Pilot Study of ONCOS-102 and Pembrolizumab: Remodeling of the Tumor Microenvironment and Clinical Outcomes in Anti–PD-1–Resistant Advanced Melanoma

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ABSTRACT

Purpose: Intratumoral oncolytic virotherapy may overcome anti–PD(L)-1 resistance by triggering pro-inflammatory remodeling of the tumor microenvironment. This pilot study investigated ONCOS-102 (oncolytic adenovirus expressing GM-CSF) plus anti–programmed cell death protein 1 (PD)-1 therapy in anti–PD-1–resistant melanoma.

Patients and Methods: Patients with advanced melanoma progressing after prior PD-1 blockade received intratumoral ONCOS-102 either as priming with 3 doses (3×10^{11} viral particles) during Week 1 [Part 1 (sequential treatment)] or as 4-dose priming and 8 booster doses every 3 weeks [Part 2 (combination treatment)]. From Week 3, all patients received pembrolizumab every 3 weeks (≤8 doses). The primary endpoint was safety. Objective response rate (ORR), progression-free survival, and immunologic activation in repeat biopsies were also investigated.

Results: In 21 patients (Part 1, $n = 9$; Part 2, $n = 12$) ONCOS-102 plus pembrolizumab was well tolerated: most adverse events (AE) were mild/moderate in severity. Pyrexia (43%), chills (43%), and nausea (28%) were the most common ONCOS-102–related AEs. There were no dose-limiting toxicities. ORR was 35% [response evaluation in solid tumors (RECIST) 1.1, irRECIST]. Reduction in size of ≥1 non-injected lesions observed in 53% patients indicated a systemic effect. In injected tumors, persistent immune-related gene expression and T-cell infiltration were associated with clinical benefit. Viral persistence and efficacy in injected and non-injected lesions without additional toxicity supported Part 2 dosing regimen in future studies.

Conclusions: ONCOS-102 plus pembrolizumab was well tolerated and led to objective responses in patients with anti–PD-1–resistant advanced melanoma. ONCOS-102 promoted T-cell infiltration, particularly cytotoxic $CD8^+$ T cells, which persisted at Week 9, driving clinical benefit. Further investigation of ONCOS-102 plus PD-1 blockade is warranted.

See related commentary by Levi and Boland, p. 3

Introduction

Antibodies blocking programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte–associated antigen (CTLA-4) have improved the prognosis of patients with unresectable advanced melanomas, with combined therapy doubling median overall survival (mOS) versus single-agent treatment (1, 2). However, 36% to 66% of patients experience disease progression (PD) after combined or single-agent anti–PD-1 and anti–CTLA-4 therapy and require further treatment (1, 3). Anti–PD-1–refractory melanomas are challenging to treat: in the second-line setting mOS of 8.8 months and 20.4 months is reported with anti–CTLA-4 monotherapy and in combination with anti–PD-L1 treatment, respectively, while mOS was 24.7 months in patients treated with pembrolizumab plus ipilimumab followed by pembrolizumab monotherapy (4, 5). While recent FDA approval of combined PD-1 and LAG-3 blockade with nivolumab plus relatlimab provides a novel option for first-line therapy, approximately half of patients experience PD at 12 months, with a median progression-free survival (PFS) of 10 months (6). Data from an early patient series also suggests individuals who experience progression on this treatment combination are unlikely to benefit from subsequent PD-1 plus CTLA-4 blockade (7). Therefore, new treatment strategies are needed for patients with PD-1–refractory melanomas.

Resistance mechanisms for anti–PD-1 agents in melanoma and other malignancies are diverse and may involve immune exclusion, T-cell exhaustion, expression of alternate immune checkpoints, and lack of antigen presentation, which can be caused by *B2M* mutations (8–12). Oncolytic viruses are immunomodulating agents that preferentially infect and lyse tumor cells and can be used as vehicles for immunomodulatory transgenes to enhance antitumor immunity and assist in immune checkpoint blockade (13). Oncolytic viruses have shown efficacy in first-line therapy as single agents and combined with anti–PD-1 or anti–CTLA-4 agents in advanced melanoma (14–16). To date, talimogene laherparepvec (T-VEC), an engineered herpes-simplex virus 1 expressing GM-CSF, is the only FDA-approved oncolytic virotherapy indicated for local treatment of unresectable lesions in patients with melanoma (13). While oncolytic viruses have potential to improve outcomes with anti–PD-1 agents, a phase III study of first-line T-VEC plus pembrolizumab failed to meet its co-primary endpoints of PFS and OS (17).

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Clinical trial registry: ClinicalTrials.gov NCT03003676.

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Translational Relevance

We report a pilot trial of ONCOS-102, a novel oncolytic adenovirus encoding GM-CSF, administered intratumorally either sequentially or in combination with pembrolizumab in patients with melanoma resistant to prior PD-1 blockade. Treatment was well tolerated. Objective responses were seen in 7 of 20 patients, and size reductions in non-injected lesions suggested local delivery of ONCOS-102 can drive a systemic antitumor effect. Serial biopsies of injected tumors at baseline, Week 3 (following ONCOS-102 and prior to pembrolizumab), and Week 9 (following ONCOS-102 and pembrolizumab) indicated that while most tumors experience $CD8^+$ and $CD4^+$ infiltration after ONCOS-102 injection, sustained infiltration at Week 9 was associated with clinical benefit. Future trials of ONCOS-102 and checkpoint inhibition are warranted in anti–PD-1–resistant melanoma. These findings suggest trials utilizing viral agents in anti–PD-1–resistant disease should not rely solely on early onset of cytotoxicity to predict clinical response.

ONCOS-102 (Ad5/3-D24-GM-CSF) is a chimeric oncolytic adenovirus expressing human GM-CSF (18). Compared with herpes simplex virus, which establishes latency and has diverse mechanisms to overcome immune surveillance, adenovirus is primarily lytic and possess a limited number of genes with known immune evasion activities (19–22). Indeed, some studies suggest adenovirus-based oncolytic virus platforms may offer superior immune activation compared with herpes simplex virus, vaccinia virus, and reovirus (23). In a phase I study, ONCOS-102 monotherapy induced innate and adaptive immune responses in patients with various treatment-refractory solid tumors, with evidence of antitumor activity (18). ONCOS-102 may help overcome anti–PD-1 resistance by mechanisms including, but not limited to, priming of antitumor T-cell responses, increasing T-cell infiltration and upregulation of Type 1 T helper cytokines in the tumor microenvironment, with concomitant upregulation of PD-L1 (18). Furthermore, the combination of ONCOS-102 and pembrolizumab provided additional antitumor effects compared with single-agent ONCOS-102 in a humanized melanoma mouse model (24).

To investigate the potential utility of combining ONCOS-102 with anti–PD-1 therapy, this pilot study assessed the safety, efficacy, and treatment-associated immune modulation of ONCOS-102 and pembrolizumab in patients with advanced melanoma and PD on prior anti–PD-1 therapy. Patients received ONCOS-102 induction during the first three weeks, followed by either pembrolizumab alone, or a combination of ONCOS-102 and pembrolizumab.

Patients and Methods

Study participants

Patients (male or female, aged ≥18 years) with advanced or unresectable histopathologically confirmed melanoma and radiographic PD despite prior anti–PD-1 therapy (with or without ipilimumab) were enrolled. Eligible patients had ≥1 tumor lesion that was measurable by response evaluation in solid tumors (RECIST) 1.1 and amenable to bedside intratumoral injection. Other inclusion criteria were Eastern Cooperative Oncology Group performance status of 0 or 1, acceptable liver, renal, and hematologic function, and had completed any local therapy ≥21 days prior to receiving study medication. Patients with clinically stable brain metastases were permitted. Exclusion criteria included severe adverse events (AE) attributable to prior anti–PD-1 therapy; active bacterial, viral, or fungal infections requiring systemic therapy; symptomatic autoimmune disease requiring >10 mg prednisolone equivalent per day; and use of chronic immunosuppressants.

The study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonisation Guidelines for Good Clinical Practice, and local regulatory requirements. The study protocol was approved by the institutional review boards at each participating study site. All participants provided informed, written consent.

Study design and treatment

This open-label, multicenter, pilot study (randomization and blinding to study medication were not applicable) comprised a 24-week treatment period followed by 3-week follow-up and was conducted in two parts. To enhance the effect of GM-CSF–induced natural killer and cytotoxic T cells by reducing regulatory T cells (Treg), all patients received a single priming dose of cyclophosphamide 300 mg/m^2 intravenously 1 to 3 days prior to starting ONCOS-102 and pembrolizumab [either as sequential (Part 1) or combination (Part 2) treatment; ref. 25].

ONCOS-102 [1.2 × 10 (11) viral particles per mL] was administered intratumorally into cutaneous/subcutaneous or lymph node lesions. One to 5 lesions were injected per patient at each timepoint (0.5 mL minimum injection volume per lesion) with a single point of entry or fan-like distribution, and each patient received a total dose of 3 × 10 (11) viral particles (2.5 mL). Pembrolizumab [2 mg/kg or 200-mg flat dose intravenously every 3 weeks) was administered per institutional guidelines.

In Part 1, patients received ONCOS-102 and pembrolizumab sequentially. Three doses of ONCOS-102 were given on Days 1, 4, and 8 followed by 8 doses of pembrolizumab every 3 weeks starting on Day 22. In Part 2, patients received four doses of ONCOS-102 (Days 1, 4, 8, and 15) followed by 8 doses of ONCOS-102 plus pembrolizumab every 3 weeks (**Fig. 1**).

ONCOS-102 dose reduction was not permitted (ONCOS-102 was discontinued if dose modification was considered necessary due to a treatment-related AEs). Dose delay of pembrolizumab was permitted if deemed necessary by the investigator.

Study assessments

The primary objective was to assess the safety of sequential (Part 1) or sequential and combination (Part 2) administration of ONCOS-102 and pembrolizumab. Safety was assessed throughout the study by evaluation of AEs (NCI Common Terminology Criteria for Adverse Events v4.03), vital signs, physical examination, and laboratory assessments. Dose-limiting toxicities (DLT), assessed during the first 9 weeks of study treatment, included any Grade ≥3 non-hematologic toxicity that the investigator considered related to ONCOS-102 except for pyrexia (unless Grade 4), fatigue (unless Grade 3 for >24 hours) or flu-like symptoms. Immune-related AEs were considered DLTs if Grade ≥3 (Grade ≥2 for pneumonitis and peripheral neuropathy), required hospitalization for >24 hours, or required two classes of systemic immunosuppression.

Efficacy was assessed as a secondary objective. This included objective response rate [ORR; defined as the proportion of patients with a best response of complete response (CR) or partial response (PR) per RECIST v1.1, or immune-related CR or immune-related PR per irRECIST]. Change in size of individual (injected and non-injected) tumor lesions from baseline and PFS (time from start of

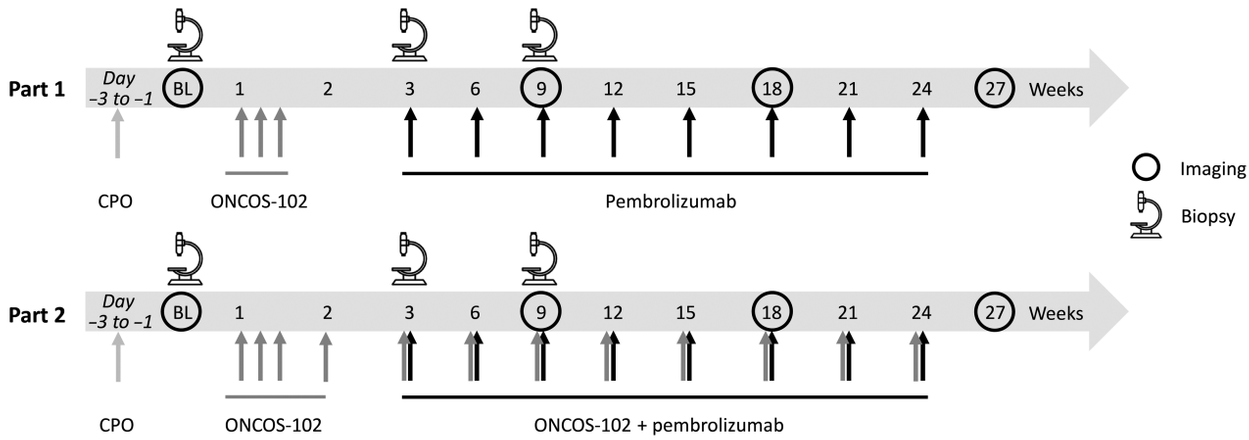

Figure 1.
Study schema. All patients received a single priming dose of cyclophosphamide. Part 1 patients received 3 intratumoral doses of ONCOS-102 followed by up to 8 sequential doses of pembrolizumab every 3 weeks. Part 2 patients received 4 intratumoral doses of ONCOS-102 followed by up to 8 doses of intratumoral ONCOS-102 in combination with pembrolizumab every 3 weeks. Tumor biopsies were obtained at baseline, Week 3 (following completion of ONCOS-102 in Part 1; ONCOS-102 ongoing in Part 2), and Week 9 [following 2 doses of pembrolizumab (all patients); ONCOS-102 ongoing in Part 2 only]. BL, baseline; CPO, cyclophosphamide.

treatment to PD or death) were also assessed. Tumor evaluations using CT or MRI were performed at Weeks 9, 18, and 27.

To investigate mechanisms of response with ONCOS-102 and pembrolizumab, tumor immune cell subsets were also assessed as secondary endpoints. Exploratory endpoints included analysis of tumor mutational burden (TMB) and tumor gene expression analysis in relation to response, including assessment of ONCOS-102 viral load by measurement of viral DNA copies and GM-CSF gene expression.

Tumor biopsies were obtained at baseline (Day 1, prior to the first injection of ONCOS-102), Week 3 [prior to the first dose of pembrolizumab (all patients) and following completion of ONCOS-102 in Part 1; ONCOS-102 ongoing in Part 2], and Week 9 [following 2 doses of pembrolizumab (all patients); ONCOS-102 ongoing in Part 2 only]. Using formalin-fixed, paraffin-embedded tumor specimens, multiplex immunofluorescence histology included detection of $CD4^+$, $CD8^+$ (including Granzyme B+) cells, calculated from whole-slide scanning. DNA and RNA, extracted from flash frozen tumor biopsies, was sequenced using NovaSeq 6000 Systems (Illumina, by Personalis Inc., CA, USA). Small variant calling (single-nucleotide variants and indels) was performed as previously described (26). RNA sequencing was processed in-house, and DNA sequencing was processed using the ImmunoID platform (Personalis Inc., CA, USA). Detailed immunofluorescence histology and next-generation sequencing methodology is available in the Supplementary Methods.

Statistical analysis

Sample size calculation was not performed; up to 24 patients (6–12 in Part 1, and 6–12 in Part 2) was considered appropriate for assessment of safety in this pilot study.

PFS was assessed using Kaplan–Meier methodology. Expression of correlative markers using immunofluorescence histology was assessed using an additive quasibinomial linear model with timepoint and best objective response (RECIST v1.1) of disease control [CR, PR, or stable disease (SD)] or PD as features.

TMB (log-transformed scores) was compared between patients with RECIST v1.1 disease control and PD using Student *t* test. Normality was assessed using the Shapiro–Wilk test. Other data were summarized using descriptive statistics. Statistical analyses were conducted in R (v 4.1.2) using RStudio.

Safety was assessed in all patients who received ≥ 1 dose of ONCOS-102 (safety population). Efficacy and immune endpoints were analyzed in patients from the safety population with repeat assessment of tumor burden and no major protocol deviations, and with repeat tumor biopsy assessment, respectively. Suspected DLTs were evaluated by the Safety Review Committee.

Data availability

The datasets used and/or analyzed in this study are available from Thomas.Hansen@targovax.com upon reasonable request from qualified researchers to conduct methodologically sound research. Sharing is subject to the protection of patient privacy and respect for patient informed consent.

Results

Patient characteristics and disposition

Between June 2016 and December 2020, 21 patients were enrolled and received study treatment at 4 sites in the U.S. and Norway: $N = 9$ in Part 1 and $N = 12$ in Part 2. Three (25%) and 4 (31%) patients completed Part 1 and Part 2, respectively. Reasons for discontinuation were loss of clinical benefit (Part 1, $n = 5$; Part 2, $n = 7$), withdrawal of consent (Part 2, $n = 1$), and hepatitis B infection (Part 1, $n = 1$).

Baseline disease characteristics of Part 1 and Part 2 patients were broadly comparable except for melanoma subtype (cutaneous subtype: Part 1, 89% vs. Part 2, 50%), disease stage (AJCC stage IV–IVM1c: 33% vs. 58%) and greater tumor burden at baseline (sum of longest diameter of target lesions: 37.5 mm vs. 73.5 mm; median number of lesions: 3 vs. 8.5) in Part 2 patients (**Table 1**). At study enrollment, all patients had received previous anti–PD-1 therapy (≥,2 cycles), 12 (57%) had also received prior anti–CTLA-4 treatment, and 3 (14%), patients with *BRAF* mutant disease had received prior BRAF and/or MEK inhibitor treatment. Most patients (15 of 21) received their last anti–PD-1 treatment ≤3 months prior to study enrollment.

Table 1. Patient characteristics at baseline (safety population).

	Part 1 ($N = 9$)	Part 2 ($N = 12$)	Total ($N = 21$)
Median age, years (range)	73 (40–87)	72 (43–83)	73 (40–87)
Sex (female/male), *n*	4/5	6/6	10/11
ECOG performance status, *n* (%)			
0	1 (11)	7 (58)	8 (18)
1	8 (89)	5 (42)	13 (62)
Melanoma subtype, *n* (%)			
Cutaneous	8 (89)	6 (50)	14 (67)
Acral	1 (11)	1 (8)	2 (10)
Mucosal	0	2 (17)	2 (10)
Unknown primary	0	3 (25)	3 (14)
AJCC stage[a], *n* (%)			
III (any stage)	6 (67)	5 (42)	11 (52)
IV			
IVM1a	2 (22)	2 (17)	4 (19)
IVM1b	0	2 (17)	2 (10)
IVM1c	1 (11)	3 (25)	4 (19)
Tumor burden at baseline			
Sum of the longest diameter of target lesions, mm (range)	37.5 (15–117)	73.5 (12–174)	43 (12–174)
Median number of lesions (range)[b]	3 (1–10)	8.5 (3–17)	7 (1–17)
Prior cancer therapy, *n* (%)			
Surgery	9 (100)	11 (92)	20 (95)
Radiotherapy	2 (22)	4 (33)	6 (29)
Chemotherapy	1 (11)	6 (50)	7 (33)
Anti–PD-1 agent	9 (100)	12 (100)	21 (100)
Anti–CTLA-4 agent	4 (45)	8 (67)	12 (57)
BRAF and/or MEK inhibitor	2 (22)	1 (8)	3 (14)
Intralesional therapy[c]	4 (45)	2 (17)	6 (29)
Driver alterations[d], *n* (%)			
BRAF V600	2 (22)	0	2 (10)
BRAF, other codon	1 (11)	1 (9)	2 (10)
NRAS Q61	2 (22)	7 (58)	9 (43)
NRAS, other codon	1 (11)	0	1 (5)
NF1	0	1 (9)	1 (5)
KIT	0	1 (9)	1 (5)
Wild-type[e]	1 (11)	1 (9)	2 (10)
Not tested/unknown	2 (22)	1 (9)	3 (14)

Abbreviations: AJCC, American Joint Committee on Cancer; ECOG, Eastern Cooperative Oncology Group.
[a]Disease stage at enrollment.
[b]Includes all target and non-target lesions at baseline.
[c]Includes T-VEC ($n = 4$), CMP-001 ($n = 1$), and PV-10 ($n = 1$).
[d]Driver alterations were identified with ≥5% allele frequency in somatic tissue and cross-referenced with the Personalis Research Cancer Gene List.
[e]Wild-type for *BRAF*, *NRAS*, *NF1*, and *KIT*.

Exposure to study treatment

In Part 1, all patients received ONCOS-102 on Days 1 and 4, and 78% received ONCOS-102 on Day 8 [ONCOS-102 was withheld in 2 patients due to a serious AE (SAE) of large intestine infection ($n = 1$) and AE of injection site swelling ($n = 1$)]. Patients received a median (range) of 6 (3–15) ONCOS-102 injections across all treated lesions (range 1–5 injected tumors per patient).

In Part 2, all patients received ONCOS-102 on Days 1, 4, 8, Week 2, and Week 3, and 92% received ONCOS-102 on Week 6 ($n = 1$ discontinued due to PD). On Weeks 9, 12, and 15, 58%, 42%, and 33% of patients received ONCOS-102. Patients received a median (range) of 23.5 (10–47) ONCOS-102 injections across all treated lesions (range 1–4 injected tumors per patient).

Patients received a median of 2 (range 1–8) and 4 (1–9) cycles of pembrolizumab in Part 1 and Part 2, respectively. No pembrolizumab dose adjustments occurred, and all patients received cyclophosphamide in accordance with the study protocol.

Safety

While all patients reported treatment-emergent AEs (TEAE) during the study, most experienced events of mild/moderate severity (Grade 1/2: 57%), 8 patients (38%) experienced Grade 3 AEs, and 1 patient (5%) experienced Grade 4 AEs (described below).

TEAEs related to ONCOS-102 were reported by 8 patients (89%) and 9 patients (75%) in Part 1 and Part 2, respectively. Overall, the most frequent ONCOS-102-related TEAEs were pyrexia (43% of patients), chills (43%), and nausea (29%; **Table 2**). The safety profile of ONCOS-102 was broadly comparable across Part 1 and Part 2. Injection site pain and injection site reaction were more frequent in Part 2, likely reflecting the higher number of ONCOS-102 injections received by this cohort. TEAEs considered related to both ONCOS-102 and pembrolizumab, and pembrolizumab alone were less frequent (**Table 2**).

No DLTs were noted during the study. SAEs occurred in 4 patients in Part 1 (11 events) and 2 patients in Part 2 (11 events). Except for pyrexia ($n = 2$), all SAE types occurred in single patients only. In Part 1, one patient experienced an SAE (Grade 3: large intestine infection) that was considered related to ONCOS-102 only. Another patient (with preexisting type 2 diabetes mellitus) experienced SAEs of Grade 4 diabetic ketoacidosis and Grade 4 type I diabetes mellitus 140 days and 13 days after last doses of ONCOS-102 and pembrolizumab, respectively, that were considered related to both drugs. SAEs related to pembrolizumab only were reported by 2 patients in Part 1: hemolytic anemia ($n = 1$) and diarrhea ($n = 1$). In Part 2, 1 patient reported an SAE related to ONCOS-102 only (pyrexia), and 2 patients reported SAEs related to ONCOS-102 and pembrolizumab: enterocolitis ($n = 1$) and pyrexia ($n = 1$).

There were no deaths during the study, and no patients discontinued ONCOS-102 due to TEAEs in Part 1. In Part 2, 1 patient discontinued ONCOS-102 and pembrolizumab due to Grade 3 aspartate aminotransferase increased considered related to pembrolizumab.

Efficacy

Overall, 35% (7 of 20) of evaluable patients achieved RECIST v1.1 objective response, including 6 PRs and 1 CR which occurred in a patient with a recurrent in-transit lesion. ORR was 38% (3 of 8 patients) in Part 1 and 33% (4 of 12 patients) in Part 2 (**Fig. 2A** and **B**). At study end (Week 27), response persisted in 5 patients (25%). Response outcomes were the same when analyzed per irRECIST. Six of the 7 patients with objective response had received their last prior dose of anti–PD-1 treatment ≤ 3 months prior to study enrollment (Supplementary Fig. S1). One patient with PD (RECIST v1.1) was treated past progression with subsequent stabilization of disease and completed the study [this patient had hallmarks of immunologic pseudoprogression (substantially increased $CD8^+$ and $CD4^+$ cells in the tumor biopsy) at Week 3 (Supplementary Fig. S2)]. Overall, of 11 patients previously treated with anti–PD-1 and anti–CTLA-4 agents (either in combination or sequentially), 7 achieved disease control (SD, PR, or CR; Supplementary Fig. S1).

Median [95% confidence interval (CI)] PFS per RECIST 1.1 was 2.1 (1.5, NA) months and 3.5 (1.8, NA) months in Part 1 and Part 2, respectively. Median (95% CI) PFS per irRECIST was 5.9 (1.5, NA) months and 3.5 (1.8, NA) months in Part 1 and Part 2, respectively.

Table 2. Treatment-related AEs (all grade) considered related[a] to ONCOS-102, pembrolizumab, or both agents (≥ 2 patients; safety population).

	AEs related[a] to ONCOS-102, only			AEs related[a] to pembrolizumab, only			AEs related[a] to ONCOS-102 and pembrolizumab		
Preferred term *n* (%)	**Part 1 (*N* = 9)**	**Part 2 (*N* = 12)**	**Total (*N* = 21)**	**Part 1 (*N* = 9)**	**Part 2 (*N* = 12)**	**Total (*N* = 21)**	**Part 1 (*N* = 9)**	**Part 2 (*N* = 12)**	**Total (*N* = 21)**
All treatment-related AEs	8 (89)	9 (75)	17 (81)	4 (44)	3 (25)	7 (33)	3 (34)	6 (50)	9 (43)
Pyrexia	3 (33)	6 (50)	9 (43)	0	0	0	1 (11)	2 (17)	3 (14)
Chills	5 (56)	4 (33)	9 (43)	0	0	0	0	0	0
Nausea	3 (33)	3 (25)	6 (29)	0	0	0	0	1 (8)	1 (5)
Injection site pain	1 (11)	3 (25)	4 (19)	0	0	0	0	0	0
Vomiting	2 (22)	2 (17)	4 (19)	0	0	0	0	0	0
Injection site reaction	0	3 (25)	3 (14)	0	0	0	0	0	0
Myalgia	3 (33)	0	3 (14)	0	0	0	0	0	0
Diarrhea	2 (22)	0	2 (10)	2 (22)	0	2 (10)	0	0	0
Pruritus	1 (11)	1 (8)	2 (10)	0	1 (8)	1 (5)	0	0	0
Rash maculo-papular	1 (11)	1 (8)	2 (10)	0	0	0	0	0	0
Hypotension	0	2 (17)	2 (10)	0	0	0	0	0	0
ALT increased	1 (11)	0	1 (5)	1 (11)	1 (8)	2 (10)	0	1 (8)	1 (5)
AST increased	0	0	0	0	1 (8)	1 (5)	1 (11)	1 (8)	2 (10)

Abbreviation: AST, aspartate aminotransferase.
[a]Possibly, probably, or definitely related to study treatment (assessed by study investigator).

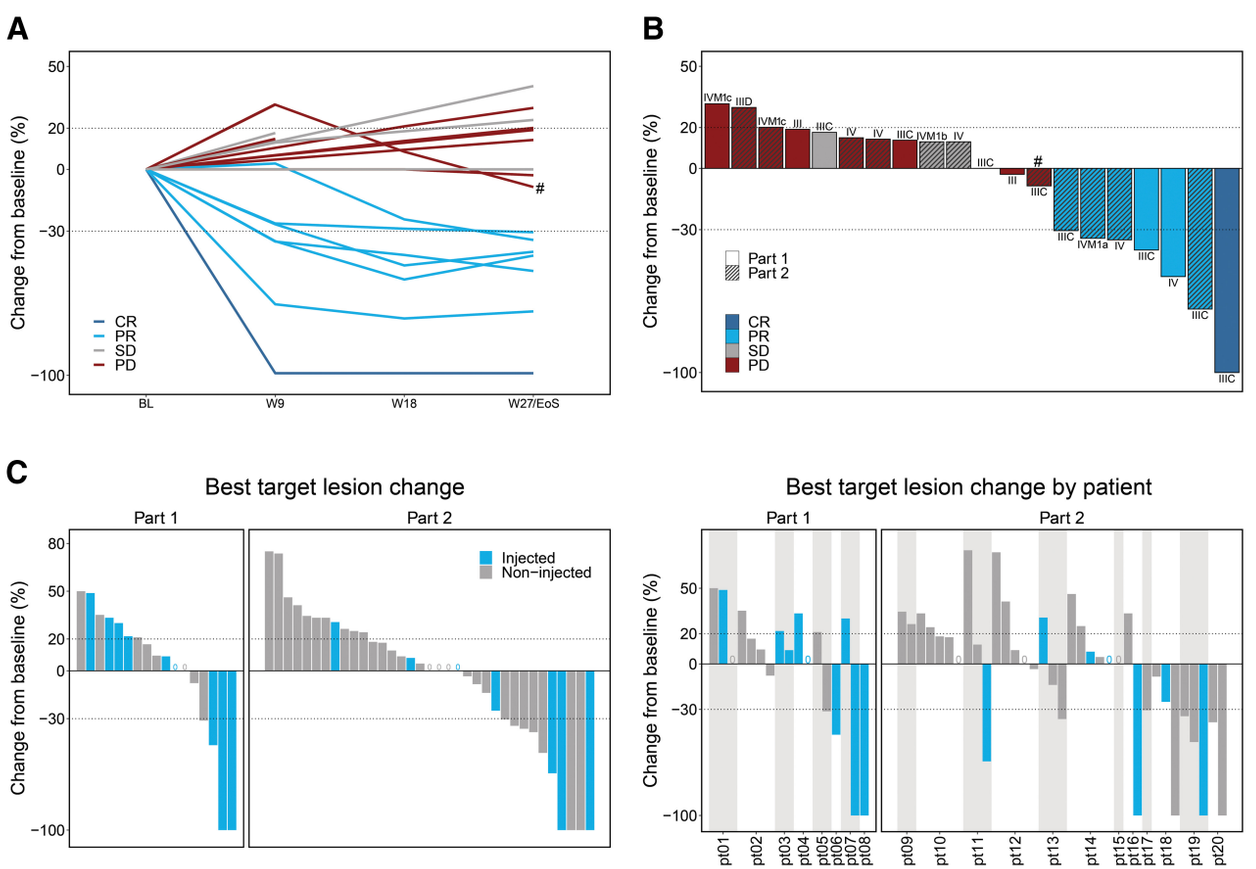

Figure 2.
Change in tumor burden (efficacy population). **A,** Spider plot of best response showing percent change from BL until EoS (Week 27). **B,** Waterfall plot for best overall response (RECIST v1.1), showing disease stage at baseline. **C,** Waterfall plot for best change in injected and non-injected individual target lesions (left) and target lesions per patient (right) stratified by cohort (Part 1 and Part 2). The alternating white and gray background groups individual patients (*x*-axis shows anonymized patient numbers). # Patient with pseudo-progression followed by tumor regression. BL, baseline; EoS, end of study; W, week.

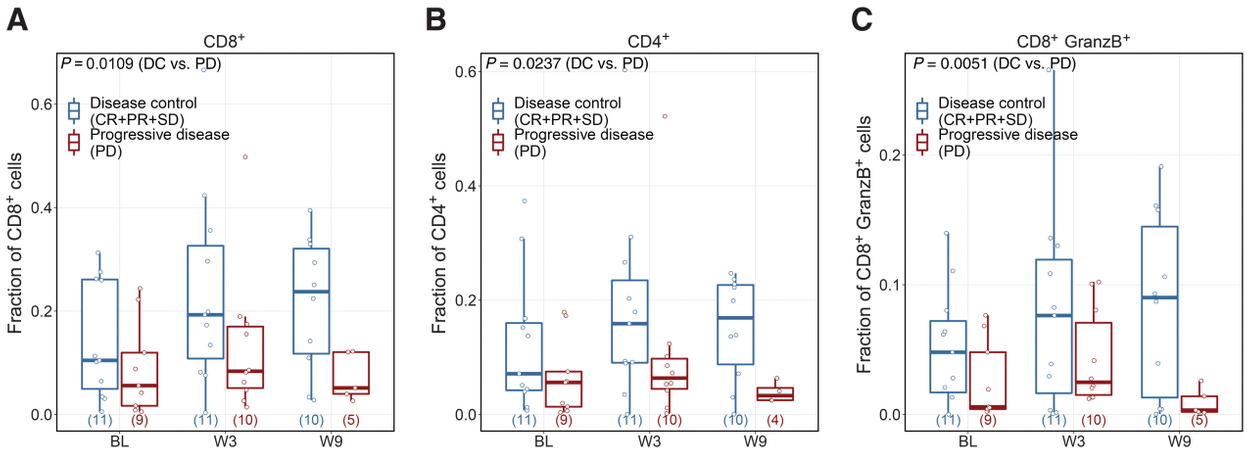

Figure 3.

Change in tumor immune cell profile over time. Immunofluorescence histology for (**A**) $CD8^+$, (**B**) $CD4^+$, and (**C**) Granzyme B–positive $CD8^+$ cells grouped by timepoint and response to treatment [RECIST v1.1 disease control (PR, CR, and SD) and PD]. Boxes show median, lower, and upper quartiles, whiskers show minimum and maximum values within the 1.5 interquartile range, and individual points denote outliers. Number of patients with available biopsies is shown in parentheses. *P* values reflect the fitted coefficient for disease control versus PD. BL, baseline; DC, disease control; W, week.

Systemic antitumor activity in non-injected lesions was assessed in 15 patients (total of 36 lesions). Of these patients, 8 (53%; Part 1, $n = 2$; Part 2, $n = 6$) had a reduction in size of ≥ 1 non-injected lesion and 6 (40%; Part 1, $n = 1$; Part 2, $n = 5$) had ≥ 30% relative reduction in tumor size (**Fig. 2C**). Shrinkage of non-injected lesions was observed more frequently in Part 2 than Part 1 (**Fig. 2C**). Notably, complete regression of injected lesions was observed in 4 patients, and 2 patients in Part 2 had complete regression of a non-injected lesion (**Fig. 2C**; Supplementary Fig. S3).

Correlative analyses of tumor biopsies

Tumor infiltration of $CD8^+$ ($P = 0.0109$) and $CD4^+$ T cells ($P = 0.0237$) differed significantly between patients with disease control and PD (**Fig. 3A** and **B**; Supplementary Fig. S2A and S2B). At baseline, higher $CD8^+$ and $CD4^+$ infiltration was seen in patients with subsequent disease control (CR+PR+SD, $n = 11$) compared with PD ($n = 10$). At Week 3 (after ONCOS-102 induction and prior to pembrolizumab), $CD8^+$ and $CD4^+$ tumor infiltration further increased and remained high at Week 9 in patients with disease control; this was not observed in patients with PD. Similar observations were seen with Granzyme B positive $CD8^+$ cells from baseline to Week 9 in patients with disease control versus PD ($P = 0.0051$; **Fig. 3C**; Supplementary Fig. S2C).

Whole transcriptome analysis, using total RNA sequencing of available biopsies, was performed in 17 patients (Supplementary Fig. S4). Overall, significant differences in the gene expression profiles were observed for patients with disease control compared with patients with progressive disease, particularly at baseline and at Week 9 (Supplementary Fig. S5A–S5C). Consistent with immunofluorescence histology analyses, tumors from patients with disease control had higher baseline T-cell infiltration based on CD3, CD4, CD6, and CD8 T-cell marker gene expression compared with PD (**Fig. 4A**). Expression of cytotoxicity genes such as perforin, Granzyme B, and other granzymes (Supplementary Fig. S6A), co-stimulatory genes such as 4–1BB, GITR, and CD27 (Supplementary Fig. S6B), and checkpoint inhibitors such as CTLA-4, LAG-3, and TIGIT (Supplementary Fig. S6C) were also generally more abundant at baseline in patients who achieved disease control versus PD. In all patients, expression of genes for T-cell markers, cytotoxicity, co-stimulatory molecules, and checkpoint inhibitors increased from baseline to Week 3. Patients with disease control showed further increases and persisting elevated expression of select immune-related genes at Week 9, whereas expression decreased below baseline for patients with PD (**Fig 4A**; Supplementary Fig. S6A–S6C). Similarly, modelling change in gene expression between patients with disease control and PD across time showed early increased expression of immune-related gene groups (**Fig. 4B**). Higher upregulation of some pathways, including cytotoxicity, was seen in patients with PD versus disease control at Week 3, suggesting a promising early response in most patients (**Fig. 4B**). Comparing Week 9 with baseline, patients with disease control maintained high expression of immune-related pathways, whereas PD was associated with declining levels (**Fig. 4B**), consistent with the immunofluorescence histology analysis.

Greater persistence of ONCOS-102 viral DNA in the tumor through Week 9 was observed with Part 2 versus Part 1 treatment regimen (**Fig. 4C**). In addition, expression of ONCOS-102-encoded GM-CSF mRNA peaked at Week 3 and was undetectable at Week 9 in patients with PD, whereas continuous expression of GM-CSF was seen in 3 of 5 patients with disease control (Supplementary Fig. S7A). This is consistent with overall high quantities of viral particles at Week 3 across patient groups and subsequent rapid clearance or less effective virus replication in patients with PD (Supplementary Fig. S7B).

In 13 patients with evaluable tumor biopsies, a statistically significant difference in geometric mean TMB was not detected in patients with disease control versus PD (7.3 mut/Mb vs. 5.7 mut/Mb; $P = 0.69$). Certain mutations of interest with known functions in PD-1 resistance were analyzed individually; notably, all patients with *B2M* somatic missense mutations ($n = 3$), indicating dysfunctional antigen presentation, did not respond to treatment (RECIST PD).

Discussion

Despite improved outcomes with first-line immune checkpoint inhibitors, a large proportion of patients with advanced melanoma ultimately do not benefit from this therapy due to primary or acquired resistance, and new treatment approaches are needed (27, 28). In this

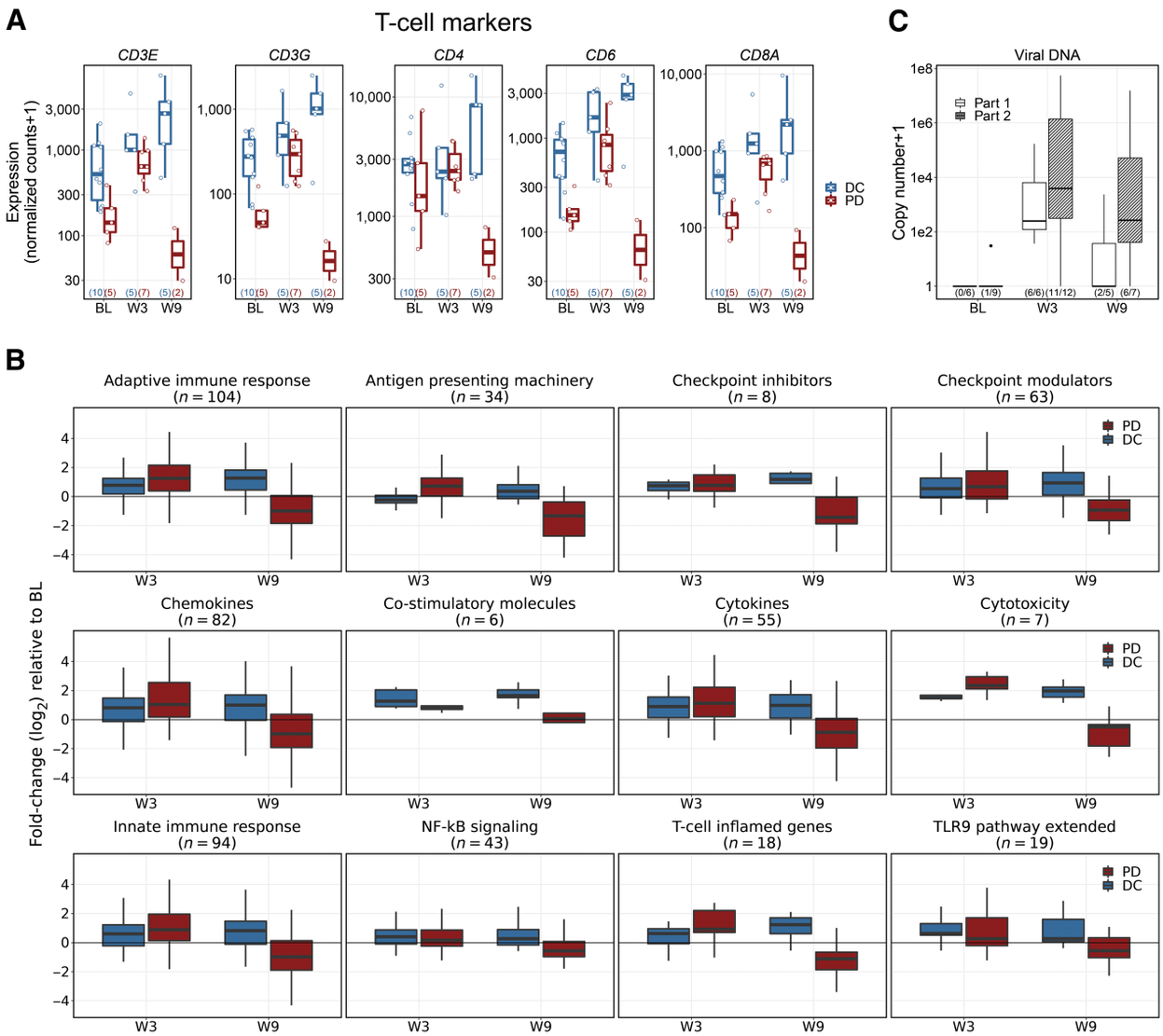

Figure 4.

Gene expression profiles according to best response to study treatment (RECIST v1.1), and viral load stratified by dosing regimen. **A,** Normalized (using DESeq2) expression for T-cell marker genes at baseline, Week 3, and Week 9 in patients with RECIST v1.1 disease control (CR, PR, or SD) and PD. **B,** Change in gene expression in select immunologic pathways (specified in Supplementary Data) from BL to Week 3 or Week 9 stratified by disease control and PD. Boxes show median, lower, and upper quartiles; whiskers show minimum and maximum values within the 1.5 interquartile range. Parentheses denote the number of patients with available biopsies (**A**) and number genes in each pathway (**B**). **C,** Detection of VPs by qPCR in tumor biopsies from patients stratified by dosing regimen [number of samples with detectable VP (numerator) and total number of samples (denominator) are noted in parentheses]; positive baseline VP (1 of 30 duplicate readings from 15 patients) shown in a Part 2 patient is likely a technical artifact. BL, baseline; qPCR, quantitative polymerase chain reaction; W, week; VP, viral particles.

pilot study, ONCOS-102 in combination with pembrolizumab was well tolerated across two dosing schedules [Part 1: ONCOS-102 priming (3 total doses) and sequential pembrolizumab; Part 2: ONCOS-102 priming and booster doses (12 total doses) in combination with pembrolizumab]. There were no DLTs and no safety concerns were identified that are likely to impact further development of this treatment combination. While the SAEs of diabetes mellitus and diabetic ketoacidosis, occurring in a single patient, were reported by the investigator to be related to both ONCOS-102 and pembrolizumab, given these are AEs known to be associated with pembrolizumab and occurred shortly after (13 days) the last dose of pembrolizumab (and 140 days after the last dose of ONCOS-102), the authors consider it most likely the event was attributable to pembrolizumab (29). Most TEAEs were mild or moderate in severity, and the most frequent ONCOS-102-related TEAEs (pyrexia, chills, and nausea) were broadly in line with the anticipated safety profile for an oncolytic adenovirus. Of note, the similar safety profile observed with both dosing schedules suggests no negative impact of extended ONCOS-102 dosing and concomitant administration with pembrolizumab.

Importantly, promising efficacy signals and biomarker data were observed in this relatively short 27-week study, including in patients who were refractory to prior anti–PD-1 and anti–CTLA-4 therapy

(either sequentially or in combination), suggesting ONCOS-102 plus pembrolizumab may have potential utility in this difficult-to-treat setting. While direct comparisons of studies with differing designs cannot be made, the ORR of 35% in patients with anti–PD-1–refractory advanced melanoma is promising in light of ORR observed in other studies of tumor injectable agents in this setting, including single-agent T-VEC (26%), Toll-like receptor 9 agonist (CMP-001) plus pembrolizumab (24%), and RP1 plus nivolumab (33%; refs. 30–32). It is likely that ONCOS-102 was a strong driver of the efficacy observed in the current study, given response is infrequently reported with single-agent anti–PD-(L)1 retreatment in patients with progression on prior checkpoint inhibitor therapy when applying accepted resistance criteria (33–35).

Responses were observed in both Part 1 (38%) and Part 2 (33%), which is notable given the higher disease burden and greater incidence of Stage IV disease in Part 2 patients. This suggests the ONCOS-102 priming and booster regimen in combination with pembrolizumab was likely the more effective regimen. There was evidence that local delivery of ONCOS-102 can stimulate systemic antitumor responses. Reduction in the size of non-injected targeted lesions was seen in more than half (53%) of evaluable patients, including six with ≥ 30% relative reduction in tumor size, corresponding to PR per response criteria for intratumoral immunotherapy (itRECIST; ref. 36). Overall, a higher frequency of non-injected target lesion shrinkage was seen with the Part 2 regimen. We also acknowledge that, within the confines of the study inclusion/exclusion criteria, investigator-based selection of patients in Part 1 likely favored lower disease burden because just 3 doses of ONCOS-102 were administered, and patients in Part 2 may better reflect the population of patients typically seen with anti–PD-1 resistant melanomas.

Reports in the first-line melanoma treatment setting suggest $CD8^+$ T-cell infiltration is associated with response to anti–PD-1 therapy, as was also noted with T-VEC (10, 14, 37, 38). Tumor microenvironment factors associated with response post-anti–PD-1 treatment are less well characterized and may depend on further modulation beyond $CD8^+$ T-cell infiltration. The clinical responses observed in patients with PD-1 resistant melanoma in this study enabled us to gain insights into potential mechanisms of viral-mediated response and resistance. Serial analyses of tumors injected with ONCOS-102 using RNA expression and immunofluorescence histology revealed correlation between the level and persistence of immune cell presence and activity and patient outcome. Our findings support the notion that disease control is driven by the ability of ONCOS-102 to sustain upregulation of multiple immune response-related genes at Week 3, prior to pembrolizumab treatment, through to Week 9. In contrast, immune gene upregulation initially observed in Week 3 tumor biopsies of patients with PD was not sustained and fell below baseline levels. Similarly, increased $CD4^+$ and $CD8^+$ T-cell infiltration by Week 3 was sustained through Week 9 in patient with disease control but not PD. Importantly, this study identifies that, in addition to the initial burst of ONCOS-102 mediated T-cell infiltration and inflammation by Week 3, persistence of infiltration at Week 9 is associated with clinical response. These findings suggest that, in contrast to studies in anti–PD-1-naïve settings, trials evaluating oncolytic virotherapy in patients resistant to PD-1 blockade should not solely rely on early onset of tumor microenvironment alterations to predict clinical response. Further study is required to understand why immune infiltration seen at Week 3 in some patient was not sustained to Week 9. Our preliminary findings suggest that some baseline tumor features, such as *B2M* alterations that can lead to dysfunctional antigen presentation, may be associated with progression (9).

Persistence of ONCOS-102 may be another factor associated with clinical benefit. Presence of ONCOS-102, evidenced by viral DNA copy numbers and ONCOS-derived GM-CSF expression, increased in all patients at Week 3, but then selectively declined in patients with PD. This suggests neutralization and clearance of ONCOS-102 is generally not observed in patients who derive clinical benefit. Increased dosing frequency may help maintain ONCOS-102 levels. Indeed, the 12 doses of ONCOS-102 given in Part 2 led to higher levels of viral genomes in tumor biopsies at Week 3 and Week 9 compared with Part 1, where only 3 doses were given. Persistence of ONCOS-102 in the tumor observed in this study likely reflects sustained viral replication, given the lytic nature of the adenovirus backbone (20, 21).

Findings from this pilot study warrant further investigation to provide insight into the immunologic activation and clinical benefit observed. Future studies will utilize the Part 2 (ONCOS-102 prime and booster combination therapy) regimen, given the efficacy, including in non-injected lesions, and durable persistence of ONCOS-102 in tumor biopsies with increased dosing frequency observed, along with the observation that improved outcomes are associated with persistence of immune response. The lack of DLTs in the current study also suggests higher doses of ONCOS-102 could be considered. Longer treatment duration and follow-up in a larger cohort of patients with anti–PD-1 resistant melanoma is also needed to inform the durability of clinical responses. CPO priming was administered based on data suggesting this may prepare the tumor microenvironment for effector T-cell expansion by depleting Tregs (39). However, peripheral blood samples obtained at screening (prior to CPO administration), Day 1, and Week 3 (and at later timepoints) revealed comparable levels of circulating Tregs (Supplementary Fig. S7C). Furthermore, the association between persistence of ONCOS-102 viral DNA and response to study treatment suggests it is unlikely the single-dose of CPO contributed to the efficacy observed. Consequently, and given the practical challenges associated with CPO administration, CPO priming will not be used in future studies of ONCOS-102.

In summary, this pilot study demonstrated that ONCOS-102 co-administered with pembrolizumab is well tolerated and provides meaningful clinical benefit for patients with advanced melanoma progressing on anti–PD-1 therapy. Comprehensive profiling of the tumor microenvironment revealed sustained ONCOS-102–induced immune cell infiltration correlating with clinical outcome. Together, these findings support further evaluation of ONCOS-102 in combination with anti–PD-1 therapy in larger studies with extended follow-up.

Authors' Disclosures

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Authors' Contributions

A.N. Shoushtari: Conceptualization, supervision, investigation, visualization, methodology, writing–original draft, writing–review and editing. **A.J. Olszanski:** Investigation, writing–review and editing. **M. Nyakas:** Investigation, writing–review and editing. **T.J. Hornyak:** Investigation, writing–review and editing. **J.D. Wolchok:** Conceptualization, investigation, writing–review and editing. **V. Levitsky:** Formal analysis, visualization, methodology, project administration, writing–review and editing. **L. Kuryk:** Data curation, project administration, writing–review and editing. **T.B. Hansen:** Data curation, software, formal analysis, visualization, writing–review and editing. **M. Jäderberg:** Conceptualization, data curation, supervision, funding acquisition, methodology, project administration, writing–review and editing.

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Note

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