumab.[d]Only patients who did not show any signs of tumor progression after completion of treatment when treated with single-agent CV8102 received subsequent intratumoral CV8102 after the eight injection for the planned duration of the study at 4-weekly intervals. RCD, recommended combination dose; RD, recommended dose.

biomarkers of interest in longitudinal blood samples and immune cell infiltration and other biomarkers of interest in tumor biopsies. Initially, the study protocol included optional expansion cohorts in patients with ACC and hnSCC. These cohorts were not opened per sponsor decision since most evidence of efficacy was seen in patients with melanoma in the dose escalation part of the study.

Patients

To be eligible for any cohort, patients had to be aged ≥18 years, have adequate organ function, have an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1 and have at least one injectable tumor lesion large enough to hold the volume injected. They also had to have recovered from all toxicities from prior therapies to US National Cancer Institute-Common Terminology Criteria for Adverse Events V.4.3 (NCI-CTCAE V.4.3) Grade ≤1 or Grade ≤2. In addition, any CPI-related adverse events (AEs) had to be resolved for ≥2 weeks before enrollment. Patients with rapidly progressing multifocal metastatic or acutely life-threatening disease or who had used topical TLR-7/8 agonists within the previous 6 months were excluded. The online supplemental material includes additional eligibility criteria for all cohorts.

The combination dose expansion cohort included patients with histologically confirmed, advanced (unresectable, locally advanced or metastatic), stage IIIB-IV cMEL refractory to PD-1 inhibitor therapy with progressive disease, according to RECIST V.1.1, during or after PD-1 inhibitor therapy (alone or in combination, per the summary of product characteristics). The last PD-1 inhibitor treatment had to be within 12 weeks prior to enrollment. Patients in this cohort must have received one of the following minimum total doses of PD-1 inhibitor: 800 mg for pembrolizumab; 1,200 mg for nivolumab as monotherapy; or at least two doses of nivolumab at a minimum dose of 1 mg/kg administered with ipilimumab every 3 weeks. Patients had to have a measurable lesion, according to RECIST V.1.1, not intended for injection, no previous treatment with IT immunotherapy (eg, oncolytic virus or other TLR agonist) in the advanced setting and no history of uncontrolled CPI-related NCI-CTCAE Grade 3 or Grade 4 AEs. They also had to agree to undergo baseline (day 1) and post-baseline biopsy of the injected lesion. Inclusion criteria for each remaining cohort are described in the online supplemental material.

All patients provided written informed consent and the study was conducted in accordance with the Declaration of Helsinki, the International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice guidelines and the appropriate local regulatory requirements.

Treatment

Patients initially received either eight injections over 12 weeks of CV8102 alone (patients with cMEL, cSCC, hnSCC, or ACC) or in combination with a PD-1 inhibitor (patients with cMEL, hnSCC). The first five administrations were performed in weekly intervals on days 1, 8, 15, 22, and 29. Subsequently, patients on nivolumab received CV8102 every second week and patients on pembrolizumab received CV8102 every third week (figure 1, online supplemental material). The PD-1 inhibitor, either nivolumab or pembrolizumab, was administered according to the manufacturers' recommendations. CV8102 could be administered until disease progression requiring initiation of next-line therapy or

unacceptable toxicity whichever occurred first. Patients without signs of tumor progression after completion of the eight initial doses of CV8102 could continue treatment for the planned duration of the study (the initial study duration was 9 months from initial treatment but prolonged to 12 months in a protocol amendment). The initial cohorts received 25 µg of CV8102 (monotherapy or combination therapy) with dose escalation in subsequent cohorts following a predefined dose escalation scheme (figure 2). The total injection volume at each treatment visit depended on the planned dose and was administered to a single lesion, if possible. The volume of CV8102 to be injected into each lesion was dependent on the size of the lesion (online supplemental table 1). If the lesion was too small to receive the complete volume, the dose could be split between lesions if they were sufficiently spaced to minimize the risk of leakage. The same lesion was injected at each treatment visit unless the original lesion had regressed and was no longer accessible.

An Independent Data Monitoring Committee (IDMC) reviewed the data from the dose escalation cohorts and advised on the RD and RCD for the monotherapy dose expansion cohort and the combination dose expansion cohort, respectively.

Study assessments

Safety

All patients were monitored for at least 2 hours after each IT injection. Safety was monitored with physical examinations, vital signs, ECGs, blood sampling for hematology, coagulation, and biochemistry and urinalysis, and all AEs were recorded during the treatment phase of the study. AEs were graded according to NCI-CTCAE, V.4.03. During the follow-up phase of the study, information on AEs and concomitant medications was recorded and any ongoing AEs and concomitant medications were followed-up. Patients who prematurely discontinued study treatment underwent an end-of-treatment (EOT) visit 28 days after the last CV8102 injection and then entered the follow-up phase. Patients enrolled in the dose escalation parts of the study were evaluated for the occurrence of any dose-limiting toxicity (DLT) with a DLT evaluation period of 2 weeks from the first administration of CV8102 or 7 days after the second dose of CV8102, whichever was longer. Justification of the 2-week observation period and DLT criteria for each dose escalation cohort are described in the online supplemental material.

Efficacy

Efficacy was assessed as the percentage change in tumor burden from the start of study treatment with CV8102 until the EOT by the investigator using RECIST V.1.1 and irRECIST. Confirmation of responses was obtained during the next routinely scheduled scan. The best overall tumor response rate was defined as patients with either a complete response (CR) or partial response (PR). Tumor burden was assessed by contrast-enhanced CT or by MRI imaging and clinical examination, documented by photographs. Radiological assessments were performed at baseline and every 12 weeks (±2 weeks) in patients with cMEL or cSCC, and every 8 weeks (±2 weeks) for the first 6 months and then every 12 weeks (±2 weeks) in patients with hnSCC or ACC, unless there was indication warranting earlier radiologic assessment. DOR was measured from the first documentation of response to the first documentation of progressive disease (PD) or death. In case no event occurred, censoring was imposed at the end-of-study visit or prior to the start of another therapy, or at the last available tumor assessment. PFS was defined as the time from first dose of CV8102 to the date of the first documented progression, as determined by the investigator or death due to any cause.

Biomarker analyzes

Details on biomarker analyzes can be found in the online supplemental material.

Statistical analyzes

Data from the dose determination set that consisted of all patients in each cohort who had received at least two IT doses of CV8102 were used to determine the MTD and RD. Patients who experienced a DLT were included if they had received a single IT dose of CV8102. The CV8102 dose escalation for the monotherapy and the combination therapy cohorts was guided by a Bayesian 2/5-parameter logistic regression model with overdose control (EWOC; Escalation Without Overdose Control). The safety analysis set included all patients who had received at least one IT dose of CV8102 and had at least one safety assessment after dosing; the safety analysis set was used for all efficacy evaluations. Data from the dose escalation cohorts were combined for the analyzes presented. Where appropriate, data for patients who had received the RD or RCD in the dose escalation cohorts were combined with data for those receiving the same dose in the dose expansion cohorts and analyzed together.

Categorical data were summarized using frequency counts and percentages of patients and continuous variables were summarized using number of observation (n), mean, SD, median, minimum and maximum, unless otherwise specified. Time-to-event variables were summarized using Kaplan-Meier analyzes. Corresponding 95% CIs were calculated for efficacy endpoints. All assessed immune and biomarker data were analyzed by descriptive and multivariate analyzes.

RESULTS

Patient treatment exposure and characteristics

Between September 25, 2017, and October 11, 2022, 124 patients were screened and 98 were enrolled and followed-up in the study (online supplemental table 2, **figure 2**). Patient demographics and baseline characteristics are summarized in table 1. The most frequent type of cancer was cMEL (58%) and the most common prior therapies were PD-1/programmed death-ligand-1

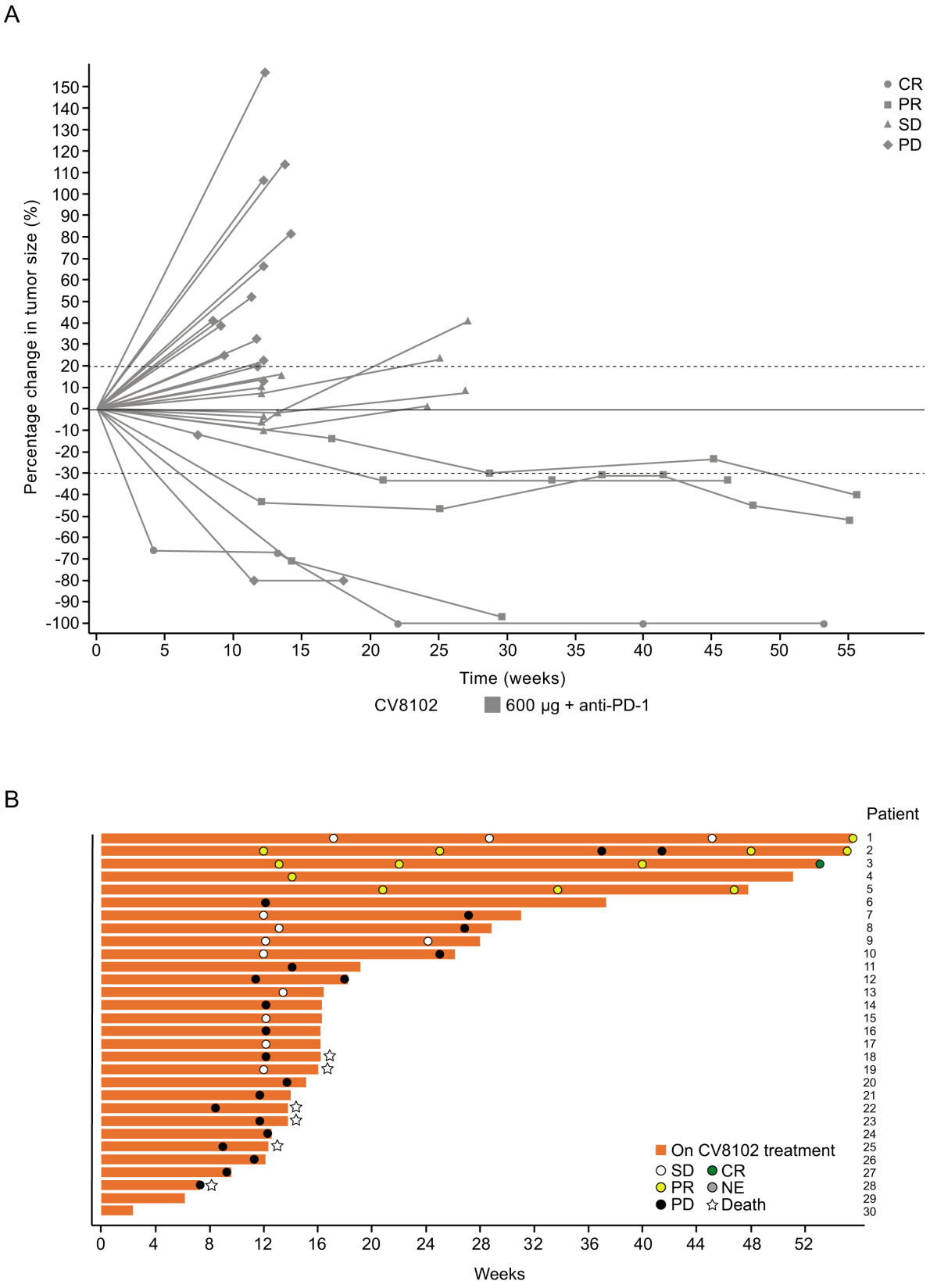

Figure 2 Treatment response per Response Evaluation Criteria in Solid Tumors V.1.1 in patients with anti-PD-1 therapy-refractory cutaneous melanoma in the combination therapy dose expansion cohort at the recommended dose of 600 µg (N=30). (A) Longitudinal change of target lesions from baseline. The per cent change in the sum of diameter from the baseline is shown. The dashed lines at 20% and −30% represent the boundary for the determination of PD and PR. (B) Overall response related to time on treatment. Each patient is represented by a bar and the treatment responses from baseline in target lesions are shown as colored dots. CR, complete response; NE, not evaluable; PD, progressive disease; PD-1, programmed cell death protein-1; PR, partial response.

Table 1 Demographics and baseline characteristics by cohort in the safety analysis set

Characteristic	Monotherapy		Combination therapy	
	Dose escalation (non-RD levels) N=27	Dose expansion+RD (600 µg) (N=16)	Dose escalation (non-RCD levels) (N=21)	Dose expansion+RCD (600 µg) (N=34)
Demographics				
Mean age (SD)	65.7 (14.56)	64.4 (14.11)	69.1 (12.64)	60.7 (14.82)
Female, n (%)	16 (59.3)	5 (31.3)	10 (47.6)	13 (38.2)
Mean height (SD)	167.33 (9.83)	170.04 (6.07)	170.60 (10.03)	175.21 (11.72)
Mean weight (SD)	69.28 (17.07)	74.88 (19.53)	77.01 (17.98)	85.98 (21.54)
Mean BMI (kg/m^2) (SD)	24.79 (6.48)	25.80 (6.15)	26.37 (5.23)	27.80 (5.32)
ECOG performance status, n (%)*				
Grade 0	16 (59.3)	8 (50.0)	16 (76.2)	21 (61.8)
Grade 1	11 (40.7)	8 (50.0)	5 (23.8)	13 (38.2)
Tumor type, n (%)				
hnSCC	3 (11.1)	2 (12.5)	4 (19.0)	1 (2.9)
cMEL	11 (40.7)	11 (68.8)	16 (76.2)	19 (55.9)
cMEL naive	1 (3.7)	0	1 (4.8)	4 (11.8)
cMEL refractory	0	1 (6.3)	0	10 (29.4)
cSCC	3 (11.1)	2 (12.5)	0	0
ACC	9 (33.3)	0	0	0
Tumor stage, n (%)				
Locally advanced	2 (7.4)	2 (12.5)	2 (9.5)	3 (8.8)
Metastatic	24 (88.9)	13 (81.3)	18 (85.7)	31 (91.2)
Missing	1 (3.7)	1 (6.3)	1 (4.8)	0
Diagnosis of most recent progression, n (%)				
Radiologically	23 (85.2)	16 (100)	16 (76.2)	29 (85.3)
Clinically	3 (11.1)	0	5 (23.8)	4 (11.8)
Missing	1 (3.7)	0	0	1 (2.9)
Median time since most recent progression, months (range)	3 (11.1)	0	5 (23.8)	4 (11.8)
Prior anticancer therapy, n (%)	1 (3.7)	0	0	1 (2.9)
PD-1/PD-L1 inhibitor	14 (51.9)	16 (100)	17 (81.0)	34 (100)
Other monoclonal antibodies and ADCs	2 (7.4)	8 (50)	8 (38.1)	9 (26.5)
EGFR inhibitors	4 (14.8)	1 (6.3)	4 (19.0)	0
Combination of antineoplastic agents	1 (3.7)	0 (0)	1 (4.8)	8 (23.5)
Platinum-based agents	2 (7.4)	1 (6.3)	4 (19.0)	1 (2.9)
MEK inhibitors	4 (14.8)	5 (31.3)	4 (19.0)	3 (8.8)
BRAF inhibitors	4 (14.8)	5 (31.3)	4 (19.0)	2 (5.9)
CTLA-4 inhibitors	2 (7.4)	6 (37.5)	8 (38.1)	16 (47.1)

*Grade 0=fully active, able to carry on all pre-disease performance without restriction; Grade 1=restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, that is, light housework, office work.
†Patients could have more than one prior treatment.
ACC, adenoid cystic carcinoma; ADC, antibody-drug conjugate; BMI, body mass index; BRAF, B-RAF serine-threonine kinase; cMEL, cutaneous melanoma; cSCC, cutaneous squamous cell carcinoma; CTLA-4, cytotoxic T-lymphocyte associated protein 4; ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor; hnSCC, head and neck squamous cell carcinoma; Max, maximum; MEK, mitogen-activated protein kinase; Min, minimum; N, number of patients; n, number of patients with data available; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand-1; RCD, recommended combination dose; RD, recommended dose.

inhibitors in 81 patients. 33% of patients had received cytotoxic T-lymphocyte associated protein 4 (CTLA-4) inhibitors as monotherapy or in combination with PD-1 inhibitors. Median age was 61 years in the combination dose expansion cohort and 68–71 years in the other cohorts. 61 (62%) and 37 (38%) patients, respectively, had ECOG performance status scores of Grade 0–1.

The patients' disposition, study treatment received, discontinuation and completion are summarized in online supplemental table 2. All patients received at least one dose of study treatment and 59 (60%) received at least eight doses. The median duration of CV8102 treatment was 10.1 weeks (range, 1.1–51.4 weeks). Study treatment was discontinued in 38 patients (39%), with the most common reason being disease progression (17 patients), followed by withdrawal of consent (5 patients). Death due to progression occurred in two patients. The most common reason for study withdrawal was death in 25 patients (26%) with reported causes of disease progression (21 patients), atypical pneumonia (1 patient), tumor bleeding (1 patient), non-Hodgkin's lymphoma (1 patient), and unknown in a patient who had stopped treatment due to disease progression (1 patient).

Safety outcomes

Overall safety population

All 98 patients experienced at least one treatment-emergent AE (TEAE) of any grade during the study, with fever (57% of patients), chills (37%), and fatigue (25%) being the most common (table 2). TEAEs considered CV8102-related by the investigators were reported in 90 patients (92%) (online supplemental table 3). Two events met the criteria for DLTs in the 900 µg cohort, one of which was outside the DLT period, but there was no apparent dose-dependency of TEAEs at the lower dose levels (online supplemental table 4a,b). Overall, there were no notable difference in the incidence of TEAEs between monotherapy and combination therapy cohorts. 34 patients (35%) experienced Grade 3 or higher AEs (table 2) with CV8102-related AEs per investigator judgment reported in 10 patients (10%). The related AEs included Grade 3 increases in alanine aminotransferases (ALT), gamma-glutamyltransferase and lipase (in two patients each) and amylase, aspartate aminotransferase (AST) and transaminases (in one patient each). In addition, one patient experienced Grade 3 increase in blood pressure together with Grade 1 tachycardia, fever and chills.

31 patients (32%) experienced any SAE (Serious Adverse Event) and 12 patients (12%) had SAEs that were considered related to CV8102. SAEs that occurred in more than one patient included cytokine release syndrome (CRS) in six patients (four Grade 1, two Grade 2), fever in three patients (one Grade 1, two Grade 2), chills in three patients (two Grade 1, one Grade 2) and tumor pain in two patients (one Grade 2, one Grade 3). Although most of these AEs were of mild-to-moderate grade, they fulfilled SAE criteria since they resulted in inpatient monitoring, as recommended in the protocol. Four patients experienced serious TEAEs that the investigator considered to be related to the PD-1 inhibitor. These included Grade 3 autoimmune nephritis, Grade 3 immune-mediated nephritis, Grade 2 CRS and Grade 3 hypertension with Grade 1 tachycardia, fever and chills requiring inpatient observation on the day of anti-PD-1 infusion (one patient each). In the seven reported CRS cases, symptoms started between 3 and 6 hours after the CV8102 injection. For more details on these cases see online supplemental table 5.

Eight patients (8%) experienced treatment-related AEs leading to study discontinuation, interruption or discontinuation of study drug or dose modifications. Those occurring in more than one patient included ALT increases in four patients, AST increase in three patients, gamma-glutamyl transferase increased in two patients and blood alkaline phosphatase increased in two patients.

Dose-limiting toxicities

Two patients experienced DLTs in the 900 µg monotherapy cohort. One patient with ACC experienced a DLT of Grade 3 AST/ALT elevations in the context of a Grade 2 CRS after the second injection of CV8102. After the dose was reduced to 600 µg, the patient completed the full course of the study treatment without recurrence. One patient with cMEL experienced Grade 3 immune-mediated pneumonitis 1 week after the second injection, which resolved within 1 week after oxygen and corticosteroid therapy. The patient stopped the study treatment and was withdrawn from the study. Although this event occurred outside of the predefined DLT period, it was considered to be a relevant and potentially DLT by the IDMC who therefore recommended not to assess the 1,200 µg dose. Given the observed toxicity at the 900 µg dose level, the IDMC recommended the 600 µg dose for the monotherapy and combination therapy expansion cohorts. The MTD per-protocol was not formally reached.

Efficacy outcomes

Out of 43 evaluable patients in the CV8102 monotherapy cohorts and according to RECIST V.1.1, one patient with PD-1-naïve cMEL achieved a CR at a dose of 150 µg (after achieving a PR at 12 weeks) and two patients achieved a PR (one patient with anti-PD-1-therapy-refractory melanoma at 450 µg and one patient with anti-PD-1 therapy-refractory SCC of the skin at 600 µg) that were confirmed within a consecutive routinely scheduled scan. Out of 55 evaluable patients in the CV8102 combination cohorts, one patient with anti-PD-1 therapy-refractory melanoma achieved a CR at a CV8102 dose of 600 µg and six anti-PD-1 therapy-refractory patients with melanoma achieved a PR (five patients at 600 µg and one patient at 900 µg), two of them were confirmed within consecutive routinely scheduled scans (figure 3A, table 3 and online supplemental tables 6-9). There was no difference in the assessment of the best overall tumor response by RECIST V.1.1 or irRECIST assessment. An example response is shown in

Table 2 Treatment-emergent adverse events that occurred in ≥5% of patients overall, by preferred term, by cohort in the safety analysis set

Event, n (%)	Monotherapy		Combination therapy	
	Dose escalation at non-RD levels (N=27)	Dose expansion and RD (600 µg) from dose escalation (N=16)	Dose escalation at non-RCD levels (N=21)	Dose expansion and RCD (600 µg) from dose escalation (N=34)
Patients with ≥1 TEAE*†	27 (100)	16 (100)	21 (100)	34 (100)
AE	27 (100)	16 (100)	21 (100)	34 (100)
Serious TEAE	9 (33.3)	4 (25.0)	9 (42.9)	9 (26.5)
NCI-CTCAE Grade 3 or higher TEAE‡	9 (33.3)	4 (25.0)	7 (33.3)	14 (41.2)
TEAE leading to study discontinuation, interruption or discontinuation of study drug, or dose modification	7 (25.9)	2 (12.5)	3 (14.3)	8 (23.5)
CV8102-related TEAE‡	27 (100)	14 (87.5)	18 (85.7)	31 (91.2)
CV8102-related NCI-CTCAE Grade ≥3 TEAE†‡	5 (18.5)	1 (6.3)	2 (9.5)	2 (5.9)
CV8102-related serious TEAE‡	5 (18.5)	1 (6.3)	3 (14.3)	3 (8.8)
CV8102-related TEAE leading to study discontinuation, interruption or discontinuation of study drug, or dose modification	6 (22.2)	0	0	2 (5.9)
TEAEs by preferred term				
Fever	13 (48.1)	11 (68.8)	9 (42.9)	23 (67.6)
Chills	6 (22.2)	6 (37.5)	8 (38.1)	16 (47.1)
Fatigue	12 (44.4)	0	7 (33.3)	5 (14.7)
Nausea	8 (29.6)	1 (6.3)	2 (9.5)	8 (23.5)
Injection site pain	8 (29.6)	1 (6.3)	4 (19.0)	5 (14.7)
Headache	8 (29.6)	1 (6.3)	3 (14.3)	5 (14.7)
Influenza-like illness	7 (25.9)	0	2 (9.5)	6 (17.6)
Asthenia	3 (11.1)	1 (6.3)	2 (9.5)	9 (26.5)
Anemia	4 (14.8)	2 (12.5)	2 (9.5)	5 (14.7)
Urinary tract infection	3 (11.1)	3 (18.8)	4 (19.0)	2 (5.9)
Pain in extremity	4 (14.8)	0	3 (14.3)	3 (8.8)
Tumor pain	2 (7.4)	5 (31.3)	0	2 (5.9)
Injection site erythema	2 (7.4)	0	4 (19.0)	2 (5.9)
Arthralgia	4 (14.8)	1 (6.3)	2 (9.5)	1 (2.9)
C-reactive protein increased	4 (14.8)	1 (6.3)	2 (9.5)	1 (2.9)
Cytokine release syndrome	1 (3.7)	1 (6.3)	2 (9.5)	3 (8.8)
Dizziness	2 (7.4)	1 (6.3)	0	4 (11.8)
Decreased appetite	3 (11.1)	1 (6.3)	3 (14.3)	0
Injection site reaction	2 (7.4)	0	2 (9.5)	2 (5.9)
Alanine aminotransferase increased	3 (11.1)	0	0	3 (8.8)
Gamma-glutamyl transferase increased	2 (7.4)	1 (6.3)	0	3 (8.8)
Tachycardia	2 (7.4)	0	1 (4.8)	3 (8.8)
Diarrhea	1 (3.7)	1 (6.3)	1 (4.8)	3 (8.8)
Vomiting	1 (3.7)	0	1 (4.8)	4 (11.8)
Hypotension	4 (14.8)	1 (6.3)	0	0
Dyspnea	2 (7.4)	0	1 (4.8)	2 (5.9)
Hypertension	2 (7.4)	0	1 (4.8)	2 (5.9)

TEAEs were defined as adverse events that started at or after the first administration of CV8102 through to the end-of-treatment visit, scheduled 28 days after the last dose of study treatment.
*TEAEs were coded using Medical Dictionary for Regulatory Activities, V.25.1.
†Patients may have TEAEs in more than one category.
‡Severity according to National Cancer Institute-Common Terminology Criteria for adverse event (NCI-CTCAE) V.4.03s.
RCD, recommended combination dose; RD, recommended dose; TEAE, treatment-emergent adverse event.

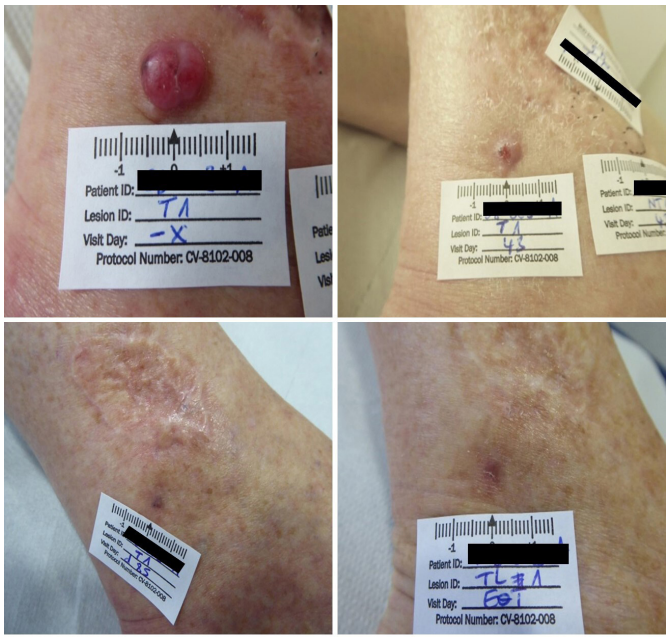

Figure 3 Melanoma lesions in an anti-programmed cell death protein-1 therapy-naive patient with stage IIIC melanoma with multifocal in-transit metastases in the 150 µg monotherapy dose escalation group at: (A) Pretreatment, (B) week 6 post-treatment (five injections of CV8102), (C) week 12 post-treatment (eight injections of CV8102), and (D) at end of study.

figure 3A–D, which shows complete regression of multifocal in-transit metastases and complete regression of all skin metastases at week 12 in a female patient (Patient 23) in her early 70s with stage IIIC melanoma in the 150 µg monotherapy dose escalation group. After the first IT injection, a marked transient rise in serum interleukin-6 (IL-6) and C-reactive protein was observed. After five injections of CV8102, partial regression of the injected tumor lesion was observed (figure 3B). Complete regression of in-transit metastases on MRI and complete regression of all skin metastases with minimal residual palpable induration of the injected lesion was seen at week 12 (figure 3C). The patient continued to receive injections of CV8102 at monthly intervals without locoregional recurrence (figure 3D) until a new intra-abdominal soft tissue lesion was observed after 9 months.

1 of the 10 patients with a CR/PR developed subsequent PD, the remaining nine patients were alive without PD at their last tumor assessment (four patients with last tumor assessment at 3 months, one patient at 9 months, and four patients at 12 months; figure 2A and online supplemental figure 2A and C. The overall median DOR was 6.0 months (range 0–9.2 months) including censored observations, and 5.8–6.0 months excluding censored observations. The median overall PFS based on both RECIST V.1.1 and irRECIST was 2.8 (95% CI: 2.8; 3.1) months (range, 0.5–12.8 months) including censored observations and 0.5–9.7 months excluding censored observations.

DORs for the combination therapy cohort dose expansion are shown in figure 2B. The best overall responses and DOR for the remaining cohorts are shown in online supplemental figures 1,2.

Median PFS was 3.1 months (95% CI: 1.9; 8.5) in the monotherapy dose escalation cohort, 2.8 months (95% CI: 2.1; 2.9) in the combination dose escalation cohort, 2.8 months (95% CI: 0.6; 2.8) in the monotherapy dose expansion cohort and 3.2 months (95% CI: 2.8; 6.2) in the combination dose expansion cohort.

Regression of lesions at non-injected sites

Some evidence of tumor regression of non-injected lesions was observed in both monotherapy and combination cohorts at different dose levels (online supplemental figure 3); corresponding figures for injected lesions are shown in online supplemental figure 4. A >30% regression of non-injected target lesions, determined by the sum of the longest diameter per RECIST V.1.1, was observed in eight patients (two patients in the monotherapy cohorts treated at 100 µg and 450 µg and six patients in the combination cohorts treated at 600 µg and 900 µg).

Exploratory outcomes: biomarker analyzes

Tissue immunofluorescence (IF) analysis results were available for paired biopsy samples from 10 patients

Table 3 Treatment response to CV8102 assessed by RECIST V.1.1 in the monotherapy and combination therapy dose escalation and dose expansion cohorts by dose levels in the safety analysis set

	Monotherapy		Combination therapy		
Treatment response, n, (%)	Dose escalation non-RD levels (N=27)	Dose escalation RD and dose expansion (600 µg) (N=16)	Dose escalation non-RCD levels (N=21)	Dose escalation RCD and dose expansion (600 µg) (N=34)	Total (N=98)
Complete response	1 (3.7)	0	0	1 (2.9)	2 (2.0)
Partial response	1 (3.7)	1 (6.3)	1 (4.8)	5 (14.7)	8 (8.2)
Stable disease	11 (40.7)	3 (18.8)	2 (9.5)	9 (26.5)	25 (25.5)
Progressive disease	10 (37.0)	11 (68.8)	11 (52.4)	16 (47.1)	48 (49.0)
Non-evaluable	4 (14.8)	1 (6.3)	7 (33.3)	3 (8.8)	15 (15.3)

RCD, recommended combination dose; RD, recommended dose.

treated with CV8102 monotherapy (9 with cMEL, 1 with hnSCC; 3 with best response of SD [stable disease], 7 with PD) and from 20 patients treated with combination therapy (19 patients with cMEL, 1 with hnSCC; with 1 CR, 2 PRs, 7 SDs, 9 PDs and 1 non-evaluable for efficacy) (see online supplemental table 7) for indication, dose received and biopsy details for each).

Results from three objective responders in the combination cohorts from whom paired biopsies were available are shown in figure 4. Immune cell infiltration analysis by

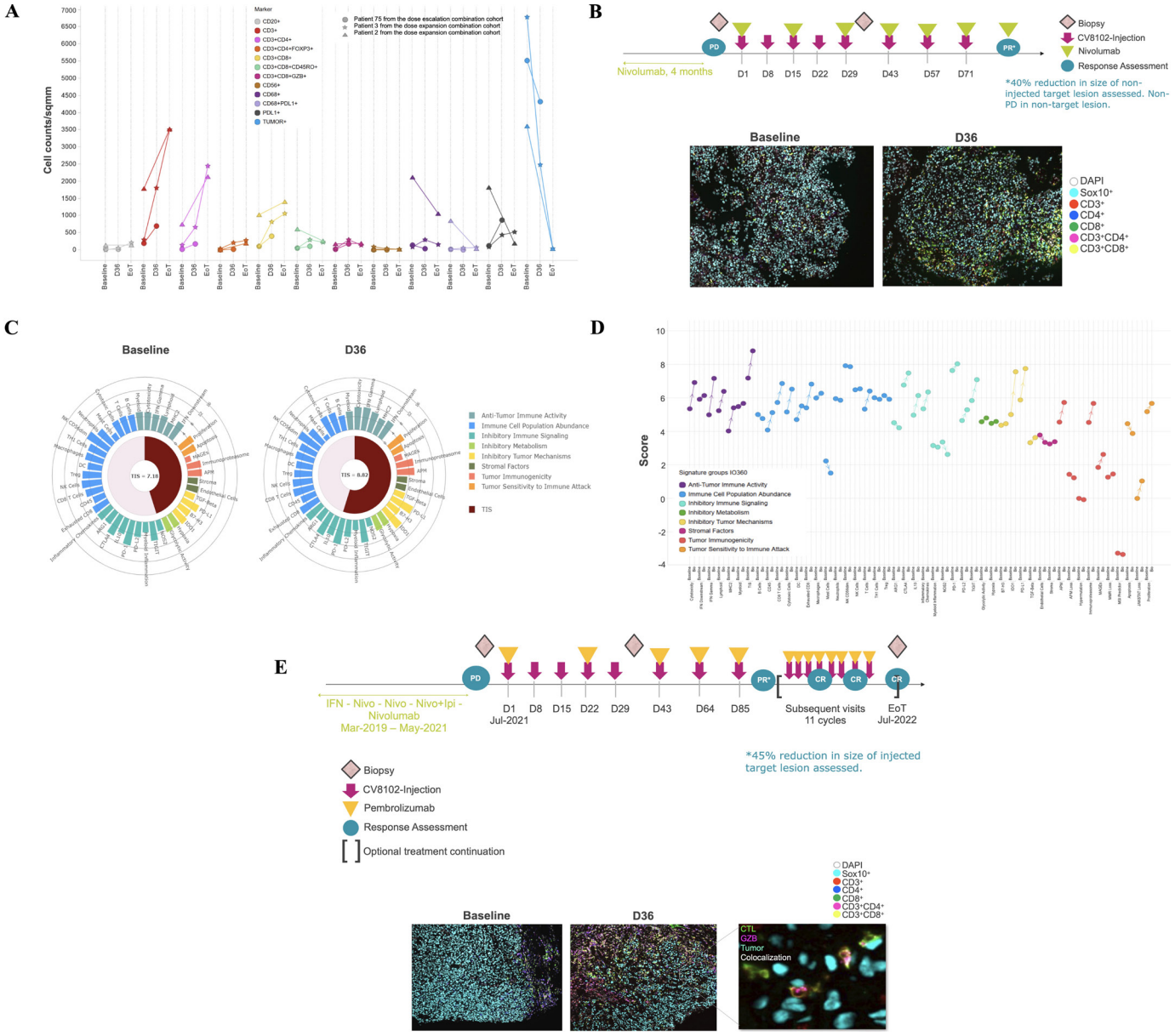

Figure 4 Analysis of the tumor microenvironment in paired biopsies from responders before and after CV8102 treatment. (A) Quantification of tumor and immune cell populations at baseline, Day 36 and EOT (all paired samples obtained from responders are shown, n=3). (B) Clinical evolution and treatments received by a patient with partial response melanoma in the dose escalation (combination cohort) and representative ROIs from biopsies collected at baseline and Day 36, from a non-injected subcutaneous lesion of the right upper limb. The patient was anti-PD-1 therapy-refractory prior to entering the trial and received 900 µg of CV8102 in combination with nivolumab for the complete treatment period of 8 injections. (C) Targeted gene expression profiling in paired samples of the same patient as in B, wheel plots showing the IO360 signatures at baseline and after treatment. (D) Results for all signature scores calculated for the sample in C. (E) Clinical evolution and treatments received by a patient with complete response melanoma in the dose expansion (combination cohort) and representative ROIs from biopsies collected at baseline and Day 36, from an injected lesion on the medial surface of the right lower leg (skin). The anti-refractory patient received 600 µg of CV8102 in combination with pembrolizumab and remained on treatment for a total of 19 injections. The zoom-in image in the post-treatment sample shows activated T cells with Granzyme B (GZMB) granules oriented towards tumor cells. DAPI, 4',6-diamidino-2-phenylindole; EOT, end of treatment; PD, progressive disease; PD-L1, programmed death-ligand-1; PR, partial response; TIS, tumor inflammation signature.

multiplex IF showed increased T-cell infiltration mostly in the $CD4^+$ and $CD8^+$ compartment of the total $CD3^+$ population after treatment with at least five IT injections of CV8102. Tumor cell density was also reduced (figure 4A). Representative images from the non-injected lesion (PR; figure 4B) and injected lesion (CR, figure 4E) are shown. A trend for increased T-cell infiltration was also observed in nearly half of the patients with SD and nearly one-third of patients with PD; overall, 16 patients showed increased T-cell infiltration, 8 showed decreased T cells and 6 showed no change (online supplemental figure 5).

Due to sample quantity requirements for nCounter measurements, NanoString analysis from paired biopsies was only possible for 25 patients, 15 of them PD, 8 SD, 1 PR and 1 non-evaluable (9 patients treated with monotherapy, 16 patients treated with combination therapy). Four of the patients with PD showed an increase in the tumor inflammation signature (TIS) score, three a decrease, and eight had no change when comparing the biopsy sample after five IT injections with the baseline biopsy. Of the patients with SD, four showed an increase in the TIS score, while one showed a decrease and three showed no change. The NanoString analysis of the samples from the one available PR patient showed increase in TIS score and other inflammatory signatures (figure 4C,D).

Cytokine and chemokine profiling showed transient changes in the peripheral blood shortly after CV8102 administration (3–24 hours). Systemic levels of interferon (IFN)-a, IFN-g, IL-10, IL-6 and IFN-g-induced protein 10 were significantly increased shortly after the first CV8102 IT administration compared with baseline, while macrophage inflammatory protein-1 beta decreased in the combination dose expansion cohort (online supplemental figure 6C). These differences were not significant in the monotherapy expansion cohort, although there was a similar trend for some patients, online supplemental figure 5B. No cytokine changes correlated with dose in the dose escalation cohorts (online supplemental figure 6A) or with response in the dose expansion cohorts (online supplemental figure 6B,C). There were no statistically significant differences between the monotherapy and the combination cohorts at any given time point.

DISCUSSION

In this phase I study, CV1802 was well tolerated as monotherapy and in combination with PD-1 inhibitors up to the RD of 600 µg in patients with advanced cMEL, cSCC, hnSCC and ACC. The most common any-grade TEAEs were fever, chills, and fatigue. Up to the RD of 600 µg there were no notable differences in incidence or severity of TEAEs between individual dose levels or between the monotherapy and combination cohorts. Thus, indicating that concomitant treatment with a PD-1 inhibitor does not impair the tolerability of IT CV8102 or require dose adjustments. At the 900 µg dose level in the monotherapy dose escalation cohort, one patient experienced a dose-limiting ALT/AST increase in the context of a moderate CRS which was successfully treated with steroids and did not reoccur after dose reduction. A second patient experienced Grade 3 pneumonitis after the DLT evaluation period which recovered after treatment discontinuation and steroid treatment. No DLTs were observed in the combination therapy dose escalation cohort. Despite the fact that the MTD was not formally reached per-protocol, since one of the Grade 3 events occurred after the DLT evaluation period, the IDMC recommended to proceed with a dose of 600 µg of CV8102 for the dose expansion cohorts. Notably, there was overall a low rate of Grade 3 TEAEs observed beyond 2 weeks and only a limited number of patients discontinued due to AEs (see online supplemental table 2). Therefore, the selection of the RD seems well justified despite the short DLT evaluation period of 2 weeks.

Overall, the safety profile of CV8102 appears comparable with those seen with other IT TLR agonists, which include transient mild-to-moderate influenza-like symptoms and injection-site reactions including cases of mainly mild-to-moderate CRS.[14 23–26] High-grade CRS was rarely described after IT treatments but was found to be dose-limiting in a phase I trial of the TLR 7/8 agonist MEDI9197.[27] In a phase I trial of the TLR7/8 agonist, EIK1001, tested in combination with pembrolizumab, manageable CRS was reported in 10% of patients.[26]

The overall population in the current study had advanced-staged disease and the majority had experienced progressive disease on PD-1 inhibitors. Despite this, CV8102 showed preliminary signs of efficacy as monotherapy and in combination with PD-1 inhibitors. Notably, in an expansion cohort of patients with anti-PD-1 therapy-refractory melanoma treated at the RD, an overall response rate (ORR) of 17% was observed after treatment with CV8102 in combination with PD-1 inhibitors. This ORR is comparable to the ORR of 22% reported in a phase I/II study of the IT TLR agonist tilsotolimod in combination with anti-CTLA-4 therapy in patients with melanoma refractory to prior PD-1 inhibitor therapy.[23] Tumor shrinkage was observed in injected and non-injected lesions suggesting that IT CV8102 induces a systemic T-cell response against tumor antigens that can also infiltrate and control distant non-injected lesions.

Given the heterogeneity of the patient population and the small number of patients in each dose cohorts, the study was not designed to assess dose dependency in terms of efficacy. While responses were mainly seen at dose levels ≥450 µg, one patient with anti-PD-1-naïve melanoma experienced a CR at the 150 µg dose level indicating responses can occur after low doses of CV8102. Exploratory analysis of systemic cytokine/chemokine levels 3–24 hours after IT injection of CV8102 showed transient and variable changes without apparent dose dependency. This indicates that individual factors such as tumor vascularization or immunosuppressive factors in the TME may impact the level of innate immune activation and systemic cytokine release after IT injection of CV8102. Of note, we

used a fixed concentration of CV8102 in our study and increased the dose by increasing the volume of the injection. Also, investigators were allowed to split the administration between lesions if needed. A possible limitation of our study might be that we did not systematically assess whether the impact of the ratio of the locally injected CV8102 volume to the volume of the injected lesion plays a role in the biological or clinical response. This should be considered for further studies to better understand the dose dependency of clinical efficacy.

Analysis of the TME in the available paired biopsies showed increased T-cell infiltration and reduced tumor cell content in the three responders. This trend was also observed in nearly half of SD patients and one-third of PD patients, suggesting that the increase in T-cell infiltration is necessary, but not sufficient, to induce a clinical response and that additional factors are determinants of clinical efficacy, such as immunosuppressive factors inhibiting T-cell function in the tumor environment or resistance of cancer cells to T cell-mediated killing.

There were several limitations of this study, including that the patient population in the dose escalation part was heterogeneous comprising four different tumor entities. Furthermore, patients in the monotherapy dose escalation cohort were less frequently pretreated with CPIs, which may have impacted the biological and clinical response. Also, there was only one investigator assessment of response and no central tumor response readout and not all responses were confirmed. In addition, because baseline and on-treatment biopsies were only available from a subgroup of 30 patients and the ORR was low, it was not possible to draw robust final conclusions about molecular or immunological patterns in the TME that may be associated with responses.

Although IT administration of TLR agonists have shown clear evidence of efficacy in certain patients,[23–25] it is not known which patients are most likely to benefit from such treatment. This has been demonstrated with the investigational IT TLR-9 agonist, tilsotolimod and the oncolytic herpes virus talimogene laherparepvec which have shown promising clinical efficacy in PD-1 inhibitor-refractory patients in combination with ipilimumab or pembrolizumab, respectively,[23] however the primary endpoints in the phase III trials were not met.[28] Investigation of the TME at baseline, and collection of serial biopsies from patients in future phase I/II studies of IT agents may improve our understanding of the factors predicting clinical benefit from IT immunomodulators and help to enrich patient populations most likely to benefit in upcoming trials.

CONCLUSIONS

The RD of CV8102 of 600 µg was generally well tolerated across all tumor types, with no notable differences in incidence or maximum severity of the TEAEs between the cohorts. CV8102 combination therapy with a PD-1 inhibitor showed preliminary signs of efficacy in patients with anti-PD-1 therapy-refractory cMEL. Preliminary signs of efficacy of monotherapy were also observed with objective responses in two patients with melanoma and one patient with SCC of the skin. In addition, CV8102 showed modulation of systemic immune-mediators and local activation and enrichment of T cells in tumor in both treated and untreated lesions, suggesting an immunomodulatory effect on the TME in the injected lesion as well as in distant untreated lesions.

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Acknowledgements Medical writing support was provided by Mark English, PhD, and Margaret Haugh, PhD, of CureVac. The authors would like to thank Sarah Katharina Kays, PhD, for her contribution to conceptualization and study implementation of biomarker strategy; Fatma Funkner, PhD, for her contribution to

conceptualization of biomarker strategy and validation of bio-analytical tools; and Dominik Vahrenhorst, PhD, for his contribution and discussion on bioinformatical analysis.

Contributors Study design and conception: UG-V, BS-B, OS-K and TS. Clinical investigators: TE, IT, IS, FGB, ME, LH, SO, JK, AO, CR, CL, LK, JM-L, ER, PT, JM-L, CW, PM, YS, PB, MF, AP, MS and AS. Clinical trial statistical analysis: OS-K. Biomarker data analysis: PC, MG, PW and JH. All authors drafted the initial version of this manuscript. TE, CR, JM-L, ER, PT, PM, CLP, BS-B, UG-V, MF, GQ, PC, MG, PW, JH and TS interpreted the data. All authors participated in review and revision of the manuscript. All authors read and approved the final manuscript. UG-V is the study guarantor.

Funding This study was funded by CureVac SE.

Competing interests TE and IT have received institutional funding for the work in this study. YS, IS, ME, LH, JK, AO, CW, PM, CLP, PB, MF, AP, MS and AS have no competing interests. CR is an occasional consultant for Roche, BMS, MSD, Sanofi, Pierre Fabre, AstraZeneca, and Novartis. CL has received consulting fees from BMS, MSD, Sanofi, Immunocore, and Pierre Fabre. JM-L has received consulting fees from Bristol-Myers Squibb, Highlight Therapeutics, Novartis, Pierre Fabre, Roche, and Sanofi. ER has received consulting fees from BMS, MSD, Novartis, and Pierre Fabre; speakers' fees from Amgen, BMS, Delcath, MSD, Merck, Novartis, Pierre Fabre, Sanofi, Amgen, BMS, Curevac, Delcath, Incyte, MSD, Merck, Novartis, Pierre Fabre, Regeneron and Roche. PT has received consulting fees from Almirall, BMS, Kyowa Kirin, Merck, Novartis, Pierre Fabre, Sanofi, 4SC, and honorarium from Almirall, BMS, Biofrontera, Kyowa Kirin, Merck, Novartis, Pierre Fabre, Roche, Sanofi, and 4SC. BS-B, MG, JH, PW, TS, SDK, GQ, PC, MF, and UG-V are employees of CureVac SE. OS-K and SO have received consulting fees from CureVac SE.

Patient consent for publication Not applicable.

Ethics approval The reference number for our study at the leading EC (Universität Tübingen Dermatologische Onkologie) is 785/2016AMG1, and in order to provide opinion based on German drug law the EC is registered with following reference: 2017-385-15954. Ethik-Kommission der Medizinischen Fakultät der Eberhard-Karls-Universität und am Universitätsklinikum Tübingen Gartenstraße 47 72074 Tübingen, Germany. Additional IRB/IEC name and addresses: Ethik-Kommission bei der Ärztekammer Niedersachsen Berliner Allee 20 30175 Hannover, Germany; Ethik-Kommission I der Medizinischen Fakultät Heidelberg Alte Glockengießerei 11/1 69115 Heidelberg, Germany; Ethik-Kommission der Universität Lübeck Ratzeburger Allee 160 23538 Lübeck, Germany; Ethik-Kommission der Ärztekammer Westfalen-Lippe und der Westfälischen Wilhelms-Universität Münster Gartenstraße 210-214 48147 Münster, Germany; Ethik-Kommission der Medizinischen Fakultät Friedrich-Alexander-Universität Erlangen-Nürnberg Krankenhausstraße 12 91054 Erlangen, Germany; Ethik-Kommission des Landes Berlin (LaGeSo) Turmstraße 21 10559 Berlin, Germany; Ethik-Kommission der Ärztekammer Westfalen-Lippe und der Westfälischen Wilhelms-Universität Münster Gartenstraße 210-214 48147 Münster, Germany; Ethikkommission an der Medizinischen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn Biomedizinisches Zentrum Sigmund-Freud-Str. 25 53105 Bonn, Germany; CEIm Hospital Universitari Vall d'Hebron Planta 13 Hospital Infantil y de la Mujer Paseo Vall d'Hebron, núm. 119-129 08035 Barcelona CHU de Saint-Etienne (Saint-Etienne Hospital Complex) - Direction des Affaires Médicales et de la Recherche (Directorate of Medical Affairs and Research) Hôpital Bellevue - Pavillon 31 - 42055 SAINT- ETIENNE Cedex 2 - France; Ethikkommission der Medizinischen Universität Graz LKH-Universitätsklinikum – Eingangsgebäude Auenbruggerplatz 2, 3. OG 8036 Graz, Austria; Ethics Committee at FSBI "NMRC of Oncology n.a. N.N. Blokhin", 115478, 24 Kashirskoye shosse, Moscow, Russia; Local Ethics Committee at FSAEI HE I. M. Sechenov First Moscow State Medical University (Sechenov University) 119991, 8 Trubetskaya st., Moscow, Russia; Biomedical ethics committee at the Clinic of advanced medical technologies n. a. Nicolay I. Pirogov, SPbU. 190103, Fontanka river emb., 154, St. Petersburg, Russia; Ethics Committee at FSBI "NMRC of Oncology n.a. N.N. Petrov" of the Ministry of Healthcare of Russia, 68 Leningradskaya street, Pesochny, Saint Petersburg, 197758, Russia. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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