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Intratumoral oncolytic virus V937 plus ipilimumab in patients with advanced melanoma: the phase 1b MITCI study

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ABSTRACT

Background Intratumoral administration of V937, a bioselected, genetically unmodified coxsackievirus A21, has previously demonstrated antitumor activity in patients with advanced melanoma as monotherapy and in combination with the programmed cell death 1 (PD-1) antibody pembrolizumab. We report results from an open-label, single-arm, phase 1b study (NCT02307149) evaluating V937 plus the cytotoxic T-lymphocyte antigen 4 inhibitor ipilimumab in patients with advanced melanoma.

Methods Adult patients (aged ≥18 years) with histologically confirmed metastatic or unresectable stage IIIB/C or IV melanoma received intratumoral V937 on days 1, 3, 5, 8, and 22 and every 3 weeks (Q3W) thereafter for up to 19 sets of injections plus intravenous ipilimumab 3 mg/kg Q3W administered for four doses starting on day 22. Imaging was performed at screening, on days 43 and 106 and every 6 weeks thereafter; response was assessed by immune-related response criteria per investigator assessment. Primary endpoints were safety in all treated patients and objective response rate (ORR) in all treated patients and in patients with disease that progressed on prior anti-PD-1 therapy.

Results Fifty patients were enrolled and treated. ORR was 30% (95% CI 18% to 45%) among all treated patients, 47% (95% CI 23% to 72%) among patients who had not received prior anti-PD-1 therapy, and 21% (95% CI 9% to 39%) among patients who had experienced disease progression on prior anti-PD-1 therapy. Tumor regression occurred in injected and non-injected lesions. Median immune-related progression-free survival was 6.2 months (95% CI 3.5 to 9.0 months), and median overall survival was 45.1 months (95% CI 28.3 months to not reached). The most common treatment-related adverse events (AEs) were pruritus (n=25, 50%), fatigue (n=22, 44%), and diarrhea (n=16, 32%). There were no V937-related dose-limiting toxicities and no treatment-related grade 5 AEs. Treatment-related grade 3 or 4 AEs, all of which were considered related to ipilimumab, occurred in 14% of patients (most commonly dehydration, diarrhea, and hepatotoxicity in 4% each).

Conclusions Responses associated with intratumoral V937 plus ipilimumab were robust, including in the subgroup of patients who had experienced disease progression on prior anti-PD-1 therapy. Toxicities were manageable and consistent with those of the individual monotherapies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Intratumoral administration of V937, a bioselected, genetically unmodified coxsackievirus A21, has previously demonstrated antitumor activity in patients with advanced melanoma as monotherapy and in combination with the programmed cell death 1 (PD-1) antibody pembrolizumab

WHAT THIS STUDY ADDS

⇒ We report results from an open-label, single-arm, phase 1b study evaluating intratumoral V937 (a bioselected, genetically unmodified coxsackievirus A21) plus intravenous ipilimumab (a cytotoxic T-lymphocyte antigen 4 inhibitor) in patients with advanced melanoma.

⇒ Responses associated with intratumoral V937 plus ipilimumab were robust, including in the subgroup of patients who had experienced disease progression on prior anti-PD-1 therapy.

⇒ Objective response rate was 30% (95% CI 18% to 45%) among all treated patients, 47% (95% CI 23% to 72%) among patients who had not received prior anti-PD-1 therapy, and 21% (95% CI 9% to 39%) among patients who had experienced disease progression on prior anti-PD-1 therapy.

⇒ Toxicities were manageable and consistent with those of the individual monotherapies.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ In this phase 1b study, combination therapy with the oncolytic virus V937 administered intratumorally plus ipilimumab had manageable toxicity, and responses were robust and durable in patients with advanced melanoma, including patients with melanoma progression after anti-PD-1 therapy

INTRODUCTION

Oncolytic viruses are an emerging class of anticancer therapeutics that function both by killing tumor cells directly (virus-mediated oncolysis) and by inducing systemic antitumor immune responses.[1] Talimogene laherparepvec (T-VEC), a genetically engineered, live attenuated herpes simplex virus

1, became the first such agent to be approved by the US Food and Drug Administration for the treatment of unresectable melanoma recurrent after initial surgery[2] based on durable responses demonstrated in a phase 3 study.[3] Coxsackievirus is a type of non-enveloped, single-stranded RNA enterovirus that typically causes asymptomatic infections or common cold-like symptoms.[4] Coxsackievirus undergoes cytosolic replication without a DNA phase and is not associated with risk of insertional mutagenesis during infection.[4] As such, it does not require genetic modification for safety.

The oncolytic virus V937 (previously known as Cavatak and CVA21) is a bioselected and genetically unmodified coxsackievirus A21.[5] It gains cellular entry via intercellular adhesion molecule 1 and decay-accelerating factor receptors, which are overexpressed by certain cancer cells, including melanoma.[6–8] In a phase 2 study of patients with advanced melanoma, intratumoral V937 monotherapy demonstrated systemic antitumor activity with reductions in the size of both injected and non-injected (eg, liver and lung) lesions.[5] The unconfirmed objective response rate (ORR; complete response (CR) or partial response (PR)) was 38.6%, and the confirmed ORR was 28.1% based on immune-related Response Evaluation Criteria in Solid Tumors (irRECIST); a response lasting ≥6 months was observed in 21.1% of patients.

Table 1 Patient demographics and baseline characteristics*

Characteristic	All treated patients N=50	Progressed on prior anti-PD-1 therapy n=33	No prior anti-PD-1 therapy n=17
Age, median (range), year	64.5 (28–88)	65.0 (28–84)	64.0 (35–88)
Sex			
Men	31 (62)	22 (67)	9 (53)
Women	19 (38)	11 (33)	8 (47)
White	50 (100)	33 (100)	17 (100)
ECOG PS			
0	34 (68)	21 (64)	13 (76)
1	16 (32)	12 (36)	4 (24)
Stage			
III	14 (28)	7 (21)	7 (41)
IVM1a	7 (14)	4 (12)	3 (18)
IVM1b	6 (12)	4 (12)	2 (12)
IVM1c(1)†	12 (24)	11 (33)	1 (6)
IVM1c(2)‡	11 (22)	7 (21)	4 (24)
Baseline tumor burden, median (range), mm^2	1479 (209–18,218)	1425 (225–18,218)	2750 (209–7348)
BRAF mutation status			
Mutant	17 (34)	12 (36)	5 (29)
Wild-type	26 (52)	18 (55)	8 (47)
Unknown	7 (14)	3 (9)	4 (24)
Received any prior systemic therapy	40 (80)	33 (100)	7 (41)
1 line	16 (32)	10 (30)	6 (35)
2 lines	8 (16)	8 (24)	0
≥3 lines	16 (32)	15 (45)	1 (6)
Select prior pharmacologic therapy§			
Chemotherapy	8 (16)	7 (21)	1 (6)
Hormone therapy	1 (2)	1 (3)	0
Immunotherapy	29 (58)	27 (82)	2 (12)
Targeted therapy	4 (8)	4 (12)	0

*Data are presented as n (%), unless otherwise noted.
†Metastases to all other visceral metastases with a normal lactate dehydrogenase.
‡Any distant metastases with an elevated lactate dehydrogenase.
§Patients could have been counted in more than one row.
anti-PD-1, anti-programmed death 1; ECOG PS, Eastern Cooperative Oncology Group performance status.

Table 2 Treatment-related AEs*†

	All treated patients, N=50		Progressed on prior anti-PD-1 therapy, n=33		No prior anti-PD-1 therapy, n=17	
Treatment-related AEs	47 (94)		30 (91)		17 (100)	
Grade ≥3 V937-related AEs	0		0		0	
Grade 3 or 4 ipilimumab-related AEs‡§	7 (14)		4 (12)		3 (18)	
Treatment-related AEs occurring in ≥10% of all treated patients	**Any grade**	**Grade 3 or 4**	**Any grade**	**Grade 3 or 4**	**Any grade**	**Grade 3 or 4**
Pruritus	25 (50)	1 (2)	17 (52)	0	8 (47)	1 (6)
Fatigue	22 (44)	1 (2)	15 (45)	0	7 (41)	1 (6)
Diarrhea	16 (32)	2 (4)	13 (39)	2 (6)	3 (18)	0
Nausea	11 (22)	0	9 (27)	0	2 (12)	0
Rash	10 (20)	0	5 (15)	0	5 (29)	0
Pyrexia	8 (16)	0	5 (15)	0	3 (18)	0
Chills	7 (14)	0	6 (18)	0	1 (6)	0
Influenza-like illness	7 (14)	0	6 (18)	0	1 (6)	0
Decreased appetite	6 (12)	0	5 (15)	0	1 (6)	0
Injection site reaction	6 (12)	0	3 (9)	0	3 (18)	0
Transaminase increase	6 (12)	0	5 (15)	0	1 (6)	0
Headache	5 (10)	0	5 (15)	0	0	0
Injection site pain	5 (10)	0	2 (6)	0	3 (18)	0

*Data are presented as n (%).
†Treatment-related AEs were those events with a V937 or ipilimumab relationship of possibly, probably, or definitely.
‡There were no grade 5 treatment-related AEs.
§The most common grade 3 and 4 treatment-related AEs were dehydration, diarrhea, and hepatotoxicity (two patients (4%) each).
AE, adverse event; anti-PD-1, antiprogrammed death 1.

Treatment was well tolerated in the study, with no grade ≥3 treatment-related adverse events (AEs).[5]

Oncolytic viruses have been shown to alter the tumor microenvironment (eg, increased $CD8^+$ T cells and programmed death ligand 1 (PD-L1) expression, reduced suppressor T cells),[9 10] which provides the rationale for combination therapy with immune checkpoint inhibitors. In a phase 1b study of patients with advanced melanoma, the

Table 3 Immune-related responses*

	All treated patients N=50	Progressed on prior anti-PD-1 therapy n=33	No prior anti-PD-1 therapy n=17
Objective response rate, % (95% CI)	30 (18 to 45)	21 (9 to 39)	47 (23 to 72)
Complete response, n (%)	5 (10)	2 (6)	3 (18)
Partial response, n (%)	10 (20)	5 (15)	5 (29)
Stable disease, n (%)	17 (34)	15 (45)	2 (12)
Progressive disease, n (%)	16 (32)	11 (33)	5 (29)
Not evaluable, n (%)	2 (4)	0	2 (12)
Time to initial response, median (range), mo	3.4 (0.7–5.1)	3.5 (3.2–5.1)	3.4 (0.7–3.4)
Disease control rate†, n (%)	32 (64)	22 (67)	10 (59)
Durable response rate‡, % (95% CI)	14 (6 to 27)	12 (3 to 28)	18 (4 to 43)
Duration of response			
Median (95% CI), months	8.8 (5.9 to 8.8)	8.8 (5.9 to 8.8)	NR (4.9 to NR)
≥6 months, %	81	80	86

*Data analyzed in the safety analysis set; responses are based on investigator assessment per immune-related response criteria.
†Complete response+partial response+stable disease.
‡Response lasting ≥6 months.
anti-PD-1, antiprogrammed death 1; NR, not reached.

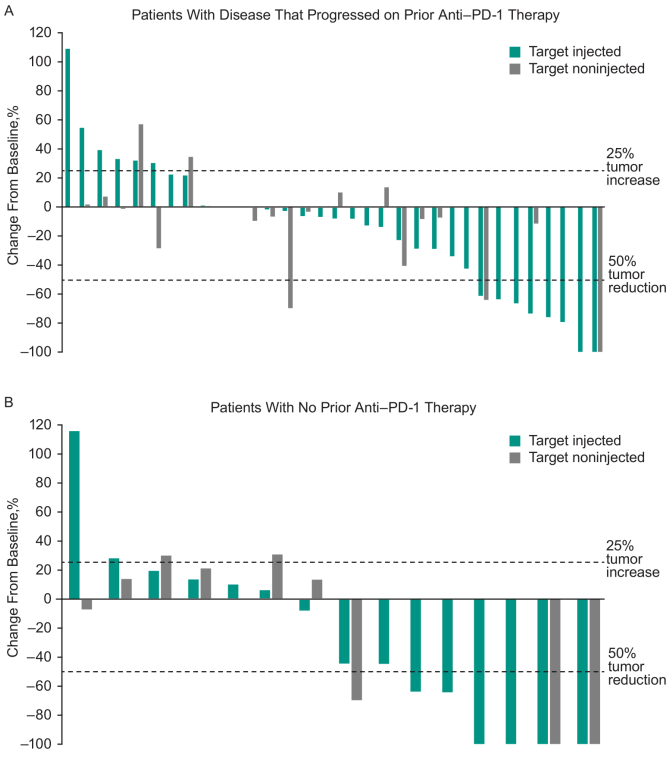

Figure 1 Best percentage change from baseline in target injected and non-injected lesions among (A) patients who had progressed on prior anti-PD-1 therapy and (B) patients with no prior anti-PD-1 therapy. Data include patients with ≥1 postbaseline tumor assessment after start of study treatment. anti-PD-1, antiprogrammed cell death 1.

combination of intratumoral V937 plus the programmed cell death 1 (PD-1) antibody pembrolizumab resulted in a confirmed ORR of 47% per irRECIST and a response lasting ≥12 months in 74% of patients.[11] No dose-limiting toxicity (DLT) occurred, and no new safety signals were identified. Other combinations of V937 with immune checkpoint inhibitors may have the potential to result in improved outcomes. Here, we report results from the phase 1b Melanoma Intra-Tumoral Cavatak and Ipilimumab (MITCI) study (ClinicalTrials.gov, NCT02307149), which evaluated the combination of V937 plus the cytotoxic T-lymphocyte antigen 4 antibody ipilimumab in patients with metastatic or unresectable stage IIIB/C or IV melanoma. At the time this study opened in 2015, the use of PD-1 inhibitors was just beginning to transform the care of patients with advanced melanoma, and the concept of anti–PD-1-refractory melanoma was not established. This trial was modified to include a cohort of patients with melanoma progression after anti-PD-1 therapy when there was greater appreciation that anti–PD-1-refractory melanoma represented a significant proportion of patients and for whom prognosis is poor[12] and responses to second-line therapies are infrequent.[13 14]

METHODS

Study design and patients

MITCI was an open-label, single-arm, phase 1b study. Eligible patients were adults (aged ≥18 years) with histologically confirmed metastatic or unresectable stage IIIB/C or IV melanoma per American Joint Committee on Cancer 7th edition, an Eastern Cooperative Oncology Group performance status of 0 or 1, ≥1 cutaneous or subcutaneous tumor (0.5–5.0 cm in longest diameter) or a palpable lymph node amenable to intratumoral injection, and ≤3 visceral metastases (excluding pulmonary lesions) with no lesions >3.0 cm. A protocol amendment was implemented approximately 2.5 years after study initiation requiring patients to have disease that progressed, per RECIST v1.1, on prior anti-PD-1 therapy. Exclusion criteria included prior treatment with chemotherapy, radiation therapy, or immunotherapy within 28 days before initiation of study treatment, previous receipt of V937 or ipilimumab, and untreated brain metastases. A complete list of inclusion and exclusion criteria can be found in the study protocol (see online supplemental file 2).

Treatment

All patients were treated with the combination of V937 plus ipilimumab. On days 1, 3, 5, 8, and 22 and every 3 weeks thereafter, patients received intratumoral V937 at a maximum total dose of 3×10^8 $TCID_{50}$ (50% tissue culture infectious dose) in a volume of up to 4.0 mL. At each injection visit, multiple lesions were injected, starting with the largest lesion(s), using a volume of 2.0 mL for tumors >25 mm in diameter, 1.0 mL for tumors 15 to 25 mm in diameter, and 0.5 mL for tumors 5 to <15 mm in diameter. Injected lesions that decreased to <5 mm in diameter were injected with 0.1 mL of V937 until complete resolution.

Patients with clinical benefit continued treatment with V937 up to a maximum of 19 sets of injections or until confirmed disease progression, CR, or unacceptable toxicity. V937 dose modifications were not permitted. Patients who stopped treatment with V937 could receive any remaining planned ipilimumab doses as clinically indicated.

Ipilimumab was administered intravenously at 3 mg/kg on days 22, 43, 64, and 85, which is the standard dose and schedule for this agent.[15] Patients who discontinued or delayed ipilimumab dosing could continue to receive planned V937 doses.

Assessments and endpoints

Safety was assessed based on the occurrence of DLTs and AEs. DLTs were defined as any grade ≥3 V937-related toxicities with onset on or before day 85; an exception was that lymphopenia was not considered a DLT. If a DLT occurred in two of the first six patients treated, the study was to be terminated. If no DLTs occurred or if the proportion of DLTs was <30% of the patients enrolled, study accrual was to continue. The accumulated safety data after 6, 12, and 18 patients had been treated and followed through at least day 85 were reviewed by the sponsor and investigators to identify the rate of DLTs and determine whether enrollment should be continued from a safety perspective. AEs were reported from the time of initiation of study treatment through 30 days after cessation of study treatment and were graded according

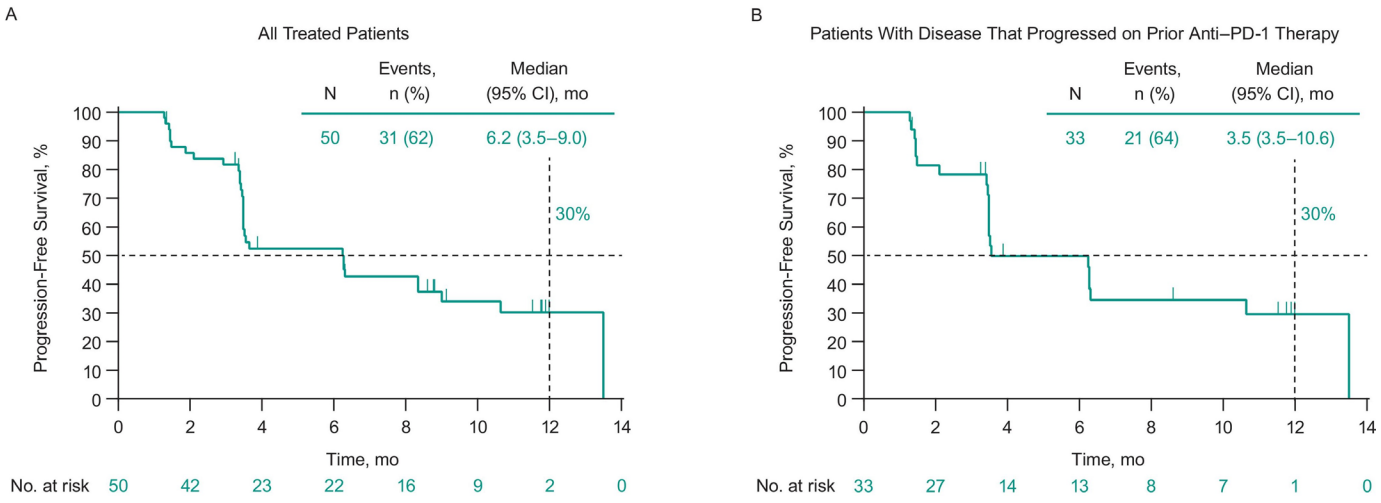

Figure 2 Progression-free survival based on investigator assessment per immune-related response criteria among (A) all treated patients and (B) patients who had progressed on prior anti-PD-1 therapy. Median progression-free survival was 8.3 months (95% CI 3.4 months to not reached) in patients with no prior anti-PD-1 therapy. anti-PD-1, antiprogrammed death 1.

to the National Cancer Institute Common Terminology Criteria for Adverse Events, V.4.03.

Disease status was assessed at screening, on days 43 and 106, and every 6 weeks thereafter by CT or MRI scans. Responses were based on immune-related response criteria[16 17] per investigator assessment.

The primary endpoints were safety in all treated patients and ORR (best response of CR or PR) in all treated patients and in patients with disease that progressed on prior anti-PD-1 therapy assessed using immune-related response criteria (modified WHO criteria). Secondary endpoints included time to initial response (TTR), durable response rate (DRR; defined as the percentage of patients with best response of CR or PR lasting ≥6 months), response of injected and non-injected melanoma lesions, progression-free survival (PFS), and overall survival (OS). The disease control rate (best response of CR, PR, or stable disease (<50% decrease to <25% increase in index lesion and new measurable lesions)) was also reported.

Statistical analyses

A sample size of 26 patients with disease that progressed on prior anti-PD-1 therapy was estimated to provide 90% power to test the null hypothesis (11% ORR) versus the alternative hypothesis (31% ORR) for the primary efficacy endpoint using a one-sided test at a significance level of 0.10. The ORR threshold of 11% was selected based on a previous study of ipilimumab in patients with advanced melanoma.[18]

Safety and efficacy analyses were conducted in all patients who received ≥1 dose of study treatment (V937 and/or ipilimumab). Data were analyzed separately for patients with disease that progressed on prior anti-PD-1 therapy and patients who were not previously treated with anti-PD-1 therapy. Two-sided exact 95% CIs were provided for ORR and DRR. PFS and OS were analyzed using the Kaplan-Meier method. Best per cent change from baseline in target injected and non-injected lesions were analyzed using a double waterfall plot.

RESULTS

Patients

The study was conducted between May 5, 2015 and November 5, 2019, at 11 sites in the USA. At the cut-off date (February 21, 2020), 50 patients were enrolled and received ≥1 dose of study treatment. Patient demographics and baseline characteristics are shown in table 1 for all treated patients and based on previous receipt of anti-PD-1 therapy. Most patients (62%) were men, and median age was 64.5 years. The majority of patients (72%) had stage IV disease, with a median baseline tumor burden of 1479 mm^2 (range: 209–18 218 mm^2). *BRAF* mutations were detected in 34% of the population. Most patients (80%) had received previous systemic therapy (≥3 lines in 32%). Patients with disease that progressed on prior anti-PD-1 therapy (60%) had disease that was more heavily pretreated with systemic therapy than patients who were not previously treated with anti-PD-1 therapy (34%) (≥3 lines in 45% vs 6%, respectively).

The median numbers of intratumoral V937 injections and ipilimumab infusions were 9 (range: 5–19) and 4 (range: 1–4), respectively. The median cumulative dose of V937 administered was 1.5×10^9 (range: 4.31×10^8 to 5.54×10^9) $TCID_{50}$. All patients had stopped study treatment as of the cut-off date (online supplemental figure S1); 60% of patients had discontinued because of disease progression and 22% had completed the study.

Safety

Treatment-related AEs, as assessed by the investigator, are summarized in table 2. No DLTs or treatment-related grade 5 AEs occurred in the overall population.

Thirty-seven patients (74%) experienced ≥1 investigator-assessed V937-related AE; no patients had grade 3 or 4

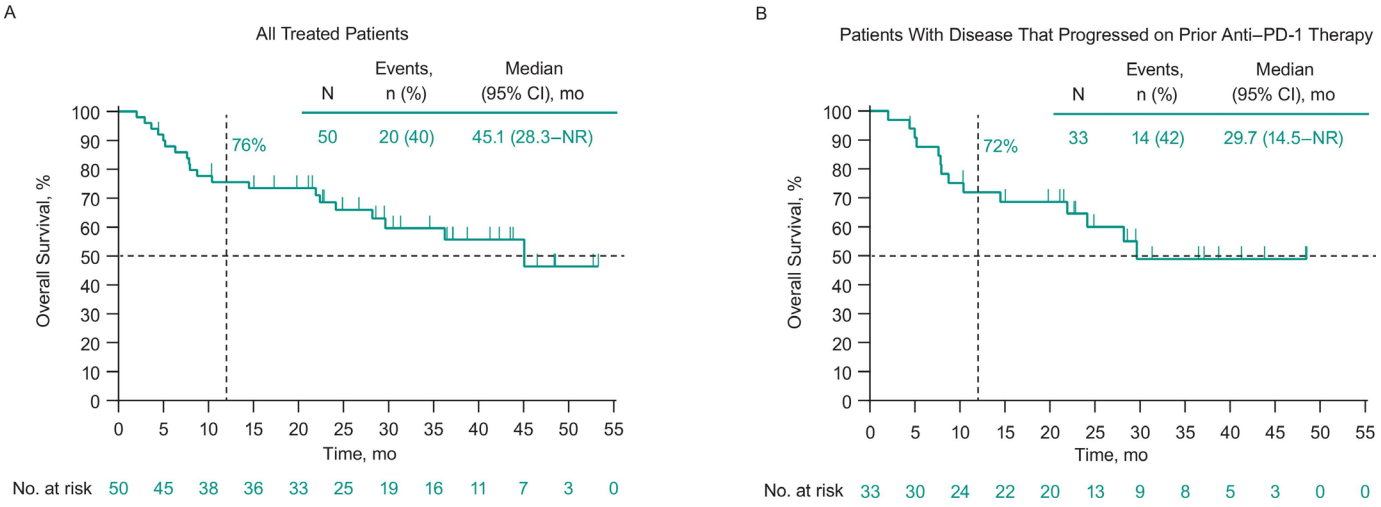

Figure 3 Overall survival among (A) all treated patients and (B) patients who had progressed on prior anti-PD-1 therapy. Median overall survival was 45.1 months (95% CI 22.4 months to not reached) in patients with no prior anti-PD-1 therapy. anti-PD-1, antiprogrammed death 1; NR, not reached.

V937-related AEs. Although attribution of toxicity to an individual component of a combination therapy is difficult, two patients (4%) discontinued V937 because of grade 2 pruritus (assessed by investigator as probably related to V937 and possibly related to ipilimumab) and grade 2 adrenal insufficiency (assessed by investigator as possibly related to V937 and probably related to ipilimumab). One patient (2%) had treatment with V937 interrupted because of an investigator-assessed V937-related AE (grade 1 fever). Six patients had an injection site reaction, which was deemed related to V937 treatment, and five patients had V937-related injection site pain.

Forty-two patients (84%) experienced ≥1 investigator-assessed ipilimumab-related AE. Seven patients (14%) had grade 3 or 4 ipilimumab-related AEs; these patients experienced dehydration, diarrhea, and hepatotoxicity (two patients (4%) each), hyperglycemia, hypokalemia, hyponatremia, colitis, fatigue, and pruritus (one patient (2% each)). Three patients (6%) discontinued ipilimumab because of grade 3 hepatotoxicity, grade 1 or 2 transaminitis, and grade 3 diarrhea (all investigator-assessed as related to ipilimumab). Eleven patients (22%) had treatment with ipilimumab interrupted because of investigator-assessed ipilimumab-related AEs, the most common of which was grade 1 or 2 diarrhea in 3 patients (6%).

Two patients (4%) died because of events (central nervous system hemorrhage (duration: 11 days), hepatic failure (duration: 29 days)) that were unrelated to treatment. Safety results were generally consistent regardless of prior receipt of anti-PD-1 therapy (table 2).

Efficacy

Across all treated patients, ORR based on investigator assessment per immune-related response criteria was 30% (95% CI 18% to 45%), with five patients experiencing CR and 10 patients experiencing PR (table 3). An additional 17 patients had stable disease for a disease control rate (CR, PR, or SD) of 64%. ORR was 21% (95% CI 9% to 39%) in patients who progressed on prior anti-PD-1 therapy and 47% (95% CI 23% to 72%) in patients with no prior anti-PD-1 therapy. In all treated patients, median TTR was 3.4 months (range: 0.7–5.1 months) among the 15 responders. Median duration of response was 8.8 months (95% CI 5.9 to 8.8 months), and the DRR (ie, the rate of CR or PR lasting ≥6 months) was 14% (95% CI 6% to 27%). Reductions from baseline in tumor burden were observed in most patients. Reductions in tumor size were observed in both target injected and non-injected lesions in patients who progressed on prior anti-PD-1 therapy and in patients with no prior anti-PD-1 therapy (figure 1).

Thirty-one patients (62%) experienced disease progression or death. Median PFS in all treated patients was 6.2 months (95% CI 3.5 to 9.0 months), and the 6-month and 1-year PFS rates were 52% and 30%, respectively (figure 2A). Twenty patients (40%) died. Median OS was 45.1 months (95% CI 28.3 months to not reached), and the 6-month and 1-year OS rates were 88% and 76%, respectively (figure 3A).

In patients with disease that progressed on prior anti-PD-1 therapy, ORR was 21% (95% CI 9% to 39%; table 3). Median PFS in these patients was 3.5 months (95% CI 3.5 to 10.6 months), and the 6-month and 1-year PFS rates were 50% and 30%, respectively (figure 2B). Median OS was 29.7 months (95% CI 14.5 to not reached), and the 6-month and 1-year OS rates were 88% and 72%, respectively (figure 3B). Photos from a representative patient with disease that progressed on prior anti-PD-1 therapy but experienced a durable response in our study are shown in figure 4.

DISCUSSION

In this phase 1b study, combination therapy with the oncolytic virus V937 administered intratumorally plus

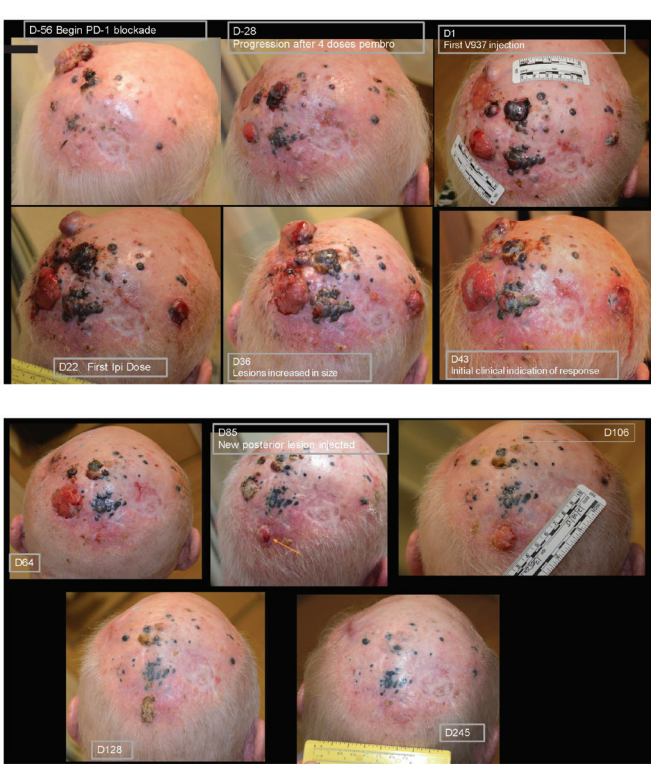

Figure 4 Photos of a representative patient who had progressed on prior anti-PD-1 therapy but experienced a complete response after treatment with intratumoral V937 plus ipilimumab. This patient was resistant to pembrolizumab and had an area of isolated progression (day −28). He was subsequently started on combination therapy with intratumoral V937 plus ipilimumab. After an initial increase in lesion size (day 36), a durable response was achieved. On day 85, a new lesion developed while on therapy (see orange arrow). As injections were no longer needed at the other sites, the new lesion was injected starting on day 85 and then completely regressed. anti-PD-1, antiprogrammed death 1; D, day.

ipilimumab had manageable toxicity, and responses were robust and durable in patients with advanced melanoma, including patients with melanoma progression after anti-PD-1 therapy. Responses seen in non-injected metastases provide evidence of probable systemic immune activation.

The safety profile of intratumoral V937 plus ipilimumab in our study was consistent with that anticipated for the individual treatment components. In previous studies of patients with advanced melanoma, the most common treatment-related AEs were injection site pain, fatigue, and chills with intratumoral V937 monotherapy[5] and pruritus, diarrhea, rash, and fatigue with ipilimumab monotherapy.[19 20] Notably, the rates of V937 injection site pain and injection site reactions in the current analysis were lower than a prior report of intratumoral V937 monotherapy in patients with melanoma.[5]

Ipilimumab-related AEs are well characterized and generally tolerated with prompt detection and appropriate management.[21] Importantly, no grade ≥3 V937-related AEs occurred with intratumoral V937 monotherapy in a phase 2 study[5] or in combination with ipilimumab in our study. The rate of grade 3–5 ipilimumab-related AEs was lower with combination therapy in our study (14%) than with ipilimumab monotherapy in phase 3 studies (20%–27%),[19 20] possibly due to differences in trial designs and patient populations. Taken together, these data suggest good tolerability with no added toxicity from combination therapy. The safety profile was similar irrespective of whether patients had received prior anti-PD-1 therapy.

Few patients with advanced melanoma respond to standard second-line therapies after progression on anti-PD-1 therapy. In our study, combination therapy resulted in an ORR of 21% in this subgroup of patients, which is higher than the rates reported with ipilimumab monotherapy in this population (10%–13%).[13 14] No comparable data exist for intratumoral V937 monotherapy because the only other study that has been published did not include any patients who previously received PD-1 or PD-L1 inhibitors.[5] Efficacy was also observed in the subgroup of patients who were not previously treated with anti-PD-1 therapy; as expected, ORR was higher in patients who were not previously treated with anti-PD-1 therapy than in patients with disease that progressed on prior anti-PD-1 therapy. The ORR was 47% in patients who were not previously treated with anti-PD-1 therapy, which compares favorably with the ORRs of intratumoral V937 monotherapy (28%)[5] and ipilimumab monotherapy (12%)[19] in this population and suggests at least an additive benefit with the combination.

In our study, we observed responses in both injected and non-injected melanoma lesions (non-injected lesions were not a study entry requirement). The antitumor activity of V937 in non-injected lesions (lung and liver metastases) has been reported in a previous phase 2 study of intratumoral V937 monotherapy in patients with advanced melanoma,[5] but it is not possible to determine in the current study whether this effect was attributable to V937 and/or ipilimumab given that all patients received both agents.

Our results are similar to those observed with the oncolytic virus T-VEC. In a phase 2 study, intratumoral T-VEC plus ipilimumab was associated with an ORR of 39% in patients with advanced melanoma, compared with 18% with ipilimumab monotherapy, and no additional safety concerns were observed with the combination.[22] Although differences in study designs and patient populations between that study and ours preclude direct comparisons (eg, only 27% received prior anticancer therapy (<3% PD-1 inhibitors) in the T-VEC study versus 80% in our study (66% PD-1 inhibitors)), the overall conclusions are similar and supportive of combination therapy.[3 22]

Limitations of our study include the relatively small sample size and lack of a control group. Although prior studies have reported associations between biomarkers including PD-1, PD-L1, tumor mutation burden, and other inflammatory gene expression signatures and response during immune checkpoint inhibitor therapy in patients with melanoma,[23 24] biomarkers were not evaluated in this study, and thus, their potential relationship

with responses could not be assessed. In addition, all participants were white and from the USA, potentially limiting the generalizability of the results.

In conclusion, combination therapy with intratumoral V937 plus ipilimumab had manageable toxicities that were consistent with those of the individual monotherapies. Responses were robust, including in the subset of patients with disease that progressed on prior anti-PD-1 therapy.

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Contributors BDC: conception, design or planning of the study; analysis of the data; acquisition of the data; interpretation of the results; drafting of the manuscript; reviewing or revising the manuscript for important intellectual content; provision of study materials/patients; approval of the final submitted version; accountable for all aspects of the work; guarantor. JR: acquisition of the data; interpretation of the results; drafting of the manuscript; reviewing or revising the manuscript for important intellectual content; approval of the final submitted version; accountable for all aspects of the work. JRH: acquisition of the data; interpretation of the results; drafting of the manuscript; approval of the final submitted version; accountable for all aspects of the work. GAD: acquisition of the data; interpretation of the results; reviewing or revising the manuscript for important intellectual content; approval of the final submitted version; accountable for all aspects of the work. MF: analysis of the data; acquisition of the data; interpretation of the data; reviewing or revising the manuscript for important intellectual content; approval of the final submitted version; accountable for all aspects of the work. LF: acquisition of the data; interpretation of the data; reviewing or revising the manuscript for important intellectual content; approval of the final submitted version; accountable for all aspects of the work. KAM: analysis of the data; acquisition of the data; reviewing or revising the manuscript for important intellectual content; approval of the final submitted version; accountable for all aspects of the work. SH: interpretation of the results; reviewing or revising the manuscript for important intellectual content; provision of study materials/patients; approval of the final submitted version; accountable for all aspects of the work. MG: acquisition of the data; reviewing or revising the manuscript for important intellectual content; administrative, logistical or technical support; approval of the final submitted version; accountable for all aspects of the work. YZ: analysis of the data; interpretation of the results; reviewing or revising the manuscript for important intellectual content; approval of the final submitted version; accountable for all aspects of the work. AL: conception, design or planning of the study; reviewing or revising the manuscript for important intellectual content; approval of the final submitted version; accountable for all aspects of the work. RHIA: conception, design or planning of the study; analysis of the data; interpretation of the results; drafting of the manuscript; reviewing or revising the manuscript for important intellectual content; approval of the final submitted version; accountable for all aspects of the work.

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Patient consent for publication Not applicable.

Ethics approval This was a multicenter study. The study protocol and amendments were approved by institutional review boards or independent ethics committees at each study site. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. Merck Sharp & Dohme LLC, a subsidiary of Merck & Co, Inc, Rahway, New Jersey, USA (MSD) is committed to providing qualified scientific researchers access to anonymized data and clinical study reports from the company's clinical trials for the purpose of conducting legitimate scientific research. MSD is also obligated to protect the rights and privacy of trial participants and, as such, has a procedure in place for evaluating and fulfilling requests for sharing company clinical trial data with qualified external scientific researchers. The MSD data sharing website (available at: http://engagezone.msd.com/ds_documentation.php) outlines the process and requirements for submitting a data request. Applications will be promptly assessed for completeness and policy compliance. Feasible requests will be reviewed by a committee of MSD subject matter experts to assess the scientific validity of the request and the qualifications of the requestors. In line with data privacy legislation, submitters of approved requests must enter into a standard data-sharing agreement with MSD before data access is granted. Data will be made available for request after product approval in the US and EU or after product development is discontinued. There are circumstances that may prevent MSD from sharing requested data, including country-specific or region-specific regulations. If the request is declined, it will be communicated to the investigator. Access to genetic or exploratory biomarker data requires a detailed, hypothesis-driven statistical analysis plan that is collaboratively developed by the requestor and MSD subject matter experts; after approval of the statistical analysis plan and execution of a data-sharing agreement, MSD will either perform the proposed analyses and share the results with the requestor or will construct biomarker covariates and add them to a file with clinical data that is uploaded to an analysis portal so that the requestor can perform the proposed analyses.

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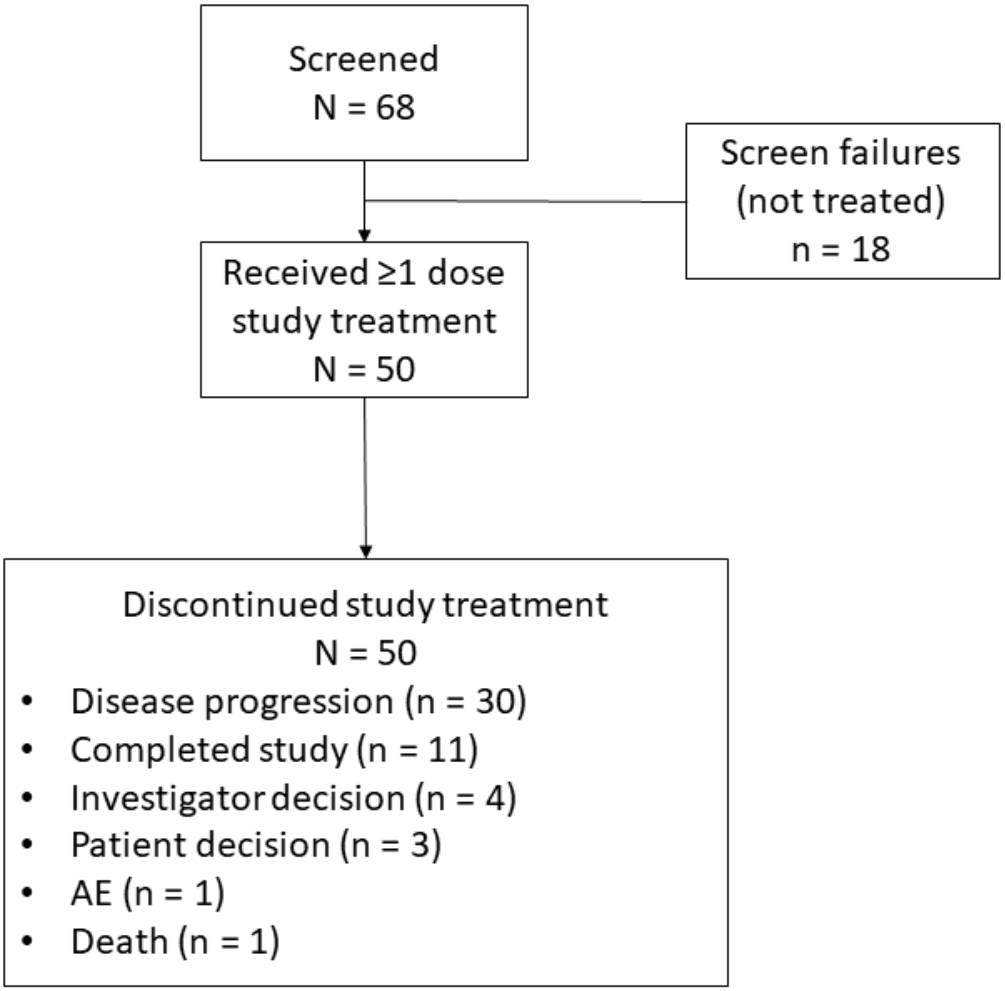
Supplemental Material

Supplemental Figure S1. Patient disposition. AE, adverse event.

Supplemental Figure S1. Patient disposition. AE, adverse event.



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CLINICAL PROTOCOL

A PHASE Ib STUDY OF INTRATUMORAL CAVATAK (COXSACKIEVIRUS A21) AND IPILIMUMAB IN PATIENTS WITH ADVANCED MELANOMA

Protocol Number:	VLA-013
Protocol Version:	8.0
Protocol Date:	7 December 2017
Development Phase:	Ib
Study Sponsor:	Viralytics Limited Suite 305, Level 3 66 Hunter Street Sydney NSW 2000 Australia
FDA IND Number:	14547

CONFIDENTIAL

SPONSOR APPROVAL SIGNATURE PAGE

Viralytics Ltd has approved of this protocol and assures that that this study will be conducted according to the stipulations described herein.

PPD

PPD

Chief Scientific Officer

Viralytics Limited

23-Jan-2018 | 6:31 AM AEDT

Date

PPD

PPD

Medical Monitor

Consultant to Viralytics Limited

09-Dec-2017 | 8:38 AM AEDT

Date

PPD

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Project Manager

Consultant to Viralytics Limited

09-Dec-2017 | 1:55 AM AEDT

Date

INVESTIGATOR PROTOCOL AGREEMENT SIGNATURE PAGE

I agree:

To assume responsibility for the proper conduct of the study at this site.

To conduct the study in compliance with this protocol, any future amendments, and with any other study conduct procedures provided by Viralytics Ltd. (Viralytics).

Not to implement any changes to the protocol without written agreement from Viralytics and prior review and written approval from the Research Ethics Committee except where necessary to eliminate an immediate hazard to subjects.

That I am thoroughly familiar with the appropriate use of CVA21 and ipilimumab, as described in this protocol and any other information provided by Viralytics including, but not limited to, the current CVA21 Investigator's Brochures (IB) and ipilimumab Package Insert.

That I am aware of, and will comply with, good clinical practices (GCP) and all applicable regulatory requirements.

To ensure that all persons assisting me with the study are adequately informed about CVA21, ipilimumab and all study-related duties and functions as described in the protocol.

Principal Investigator Signature Date

Principal Investigator

TABLE OF CONTENTS

085W6K 0586K3

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic Hormone
ADL	Activities of Daily Living
AE	Adverse Event
ALT	Alanine Aminotransferase
AR	Adverse Reaction
AST	Aspartate Aminotransferase
BRAF	A human gene that encodes a protein called B-Raf
BRAF-wt	B-Raf wild type
BSL	Bio-Safety Level
CFR	Code of Federal Regulations (FDA)
cGMP	Current Good Manufacturing Practices
CRO	Contract Research Organization
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-Lymphocyte-Associate Protein 4
CVA21	Coxsackievirus A21
DAF	Decay-accelerating Factor
DLT	Dose Limiting Toxicity
DMC	Data Monitoring Committee
DOR	Duration of Response
DRR	Durable response rate
DSUR	Development Safety Update Report
DTAP	Diethylenetriaminepentaacetic Acid
EACRI	Earl A. Chiles Research Institute
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FACT-BRM	Functional Assessment of Cancer – Biological Response Modifier (QOL)
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GM-CSF	Granulocyte Macrophage Colony-Stimulating Factor
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HLA	Human Leucocyte Antigen
HLP	Hind Limb Paralysis
IBC	Institutional Biosafety Committee (or equivalent)
ICAM-1	Intercellular Adhesion Molecule-1
ICH	International Conference on Harmonization
IND	Investigational New Drug
INR	International Normalized Ratio
IP	Investigational Product
IRB	Institutional Review Board
irCR	Immune-related Complete Response
irPD	Immune-related Progressive Disease
irPR	Immune-related Partial Response
irRC	Immune-related Response Criteria
irSD	Immune-related Stable Disease
ISF	Investigator Site File
IT	Intratumoral
IUD	Intrauterine Device
IV	Intravenous (sometimes also Roman numeral 4, as in stage IV disease).
LDH	Lactate dehydrogenase
LH	Luteinizing Hormone

LN	Lymph Node
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
NOEL	No-Observed Adverse-Effect Level
ORR	Overall Response Rate
OTC	Over the Counter
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate-Buffered Saline
PD-1	Programmed Death Receptor-1
PD-L1	Programmed Death Ligand – 1
PFS	Progression Free Survival
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QOL	Quality of Life
REC	Research Ethics Committee
RECIST	Response Criteria in Solid Tumors
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAD	Short Axis Diameter (used in measuring Lymph Nodes)
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SPD	Sum of the Product of Perpendicular Diameters (Used in measuring index lesions)
SUSAR	Suspected Unexpected Serious Adverse Reaction
$TCID_{50}$	50% tissue culture infectious dose
TEAE	Treatment Emergent Adverse Event
TMF	Trial Master File
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal
WBC	White Blood Count
WHO	World Health Organization

SYNOPSIS

Study Title	A phase Ib study of intratumoral CAVATAK (Coxsackievirus A21) and ipilimumab in patients with advanced melanoma
Protocol Number	VLA-013
Study Sites	Approximately 10 sites in the USA
Planned Sample Size	Up to 59 subjects, including 29 who meet the new criteria in Version 7 of the protocol and onwards
Study Phase	Phase Ib
Objectives	Primary Objective To evaluate the safety and efficacy of CAVATAK (CVA21) administered intratumorally in combination with the approved dose and schedule of ipilimumab. Of particular interest is to estimate the overall response rate (ORR) in the subgroup of subjects with unresectable or metastatic stage III B/C or IV melanoma who have progressed on prior anti-PD-1 therapy. Secondary Objectives 1. Assess the clinical efficacy of ipilimumab in combination with intratumoral CVA21 in terms of: • Immune-related progression-free survival (irPFS) at 6 and 12 months, • Durable response rate (DRR), • 1-year survival, • Overall survival (OS), and • Quality of life. 2. Assess the response of injected and non-injected melanoma lesions after CVA21 and ipilimumab. 3. Assess the time to initial response. [redacted]
Study Design	Open label, single arm study of intratumoral CVA21 and ipilimumab in advanced melanoma patients.

Criteria for Evaluation	**Primary Endpoints:** 1. Best response of complete response (CR) or partial response (PR) as determined by using irRC (modified WHO criteria). 2. Occurrence of DLTs, adverse events and changes from baseline in clinical chemistry and hematology laboratory values. **Secondary Endpoints:** 1. Response as assessed by irRC: • irPFS, • response of CR or PR lasting 26 weeks or longer, • 1-year survival, • OS, and • change in quality of life assessments. 2. Response of injected and non-injected melanoma lesions following treatment 3. Time to initial response. [redacted]
Eligibility Criteria	**Inclusion Criteria** 1. Patients with unresectable or metastatic stage III B/C or IV melanoma. Patients enrolled under this version of the protocol must also have progressed on prior anti-PD-1 therapy, according to RECIST 1.1 criteria. Patients who progressed within 3 months of treatment start are excluded. 2. Patients must have at least one cutaneous or subcutaneous tumor, measuring 0.5 to 5.0 cm in the longest diameter, or a palpable lymph node. At least one tumor must qualify as an index lesion that can be accurately and reproducibly measured in two dimensions for which the longest diameter is ≥10 mm (≥15 mm in short axis diameter [SAD] for lymph nodes), and be amenable to intratumoral injection. 3. Histological confirmation of melanoma will be required by previous biopsy or cytology. 4. Patients who have received prior ipilimumab treatment for metastatic melanoma are not eligible.

	5. Patients with ≤3 visceral metastases (excluding pulmonary lesions), with no lesions >3.0 cm. Patients with substantial tumor burden of non-measurable disease may not be good candidates for an immunotherapy and should be discussed with the Medical Monitor. 6. Patients must be ≥18 years of age. 7. ECOG performance status of 0-1 (Appendix 15.2). 8. Women of childbearing potential must have a serum pregnancy test performed within 72 hours prior to the start of protocol treatment. The results of this test must be negative for the patient to be eligible. In addition, women of childbearing potential as well as male patients must agree to take appropriate precautions to avoid pregnancy. 9. No active bleeding. 10. Anticipated lifespan greater than 12 weeks. 11. Patients must be able and willing to sign a study-specific informed consent document. 12. No chemotherapy, radiation therapy, hormonal treatment or immunotherapy within 28 days prior to initiation of treatment. Subjects must have resolution of toxic effects of the most recent chemotherapy to grade 1 or less (except alopecia). If subject received major surgery or radiation therapy of >30 Gy, they must have recovered from the toxicity and/or any complications. **Exclusion Criteria** 1. Patients with tumors to be injected lying close to an airway, major blood vessel or spinal cord that, in the opinion of the Investigator, could cause occlusion or compression in the case of tumor swelling or erosion into a major vessel in the case of necrosis. Patients with lesions in mucosal areas (vulvar, anus, oral cavity, etc.), are eligible, as long as the subject has at least one lesion suitable for injection; consult Medical Monitor for confirmation. 2. Patients with active, known or suspected autoimmune disease except for autoimmune thyroiditis or vitiligo (see Appendix 15.3). Thyroiditis patients must be asymptomatic, on adequate thyroid replacement and have normal thyroid function tests. 3. Patients with active colitis or immune-mediated colitis that has not resolved to grade 1 or less. 4. Patients with untreated brain metastases. Patients with treated brain metastases who are off corticosteroids for at least two weeks and who demonstrate control of brain metastases with

	follow-up imaging 4 or more weeks after initial therapy are eligible. 5. Patients previously treated with CVA21. 6. Other active metastatic cancer requiring treatment. 7. Patients with active infection requiring antibiotics (systemic therapy). 8. Pregnant or lactating women, as treatment involves unforeseeable risks to the embryo or fetus. 9. Need for chronic steroids. Inhaled corticosteroids are acceptable. 10. Laboratory exclusions (to be performed within 28 days of enrollment): a. WBC <3.0 x 10^9/L b. Hgb <9.0 g/dL c. AST or ALT >2.5 x upper limit of normal (ULN), or >5 x ULN for subjects with liver metastases d. total bilirubin >1.9 mg/dL (unless due to Gilbert's Syndrome) e. INR >1.5 x ULN f. Patients with a known history of HIV or active Hepatitis B or Hepatitis C are excluded but disease status does not require confirmation by laboratory testing. 11. Inability to give informed consent and comply with the protocol. Patients with a history of psychiatric illness must be judged able to understand fully the investigational nature of the study and the risks associated with the therapy. 12. Requiring or using other investigational agents while on treatment in this study. 13. Known sensitivity to any of the products or components to be administered during dosing. 14. Subjects likely to be unavailable or capable of completing all protocol-required visits or procedures. 15. History of evidence of other clinically significant disorders, condition, or disease that, in the opinion of the investigator or Viralytics Medical Monitor, would pose a risk to subject safety or interfere with the planned protocol evaluation.
Investigational Therapy	CAVATAK (Coxsackievirus A21, CVA21)

Reference Therapy	Historical ipilimumab alone data
Treatment Duration	Subjects will receive up to 19 sets of CVA21 injections and 4 doses of ipilimumab per current prescribing standards for up to 1 year.
Statistical Methods and Planned Analyses	Prior to Version 6 of the protocol, Simon's optimal two-stage design was utilized with the objective of estimating response rate. Safety of CVA21 was assessed and there was provision for the study to be stopped early for subject safety according to stopping rules. In this version of the protocol, the focus is on subjects who have progressed on prior anti-PD-1 therapy and the description in this paragraph relates to the set of patients who fulfil these criteria. A sample size of 26 subjects who have progressed on prior anti-PD-1 therapy, will provide 90% power for H_0: ORR=0.11 versus H_a: ORR=0.31 using a one-sided test at a significance level of 0.10. The sample size of 26 subjects may be increased by up to 3 additional subjects (10%) to adjust for subjects who do not complete the Day 106 lesion assessment. Thus, the total maximum sample size for the population of subjects who have progressed on prior anti-PD-1 therapy is estimated to be 29 subjects. The primary population of interest for all summaries of study data is the Safety Population, defined as all subjects who receive any amount of study drug (CVA21 and/or ipilimumab). In addition, of interest for the efficacy summaries and analyses is the subgroup of subjects who have progressed on prior anti-PD-1 therapy. Summaries of disposition, demographics, and baseline data will be provided. Summaries of the efficacy data, including ORR, DRR, irPFS, OS, time to response, response in injected versus non-injected lesions, and the QoL data (FACT-BRM) will be provided for the Safety Population and for the subgroup of subjects who have progressed on prior anti-PD-1 therapy. The null hypothesis, H_0: ORR=0.11, versus H_a: ORR>0.11 will be tested using a one-sided binomial test at a 0.10 and 0.05 significance level. Exploratory measures of immune activation in tumor tissue will be summarized. Additional exploratory analyses may be performed. Safety assessments, including AEs, DLTs, laboratory values, and vital signs, will be summarized for the Safety Population.

1. INTRODUCTION

1.1. Background

This trial intends to explore the use of Coxsackievirus A21 (CVA21, CAVATAK), a wild-type, non-genetically altered virus as a therapeutic agent when administered via intratumoral injection to melanoma lesions. CVA21 will be administered in combination (as described below) with ipilimumab administered intravenously to treat advanced melanoma. Melanoma cells are known to express ICAM-1, the entry receptor targeted by CVA21. The major focus of this study will be to evaluate the safety and tolerance of the combination of intratumoral CVA21 and intravenous ipilimumab in terms of anti-tumor effect and preliminary evidence of efficacy.

1.1.1. Advanced Melanoma

The American Cancer Society estimates that there will be over 76,000 new diagnoses and 9,700 deaths from melanoma in the United States in 2014.[1] Metastatic melanoma has a poor prognosis with less than 5% of patients surviving five years from the manifestation of visceral organ involvement. Disease-specific survival curves in all stages of melanoma have a negative slope, indicative that metastatic disease can develop many years after the initial diagnosis even from thin primary lesions. For instance, in survival data compiled by Balch et al, up to 10% of patients presenting with stage I melanoma (primary site less than 1 mm in depth and no nodal involvement) will die as a consequence of metastatic disease within 10 years.[2] Disease recurrence can manifest years or even decades after the initial diagnosis. The potential lethality of early stage melanoma distinguishes it from other solid tumors.

Immunotherapy has been studied for decades to treat melanoma and the only FDA-approved immunotherapeutic agent for metastatic melanoma was interleukin-2 until 2011. There have been recent significant advances in the treatment of melanoma. Two randomized phase III studies have shown improved survival for patients with advanced melanoma treated with ipilimumab.[3,4] The FDA approved the use of ipilimumab for first or second-line treatment of metastatic melanoma in March 2011. Combined PD-1/PD-L1 blockade has significant activity and combinations of T-cell checkpoint inhibitors are also showing significant clinical promise. The combination of nivolumab (anti-PD-1) and ipilimumab showed an overall response rate of 40% in patients with metastatic melanoma.[5] Targeted therapy in melanoma has also shown promise. Vemurafenib, which targets the BRAF V600E mutation, has an overall response rate of approximately 50%.[6] A phase III study comparing vemurafenib to dacarbazine showed a significant increase in survival for patients receiving vemurafenib. The median progression-free survival was 5.3 months in the vemurafenib group, leading to FDA approval in August 2011.[7] Similar findings have been observed with another BRAF inhibitor, dabrafenib, used alone or in conjunction with trametinib. Both agents have recently garnered FDA approval as single agents or in combination.[8]

Even with the recent FDA approval of ipilimumab showing a 4-month improvement in median survival[3] and targeted agents such as vemurafenib having a high initial response rate,[6,9] there is still a substantial unmet need for new successful therapies in patients with widespread melanoma.

1.1.2. CVA21

CVA21 is a naturally occurring virus that induces mild upper respiratory symptoms during natural infection of humans. CVA21 naturally infects cells in the respiratory tract, which are known to express ICAM-1, thereby resulting in the development of "common cold"-like symptoms frequently observed during natural and experimental infection.[14-17]

CVA21 also displays potent oncolytic activity against both in vitro cultures of human cancer cells and against in vivo xenografts of human cancers in mouse models (melanoma[18], prostate cancer[19], breast cancer[20], and multiple myeloma[21]). In the mouse xenograft CVA21 challenge models, progeny virus released from infected cells is capable of infecting adjacent cells, entering the systemic circulation, and targeting micrometastatic foci. Furthermore, tumor antigens released following CVA21-induced cancer cell lysis may potentially stimulate the host immune system against such neoplastic cells.

CVA21 is a live bio-selected oncolytic virus preparation derived from the non-genetically altered prototype Kuykendall strain of Coxsackievirus A21. Coxsackieviruses are non-enveloped viruses with positive single-stranded RNA and 4 capsid proteins. The CVA21 capsid contains a single-stranded RNA positive sense genome of 7405 nucleotides, encoding an open reading frame of 2208 codons. Also within the viral genome is a 713 nucleotide 5' non-coding region and 3' non-coding region of 68 nucleotides attached to a poly (A) tract. The CVA21 capsid is approximately 28 nm in diameter, allowing the purified virus to pass through a sterilizing (bacterial and fungal) 0.2-µm filter for aseptic processing and dispensing of the finished product.

Relevant Findings from Previous Preclinical Studies

A series of preclinical studies have been completed. Animal data confirm that CVA21 can cause oncolysis of tumors at sites distant to the primary site of viral administration (abscopal effect). Intratumoral and IV administration of CVA21 into mice bearing ICAM-1 and/or DAF-expressing xenografts showed significant decreases in the injected/ primary tumor volumes of virus-treated mice compared to PBS-treated control mice.

Based on the results from the repeat-dose toxicology study on CVA21 administered subcutaneously (SC) to male and female chimeric Hu/Mu ICAM-1 transgenic Balb/C mice, the administered SC dose of about 1.25 x 10^9 $TCID_{50}$/kg caused no apparent toxic effect, including any signs of hind-limb paralysis (HLP) or other forms of myositis, and was considered to be the no-observed adverse-effect level (NOAEL) dose for the study. This CVA21 SC dose in mice has a human equivalent dose of about 1 x 10^8 $TCID_{50}$/kg, which provides a safety margin of about 20-fold for the human CVA21 dose of about 4.5 x 10^6 $TCID_{50}$/kg (for a 70-kg patient) to be evaluated during the present clinical trial. Furthermore, Hu/Mu ICAM-1 transgenic mice tolerated systemic exposure to CVA21 at levels of approximately 8 x 10^4 $TCID_{50}$/mL equivalents post-primary SC injection (30 minutes after dosing), which is approximately 50 times the level (1.8 x 10 to 1.2 x 10^3 $TCID_{50}$/mL equivalents) observed in the serum of patients in earlier clinical trials following the initial intratumoral (IT) injection with CVA21 at 3.2 x 10^8 $TCID_{50}$.

Prior Human Experience

To date there are 3 completed phase I clinical studies assessing the IT administration of CVA21 in 14 patients with stage IV melanoma. With respect to previous CVA21 clinical experience, 14 patients with stage IV malignant melanoma have been administered either 1 or 2 IT injections of CVA21 in Australian hospitals within 3 separate phase I studies.

The first clinical use of CVA21 as an oncolytic agent was initiated by the sponsor late in 2003 with the IT administration of CVA21 to 2 patients with late-stage melanoma (PSX- X01). Subsequently, an additional small single-dose study was performed to formally assess the safety of IT CVA21 administration and to gather further preliminary information about viral persistence and anti-CVA21 antibody production (PSX-X02). A Phase I dose escalation study was undertaken to assess the safety of multiple IT administrations of increasing concentrations of CVA21 in patients with late-stage melanoma. Secondary objectives of that study also addressed the response of tumor size, systemic exposure, viral persistence, and anti-CVA21 antibody production (PSX-X03). In addition, the sponsor conducted 2 phase 1 studies involving administration of CVA21 to patients with late-stage cancer. In the first study, the IV administration of CVA21 was investigated in 10 patients with late-stage melanoma, breast, colon, or prostate cancer (PSX-X04). In the second study, the IT administration of CVA21 was assessed in 4 patients with recurrent squamous cell carcinoma of the head and neck (VLA-X06). In these phase I studies, CVA21 administration by either IV or IT routes was generally well tolerated, with evidence of disease stabilization in some patients.

An open-label multi-center phase II study of the IT administration of CVA21 to seventy (70) patients with stage IIIC and stage IV melanoma (VLA-007: ClinicalTrials.gov Identifier: NCT01227551) was completed in 2015.[32] CVA21 was administered on Days 1, 3, 5, 8, 22, 43, 64, 85, 106, and 127. Responding patients were eligible to receive maintenance administrations (VLA-008: ClinicalTrials.gov Identifier: NCT01636882). A total of 70 patients (57 from the original study and 13 from a biopsy sub-study) received IT administration. Patients received an average 8.3 sets of injections, with the most common side effects being grade 1 fatigue, chills, local injection site reactions, and fever. There have been no reports of grade 3 or 4 toxicities related to CVA21. The final results of this study were presented in abstract form indicating that the primary endpoint of immune-related progression-free survival at 6 months of greater than 22.5% had been achieved (38.6%). Furthermore, the study displayed an overall tumor response of 28.1% using immune-related response evaluation in solid tumor (RECIST 1.1) criteria. Abscopal responses in cutaneous, subcutaneous, lymph node, and lung lesions have been observed in a number of patients. The mechanism of response is currently being investigated, but likely involves a combination of viral-mediated oncolysis and host-immune cell activation involving interferon-γ production by T cells. Biopsy studies are ongoing to investigate injected and non-injected melanoma tumors after CVA21 to ascertain if phenotypic or functional changes occur in tumor infiltrating T cells and whether the up- regulation of checkpoint inhibitor molecules such as PD-1 may influence response.

1.1.3. Ipilimumab

Ipilimumab is a fully human IgG1 monoclonal antibody that binds to the CTLA-4 receptor expressed on activated T cells. The normal function of CTLA-4 is to down-regulate activated T-

cells. The normal ligand to CTLA-4 is B7.2 (CD86).[10] It was hypothesized that an antagonistic antibody would promote T-cell survival and activation resulting in greater anti- tumor effects.

Two phase III randomized studies with ipilimumab have been performed in patients with advanced melanoma. The first study was conducted in patients with widely metastatic melanoma who expressed the HLA-A*0201 phenotype and who had failed prior systemic therapies.[11] The eligibility criterion for HLA-A*0201 expression was to allow comparison of ipilimumab to a gp100 peptide vaccine. The specific gp100 peptides that comprised the vaccine are recognized only in the context of HLA-A*0201 expression. Patients were assigned randomly to treatment groups in a proportion of 3:1:1 to ipilimumab (3 mg/kg intravenously [IV] q 3 weeks x 4 doses) and gp100 vaccine, ipilimumab monotherapy + placebo, or gp100 vaccine monotherapy + placebo, respectively. There was a statistically significant improvement in survival in the patients who received ipilimumab (Table 1).

Table 1: Results of the Pivotal Trial Leading to FDA Approval of Ipilimumab

	A **Ipilimumab**	**B** **Ipilimumab +**	**C** **gp100**
Survival at 1 year (%)	44	46	25
Survival at 2 years (%)	22	24	14
Best Overall Response (%)	5.7	10.9	1.5
Disease control rate (%)	20.1	28.5	11
P value A versus C	0.0179		
P value B versus C	0.0002		

A second randomized phase III study has been performed in patients with advanced melanoma comparing ipilimumab (10 mg/kg IV q 3 weeks x 4 doses) and dacarbazine with dacarbazine and placebo in patients as first-line therapy in metastatic disease.[4] Patients were randomized in a 1:1 proportion to treatment group. Overall survival was better in the patients receiving ipilimumab (11.1 months versus 9.1 months for placebo) and a higher proportion of patients were alive at 1, 2, and 3 years in the ipilimumab group. The toxicities of ipilimumab have been extensively reviewed[12] and include rash, diarrhea, and colitis with perforation, endocrinopathies, hepatocellular injury, fatigue, and pyrexia.

The toxicities are presumed to be due to T-cell activation induced by CTLA4-blockade by ipilimumab, although the precise mechanism is not well understood. These toxicities are generally ameliorated by steroids and the use of steroids does not seem to diminish the therapeutic effect.[12] There are well-established guidelines for the management of ipilimumab toxicity and these shall be employed in this protocol. Appendix 15.7 contains a link to current flow diagrams for management of ipilimumab toxicity by grade.

1.1.4. Other Oncolytic Virus-Based Vaccines and Combinations in Melanoma

There have been many studies of oncolytic viruses in melanoma. Some examples include herpes simplex virus, poxvirus, vaccinia, and reovirus.[22-25] The results of a phase III trial with a genetically modified herpes simplex virus (HSV) known as Talimogene laherparepvec (T-VEC) were reported at the 2014 American Society of Clinical Oncology Annual Meeting. The genetic modifications incorporated into T-VEC include the ability of the HSV to secrete GM-CSF. The genes encoding ICP34.5 and ICP47 were deleted, which enhance the tumor lytic properties of this virus. The trial had a 2:1 randomization to T-VEC versus GM-CSF; 436 patients were in the intent-to-treat group with 295 assigned to receive T-VEC and 141 GM-CSF[26]. Table 2 summarizes the results of this trial.

Table 2: Results of the Phase 3 T-VEC versus GM-CSF study

	GM-CSF	T-VEC
Overall Response	5.7 %	26.4 %
CR	0.7 %	10.8 %
PR	5.0 %	15.6 %
Durable Response	2.1 months	16.3 months (p <0.0001)
Overall Survival	18.9 months	23.3 months (p = 0.51)

A phase 1b study was also presented studying the combination of T-VEC with anti-CTLA-4 ipilimumab.[27] The rationale for combining an oncolytic vaccine with a T-cell checkpoint inhibitor was the hypothesis that existing T cell anti-tumor responses are expanded by ipilimumab[28]. T-VEC given before ipilimumab would expand the T-cell repertoire for melanoma antigens and subsequent ipilimumab could increase the lifespan and activity of these melanoma-specific T cells. T-VEC was given on weeks 1 and 4, then every 2 weeks thereafter. Ipilimumab (3 mg/kg IV) started at week 6 (after 3 T-VEC doses) and continued every 3 weeks for a total of 4 doses, which is the standard dose and schedule for this agent. Nineteen patients were enrolled and 10 had stage IV M1b/c disease. All patients had at least one tumor deposit amenable to IT injection. Toxicities were as anticipated with 32% of patients experiencing grade 3 or 4 toxicities attributable to ipilimumab. The overall response was 57% with 6 patients achieving complete response, 5 with partial response, and 6 with disease stability. The median time to response was 2.9 months, which is shorter than anticipated with ipilimumab or T-VEC. There was significant expansion in the peripheral blood of CD8+ T cells of 1.8-fold after T-VEC and 2.9-fold after ipilimumab was completed.

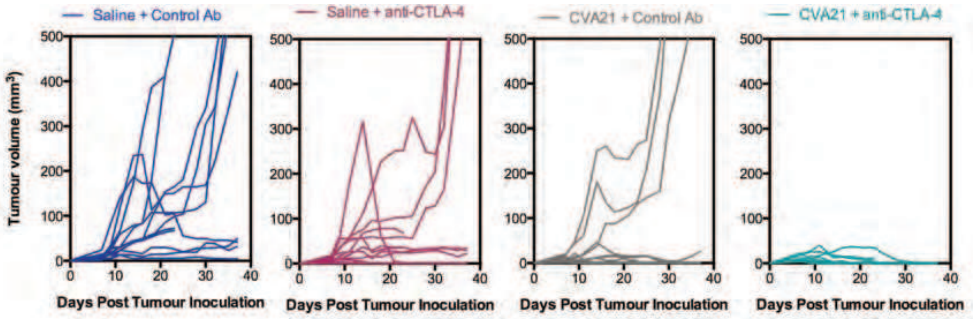
Although the combination study was small, these results help to establish the proof of concept that oncolytic viruses injected into subcutaneous melanoma deposits can initiate a cellular immune response that can be amplified by anti-CTLA-4, resulting in clinically meaningful responses and a much higher objective response than anticipated with either agent used as monotherapy.

1.2. Rationale for Study

As summarized above, there is a strong rationale for combining oncolytic virus vaccination with anti-CTLA-4 and initial clinical experience with this approach is promising. This result also supports the growing consensus that the effectiveness of extant T-cell enhancing

immunotherapies such as ipilimumab and interleukin-2 are dependent on pre-existing T-cell recognition of melanoma. In recent preclinical studies performed in a fully immune-competent mouse model of melanoma, enhanced anti-tumor activity was observed in mice receiving a combination of IT CVA21 and immune-checkpoint antibodies (anti-CTLA-4 and anti-PD-1) compared to activity displayed from single agent use alone (Figure 1).

Figure 1: Immune competent mouse model of melanoma of CVA21 in combination with anti-CTLA-4, spider plot of individual tumor growth



The present study will employ a phase Ib design using the established dose of CVA21 with ipilimumab in patients with advanced melanoma for whom ipilimumab would be considered standard of care. Our hypothesis is that oncolysis of melanoma cells by CVA21 will be important in amplifying the T-cell potentiating effects of ipilimumab, thus CVA21 treatment will precede ipilimumab with IT administration on Days 1, 3, 5, and 8 before ipilimumab and then both agents will be co-administered on Days 22, 43, 64, and 85. Patients with clinical benefit can continue CVA21 every 3 weeks for up to one year. In addition to monitoring for toxicity and clinical response, blood samples will be obtained to assess immunologic measures relevant to melanoma immune responses and ipilimumab T- cell checkpoint inhibition (see Section 6.3 for more details about immune monitoring).

2. STUDY DESIGN

2.1. Summary

This is an open label single arm study intended to enroll up to 59 subjects, including 22 who meet the new criteria in Version 7 of the protocol and onwards. Subjects will start treatment with IT CVA21 administered on Days 1, 3, 5, and 8 followed by administration of both CVA21 and ipilimumab (intravenous) on Days 22, 43, 64, and 85. On days where both ipilimumab and CVA21 are given, ipilimumab will be administered first. Additional CVA21 injections will be administered on Days 106 and every 3 weeks thereafter to a maximum of 19 sets of injections.

The tumors to be injected may be designated as index, non-index or (after Visit 1) new lesions, as long as they meet the minimum size for injection (5 mm). Note: following initial treatment with CVA21, any lesion that reduces in diameter to <5 mm may be injected with 0.1 mL of CVA21 per Appendix 15.4. Tumors lying close to an airway, major blood vessel, or spinal cord that, in the opinion of the Investigators, could cause occlusion or compression in the case of tumor

swelling or erosion into a major vessel in the case of necrosis are NOT to be injected with CVA21. Subjects with clinical benefit can continue CVA21 (up to a maximum of 19 sets of injections) until complete response, all injectable tumors have disappeared, or intolerance of study treatment, whichever occurs first. Ipilimumab will be administered at a dose of 3 mg/kg on Days 22, 43, 64, and 85, which is the standard dose and schedule for this agent.

2.2. Study Objectives

2.2.1. Primary Objective

To evaluate the safety and efficacy of CAVATAK (CVA21) administered intratumorally in combination with the approved dose and schedule of ipilimumab. Of particular interest is to estimate the overall response rate (ORR) in the subgroup of subjects with unresectable or metastatic stage III B/C or IV melanoma who have progressed on prior anti-PD-1 therapy.

2.2.2. Secondary Objectives

1. Assess the clinical efficacy of ipilimumab in combination with intratumoral CVA21 in terms of:
   - Immune-related progression-free survival (irPFS) at 6 and 12 months,
   - Durable response rate (DRR),
   - 1-year survival,
   - Overall survival (OS), and
   - Quality of life.
2. Assess the response of injected and non-injected melanoma deposits after CVA21 and ipilimumab.
3. Assess the time to initial response.

2.2.3. [redacted]

[redacted]

2.3. Criteria for Evaluation

2.3.1. Primary Endpoints

1. Best response of CR or PR as determined by using irRC (modified WHO criteria).
2. Occurrence of DLTs, adverse events and changes from baseline in clinical chemistry and hematology laboratory values.

2.3.2. Secondary Endpoints

1. Response as assessed by irRC:
   - irPFS,

- response of CR or PR lasting 26 weeks or longer,
- 1-year survival,
- OS, and
- change in quality of life assessments.

2. Response of injected and non-injected melanoma lesions following treatment.
3. Time to initial response.

2.3.3. [redacted]

2.4. Dose Escalation

Dose escalations in individual subjects will not be permitted

2.5. Randomization and Blinding

Not applicable; this is an open-label, non-randomized study.

3. STUDY POPULATION

3.1. Number of Centers and Participants

This study will involve approximately 10 clinical study sites selected in the United States and will enroll up to 59 subjects, including 29 who meet the new criteria in Version 7 of the protocol and onwards.

3.2. Inclusion Criteria

1. Patients with unresectable or metastatic stage III B/C or IV melanoma. Patients enrolled under this version of the protocol must also have progressed on prior anti-PD-1 therapy, according to RECIST 1.1 criteria. Patients who progressed within 3 months of treatment start are excluded.
2. Patients must have at least one cutaneous or subcutaneous tumor, measuring 0.5 to 5.0 cm in the longest diameter, or a palpable lymph node. At least one tumor must qualify as an index lesion that can be accurately and reproducibly measured in two dimensions for which the longest diameter is ≥10 mm (≥15 mm in [SAD] for lymph nodes), and be amenable to intratumoral injection.
3. Histological confirmation of melanoma will be required by previous biopsy or cytology.
4. Patients who have received prior ipilimumab treatment for metastatic melanoma are not eligible.
5. Patients with ≤3 visceral metastases (excluding pulmonary lesions), with no lesions >3.0 cm. Patients with substantial tumor burden of non-measurable disease may not be good candidates for an immunotherapy and should be discussed with the Medical Monitor.

6. Patients must be ≥18 years of age.
7. ECOG performance status of 0-1 (Appendix 15.2).
8. Women of childbearing potential must have a serum pregnancy test performed within 72 hours prior to the start of protocol treatment. The results of this test must be negative for the patient to be eligible. In addition, women of childbearing potential as well as male patients must agree to take appropriate precautions to avoid pregnancy.
9. No active bleeding.
10. Anticipated lifespan greater than 12 weeks.
11. Patients must be able and willing to sign a study-specific informed consent document.
12. No chemotherapy, radiation therapy, hormonal treatment or immunotherapy within 28 days prior to initiation of treatment. Subjects must have resolution of toxic effects of the most recent chemotherapy to grade 1 or less (except alopecia). If subject received major surgery or radiation therapy of >30 Gy, they must have recovered from the toxicity and/or any complications.

3.3. Exclusion Criteria

1. Patients with tumors to be injected lying close to an airway, major blood vessel or spinal cord that, in the opinion of the Investigator, could cause occlusion or compression in the case of tumor swelling or erosion into a major vessel in the case of necrosis. Patients with lesions in mucosal areas (vulvar, anus, oral cavity, etc.), are eligible, as long as the subject has at least one lesion suitable for injection; consult Medical Monitor for confirmation.
2. Patients with active, known or suspected autoimmune disease except for autoimmune thyroiditis or vitiligo (see Appendix 15.3). Thyroiditis patients must be asymptomatic, on adequate thyroid replacement and have normal thyroid function tests.
3. Patients with active colitis or immune-mediated colitis that has not resolved to grade 1 or less.
4. Patients with untreated brain metastases. Patients with treated brain metastases who are off corticosteroids for at least two weeks and who demonstrate control of brain metastases with follow-up imaging 4 or more weeks after initial therapy are eligible.
5. Patients previously treated with CVA21.
6. Other active metastatic cancer requiring treatment.
7. Patients with active infection requiring antibiotics (systemic therapy).
8. Pregnant or lactating women, as treatment involves unforeseeable risks to the embryo or fetus.
9. Need for chronic steroids. Inhaled corticosteroids are acceptable.
10. Laboratory exclusions (to be performed within 28 days of enrollment):
    a. WBC <3.0 x 10^9/L

b. Hgb <9.0 g/dL

c. AST or ALT >2.5 x upper limit of normal (ULN) or >5 X ULN for subjects with liver metastases

d. total bilirubin >1.9 mg/dL (unless due to Gilbert's Syndrome)

e. INR >1.5 x ULN

f. Patients with a known history of HIV or active Hepatitis B or Hepatitis C are excluded but disease status does not require confirmation by laboratory testing.

11. Inability to give informed consent and comply with the protocol. Patients with a history of psychiatric illness must be judged able to understand fully the investigational nature of the study and the risks associated with the therapy.
12. Requiring or using other investigational agents while on treatment in this study.
13. Known sensitivity to any of the products or components to be administered during dosing.
14. Subjects likely to not be available or capable of completing all protocol required visits or procedures.
15. History or evidence of other clinical significant disorders, condition or disease that, in the opinion of the investigator or Viralytics Medical Monitor, would pose a risk to subject safety or interfere with the planned protocol evaluation.

4. SUBJECT SELECTION AND ENROLMENT

4.1. Identifying Participants

Eligible subjects, enrolled under version 7 of the protocol and onwards, will be those diagnosed with histologically confirmed, metastatic or unresectable stage III B/C or IV melanoma who have progressed following prior treatment with anti-PD-1 inhibitor. Subjects must meet all eligibility criteria and have at least one cutaneous, subcutaneous tumor or palpable lymph node that is amenable to intratumoral injection.

4.2. Consenting Participants

All subjects must receive, review and sign an Institutional Review Board/Research Ethics Committee-approved informed consent form prior to any study-specific procedures or assessments being conducted. Consenting of subjects must be conducted according to Good Clinical Practices. Only subjects who are capable of understanding and consenting to the trial will participate.

4.2.1. Subject Registration and Numbering

For Providence Portland Medical Center only:

To register a subject, the investigator will call the Data Management Office of the Robert W. Franz Cancer Research Center at (503) 215-2613 and speak to one of the nurse coordinators for the trial. The following information will be requested:

Investigator's name

Subject's Identification

Subject's name or initials and chart number

Subject's Social Security number

Eligibility Verification

For all other sites, the next available subject number will be used.

Study Numbers will be assigned using the following formula: last 2-digits of Viralytics protocol number + 2-digit site number + 3-digit subject number. For example:

- The first subject at site 01 would be 1301001

All case report forms, study reports, and laboratory samples for research tests, including immune parameters or pharmacokinetics, will be labeled with the full subject Study Number.

4.3. Screening for Eligibility

After written informed consent is obtained, demographic data, a complete medical and medication history, physical exam and laboratory samples will be obtained to confirm all eligibility criteria have been met. Subject eligibility screening procedures must be completed within 28 days prior to study treatment initiation (Day 1), but procedures to determine eligibility may be conducted over several days during this period. **Eligibility must be confirmed by the Viralytics Medical Monitor prior to enrollment.**

4.4. Ineligible and Non-Recruited Subjects

Subjects who have signed an Informed Consent Form and who are subsequently deemed ineligible will be considered screen failures. If a subject is re-screened, the original screening number will be maintained. A new screen number will not be assigned to a subject previously screened.

4.5. Withdrawal Procedures

4.5.1. Study Treatment Discontinuation

All reasons for discontinuation of treatment must be documented. All subjects will be followed for survival or until death post-treatment.

Criteria for removal from study treatment are:

- Symptomatic disease progression after CVA21 and ipilimumab or the follow-up period; the subject should be re-staged and sites of recurrence and/or progression documented.
- Unacceptable toxicity – defined as:
  - Grade ≥3 allergic reaction to the treatment medication or its excipients;
  - Any grade 2-3 AE possibly related to CVA21 that does not resolve to grade 1 or less by the next dose allowing for a delay for toxicity of no longer than 21 days (missed doses will NOT be made up);

- Any grade 4 AE considered possibly related to CVA21.

- The subject may elect to withdraw from study treatment at any time for any reason. NB: Subjects who withdraw from study treatment may elect to continue participation in the study for survival assessments.
- Development of intercurrent, non-cancer related illnesses that prevent continuation of therapy or regular protocol-defined visits.
- Pregnancy

In the absence of symptomatic disease progression requiring alternate therapy or withdrawal of consent from the study, or withdrawal by the Investigator, subjects may continue with protocol scheduled visits and will be followed for survival or until death post-treatment.

4.5.2. Study Discontinuation

Participation in this study is voluntary and subjects may be withdrawn from the study at any time without any loss of benefits to which they are otherwise entitled. For every subject a reason for discontinuation of the study is required. The following list provides possible reasons for discontinuation and the list is recorded in order of priority.

Reasons for discontinuation from the study include the following:

- Completed Study – Subject completed all visits to Day 358
- Death
- Lost to follow-up
- Investigator decision, after consultation with the Sponsor
- Subject Withdrawal of Consent
- Termination of study by sponsor
- Other

Where possible, subjects who are withdrawn prior to the final visit must be brought in for an Early Termination visit, and visit procedures outlined in the Schedule of Procedures conducted with the exception of subjects who withdraw consent for further procedures, are lost to follow-up or in the case of death. Subjects who progress or who elect to discontinue tumor assessments will be followed for survival only, every three months for the first year, then every six months until study closure.

5. INVESTIGATIONAL MEDICINAL PRODUCT

5.1. CVA21

CVA21 is a gradient-purified preparation of the Coxsackievirus A21 prototype strain consisting of infectious and noninfectious viral particles and free from mycoplasmal, viral and other contaminants of microbiological origin. CVA21 is produced in a cGMP-compliant environment

utilizing gradient purification and submitted to sterile filtration. The investigational agent consists of drug substance diluted in 4% w/v sucrose in phosphate-buffered saline (PBS) at pH 7.2.

Storage: CVA21 will be stored frozen at <-70°C in sealed glass vials.

Toxicity: The main toxicities of CVA21 are local injection site reactions including erythema and pain, low-grade temperatures, transient flu-like symptoms, arthralgias, and myalgias. To date, these toxicities have been mild or moderate in grade. Further information is available in the CVA21 Investigator Brochure.

5.1.1. Dose Preparation

To load the syringe with CVA21, remove vial from individual carton and thaw at room temperature (18-25°C). Do not leave the vial at room temperature for longer than is necessary to thaw the contents. A lab coat, safety glasses, sterile gloves, and mask should be worn while loading the syringe with CVA21. Gently mix the vial for 5 seconds and tear off the plastic top. Use a luer-lock syringe of appropriate volume and 21-gauge needle to draw up the required volume. Remove air bubbles. Remove the withdrawal needle and replace with a 25-gauge capped needle. Hold on ice until required (2-8°C). Administer within 3 hours from loading the syringe distributed into the tumors as described in Appendix 15.4.

5.1.2. Dose Modifications

CVA21 administration will stop for any individual subject experiencing DLT from this agent. There will be no CVA21 dose modifications for individual subjects. If CVA21 dosing is stopped in an individual subject, any remaining planned ipilimumab doses can be administered if clinically indicated.

5.1.3. Administration

Each subject will receive CVA21 up to a total dose of 3 $x10^8$ $TCID_{50}$ (about 4.5 x 10^6 $TCID_{50}$/kg for a 70-kg subject) in a maximum volume of 4.0 mL by IT administration on Days 1, 3, 5, 8, 22, 43, 64, and at further 3-weekly intervals (up to a maximum of 19 sets of injections) until confirmed disease progression, complete response or development of excessive toxicity.

At each scheduled injection visit, if possible, multiple lesions are injected in a dose hyper-fraction pattern, starting with the largest lesion(s) to a maximum of 4.0 mL using Table 3 to determine volume injected.

The largest diameter of each tumor to be injected is measured and the volume of CVA21 to be injected into each tumor determined. The sum of these volumes is the total volume of CVA21 required for the administration. The maximum volume of CVA21 to be administered is 4.0 mL. Following initial injection with CVA21, any injected lesion that reduces in diameter to <5 mm will be injected with 0.1 mL of CVA21 as per the stated treatment schedule until the lesion completely resolves.

For details of the priority for selecting tumors to be injected, please refer to Appendix 15.4: CVA21 Intratumoral Injection Technique.

Table 3: CVA21 Administration Volume by Tumor Diameter

Tumor Diameter	Volume of CVA21
>25 mm	2.0 mL
15 – 25 mm	1.0 mL
5 <15 mm	0.5 mL
<5 mm*	0.1 mL

* only for lesions previously treated with CVA21

Following CVA21 administration, injections sites should be wiped with sterile tissue and an occlusive dressing applied to completely cover the injection site. Used tissues should be placed in appropriate infectious waste containers.

In laboratory studies, the major side effect toxicity observed in mice injected with CVA21 is the development of hind-limb paralysis resulting from inflammation in the muscles of the leg, in particular following intramuscular injection. However, in the clinical experience of Viralytics to date, no signs of the development of myositis have been observed in human subjects administered CVA21 by either IT or IV routes. Therefore, CVA21 is NOT to be administered via the intramuscular route in this study.

Disposal of Contaminated Materials and Management of Accidental Spills:

Viral administration should be performed in a manner to minimize potential exposure to other cancer suffering or immunocompromised individuals. Furthermore, systematic decontamination of surfaces that may directly come into contact with viral inoculum or excretion samples must be undertaken, with an appropriate anti-viral agent (e.g., Virkon®, a sodium hypochlorite solution [5-6%] or formaldehyde [3%]). All materials utilized in the viral administration must be disposed of in appropriate infectious waste containers.

Any mishap such as accidental spillage or inoculation might expose a staff member to virus in ways not occurring naturally, although there is nothing to suggest they would be at particular risk as a result. Nevertheless, because of the above, it would be advisable that handling and inoculation of virus be done by staff trained in handling infectious agents and disposal of potentially infectious waste. Accidental spills from samples and operations should be adsorbed with paper tissues soaked with an appropriate anti-viral agent (e.g., Virkon®, a sodium hypochlorite solution [5-6%] or formaldehyde [3%]) and then with water. Tissues should then be discarded in proper containers designated for infectious laboratory/hospital waste.

5.1.4. Intra-Subject Dose-Escalation

All subjects will receive the same dose of CVA21; no intra-subject dose escalation will occur.

5.2. Ipilimumab

Please see a link to the full package insert in Appendix 15.6. Ipilimumab (Yervoy®) is a recombinant, human monoclonal antibody that binds to the cytotoxic T-lymphocyte-associated

antigen 4 (CTLA-4). Ipilimumab is an IgG1 kappa immunoglobulin with an approximate molecular weight of 148 kDa. Ipilimumab is produced in mammalian (Chinese hamster ovary) cell culture.

Toxicity: Side effects of ipilimumab are immune-mediated and include fatigue, diarrhea, pyrexia, enterocolitis, hepatitis, dermatitis, neuropathies, endocrinopathies, and ocular manifestations.

How Supplied: Ipilimumab is a sterile, preservative-free, clear to slightly opalescent, colorless to pale yellow solution for intravenous infusion, which may contain a small amount of visible translucent-to-white, amorphous ipilimumab particulates. It is supplied in single- use vials of 50 mg/10 mL (5mg/ml) and 200 mg/40 mL (5mg/mL). Each milliliter contains 5 mg of ipilimumab and the following inactive ingredients: diethylene triamine pentaacetic acid (DTPA) (0.04 mg), mannitol (10 mg), polysorbate 80 (vegetable origin) (0.1 mg), sodium chloride (5.85 mg), tris hydrochloride (3.15 mg), and Water for Injection, USP at a pH of 7. Commercial ipilimumab supply will be used for this study. The study sponsor will not cover the costs for ipilimumab.

Storage: Store Ipilimumab under refrigeration at 2°C to 8°C (36°F to 46°F). Do not freeze. Protect vials from light.

5.2.1. Dose Preparation

Do not shake product. Inspect the ipilimumab vial visually for particulate matter and discoloration prior to administration. Discard the vial if the solution is cloudy, there is pronounced discoloration (solution may have pale yellow color), or there is foreign particulate matter other than translucent-to-white, amorphous particles. Allow the vials to stand at room temperature for approximately 5 minutes prior to preparation of infusion. Withdraw the required volume of YERVOY® and transfer into an intravenous bag. Dilute with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to prepare a diluted solution with a final concentration ranging from 1 mg/mL to 2 mg/mL. Mix diluted solution by gentle inversion. Store the diluted solution for no more than 24 hours under refrigeration (2°C to 8°C, 36°F to 46°F) or at room temperature (20°C to 25°C, 68°F to 77°F). Discard partially used vials or empty vials of ipilimumab.

5.2.2. Dose Modifications

For subjects experiencing ipilimumab toxicity, these toxicities will be managed per established guidelines (included in Appendix 15.6 and Appendix 15.7). The general strategy to abrogate immune-mediated toxicities is to administer high-dose steroids (e.g., prednisone) until the toxicity resolves, followed by a taper of the steroid dose. If the toxicity does not recur during the taper and no other adverse events ensue, then ipilimumab dosing can continue. If ipilimumab dosing is stopped or delayed, any remaining planned CVA21 doses may be administered. If a dose of ipilimumab is skipped, it may be given at the next study visit after approval from the Medical Monitor.

5.2.3. Administration

Ipilimumab will be administered at the recommended dose of 3 mg/kg IV every 3 weeks for a total of 4 doses. Ipilimumab will not be not mixed with, or administered as an infusion with other medicinal products. Flush the intravenous line with 0.9% Sodium Chloride Injection, USP or 5%

Dextrose Injection, USP after each dose. Administer diluted solution over 90 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein-binding in-line filter.

5.2.4. Intra-Subject Dose Escalation

Dose escalations in individual subjects will not be permitted. Standard supportive medications including anti-emetics and pain medications will be offered during treatment. Steroids will not be used for the treatment of nausea, but can be used to ameliorate ipilimumab-induced immune related toxicity as detailed in Appendix 15.7.

5.3. Dose-Limiting Toxicity (if applicable)

Toxicities will be assessed using CTCAE v. 4.03. Dose-limiting toxicity (DLT) is defined as any grade ≥3 toxicities believed related or possibly related to CVA21 with onset on or before the Day 85 visit, with the exception of lymphopenia, which will not be considered a DLT. If a CVA21-related DLT is observed in 2 subjects among the first 6 treated, then the study will be terminated. If no DLTs are encountered or if the proportion of CVA21-related grade ≥3 toxicity is less than 30% of the subjects accrued, then the study accrual may continue. The accumulated safety data after 6, 12, and 18 subjects have been treated and followed for until at least Day 85 will be reviewed by the sponsor and investigator to identify the rate of DLTs and determine if enrollment should be continued from a safety perspective.

For ipilimumab, use the guidelines for administration and toxicity management as defined in the Yervoy® Package Insert (Appendix 15.6), which are in accordance with the FDA labeling for this medication. Management of ipilimumab toxicity will be according to the Yervoy® Adverse Reaction Monitoring Guide (Appendix 15.7).

For non-treatment-related events that may warrant holding one or both study treatments, dosing will resume as normally scheduled at the next scheduled study visit assuming the event has resolved to an appropriate level.

5.4. Subject Compliance

Study drug compliance is not expected to be an issue as CVA21 and ipilimumab are administered by site personnel during study visits.

5.5. Overdose

Should subjects receive a dose in excess of that allowed, the Medical Monitor must be notified immediately. Any overdose should be documented in the Pharmacy records and eCRF as well as recorded as a protocol deviation.

CVA21: For this study, an overdose will be defined as administration of >4.0ml (2 vials) of CVA21.

Ipilimumab: There is currently no information on overdosage with ipilimumab.

5.6. Prior, Concomitant and Subsequent Therapy

With the exception of prohibited medications, treatment that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of care and GCP. Radiation solely for palliation of symptoms is permitted following discussion with the medical monitor. All concomitant

medication will be recorded on the case report form including all prescription, over-the-counter (OTC), herbal supplements, and IV medications. All changes that occur during the trial period will be recorded in the eCRF.

Prohibited Treatment:

Subjects are prohibited from receiving the following therapies during the Screening and Treatment periods of this study:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Other Investigational agents

All concomitant medications received within 30 days of the first dose of study drug treatment and 30 days after the last dose of study drug treatment should be recorded.

5.7. Diet/Pregnancy/Contraception/Other Considerations

Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

Pregnancy

Throughout the study and for at least 4 weeks following the last administration of study drug, women of child-bearing potential should consistently and correctly use a highly effective method of contraception such as implants, injectable, combined oral contraceptives, some intrauterine devices (IUD), barrier methods, sexual abstinence or vasectomized partner.

Male subjects with partners who are pregnant or of child-bearing potential, should practice sexual abstinence or use consistently and correctly a male condom for the duration of the study and for at least 4 weeks following the last administration of study drug. Furthermore, it is recommended that sperm donations are not to be undertaken during the study and for at least 4 weeks following the last administration of study drug.

5.8. Dispensing and Accountability

Complete and accurate records must be maintained for tracking of all shipments of investigations product (IP) received, IP dispensed to study subjects and IP returned to the appropriate party (i.e., Sponsor or other designated recipient.) All records will be made available for review to a representative of the sponsor throughout the study to confirm IP accountability.

Please refer to the Pharmacy Manual for detailed procedures.

6. STUDY ASSESSMENTS

6.1. Safety Assessments

Clinical safety assessments will be performed at study visits per the Schedule of Procedures (Appendix 15.1).

6.1.1. Physical Examination

Physical examinations will include examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities and nervous system. Full Physical Examination will be completed at Screening. All other visits will require a symptom driven physical examination. An AE eCRF must be completed for all clinically noteworthy findings identified.

6.1.2. Vital Signs and Body Weight

Height and weight without shoes will be recorded in centimeters and kilograms respectively. Vital signs will include blood pressure, heart rate, respiratory rate and temperature.

6.1.3. ECG

Standard 12-lead electrocardiogram (ECG) will be conducted and will include calculation of QTc interval.

6.1.4. Brain MRI

Standard Brain MRI is to be conducted at screening as clinically indicated. Brain MRI may also be repeated as clinically indicated.

6.1.5. Laboratory Parameters

The following laboratory tests are to be performed as indicated by the Schedule of Procedures:

Table 4: Laboratory Assessments for Safety

Hematology	Serum Chemistry	Hormones
Red Blood Cells White Blood Cells (WBC) Hemoglobin Hematocrit Platelet count Differential WBC Count: Absolute neutrophil count Absolute lymphocyte count Absolute monocyte count Absolute eosinophil count Absolute basophil count PT PTT	Alanine aminotransferase (ALT/SGPT) Albumin Alkaline phosphatase Aspartate aminotransferase (AST/SGOT) Total bilirubin Total protein Creatinine Blood urea nitrogen Bicarbonate Chloride Potassium Sodium Calcium Lactate dehydrogenase Glucose Serum pregnancy test **Thyroid Panel**: TSH, Free T4.	Total Testosterone (males only) FSH (females only) LH (females only) ACTH Cortisol

Local Lab: Laboratory samples will be analyzed by local laboratories for interpretation of results. In the event of an unexplained clinically noteworthy abnormal laboratory test value, the test should be repeated immediately and followed up until it has returned to the normal range and/or an adequate explanation of the abnormality is found.

Central Lab: In addition to the above, serum samples drawn at the same time as the local laboratory samples (prior to administration of CVA21 or ipilimumab) will be forwarded to the Central laboratory for assessing neutralizing antibodies to CVA21 and sICAM-1 and for assessing CVA21 serum load. Please refer to the Laboratory Manual for sample processing details.

6.1.6. Pregnancy Test:

Female subjects of child-bearing potential must have a negative serum pregnancy test within 72 hours prior to receiving the first dose of study medication and as indicated by the schedule of procedures.

6.1.7. CVA21 Excretion testing

Excretion testing is not being conducted in this protocol.

6.1.8. CVA21 Transmission Precautions and Procedures

Subject and Care Giver Precautions

There is a possibility that subjects in the study may excrete virus from the respiratory or gastrointestinal tract for some days after CVA21 administration. Additional information for subjects is available in the subject information sheet, "What to do on a CVA21 study". This provides simple precautions CVA21-treated subjects should take to minimize the risk of transmitting the virus to others. Subjects should be treated as if they have a community-acquired Coxsackievirus infection and do not require isolation. As such, caregivers, family members, clinical trial staff, and healthcare workers must be aware of this potential shedding and follow the guidance listed below. However, it must be noted that potential shedding is unlikely to be quantitatively different from naturally acquired infection.

Primary caregivers should be fully advised that the subject in the study may excrete infectious virus in respiratory and gastrointestinal tract secretions for a number of weeks following the initial viral injection. The caregivers should be fully informed that CVA21 is acquired naturally by the respiratory route and circulates naturally in the community from time to time. Precautions that are normally undertaken during the general prevention of respiratory infections should be followed. Hands should be washed with soap and warm water immediately following handling of feces/fecal soiled clothing, and material exposed to respiratory secretions such as tissues and handkerchiefs. Caregivers and family members should observe personal hygiene (i.e., hand washing) following contact with recently treated subjects. Caregivers who present symptoms of a cold or flu should present to a study investigator to provide a sample (as deemed appropriate) for analysis of the underlying infection and notify their treating physician (if medical attention sought) about possible exposure via this clinical trial.

Should a subject develop symptoms of a suspected respiratory infection, they should wear a mask when attending study visits to minimize any risk of aerosol generation during coughing.

Procedural Precautions

CVA21 is classified as Biosafety Level 2 (BSL-2) biohazard material and all precautions and procedures for BSL-2 laboratory should be observed when handling CVA21 or the contaminated equipment and objects that have been in contact with CVA21. Further information may be found in the Pharmacy Manual.

All waste generated during the dispensing and administration of CAVATAK (including sharps, needles, and vials as indicated) that have been in contact with the virus will be placed in a biohazard bag or sharps bin and incinerated. During clean-up, protective clothing, properly fastened, must be worn by all personnel.

Eye and face protection, including surgical masks, must be worn when applicable to guard against any splashing or aerosol droplets. Safety glasses must be decontaminated by wiping all

surfaces with disinfectant (i.e., Virkon). Gloves must be worn for all procedures that might involve direct skin contact and hands must be washed after gloves have been removed.

All equipment and work surfaces must be decontaminated with disinfectant (i.e., Virkon, 3% formaldehyde or 5-6% sodium hypochlorite).

All protective clothing must be removed before going beyond the contaminated areas and will be placed in a biohazard bag. If items are non-disposable they will then be laundered according to normal hospital policy for bio-hazardous items. Disposable items must be incinerated.

Empty or partially filled vials of CVA21 should be disposed of per local procedures after preparing the study drug administration syringes, with the empty carton kept for monitoring drug accountability.

The sponsor recommends that during the course of the study all staff involved in the viral administration and the collection of excretion samples wear protective gloves and face masks that are disposed of immediately. Although CVA21 is primarily transmitted via aerosols, CVA21 injection sites should be covered with an occlusive dressing that covers the entire lesion. This dressing should remain in place until the next scheduled visit, when it will be replaced by a new dressing until the site is dry and clear. Should a dressing be dislodged, a new one should be applied and the old one disposed of in appropriate infectious waste containers.

Viral administration should be performed in a manner to minimize potential exposure to immunocompromised individuals, i.e., viral administration should be performed at the end of a scheduled clinic or in a room distanced from or annexed to the major patient treatment room. While there is no requirement to decontaminate the bed or room where the injection was performed, systematic decontamination of surfaces that may have come into direct contact with CVA21, subject blood or excretion samples must be undertaken with an appropriate antiviral agent (e.g., Virkon® a sodium hypochlorite solution [5-6%], or formaldehyde [3%]). All materials utilized in the viral administration must be disposed of in appropriate infectious waste containers. Please refer to the CVA21 Material Safety Data Sheet for further details.

Staff injury by needles that have been in contact with the virus should be treated as per the standard occupational health guidelines on needle injuries. Staff should inform the occupational health department and both the principal investigator and research nurse involved in the trial of the injury. Any member of staff involved in direct subject care and/or sample handling who becomes unwell should report the illness to the occupational health department, principal investigator and research nurse.

Procedures for Suspected Cases of Transmission

Any mishap such as accidental spillage or inoculation might expose a staff member to virus in ways not occurring naturally, although there is nothing to suggest they would be at particular risk as a result. Nevertheless, because of the above, it would be advisable that handling and inoculation of virus be done by staff trained in handling infectious agents and disposal of potentially infectious waste.

If a healthcare worker or close contact reports illness that is possible from viral transmission of the treated subjects, the back of the throat of the healthcare worker or close contact should be swabbed with a sterile cotton swab near the tonsils. The close contact or healthcare worker should resist gagging and closing the mouth while the swab touches the back of the throat. To improve the chances of detecting infectious agents, the swab may be used to scrape the back of the throat several times. The swab should be placed immediately in viral transport media (e.g., Universal viral transport media, Becton Dickinson # 220221) and the sample stored at 4oC until transportation to the testing laboratory. The close contact or healthcare worker should wash their hands immediately. In addition or in place of the throat swab, a sputum sample may be given from the close contact or healthcare worker. The close contact or healthcare worker will be asked to cough deeply and expectorate any material that comes up from their lungs into a special collection container and the sample kept at 4oC until transportation to the testing laboratory. The close contact or healthcare worker should wash his/her hands immediately.

Please see the procedures in Appendices 15.8 and 15.9 for further details.

6.2. Efficacy Assessments

6.2.1 Imaging

Disease status will be assessed by computerized tomography (CT) or magnetic resonance imaging (MRI) scans and other appropriate measures (i.e., Physical Exam measurement) at intervals indicated in the Schedule of Procedures. Objective responses will be assessed according to immune-related response criteria (modified WHO) (Appendix 15.5).

6.2.2. Optional Photography

For visible melanoma lesions identified as Index (target) lesions, optional photographs will be obtained at Screening and at all response assessment time points per the Schedule of Procedures (Appendix 15.1). Pictures will be uploaded to the eCRF. Please refer to the Photography Manual for further details.

6.2.3 QOL/FACT-BRM

The FACT-BRM (Functional Assessment of Cancer - Biological Response Modifier) will be used before and during the planned study treatment (Appendix 15.10). This survey tool will be administered prior to performing study procedures as described in the Schedule of Procedures (Appendix 15.1).

6.3. [redacted]

7. DATA COLLECTION

7.1. Data Collection and Retrieval

Study data will be collected through entry on study specific password protected and secure electronic Case Report forms. eCRF Completion Guidelines should be reviewed for any questions on data entry or data fields.

Study site staff responsible for data entry will be assigned password-protected user-specific access once they have been trained in the use of the eCRF system.

All data entries to eCRFs must be supported by a clinical source document.

7.2. Investigator Reporting Requirements

The Investigator is responsible for the quality of the data recorded in the eCRF. Data should be entered within 72 hours of the subject's visit.

7.3. Record Retention

All study documentation will be kept by the site for at least 15 years after publication of the study data.

8. STATISTICAL AND DATA ANALYSIS

8.1. Sample Size Calculation

Prior to Version 6 to the protocol, a two-stage design often used for phase II studies was utilized with the objective of estimating response rate. Simultaneously, as a phase I trial, the safety of CVA21 was assessed and there was provision for the study to be stopped early for subject safety according to stopping rules defined in Section 5.3 "Dose-limiting Toxicity" to keep overall dose-limiting toxicity rate (grade ≥3 toxicities) ≤30%. The reported toxicity rate for CVA21 is low. In the VLA-007 study, there were no grade ≥3 toxicities observed in 57 subjects. The toxicity rate for the combined therapy was expected to be comparable to that for ipilimumab alone.

As stated in the final protocol, Simon's optimal two-stage design was used.[31] In all versions prior to Version 5 of the protocol, the null hypothesis that the overall response rate (ORR = proportion of subjects with a CR or PR according to irRC) was 0.26, H_0: ORR=0.26, was tested against a one-sided alternative, H_a: ORR=0.50. In the first stage, 12 subjects were to be accrued. If there were 3 or fewer responses in these 12 subjects within 12 months of starting treatment, the study was to be stopped due to futility of treatment. Otherwise, 14 additional subjects were to be accrued for a total of 26. In April 2016, the Data Monitoring Committee determined that 4 subjects had confirmed responses of PR or CR, and in the absence of any DLTs, stage 2 of the study was opened.

In Version 5 of the protocol, the sample size was modified to reflect a null hypothesis, H_0: ORR=0.28 versus the alternative, H_a: ORR=0.50. The required sample size for the study was increased to 45 subjects and the total enrollment was increased to a maximum sample size of 50 subject to adjust for subjects who did not complete the Day 106 lesion assessment. Using Simon's optimal two-stage design with a one-sided alpha =0.05 level of significance, the study had power of 90% to detect a difference when the true response rate was 0.5. The rationale for increasing the null ORR was that a response rate of 0.28 was reported for Viralytics study VLA-007.[32]

In this version of the protocol, the focus is on subjects who have progressed on prior anti-PD-1 therapy. The sample size has been modified in order to have approximately 29 subjects who have progressed on prior anti-PD-1 therapy, combining subjects with this phenotype who were enrolled under a prior version of the protocol who meet these criteria with subjects enrolled under this version of the protocol. The objectives of the study have been modified to focus on the estimation of ORR in this population.

A type 2 error is at least as important as a type 1 error when deciding if this combination should be investigated further in this population. Hence, the one-sided alpha has been set to 0.10 and the power has been set to 90%. An ORR of 0.11 was reported from the ipilimumab phase 3 trial[3]. A sample size of 26 subjects who have progressed on prior anti-PD-1 therapy, will provide 90% power for H_0: ORR=0.11 versus H_a: ORR=0.31 using a one-sided test at a significance level of 0.10. The sample size of 26 subjects may be increased by up to 3 additional subjects (10%) to adjust for subjects who do not complete the Day 106 lesion assessment. Thus, the total maximum sample size for the population of subjects who have progressed on prior anti-PD-1

therapy is estimated to be 29 subjects and the total sample size for the study is estimated to be approximately 59 subjects.

8.2. Randomization and Blinding

This study is a non-randomized, open-label study.

8.3. Data Analysis

A Statistical Analysis Plan (SAP) will provide the details for all summaries and analyses to be provided in the clinical study report for this study. The primary population of interest for all summaries of study data is the Safety Population, defined as all subjects who receive any amount of study drug (CVA21 and/or ipilimumab). In addition, of interest for the efficacy summaries and analyses is the subgroup of subjects who have progressed on prior anti-PD-1 therapy.

8.3.1. Disposition, Demographics and Baseline Characteristics

Summaries of disposition, demographics, and baseline data will be provided.

8.3.2. Efficacy Data

Summaries of the efficacy data will be provided for the Safety Population and for the subgroup of subjects who have progressed on prior anti-PD-1 therapy . All determinations of response and progression will be based on irRC.

ORR will be summarized. Two-sided exact 90% and 95% CIs will be constructed for ORR. The null hypothesis, H_0: ORR=0.11, versus H_a: ORR>0.11 will be tested using a one-sided binomial test at a 0.10 and 0.05 significance level.

Durable response rate (DRR), defined as the percentage of subjects with a best response of CR or PR lasting at least 26 weeks, will be summarized. Two-sided exact 90% and 95% CIs will be constructed for DRR.

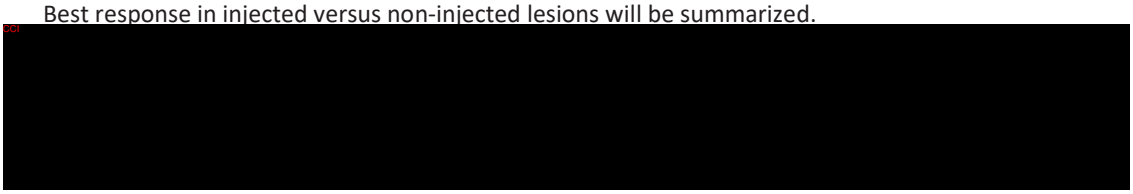
Immune-related progression-free survival (irPFS) is defined as the time from the beginning of treatment to PD or death, whichever occurs first. Summary statistics for irPFS will be provided using Kaplan-Meier methods.

Overall survival (OS) is defined as the time from the beginning of treatment to death. The one-year survival rate will be summarized along with 90% and 95% confidence intervals. Summary statistics for OS will be provided using Kaplan-Meier methods.

Time to response (TTR) is defined as the time from the beginning of treatment to initial documentation of a confirmed CR or PR. Summary statistics for TTR will be provided for the subjects with a best confirmed response of CR or PR.

Rules for handling missing or partial dates and censoring rules for irPFS and OS will be specified in the SAP.

Best response in injected versus non-injected lesions will be summarized.



8.3.3. Safety Data

Safety assessments include AEs, DLTs, laboratory values, and vital signs. The baseline value of laboratory data and vital signs is the most recent value recorded on or before the first day of study treatment.

Study drug exposure, including duration, total dose, and dose modifications will be summarized. Treatment-emergent adverse events (TEAEs) are defined as AEs that start on or after the first day of study treatment and within 30 days of the last administration of study treatment. The incidence of TEAEs will be summarized based on the number and percentage of subjects in each cohort and overall who experience events classified by MedDRA system organ class and preferred term. In the event that a subject experiences repeated episodes of the same AE, the subject will be counted once within each system organ class and once within each preferred term. Summaries of the incidence of treatment-related AEs, serious AEs, and AEs classified according to severity grade will be provided. The event with the highest severity grade and/or strongest causal relationship to treatment will be used for these summaries.

Laboratory data results (actual value and change from baseline) for selected hematology chemistry tests will be summarized for each cohort and overall at each scheduled time point. Subjects with missing data for a given time point will not contribute to the summary for that time point. Laboratory results from samples taken >30 days after the last administration of study treatment will be excluded from these summaries.

Laboratory test results will be assigned toxicity grades using the National Cancer Institute's CTCAE. Shifts in severity grades for selected laboratory tests will be summarized for each cohort by comparing the maximum toxicity grade observed after the start of study treatment to the baseline toxicity grade. All available laboratory results will be included in these summaries.

Vital sign results (actual value and change from baseline) will be summarized for each cohort and overall at each scheduled time point.

9. ADVERSE EVENTS

The Investigator is responsible for the detection and documentation of events meeting the criteria and definitions detailed below.

Full details of contraindications and side effects that have been reported following administration of the trial drug can be found in the Investigator's Brochure.

Participants should be instructed to contact their Investigator at any time after consenting to join the trial if any symptoms develop. All reported adverse events (AEs) that occur after joining the trial must be recorded in detail in the CRF. In the case of an AE, the Investigator should initiate the appropriate treatment according to their medical judgment. Participants with AEs present at the last visit must be followed up until resolution or stabilization of the event.

Progression of the cancer under study is not considered an adverse event unless it is considered to be drug-related by the investigator. All laboratory test results are recorded on the respective laboratory case report form pages but abnormal laboratory test results should only be recorded as an adverse event if they represent a clinical condition. In these cases, the AE should not record the laboratory abnormality verbatim, but instead should be recorded as an appropriate AE term which captures the adverse clinical condition which resulted from the laboratory abnormality.

All adverse events that occur after the consent form is signed but before treatment initiation (Day 1) must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

From the time of treatment initiation (Day 1) through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for adverse events meeting any serious criteria is described in section Immediate Reporting of Adverse Events to the Sponsor. The investigator will make every attempt to follow all subjects with non-serious adverse events for outcome.

9.1. Definitions

An **adverse event** (AE) is any untoward medical event affecting a clinical trial participant. Each initial AE will be considered for severity, causality or expectedness and may be reclassified as a serious event or reaction based on prevailing circumstances.

An **adverse reaction** (AR) is where it is suspected that an AE has been caused by a reaction to a trial drug

A **serious adverse event** (SAE), **serious adverse reaction** (SAR) or **suspected unexpected serious adverse reaction** (SUSAR) is any AE, AR or UAR that at any dose:

- results in death;
- is life threatening (i.e., the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- requires hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect.

Note: Hospitalizations for treatment planned prior to randomization and hospitalization for elective treatment of a pre-existing condition will not be considered as an AE. Complications occurring during such hospitalization will be AEs or SAEs as appropriate.

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to the Sponsor in the same timeframe as SAEs to meet certain local requirements.

Therefore, these events are considered serious by the Sponsor for collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

Unexpected adverse event:

Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

Associated with the use of the drug / intervention:

There is a reasonable possibility that the experience may have been caused by the drug.

Disability:

A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse event:

Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Unanticipated Problem

An unanticipated problem is an adverse event that is (i) unexpected; (ii) serious; and (iii) felt by the investigator to be possibly, probably, or definitely related to the research intervention. Only adverse events that meet this definition need be reported to the IRB.

For more information on the definition of an unanticipated problem and reporting requirement, consult the current IRB.

9.2. Reporting of Overdoses

All overdoses should be reported to the sponsor and medical monitor as soon as the overdose is noted.

In the event of an overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

9.3. Reporting of Pregnancy

Although pregnancy is not considered an adverse event, it is the responsibility of investigators or their designees to report any pregnancy in a study subject (spontaneously reported to them) that occurs during the trial.

Pregnancies that occur after the consent form is signed but before treatment must be reported by the investigator if they cause the subject to be excluded from the trial.

Pregnancies that occur from the time of enrollment through 120 days following cessation of study treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, must be reported by the investigator. All reported pregnancies must be followed to completion/termination of the pregnancy.

Such events must be reported within 24 hours to the Sponsor. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated.

9.4. Recording AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital notes, laboratory and diagnostic reports) related to the event. The Investigator or their delegate should then record all relevant information in the eCRF and on the SAE form.

Information to be collected includes dose, type of event, onset date, Investigator assessment of severity and causality, date of resolution as well as treatment required, investigations needed and outcome.

9.5. Evaluation of AEs and SAEs

The reporting period for adverse events is from the time of study enrolment to the final study visit, and for those who withdraw before the final planned study visit, within 30 days after the last dose of CVA21 or ipilimumab.

Seriousness, causality, severity and expectedness should be evaluated as though the participant is taking active drug.

9.5.1. Assessment of Seriousness

The Investigator should make an assessment of seriousness as defined in **Section 9.1**.

9.5.2. Assessment of Causality

The Investigator must make an assessment of whether the AE/SAE is likely to be related to treatment according to the following definitions:

Unrelated: This category applies to those AEs that are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).

Unlikely: This category applies to those AEs that are judged to be unrelated to the test drug, but for which no extraneous cause may be found. An AE may be considered unlikely to be related to study medication when it meets two of the following criteria: (1) it does not follow a reasonable temporal sequence from administration of the test drug; (2) it could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it does not follow a known pattern of response to the test drug; or (4) it does not reappear or worsen when the drug is re-administered.

Possibly: This category applies to those AEs for which a connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possibly related when it meets two of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; or (3) it follows a known pattern of response to the test drug.

Probably: This category applies to those AEs that the investigator feels with a high degree of certainty are related to the test drug. An AE may be considered probably related when it meets

3 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it disappears or decreases on cessation or reduction in dose (note that there are exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; for example, as in bone marrow depression, fixed drug eruptions, or tardive dyskinesia); or (4) it follows a known pattern of response to the test drug.

Definitely: This category applies to those AEs that the investigator feels are incontrovertibly related to test drug. An AE may be assigned an attribution of definitely related when it meets all of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it disappears or decreases on cessation or reduction in dose and recurs with re-exposure to drug (if rechallenge occurs); and (4) it follows a known pattern of response to the test drug.

All AEs/SAEs judged as having a reasonable suspected causal relationship (e.g., possibly, probably, definitely) to the study drug will be considered as ARs/SARs. All AEs/SAEs judged as being related (e.g., possibly, probably, definitely) to an interaction between the study drug and another drug will also be considered to be ARs/SAR.

Alternative causes such as natural history of the underlying disease, concomitant therapy, other risk factors and the temporal relationship of the event to the treatment should be considered.

9.5.3. Assessment of Severity

The severity of AEs will be assessed according to the NCI CTCAE, version 4.03. If the AE is not defined in the CTCAE, the Investigator will determine the severity based on the following definitions:

- **Mild (Grade 1):** asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Moderate (Grade 2):** minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL*.
- **Severe (Grade 3):** medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
- **Life-threatening (Grade 4):** urgent intervention indicated.
- **Death (Grade 5):** death related to AE.

* *Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.*

** *Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.*

9.5.4. Assessment of Expectedness

If an event is judged to be an AR/SAR, the evaluation of expectedness should be made based on knowledge of the reaction and the relevant product information documented in the Package Insert and Investigator's Brochure.

9.6. Reporting of SAE/SARs and SUSARS

Once the Investigator becomes aware that an SAE has occurred in a study participant, they must report the information to the Sponsor within 24 hours of becoming aware of the event.

The Investigator must complete and submit the Adverse Event Form and Complementary Page for SAEs in the Electronic Data Capture (EDC) system. The EDC system will be the primary method used for SAE reporting to Precision Oncology Drug Safety and every attempt should be made by the Investigator or designee to enter all SAE data into the system. In the event the EDC system is not operational then the Paper SAE Report Form must be filled out and faxed to Precision Oncology Drug Safety. All SAE reports that have the minimum data set for reporting should be submitted via the EDC system, even if only limited information is available. This must occur within 24 hours of discovery of the event regardless of the relationship (or lack thereof) of the SAE to the CVA21 or ipilimumab. If all the required information is not available at the time of reporting, the Investigator must ensure that any missing information is provided as soon as this becomes available. It should be indicated on the report that this information is follow-up information of a previously reported event.

All serious adverse events that occur during the study will be reviewed by Viralytics Medical Monitor, Dr. Seymour Fein by email (seymour.fein@markusresearch.com) or telephone 845-639-1830 Ext. 17.

Report SAE within 24 hours of discovery to:

- **Fax Number: 215-392-3397**
- **Precision Oncology Drug Safety e-mail: drugsafety@actoncology.com**

9.7. Regulatory Reporting Requirements

9.7.1. Annual Report

An annual report which includes the Development Safety Update Report (DSUR; listing, for example, all SARs and SUSARs) will be submitted to the US Food and Drug Administration (FDA), and any other applicable regulatory authorities. The Sponsor, or Sponsor's authorized representative or designee, is responsible for submitting this annual report.

9.7.2. Expedited Reporting

The Sponsor, or Sponsor's authorized representative or designee, is responsible for informing the FDA and the main IRB/REC (if applicable) of safety issues. For the FDA, the timelines for reporting are as follows:

- Fatal or life threatening SUSARs will be reported to the agency no later than 7 calendar days, and
- All other SUSARs will be reported no to the agency later than 15 calendar days.

Other regulatory agencies may be informed as appropriate.

9.8. Follow-up Procedures

After initially recording an AE or recording and reporting an SAE, the Investigator is required to follow each participant until resolution. Follow up information on an SAE should be reported to the Sponsor or designee.

Unless otherwise stated in the protocol, AEs and SAEs should be followed up until resolution or death of the trial subjects. An unanticipated event that is serious and definitely or probably caused by the study treatment (drugs or device) will be reported to the IRB in accordance with their guidelines and within their timelines.

10. TRIAL MANAGEMENT AND OVERSIGHT

10.1. Data Monitoring Committee (or Cohort Review Committee)

This study will utilize an independent Data Monitoring Committee (DMC). The DMC will meet for the following circumstances:

1. If there are 5 or fewer confirmed responses in the first 18 subjects, as confirmed by the DMC, the study will be stopped due to futility of treatment.
2. If a Grade 3 or greater, CVA21 related DLT (except lymphopenia) is observed in 2 subjects among the first 6 treated.
3. At the discretion of the medical monitoring team or if overall DLT rate exceeds 30% of treated subjects.

The Medical Monitor will review safety data following enrollment and completion of Day 85 by 6, 12 and 18 subjects. The DMC will review any findings to determine whether enrollment should be continued from a safety perspective.

The DMC will continue until the database is locked prior to preparation of the clinical study report.

10.2. Inspection of Records

All case report forms will undergo quality assurance review. All quality assurance reviews will include verification of the accuracy and integrity of data entered to case report forms. Incorrect data will be identified and corrected. The existence of adequate source documents for all data will be verified. All annual reports (continuing reviews) or publications will be submitted to the IRB for review and approval.

10.3. Monitoring

Study monitoring activities (Quality Control Reviews) are performed by a Contract Research Organization (CRO). Individuals who perform study monitoring activities do not report to Principal Investigators or research scientists and may not monitor studies for which they have direct responsibility.

Study monitoring activities are conducted regularly and include (but are not limited to) review and verification of the following:

- Eligibility
- Informed Consent process
- Adherence to protocol treatment plan
- Case Report Forms (CRFs)
- Source Documentation
- Adverse Events
- Regulatory Reporting

Results of study monitoring activities will be reported to applicable study personnel.

10.4. Risk Management

10.4.1. Potential Risks

There are three main potential risks:

- The most common potential risk for subjects in this study is an adverse event of CVA21-related “flu-like” illness. This is usually mild-moderate in severity and usually resolves within a few days.
- Signs and symptoms of transient septicemia such as fever, chills, rigors and tachycardia
- Although not observed in current studies, there is also a possible risk of CVA21 transmission to healthcare workers associated with subject care and conducting study assessments, and close contacts of the subjects themselves. Should a case of CVA21 transmission be suspected, details should be recorded per the procedures outlined in Appendix 15.8 and 15.9.

Further information on potential risks and guidance for the Investigator may be found in the current CAVATAK Investigator’s Brochure.

10.4.2 Minimizing Risks

The risks identified in Section 10.4.1 may be minimized as follows:

- While this risk is unlikely to be minimized, subject’s symptoms may be addressed through the administration of antipyretics and non-steroidal anti-inflammatory drugs.
- Inadvertent transmission of CVA21 from treated subjects to healthcare workers can be minimized if procedures used in the handling of Biosafety Level 2 agents are followed.

Viral administration should be performed in a manner to minimize potential exposure to other cancer suffering or immunocompromised individuals. Furthermore, systematic decontamination of surfaces that may directly come into contact with CVA21 or excretion samples must be undertaken, with an appropriate anti-viral agent (e.g., Virkon®, a sodium hypochlorite solution [5-6%] or formaldehyde [3%]). All materials utilized in the viral administration must be disposed of in appropriate infectious waste containers.

Any mishap such as accidental spillage or inoculation might expose a staff member to virus in ways not occurring naturally, although there is nothing to suggest they would be at particular risk as a result. Nevertheless, because of the above, it would be advisable that handling and inoculation of virus be done by staff trained in handling infectious agents and disposal of potentially infectious waste. Accidental spills from samples and operations should be adsorbed with paper tissues soaked with an appropriate anti-viral agent (e.g., Virkon®, a sodium hypochlorite solution [5-6%] or formaldehyde [3%]) and then with water. Tissues should then be discarded in proper containers designated for infectious laboratory/hospital waste. Please refer to the CVA21 Material Safety Data Sheet.

Inadvertent transmission of CVA21 from treated subjects to close contacts may be minimized through thorough subject education of potential transmission routes. Precautions are highlighted in a specific subject information sheet “What to do on a CAVATAK study”.

11. GOOD CLINICAL PRACTICES

11.1. Ethical Conduct of the Study

The study will be conducted in accordance with local laws and the principles of good clinical practice (GCP) and research for human patients (Declaration of Helsinki).

A favorable ethical opinion will be obtained from the appropriate IRB/REC prior to commencement of the study.

11.2. Regulatory Compliance

The study will be performed in accordance with United States IND regulations (21 CFR 56) or local national laws (as applicable), the guidelines of the International Conference on Harmonization (ICH), and the guidelines of the Declaration of Helsinki adopted by the 18th World Medical Assembly in Helsinki, Finland in 1964 and amended by subsequent assemblies in Tokyo, Japan in 1975; Venice, Italy in 1983; Hong Kong in 1989; Somerset West, South Africa in 1996, and in Edinburgh, Scotland in 2000; notes of clarification were added in 2002 and 2004.

11.3. Investigator Responsibilities

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff. Delegated tasks must be documented on a Delegation Log and signed by all those named on the list.

11.3.1. Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants must receive adequate oral and written information; appropriate Participant Information and Informed Consent Forms will be provided. The oral explanation to the participant should be performed by the Investigator or designated person, and must cover all the elements specified in the Participant Information Sheet/Informed Consent.

The participant must be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant must be given sufficient time to consider the information provided. It should be emphasized that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The participant should be informed and agree to their medical records being inspected by regulatory authorities but understand that inspection is undertaken by authorized personnel and their data will remain confidential.

The Investigator or delegated member of the trial team and the participant should sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The participant should receive a copy of this document and a copy should be filed in the Trial Master File (TMF) or Investigator Site File (ISF) as appropriate.

11.3.2. Study Site Staff

The Investigator must be familiar with the IP, protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the IP, protocol and their trial related duties.

11.3.3. Data Recording

The Investigator is responsible for the quality of the data recorded in the eCRF. Data should be entered within 72 hours of the subject's visit.

11.3.4. Investigator Documentation

Prior to beginning the study, each Investigator will be asked to provide particular essential documents to the Sponsor, including but not limited to:

- An original signed Investigator's Declaration (as part of the Clinical Trial Agreement documents);
- A signed copy of the Investigator's responsibilities letter issued by the Sponsor.

The Investigator, with the agreement of the Sponsor, will ensure all other documents required for compliance with the principles of GCP are retained in a TMF and that appropriate documentation is available in local ISFs.

11.3.5. GCP Training

All study staff must hold evidence of appropriate GCP training or undergo GCP training. This should be updated as appropriate throughout the trial.

11.3.6. Confidentiality

All laboratory specimens, evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee, Regulatory Authorities, or the IRB/REC. The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

11.3.7. Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Access to collated participant data will be restricted to those clinicians treating the participants.

Computers used to collate the data will have limited access measures via assigned user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

12. STUDY CONDUCT RESPONSIBILITIES

12.1. Protocol Amendments

All modifications or amendments to the protocol or informed consent document must be approved by Viralytics before being submitted to the Institutional Review Board (IRB) for review and approval. All modifications and amendments will be documented with a new version number and date. All changes to the informed consent document will include the date of the revision on the form.

No changes will be implemented until IRB/REC approval is obtained except when a potential threat to subject safety exists.

12.2. Protocol Violations and Deviations

The Investigator should not implement any deviation from the protocol without agreement from Viralytics, and appropriate IRB/REC and Regulatory Authority approval except where necessary to eliminate an immediate hazard to trial participants.

In the event that an Investigator needs to deviate from the protocol, the nature of and reasons for the deviation should be recorded in the eCRF. If this necessitates a subsequent protocol

amendment, this should be submitted to the IRB/REC and Regulatory Authority for review and approval as appropriate.

The IRB will be notified of any significant deviations from the approved protocol.

All IRB-reportable protocol deviations must be reported to Viralytics Medical Monitor, Dr. Seymour Fein by email (seymour.fein@markusresearch.com) or telephone 845-639-1830 Ext. 17.

12.3. Study Record Retention

According to 21 CFR 312.62(c), the investigator shall retain required records for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated. If no application is to be filed or if the application is not approved for such indication, the investigator shall retain these records until 2 years after the investigation is discontinued or the IND is withdrawn and the FDA is notified.

The investigator must retain protocols; amendments; IRB/IBC approvals; completed, signed, dated consent forms; subject source documents; case report forms; quality monitoring reports; drug accountability records; and all documents of any nature regarding the study or subjects enrolled. Records may be placed in long-term storage after the study is completed. The location of long-term storage will be secure and easily accessed for regulatory purposes.

It is recommended that all study documentation will be kept by the site for at least 15 years after publication of the study data per ICH guidelines.

12.4. End of Study/Study Termination by Sponsor

The end of study is defined as the last participant's last visit.

The Investigators and/or Viralytics have the right at any time to terminate the study for clinical or administrative reasons.

The end of the study will be reported to the IRB/REC and Regulatory Authority within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants and ensure that the appropriate follow up is arranged for all involved.

12.5. Audits and Inspections

The study may be subject to audit by the sponsor or by regulatory authorities. If such an audit occurs, the investigator must agree to allow access to required subject records. By signing this protocol, the investigator grants permission to personnel from the sponsor, its representatives and appropriate regulatory authorities for on-site monitoring of all appropriate study documentation, as well as on-site review of the procedures employed in CRF generation, where clinically appropriate.

13. REPORTING, PUBLICATIONS AND NOTIFICATION OF RESULTS

13.1. Authorship Policy

Ownership of the data arising from this study resides with the Sponsor. On completion of the study, the study data will be analyzed and tabulated, and a clinical study report will be prepared.

13.2. Publication

As a multi-center trial, the sponsor intends to publish clinical data from all centers participating in the investigation. A publication committee selected by the sponsor will submit draft manuscripts to all participating investigators for their comments. In conformity with the uniform requirements for manuscripts submitted to biomedical journals published by the International Committee of Medical Journal Editors (Kassirer and Angell, 1991), investigators whose contribution consists solely in the collection of data will not be named individually as authors. Rather, those investigators will receive a collective authorship.

Individual investigators and/or their associates subsequently may publish additional findings of this study in scientific journals or present them at scientific meetings, provided that the sponsor is given ample opportunity to review any proposed abstract, manuscript, or slide presentation prior to its submission. This review is required to ensure that the sponsor is aware of all written and oral presentations of the data and does not imply any editorial review or restriction of the contents of the presentation or use.

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1. APPENDICES

15.1. Schedule of Procedures

Study Days	Screening[16] (<28 days to Day 1)	Day 1	Day 3 +/- 1d	Day 5 +/- 1d	Day 8 +/- 1d	Day 22 +/- 4d	Day 26 +/- 2d	Day 43 +/- 4d	Day 64 +/- 4d	Day 85 +/- 4d	Day 106 +/- 4d	Day 127 +/- 7d	Day 148 +/- 7d	Day 169 +/- 7d	Day 190 +/- 7d	Days 211 +/- 7d	Day 232 +/- 7d	Day 253 +/- 7d	Day 274 +/- 7d	Day 295 +/- 7d	Day 316 +/- 7d	Day 358/ET[15] +/- 7d
Medical History	X	X																				
Physical exam[1]	X	X				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs, weight[1]	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CVA21 administration[2]		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Ipilimumab administration[3]						X		X	X	X												
CBC, Diff., plt	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chemistry panel [1, 4]	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LDH[1]	X										X				X				X			X
TSH/Free T4[1]	X					X		X	X	X					X				X			X
PT/PTT	X														X				X			X
Testosterone[5]	X								X		X				X				X			X
FSH/LH[6]	X								X		X				X				X			X
ACTH/Cortisol[1]	X					X		X	X	X	X				X				X			X
Immunologic monitoring[7]	(X)	(X)				(X)		(X)	(X)	(X)					(X)				(X)			(X)
CVA21 neutralizing ab[8]		X				X				X					X				X			X
CVA21 serum load[8]		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12 lead ECG	X																					
CT/MRI, response assessment[9]	X							X			X		X		X		X		X		X	X
Photography of visible lesions[10]	X							X			X		X		X		X		X		X	X
QOL assessment[11]	X	X				X		X	X	X	X		X		X		X		X		X	X
Brain MRI[12]	X																					
Pregnancy test[6]	X	X				X		X	X													X
Tumor biopsy[13]	X										X											
AE, Con Meds[14]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

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Schedule of Procedures Footnotes

1. Physical examination, vital signs, weight and laboratory samples must be done before dosing with either CVA21 or ipilimumab.
2. CAVATAK™ (CVA21) up to a dose of 3 x 10^8 $TCID_{50}$ (about 4.5 x 10^6 $TCID_{50}$/kg for a 70-kg subject) in a maximum volume of 4.0 mL by IT administration, maximum of 19 sets of injections per subject (Days 1, 3, 5, 8, 22, 43, 64, 85, 106, 127, 148, 169, 190, 211, 232, 253, 274, 295 and 316).
3. Ipilimumab will be administered at 3 mg/kg to be given over 90 minutes. On days where both ipilimumab and CVA21 are given, ipilimumab will be administered first.
4. Serum Chemistry tests include albumin, ALT, AST, alkaline phosphatase, bilirubin-total, bicarbonate, BUN, calcium, chloride, creatinine, glucose, protein-total, sodium, and potassium.
5. Total testosterone. Male subjects only.
6. Female subjects only. The pregnancy test will be repeated at Day 358/Early Termination.
7. Samples for immunologic monitoring are obtained for subjects enrolled at one site only (Portland Medical Center).
8. CVA21 neutralizing antibody titer and CVA21 serum load will be analyzed from the same serum sample. Laboratory kit for collection of these samples to be provided by central laboratory.
9. CT or MRI of chest, abdomen and pelvis to assess overall response. Other imaging studies as clinically appropriate as determined by the treating physician such as measurement with calipers of visible lesions. Bi-dimensional measurements should be recorded for both index and non-index lesions. Note: irRC requires that overall response assessments of irCR, irPR or irPD must be confirmed by a second assessment at least 4 weeks apart. However, as study visits after Day 106 occur 3 weeks apart, a second assessment 6 weeks later is preferred, with the CT/MRI and study procedures all being completed within the allowed visit window.
10. Optional pictures of visible index lesions will be obtained prior to administration of CVA21 and/or ipilimumab. Please consult the Melanoma Lesion Photography Manual for information on procedures.
11. QOL assessment using the FACT-BRM questionnaire to be filled out by the subject and data gathered by the research nurse at the time points indicated.
12. Repeat brain MRI as clinically indicated.
13. Tumor biopsy of non-index lesions greater than 1 cm if subject consents and specimen can be obtained using physical exam or ultrasound to identify lesions. Archival tissue may be used in place of a fresh biopsy at Screening.
14. Research nurse will meet with the subject as indicated during office visits to assess and grade toxicities.
15. Subjects who withdraw from the study will be followed for survival only, every 3 months for the first year and then every 6 months until study closure.
16. The Screening Period begins when the informed consent form is signed and continues until the first dose of CVA21 is administered. Screening assessments should be performed no more than 28 days prior to Day 1. Screening physical examination, vital signs and laboratory assessments can also be used as Day 1 assessments without having the need to repeat these tests, if these screening assessments were conducted within 7 days of Day 1.

15.2. ECOG Performance Status

0. Fully active, able to carry on all pre-disease activities without restriction (Karnofsky 90-100).
1. Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature. For example, light housework, office work (Karnofsky 70-80).
2. Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours (Karnofsky 50-60).
3. Capable of only limited self-care, confined to bed or chair 50% or more of waking hours (Karnofsky 30-40).
4. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair (Karnofsky 10-20).
5. Death (Karnofsky 0).

15.3. Listing of Autoimmune Diseases

Subjects should be carefully questioned about any history of acquired immune deficiencies or autoimmune disease. Subjects are not eligible for the study if there is any history of immune deficiencies or autoimmune disease. Possible exceptions could be subjects with a medical history of atopic disease or childhood arthralgias where the clinical suspicion of an autoimmune process is low. In addition, transient autoimmune manifestations of an acute infectious disease that resolved upon treatment of the infectious agent are not excluded (e.g., acute Lyme arthritis). Vitiligo or immune- mediated hypothyroidism from prior melanoma immunotherapy are not excluded. Diseases that may be autoimmune-related include but are not limited to the following:

Acute disseminated encephalomyelitis	Addison's disease
Alopecia universalis	Ankylosing spondylitis
Antiphospholipid antibody syndrome	Aplastic anemia
Asthma	Autoimmune hemolytic anemia
Autoimmune hepatitis	Autoimmune hypoparathyroidism
Autoimmune hypophysitis	Autoimmune myocarditis
Autoimmune oophoritis	Autoimmune orchitis
Autoimmune thrombocytopenic purpura	Behcet's disease
Bullous pemphigold	Celiac disease
Chronic fatigue syndrome	Chronic inflammatory demyelinating polyneuropathy
Churg-Strauss syndrome	Crohn's disease
Dermatomyositis	Dysautonomia
Eczema	Epidermolysis bullosa acquista
Gestational pemphigoid	Giant cell arteritis
Goodpasture's syndrome	Graves' disease
Guillain-Barré syndrome	Hashimoto's disease
IgA nephropathy	Inflammatory bowel disease
Interstitial cystitis	Kawasaki's disease
Lambert-Eaton myasthenia syndrome	Lupus erythematosus
Lyme disease – chronic	Meniere's syndrome
Mooren's ulcer	Morphea
Multiple sclerosis	Myasthenia gravis
Neuromyotonia	Optic neuritis

Opsoclonus myoclonus syndrome	Ord's thyroiditis
Pemphigus	Pernicious anemia
Polyarteritis nodusa	Polyarthritis
Polygrandular autoimmune syndrome	Primary biliary cirrhosis
Psoriasis	Reiter's syndrome
Rheumatoid arthritis	Sarcoidosis
Scleroderma	Sjögren's syndrome
Stiff-Person syndrome	Ulcerative colitis

15.4. CVA21 Intratumoral Injection Technique

Distribution Technique for Injecting CVA21 into the Selected Tumor

The technique has been devised to allow for a maximal amount of distribution of CVA21 throughout the tumor.

Multiple lesions will be injected in a dose hyper-fraction pattern, starting with the largest lesion(s) (2.0 mL injected into tumors >25 mm, 1.0 mL into 15 to 25 mm; 0.5 mL into 5 to 15 mm) to a 4.0 mL maximum. Measure the largest diameter of each tumor to be injected and determine the volume of CVA21 to be injected into each tumor. Sum the volumes to be injected so as to calculate the total volume of CVA21 required for the administration. The maximum volume of CVA21 to be administered is 4.0 mL. Following initial injection with CVA21, any injected lesion that reduces in diameter to <5 mm will be injected with 0.1 mL of CVA21 as per the stated treatment schedule until the lesion completely resolves. The required volume CVA21 is withdrawn into a syringe. Exchange the withdrawal needle for a new needle and distribute the diluted virus solution into the tumors as described below.

Tumor diameter	Volume of CVA21
>25 mm	2.0 mL
15 - 25 mm	1.0 mL
5 - <15 mm	0.5 mL
<5 mm*	0.1 mL

* only for lesions previously treated with CVA21

The distribution of virus is important to maximize the number of cancer cells initially infected with CAVATAK virus. Maximizing the number of cancer cells and regions throughout the tumor that are initially infected theoretically will increase the amount of cancer cells destroyed. It will also increase the amount of viral progeny produced by the tumor, and therefore increase the chance of ongoing viremia. Viremia is critical for the seeding of remote tumors, and therefore this injection technique was created to accomplish this, as well as to have a uniform technique that will be the guide for all the subjects treated.

The following guidelines should be followed regarding order of preference for lesions to be injected, if 4.0mLs is determined to be insufficient to inject all qualifying lesions:

At study day 1:

1. Select the largest injectable lesions first, followed by smaller lesions if the entire 4mL has not been used.

At subsequent study visits:

1. New lesions not present at baseline that are ≥ 5mm for non-lymph node (LN) lesions and ≥ 15mm for LN (short axis diameter),

2. Index lesions that were present at baseline but were not previously injected.

3. Lesions injected at baseline, both index and non-index.

Any newly injected lesions should continue to be injected for the remainder of the study, subject to order of preference above and without exceeding the maximum 4mL dose.

A 25-gauge needle should be used. A syringe is used that will accommodate the volume of CAVATAK required. The volume of CAVATAK to be administered will be determined based on the largest diameter of the tumor to be injected. To load the syringe with CAVATAK, remove vial from individual carton and thaw at room temperature (18-25°C). Do not leave the vial at room temperature for longer than is necessary to thaw the contents. A lab coat, safety glasses, sterile gloves and mask should be worn while loading the syringe with CAVATAK. Gently mix the vial for 5 seconds and tear off the plastic top. Use a luer-lock syringe of appropriate volume and 21-gauge needle to draw up the required volume.

Remove air bubbles. Remove the withdrawal needle and replace with a 25-gauge capped needle. Hold on ice until required (2-8°C). Administer within 3 hours from loading the syringe as distributed into the tumors as described below.

The injection will be in 9 regions within the tumor on each injection day. The regions do not overlap, and they are selected by using the following landmarks.

The distribution of the viral solution will be as follows for Day 1, the first injection:

The center of the tumor is estimated and marked.

Marks are made around the periphery of the tumor at 45 degree radians.

The site of the needle insertion is between the center mark and the 270-degree radiant. It should be at the approximate midpoint. This is the first dose injection site.

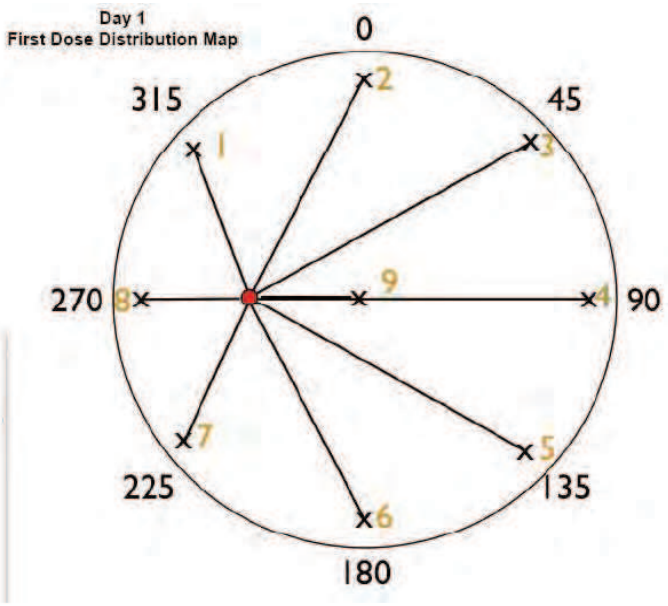
The volume of distribution is divided by 10, and will be distributed into 9 zones within the tumor.

The target zone for injection is the area within the tumor adjacent to the radiant marks, estimated to be approximately within the outer 20 % “rim” of the tumor. This will result in 8 injections. These first 8 injections should be aimed to be deep to the midline plane of the tumor.

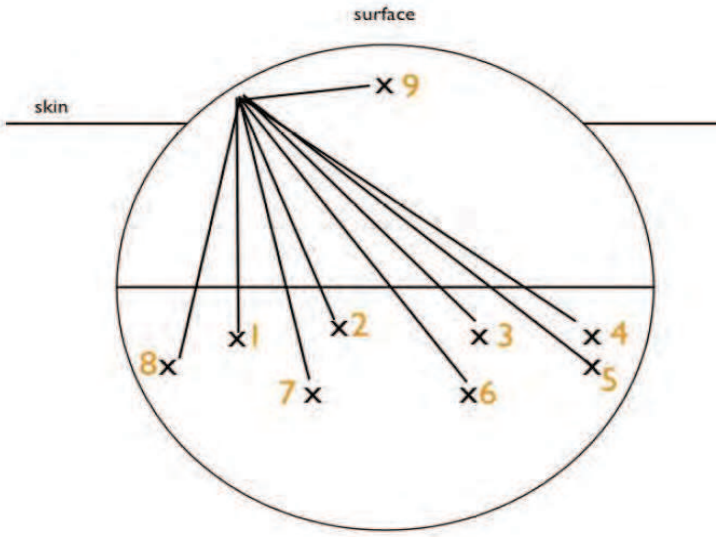
The final injection is made directly deep to the predicted center of the tumor, and on this first dose is aimed at a depth above the midline and comprises 20% of the injection volume.

This is summarized in the following diagrams.

TOP VIEW



SIDE VIEW 1st injection



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The second injection, on Day 3 (Visit 2) is as follows:

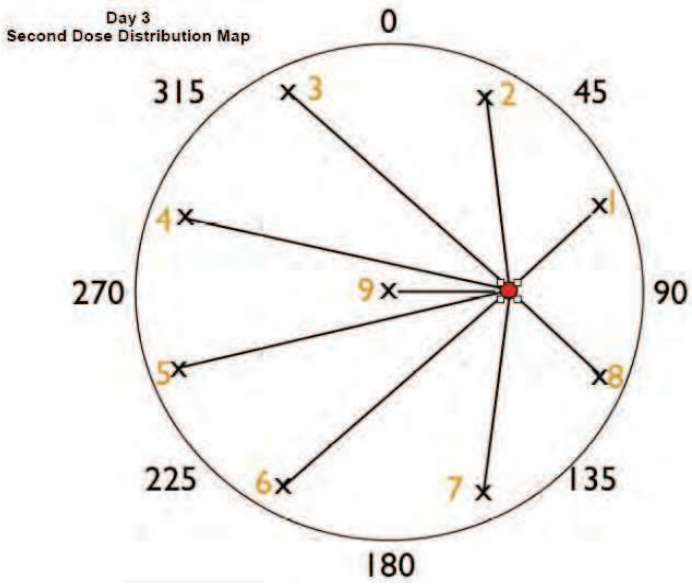
The injection area is to the right of the tumor, midway to the periphery in line with the 90 degree radiant.

The target injection zones are midway between the 45 degree radians approximated to be within the 20% outer "rim" of the tumor. These injections should be made "superficial" to the mid-line depth of the tumor (as opposed to being deep to the mid-line depth of the tumor in the first day injection.

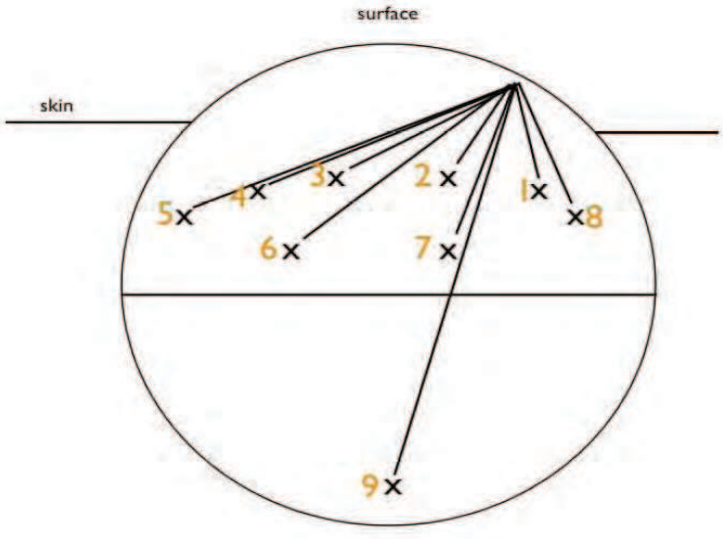
The final injection compromising 20% of the volume of distribution is targeted to the center of the tumor, deep to the mid-line, attempting to direct the solution into the outer 20% rim of the tumor.

The following diagrams demonstrate the injection target zones of the second dose of viral solution.

TOP VIEW



SIDE VIEW 2nd injection



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15.5. Evaluation of Response (irResponse Criteria)

It is expected that clinically significant tumor regressions may be delayed, or occur after a period of progression in subjects who receive ipilimumab-based immunotherapy.[27] Response and progression will be evaluated in this study using the immune-related response criteria (irRC) in solid tumors as described by Hoos et al.[29, 30]

Measurable disease: Measurability is defined as 5 x 5 mm or more on helical computer tomography scans. The sum of the product of perpendicular diameters (SPD) of index lesions at baseline is added to that of new lesions to calculate total tumor burden according to the following formula:

$$\text{Tumor Burden} = SPD_{\text{index lesions}} + SPD_{\text{new measurable lesions}}$$

Decrease in total measurable tumor burden is assessed relative to the baseline tumor burden, that is, SPD of all index lesions at baseline. The response categories of irCR, irPR and irPD should be confirmed at two consecutive time points at least 4 weeks apart. Overall, immune-related response based on two or more tumor assessments is derived as shown in the following table:

Derivation of overall immune-related response for all assessed time points*			
Measurable response	**Non-measurable response**		**Overall response**
Index and new measurable lesions (total measurable tumor burden)†	**Non-index lesions**	**New nonmeasurable lesions**	**Using irRC**
100% decrease	Absent	Absent	irCR‡
≥50% decrease	Any	Any	irPR‡
<50% decrease to <25% increase	Any	Any	irSD
≥25% increase	Any	Any	irPD‡

* After Wolchok, et al (30). irCR = immune-related complete response – complete disappearance of all index and new measurable lesions; irPR = immune-related partial response – decrease in tumor volume ≥50% relative to baseline; irSD = immune-related stable disease – not meeting criteria for irCR or irPR, in absence of irPD; iPD = immune-related progressive disease – increase in tumor volume ≥25% relative to nadir.

† Index and non-index lesions are selected at baseline. Index lesions are measurable (≥ 5 x 5 mm) and non-index lesions are not measurable (< 5 x 5 mm, ascites, bone lesions, etc). Changes are assessed relative to baseline and include measurable lesions only (> 5 x 5 mm).

‡ Assuming response and progression are confirmed by a second assessment at least 4 weeks apart. Note: as study visits after Day 106 occur 3 weeks apart, a second assessment 6 weeks later

is preferred with the CT/MRI and study procedures all being conducted within the allowed study window.

Using irRC, the appearance of new lesions alone does not constitute irPD if they do not add to the tumor burden by at least 25%. Subjects with new lesions but an overall tumor burden decrease qualifying for partial response (≥50% decrease) or qualifying for stable disease (<50% decrease to >25% increase) are considered to have irPR or irSD, respectively (same percentage changes including new lesions).

Note: irRC does not include guidelines for the assessment of response in lymph nodes when designated as index lesions. Therefore, for the purposes of this study, the rules used in RECIST1.1 will be used. Specifically,

1. *Lymph nodes are considered normal structures when less than 10 mm in short axis diameter (SAD) and pathologically enlarged if >10 mm. To be considered an index lesion and measurable, a lymph node must be ≥15 mm in SAD.*
2. *The SPD for nodal index lesions is added to the SPD of all index lesions at Baseline and at subsequent response assessments.*
3. *Lymph nodes identified as index lesions that reduce to <10 mm in SAD should be recorded but are not included in the SPD of all index lesions.*

15.6. YERVOY® (Ipilimumab) Package Insert

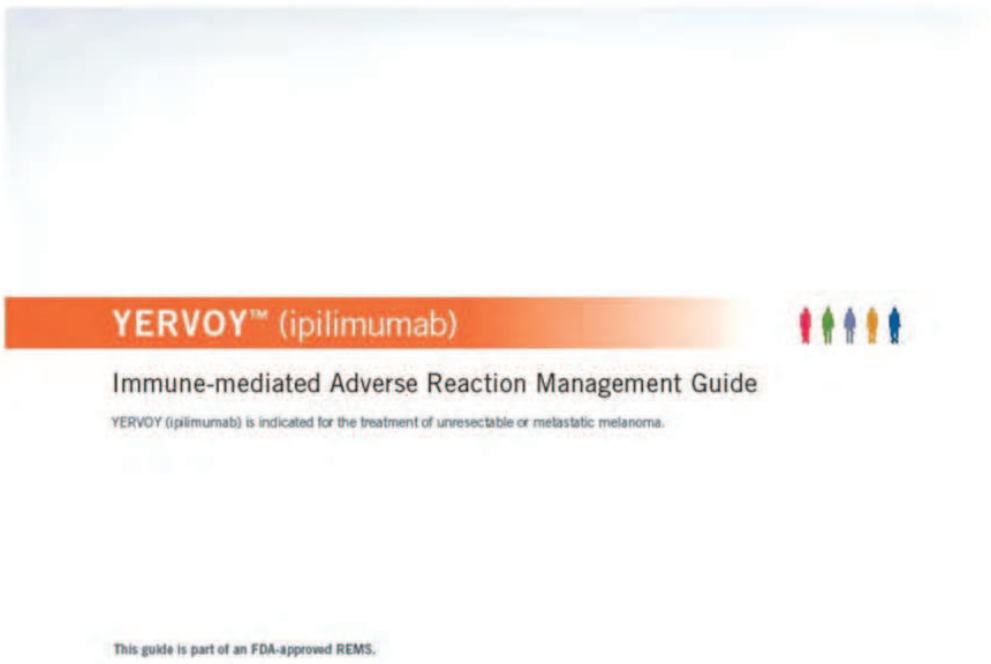
Please refer to the Yervoy package insert at the below link:

http://packageinserts.bms.com/pi/pi_yervoy.pdf

15.7. Suggested Management of Ipilimumab Toxicity

(from the Yervoy® (Ipilimumab) website)

http://www.hcp.yervoy.com/servlet/servlet.FileDownload?file=00Pi000000PI1ZVEA1



15.8. Healthcare Worker instructions in case of suspected CVA21 transmission

General Information

Coxsackievirus A21 is a naturally occurring virus that induces mild upper respiratory symptoms during natural infection of humans. CVA21 is acquired naturally by the respiratory route and circulates naturally in the community from time to time. Based on a number of small serology monitoring studies, around 20-50% of the general population possess pre-existing immunity to CVA21. CVA21 naturally infects cells in the respiratory tract that are known to express ICAM-1, thereby resulting in the development of "common cold"-like symptoms commonly observed during natural and experimental infection. During experimental human infection with CVA21 via intranasal administration, infectious virus has been recovered in nasal washing, throat swab, sputum and feces.

As such there is a possibility that subjects in the study may excrete virus from the respiratory or gastrointestinal tract for some days after CVA21 administration. As such, caregivers, family members, clinical trial staff and healthcare workers must be aware of this potential shedding and follow the guidance listed below. However, it must be noted that potential shedding is unlikely to be quantitatively different from naturally acquired infection.

Specific Instructions

- The sponsor recommends that during the course of the study all staff involved in the viral administration and the collection of excretion samples wear protective gloves and face masks that are disposed of immediately. Although CVA21 is primarily transmitted via aerosols, injected lesions should be covered with an occlusive dressing that covers the entire lesion. This dressing should remain in place until the next scheduled visit, when it will be replaced by a new dressing. Viral administration should be performed in a manner to minimize potential exposure to other subjects with cancer or immunocompromised individuals. Furthermore, systematic decontamination of surfaces that may directly come into contact with viral inoculum or excretion samples must be undertaken with an appropriate antiviral agent (e.g., Virkon®, a sodium hypochlorite solution [5-6%] or formaldehyde [3%]).). All materials utilized in the viral administration must be disposed of in appropriate infectious waste containers.
- In the event of an accidental spillage of CVA21, the healthcare worker should wear a protective laboratory gown, protective gloves and a face mask that are disposed of immediately following the decontamination process. The contaminated site should be immediately be covered with an appropriate antiviral agent (e.g., Virkon®, a sodium hypochlorite solution [5-6%] or formaldehyde [3%]) for at least 10 minutes and then adsorbent tissue/paper used to collect the antiviral solution. The contaminated site should then be thoroughly wiped with a clean tissue containing fresh antiviral solution and then wiped dry with fresh tissue. Contaminated tissues should be disposed of in appropriate infectious waste containers. If the contaminated area contains

fragments of the vial containing the CVA21 solution then they should be immediately placed in a sealed bio-hazard sharps container.

- Any mishap such as accidental spillage or inoculation might expose a staff member to virus in ways not occurring naturally, although there is nothing to suggest they would be at particular risk as a result. Nevertheless, because of the above, it would be advisable that handling and inoculation of virus be done by staff trained in handling infectious agents and disposal of potentially infectious waste.
- In the event that a health care worker accidentally injects themselves with CVA21, encourage bleeding from the skin wound and wash the injured area with copious soapy water, disinfectant, scrub solution or water and cover the site of injection with an occlusive dressing.
- If a healthcare worker experiences symptoms of a "common cold infection" following recent contact with a study subject, then the duration and severity of the symptoms should be noted on the supplied record form. Furthermore, a throat swab or sputum sample may be taken for analysis of the presence of CVA21

Record of illness by health care worker

Name of health care worker	Age of contact	Sex Male or Female	Date of first contact with subject after treatment	Date of last contact with subject prior to developing illness	High temperature Please record	Nausea (Y/N)	Sore throat (Y/N)	Diarrhea (Y/N)	Duration of illness (number of days)	Throat swab of sputum sample taken (Y/N)	Other comments about illness

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15.9. Close Contact Instructions in Case of Suspected CVA21 Transmission

General Information

Coxsackievirus A21 is a naturally occurring virus that induces mild upper respiratory symptoms during natural infection of humans. CVA21 is acquired naturally by the respiratory route and circulates naturally in the community from time to time. Based on a number of small serology monitoring studies, around 20-50% of the general population possesses pre-existing immunity to CVA21. CVA21 naturally infects cells in the respiratory tract that are known to express ICAM-1, thereby resulting in the development of "common cold"-like symptoms commonly observed during natural and experimental infection. During experimental human infection with CVA21 via intranasal administration, infectious virus has been recovered in nasal washing, throat swab, sputum and feces.

As such there is a possibility that subjects in the study may excrete virus from the respiratory or gastrointestinal tract for some days after CVA21 administration. As such, caregivers, family members, clinical trial staff and healthcare workers must be aware of this potential shedding and follow the guidance listed below. However, it must be noted that potential shedding is unlikely to be quantitatively different from naturally acquired infection.

Specific Instructions

- Subjects in the study may excrete infectious virus in respiratory and gastrointestinal tract secretions for a number of weeks following the initial viral injection. Precautions that are normally undertaken during the general prevention of respiratory infections should be followed. Hands should be washed with soap and warm water immediately following handling of feces/fecal soiled clothing, and material exposed to respiratory secretions such as tissues and handkerchiefs.
- Caregivers and family members should observe personal hygiene (i.e., hand washing) following contact with recently treated subjects. Caregivers who present symptoms of a cold or flu should present to a study investigator to provide a sample for analysis of the underlying infection.
- If the occlusive dressing(s) on the study subject is accidentally removed, then a new occlusive dressing should be placed over the sites of injection. Old or soiled occlusive dressings should be placed in a sealed plastic bag and disposed in the house-hold rubbish container
- If a close contact experiences symptoms of a "common cold infection" following recent contact with a study subject, then the duration and severity of the symptoms should be noted on the supplied record form and given to the healthcare workers of the site administering the CVA21 injections. If the close contact is concerned that they have contracted a CVA21 infection, a throat swab or sputum sample may be taken by the healthcare workers of the site administering the CVA21 injections for analysis of the presence of CVA21.

Record of illness by close contact

Name of close contact	Age of contact	Sex Male or Female	Date of first contact with subject after treatment	Date of last contact with subject prior to developing illness	High temperature Please record	Nausea (Y/N)	Sore throat (Y/N)	Diarrhea (Y/N)	Duration of illness (number of days)	Throat swab of sputum sample taken (Y/N)	Other comment about illness

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15.10. FACT-BRM Questionnaire

Below is a list of statements that other people with your illness have said are important. Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box and go to the next	0	1	2	3	4
GS7	I am satisfied with my sex life	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	EMOTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right	0	1	2	3	4

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS - Physical	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
BMT6	I get tired easily	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
BRM1	I have pain in my joints	0	1	2	3	4
BRM2	I am bothered by the chills	0	1	2	3	4
BRM3	I am bothered by fevers (episodes of high body temperature)	0	1	2	3	4
BRM10	I am bothered by sweating	0	1	2	3	4

	ADDITIONAL CONCERNS - Mental	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
HI8	I have trouble concentrating	0	1	2	3	4
HI9	I have trouble remembering things	0	1	2	3	4
BRM7	I get depressed easily	0	1	2	3	4
BRM8	I get annoyed easily	0	1	2	3	4
BRM9	I have emotional ups and downs	0	1	2	3	4
HI6	I feel motivated to do things	0	1	2	3	4