


ONCOS-102 plus pemetrexed and platinum chemotherapy in malignant pleural mesothelioma: a randomized phase 2 study investigating clinical outcomes and the tumor microenvironment

Santiago Ponce,¹ Susana Cedrés,^{2,3} Charles Ricordel,⁴ Nicolas Isambert,⁵ Santiago Viteri,⁶ Mercedes Herrera-Juarez,¹ Alex Martinez-Marti,^{2,3} Alejandro Navarro,^{2,3} Mathieu Lederlin,⁷ Xavier Serres,^{2,3} Jon Zugazagoitia,^{1,8,9} Sylvia Vetrhus,¹⁰ Magnus Jaderberg,¹⁰ Thomas Birkballe Hansen ,¹⁰ Victor Levitsky,¹⁰ Luis Paz-Ares^{1,8,9}

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For numbered affiliations see end of article.

Correspondence to

Dr Thomas Birkballe Hansen; thomas.hansen@circio.com

ABSTRACT

Background ONCOS-102, an oncolytic adenovirus expressing granulocyte-macrophage colony-stimulating factor, can alter the tumor microenvironment to an immunostimulatory state. Combining ONCOS-102 with standard-of-care chemotherapy for malignant pleural mesothelioma (MPM) may improve treatment outcomes. **Methods** In this open-label, randomized study, patients with unresectable MPM received intratumoral ONCOS-102 (3×10^{11} virus particles on days 1, 4, 8, 36, 78, and 120) and pemetrexed plus cisplatin/carboplatin (from day 22), or pemetrexed plus cisplatin/carboplatin alone. The primary endpoint was safety. Overall survival (OS), progression-free survival, objective response rate, and tumor immunologic activation (baseline and day 36 biopsies) were also assessed.

Results In total, 31 patients (safety lead-in: n=6, randomized: n=25) were enrolled. Anemia (15.0% and 27.3%) and neutropenia (40.0% and 45.5%) were the most frequent grade ≥ 3 adverse events (AEs) in the ONCOS-102 (n=20) and chemotherapy-alone (n=11) cohorts. No patients discontinued ONCOS-102 due to AEs. No statistically significant difference in efficacy endpoints was observed. There was a numerical improvement in OS (30-month OS rate 34.1% vs 0; median OS 20.3 vs 13.5 months) with ONCOS-102 versus chemotherapy alone in chemotherapy-naïve patients (n=17). By day 36, ONCOS-102 was associated with increased T-cell infiltration and immune-related gene expression that was not observed in the control cohort. Substantial immune activation in the tumor microenvironment was associated with survival at month 18 in the ONCOS-102 cohort.

Conclusions ONCOS-102 plus pemetrexed and cisplatin/carboplatin was well tolerated by patients with MPM. In injected tumors, ONCOS-102 promoted a proinflammatory environment, including T-cell infiltration, which showed association with survival at month 18.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Many patients with malignant pleural mesothelioma (MPM) are not eligible for immune checkpoint inhibitors or have disease that is refractory or progresses over time and alternate treatment approaches are needed. Oncolytic viruses such as ONCOS-102 replicate within cancer cells and alter the tumor microenvironment from an immunosuppressive to an inflammatory state.

WHAT THIS STUDY ADDS

⇒ This is the first study to investigate the addition of intratumoral therapy with an oncolytic virus to standard-of care chemotherapy (pemetrexed plus cisplatin/carboplatin) in MPM. ONCOS-102 was well tolerated, and increased T-cell infiltration and proinflammatory gene expression which was not observed with chemotherapy alone. This immune activation was associated with a survival advantage.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This early study suggests intratumoral ONCOS-102, co-administered with pemetrexed plus platinum chemotherapy, has the potential to offer an alternate immune-based treatment approach for MPM.

INTRODUCTION

Malignant pleural mesothelioma (MPM) is an aggressive disease associated with asbestos exposure; its incidence is rising in some countries despite global efforts to reduce asbestos use. This is largely due to the long latency period (approximately 40 years) between asbestos exposure and disease

presentation.^{1–3} MPM patients have a poor prognosis: 5-year survival rates of 5%–10% are reported with standard chemotherapy.^{4,5} Addition of bevacizumab to first-line chemotherapy improved overall survival (OS) by approximately 2 months with manageable adverse events (AEs), while tumor-treating fields (TTF, non-invasive delivery of alternating electric fields to tumors) in combination with platinum-based chemotherapy demonstrated activity in a phase 2 study of epithelioid MPM and was approved by the US Food and Drug Administration.^{6–9} However, bevacizumab and TTF are not routinely used in many countries due to cost and accessibility.

Chronic inflammation is observed in response to asbestos fiber deposits in the lung, with the immune system playing a key role in MPM initiation and progression.^{1,10} Several studies indicate immunosuppression in MPM tumors, and an association between expression of the T-cell inhibitory protein, programmed death-ligand 1 (PD-L1), and poor prognosis, providing rationale for using immunotherapy in this setting.^{10–12} Indeed, immune checkpoint inhibitors have provided a new standard of care for unresectable MPM.⁶ In the first-line setting, a 4-month OS advantage was seen with nivolumab plus ipilimumab versus pemetrexed plus platinum agent in the overall patient population of the phase 3 CheckMate 743 study.^{1,13} In the CONFIRM study, single-agent nivolumab improved OS by approximately 3 months versus placebo in MPM patients in whom first-line chemotherapy has failed.¹⁴ However, many patients are refractory to checkpoint inhibitor therapy or progress over time (only 14% of patients receiving first-line nivolumab plus ipilimumab in CheckMate 743 were progression free at 3 years¹⁵) or are not eligible for this treatment. Consequently, new treatment approaches are needed.

Oncolytic viruses offer an alternate immune-based approach for treating cancer. Delivered via intratumoral injection, they replicate specifically in tumor cells leading to direct lysis, and alter the tumor microenvironment from an immunosuppressive to an inflammatory state, thereby facilitating antitumor T-cell responses.¹⁶ Talimogene laherparepvec, a genetically engineered herpes simplex virus 1, was the first oncolytic viral therapy approved in Europe and the USA, and is indicated for local treatment of unresectable melanoma. Several oncolytic viruses are currently in clinical trials across a range of solid tumors.^{16,17}

ONCOS-102 (Ad5/3-D24-GM-CSF) is a chimeric oncolytic adenovirus engineered to express human granulocyte-macrophage colony-stimulating factor (GM-CSF), a potent inducer of antitumor immunity.¹⁸ Adenoviruses are primarily lytic and contain a limited number of genes for immune evasion, and some studies suggest adenovirus-based oncolytic viruses have superior immune activation compared with herpes simplex virus, vaccinia virus, and reovirus.^{19–21} In preclinical studies, ONCOS-102 induced immunogenic cell death in human mesothelioma cell lines.²² Furthermore, ONCOS-102 demonstrated antitumor activity in a mouse xenograft

model of treatment-refractory MPM: while pemetrexed plus platinum chemotherapy did not reduce tumor growth, a synergistic antitumor effect was seen with ONCOS-102 as combination therapy, including complete tumor regression in some animals.²² In a phase I dose-escalation study, ONCOS-102 monotherapy induced innate and adaptive immune responses in patients with treatment-refractory solid tumors.¹⁸ A dose–response relationship was noted between tumor infiltrating immune cells, including CD8+T cells and CD68+ macrophages, and OS, supporting further investigation of ONCOS-102 at the highest investigated dose (3×10^{11} virus particles).¹⁸

In this study, we investigated the potential utility of ONCOS-102 in combination with standard-of-care chemotherapy in MPM. The safety, efficacy, and treatment-associated immune activation of ONCOS-102 with pemetrexed plus a platinum agent were investigated in patients with unresectable MPM.

METHODS

Study design and patient population

This was an open-label, multicenter, parallel arm, two-part study (EudraCT number 2015-0051430-13). Patients with histologically confirmed, unresectable, advanced MPM were enrolled between June 17, 2016 and April 17, 2019 at study sites in Spain and France. All patients were ≥ 18 years, had measurable disease (per RECIST V.1.1), tumor accessible to intratumoral injections, and were eligible for chemotherapy (prior chemotherapy was permitted). Other inclusion criteria were Eastern Cooperative Oncology Group Performance Status of 0 or 1, and adequate liver, renal, and hematological function. Key exclusion criteria included prior oncolytic virus treatment or vaccination containing live virus ≤ 4 weeks prior to study treatment; significant immunosuppressive medication ≤ 4 weeks prior to study treatment; active bacterial, viral, or fungal infections requiring systemic therapy; history of severe cardiac disease; and known brain metastases.

The study schema is shown in [figure 1](#). Patients in the phase Ib safety lead-in cohort received a single priming dose of cyclophosphamide (CPO) 300 mg/m² intravenously 1–3 days prior to starting ONCOS-102. ONCOS-102 (3×10^{11} virus particles in 2.5 mL) could be administered in 1–5 separate lesions (≥ 0.5 mL per lesion). Intratumoral injections were performed by a radiologist under CT and/or ultrasound guidance. Patients received an ONCOS-102 priming cycle, comprising injections on days 1, 4, 8, and 36, followed by two further treatments at 6-week intervals (days 78 and 120; a second CPO dose was administered 1–3 days prior to day 78). Patients also received pemetrexed (500 mg/m²) plus cisplatin (75 mg/m²) or carboplatin (area under the concentration–time curve [AUC] 5, following a protocol amendment) in 21-day cycles, starting on day 22 for up to six cycles, plus folic acid, vitamin B₁₂, and dexamethasone per local practices. The phase II randomized cohort was enrolled

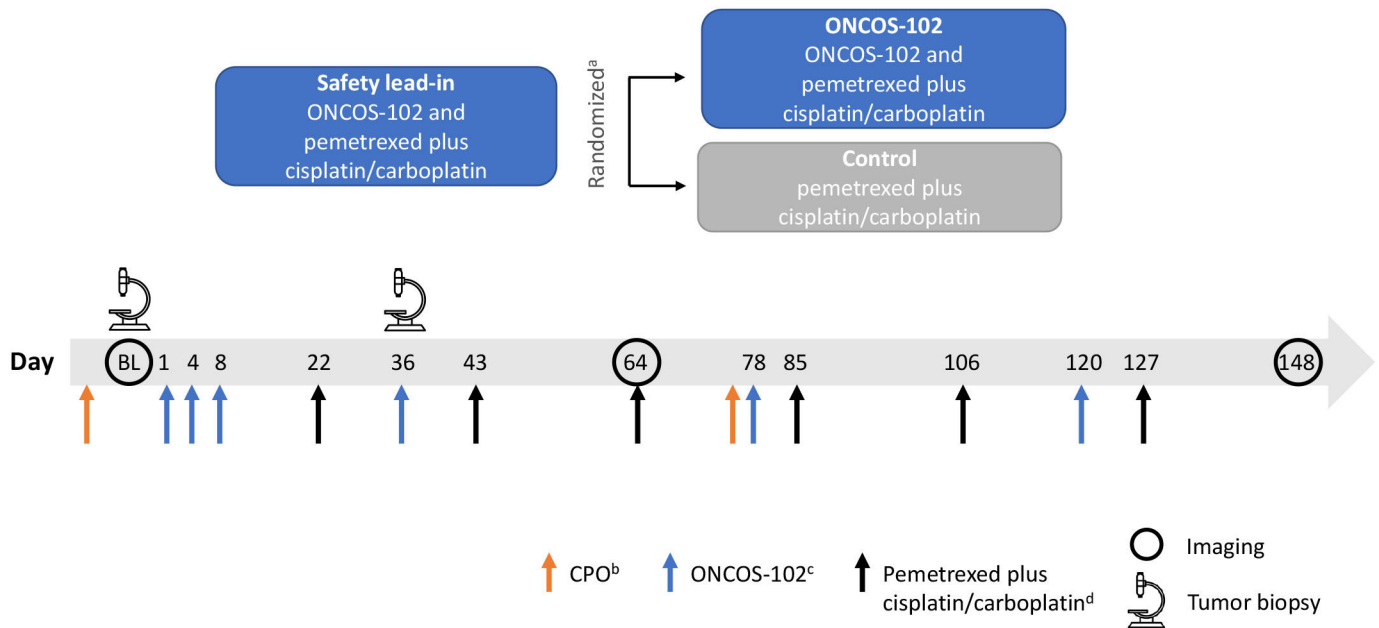


Figure 1 Study design. ^aStratified by prior chemotherapy (naïve/non-naïve). ^bCPO 300 mg/m² was administered 1–3 days prior to ONCOS-102 (days –3 to –1 and days 75 to 77). ^cSix intratumoral injections (days 1, 4, 8, 36, 78, 120) containing 3×10¹¹ virus particles in 2.5 mL across 1–5 lesions (≥0.5 mL/lesion). ^dPemetrexed (500 mg/m²) with cisplatin (75 mg/m²) or carboplatin (AUC 5, following a protocol amendment) administered in 21-day cycles starting on day 22 for up to six cycles, along with folic acid, vitamin B₁₂, and dexamethasone in accordance with local practices. Blood samples for cytokine and other analyses were obtained prior to study medication on days 1, 43, 85, and 127 in the chemotherapy-alone cohort and on days 1, 4, 8, 36, 78, and 120 in the ONCOS-102 randomized cohort, and at predose and postdose in the phase Ib safety lead-in cohort. Imaging time points are shown for ONCOS-102 cohort. Tumor imaging was performed at baseline, weeks 6 and 18 in the control group. AUC, area under the concentration–time curve; BL, baseline, CPO, cyclophosphamide.

following the data safety monitoring board review of data from the safety lead-in cohort. Patients were enrolled and assigned to study treatment by the study investigators using randomly permuted blocks generated by the study sponsor via a central web-based system, stratified by prior chemotherapy (naïve or non-naïve). Patients received either the same treatment schedule as the lead-in safety cohort or pemetrexed plus cisplatin/carboplatin alone in 21-day cycles (control group). ONCOS-102 dose reduction was not permitted. Dose reduction of pemetrexed, cisplatin/carboplatin was permitted if deemed necessary by the investigator in accordance with local practices.

Study objectives and assessments

The primary objective was to assess the safety of ONCOS-102 in combination with pemetrexed plus cisplatin/carboplatin. Evaluation of efficacy, tumor-specific immunological activation, and correlation between immunological activation and clinical outcome were secondary objectives.

Safety was assessed throughout the study by AEs, graded per National Cancer Institute Common Terminology Criteria for Adverse Events V.4.0, vital signs, physical examination, and laboratory assessments. Tumor evaluations using CT were performed at baseline, weeks 9 and 21 (ONCOS-102-treated patients) or weeks 6 and 18 (control group). Objective response rate (ORR) was defined as the proportion of patients with a best response of complete response (CR) or partial response (PR) per

RECIST V.1.1, or immune-related CR or PR per irRECIST at any time. Progression-free survival (PFS; time from start of study treatment to first documented disease progression or death) and OS (time from start of study treatment to death of any cause) were also assessed.

Pharmacodynamic immunological activation was investigated in tumor biopsies obtained at baseline (day 1, prior to the first injection of ONCOS-102) and day 36 (during cycle 1 of chemotherapy (all patients) and following three priming dose of ONCOS-102 (ONCOS-102-treated patients)). Using formalin-fixed paraffin-embedded tumor specimens, multiplex immunofluorescence histology included detection of CD4+ and CD8+ (including Granzyme B+ cells) and M1:M2 macrophage ratio, calculated from whole-slide scanning. RNA, extracted from flash frozen tumor biopsies, was sequenced using NovaSeq 6000 Systems (Illumina, by Personalis, California, USA) and processed in-house (see online supplemental methods for further details).

Statistical analysis

Formal sample size calculations were not performed for this exploratory study: 6 and 24 (n=14 ONCOS-102 plus chemotherapy, n=10 chemotherapy alone) patients were planned for the phase Ib safety lead-in and phase II cohorts, respectively. Safety and efficacy are reported in patients who received any dose of ONCOS-102 and pemetrexed plus cisplatin/carboplatin (intention-to-treat

(ITT) population). Immune endpoints were analysed in the ITT population with repeat tumor biopsy assessments.

Pooled analysis of patients who received ONCOS-102 plus chemotherapy in the phase Ib safety lead-in and phase II cohorts was performed (ONCOS-102 group). All statistical analyses were exploratory. PFS and OS (all patients were followed to death or a minimum of 30 months) were assessed using Kaplan-Meier methodology and compared between treatment groups using log-rank tests. ORR was compared between treatment groups using Fisher's exact test. Correlative marker expression was assessed using an additive quasibinomial generalized linear model for fractional (percentage) data or using linear regression on log-transformed values for relative data (value ratios) on each group (eg, chemotherapy-alone and ONCOS-102 plus chemotherapy) separately with time point and individual patient as covariates. Other data were summarized using descriptive statistics. Statistical analyses were conducted in R (V.4.1.2) using RStudio.

RESULTS

Study population

In total, 31 patients were enrolled across the safety lead-in cohort (n=6 received ONCOS-102 plus chemotherapy) and randomized phase II cohort (n=25: (ONCOS-102 plus chemotherapy n=14, chemotherapy alone n=11)). Study treatment was completed by 14 (70.0%) and 6

patients (54.5%) in the ONCOS-102 and chemotherapy-alone groups, respectively. Disease progression (n=7) was the most frequent reason for discontinuation (figure 2). Of the 20 patients who completed the study, subsequent anticancer treatments included chemotherapy (n=18), immunotherapy (n=6), radiotherapy (n=5), and thoracotomy (n=1).

Baseline demographic characteristics were broadly comparable across the treatment groups, and most individuals had epithelioid histology (77.4%) (table 1). Approximately half the patients (n=14 (45.2%) had received prior pemetrexed-platinum-based chemotherapy (n=14, 45.2%). Mean tumor diameter by RECIST V.1.1 was 87.3 mm vs 46.4 mm in patients in the ONCOS-102 and chemotherapy-alone groups, respectively, and most patients (90.0% vs 72.7%, respectively) had disease stage III/IV (table 1).

Exposure to study treatment

On day 1, ONCOS-102 was injected into one tumor lesion in 18 (90.0%) patients and two sites in two patients (10.0%). All patients also received ONCOS-102 on days 4, and 8, while 19 (95.0%), 15 (75.0%), and 13 (65.0%) patients received intratumoral injections on days 36, 78, and 120, respectively. Patients received a median (range) of 6 (3–10) ONCOS-102 injections across all treated lesions.

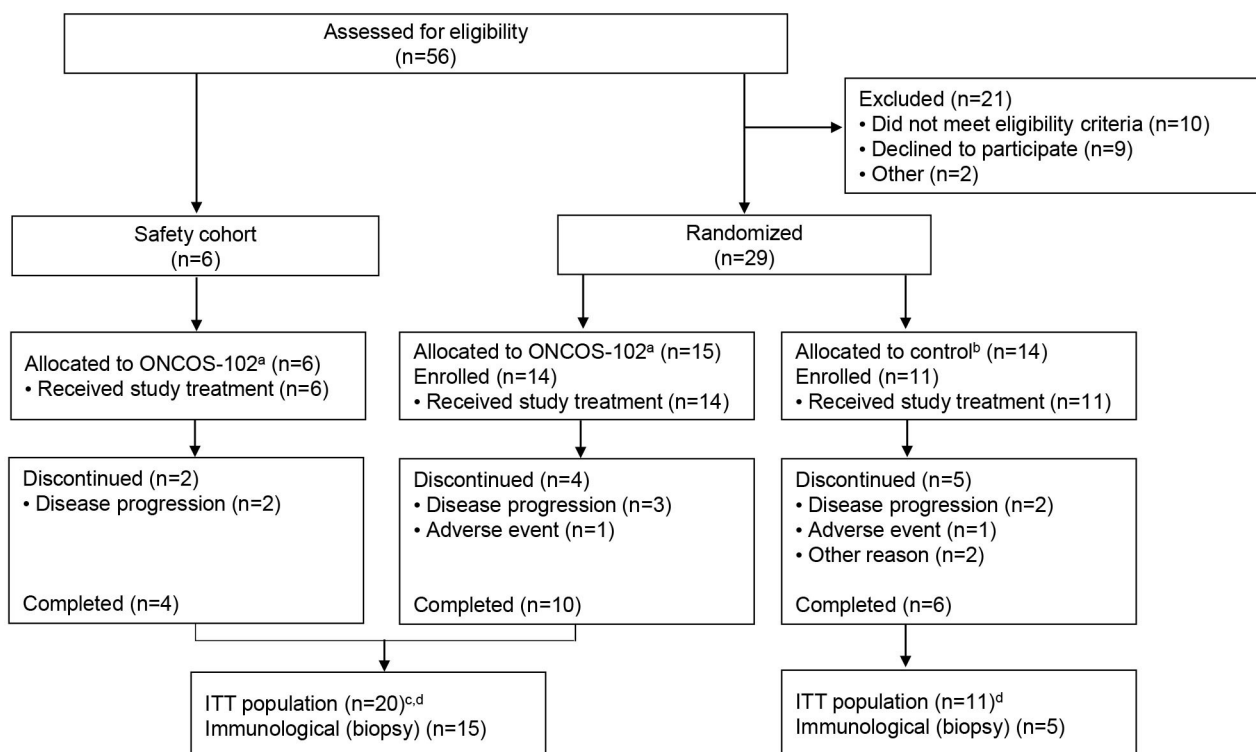


Figure 2 Patient disposition. ^aAllocated to ONCOS-102 (3×10^{11} virus particles in 2.5 mL) with pemetrexed (500 mg/m²) in combination with cisplatin (75 mg/m²) or carboplatin (AUC 5) in 21-day cycles. ^bRandomized to pemetrexed (500 mg/m²) in combination with cisplatin (75 mg/m²) or carboplatin (AUC 5) in 21-day cycles. ^cData were pooled from safety cohort and ONCOS-102 randomized cohort. ^dAs all enrolled patients received study treatment, the ITT population was used to summarize efficacy and safety outcomes. AUC, area under the concentration–time curve; ITT, intention to treat.

Table 1 Patient characteristics at baseline (ITT population)

	ONCOS-102 plus chemotherapy (N=20)	Chemotherapy alone (N=11)	Total (N=31)
Median age, years (range)	66 (36–80)	68 (61–75)	68 (36–80)
Sex (female/male), n (%)	14 (70.0)/6 (30.0)	8 (72.7)/3 (27.3)	22 (71.0)/9 (29.0)
ECOG performance status, n (%)			
0	6 (30.0)	2 (18.2)	8 (25.8)
1	14 (70.0)	9 (81.8)	23 (74.2)
No of lesions, median (range)*	4 (2–9)	4 (1–6)	4 (1–9)
Tumor diameter, mm (RECIST V.1.1), mean (SD)	87.3 (42.7)	46.4 (27.7)	N/A
Disease stage†, n (%)			
I	0	1 (9.1)	1 (3.2)
IA	1 (5.0)	0	1 (3.2)
II	1 (5.0)	2 (18.2)	3 (9.7)
III	6 (30.0)	3 (27.3)	9 (29.0)
IV	12 (60.0)	5 (45.5)	17 (54.8)
Histological type, n (%)			
Epithelioid	16 (80.0)	8 (72.7)	24 (77.4)
Sarcomatoid	4 (20.0)	2 (18.2)	6 (19.4)
Other	0	1 (9.1)	1 (3.2)
Lesion location, n (%)			
Bone	1 (5.0)	0	1 (3.2)
Liver	1 (5.0)	0	1 (3.2)
Lung	5 (25.0)	5 (45.5)	10 (32.3)
Lymph nodes	11 (55.0)	5 (45.5)	16 (51.6)
Mediastinum	3 (15.0)	3 (27.3)	6 (19.4)
Pleura	18 (90.0)	10 (90.0)	28 (90.3)
Other	6 (30.0)	2 (18.2)	8 (25.8)
Prior cancer treatment‡, n (%)			
Surgery	8 (40.0)	2 (18.2)	10 (32.3)
Radiotherapy	6 (30.0)	4 (36.4)	10 (32.3)
Chemotherapy§	9 (45.0)	5 (45.5)	14 (45.2)
Other	1 (5.0)	0	1 (3.2)

*Observed on diagnostic CT scan.

†At enrolment.

‡Patients may report multiple prior cancer treatments.

§All patients with prior chemotherapy had received pemetrexed plus platinum regimen.

ECOG, Eastern Cooperative Oncology Group; ITT, intention to treat; N/A, not available; RECIST, Response Evaluation Criteria In Solid Tumors.

Patients who started chemotherapy (one patient in the ONCOS-102 group discontinued prior to day 22) received a median of 6 (range 1–6) cycles of pemetrexed. Most (n=29 (93.5%)) received cisplatin (median (range) 5 (1–6) cycles) and, following a protocol amendment, n=12 (38.7%) went on to receive carboplatin (3.5 (1–6) cycles) due to cisplatin toxicity or medical decision. One patient received carboplatin for six cycles (without prior cisplatin). All patients received CPO treatment per the study protocol.

Safety

Hematological events (blood and lymphatics System Organ Class) were the most frequent treatment-emergent AEs (TEAEs) in the ONCOS-102 (100%) and chemotherapy-alone (90.9%) groups and included anemia (75.0% and 90.9%) and neutropenia (70.0% and 72.7%); there were no cases of febrile neutropenia. The most frequent non-hematological TEAEs were asthenia (85.0% vs 63.6%), fever (75.0% vs 18.2%), nausea (75.0% vs 45.5%) and decreased

Table 2 Frequent all-causality adverse events ($\geq 10\%$ patients in either treatment group; safety population)

Preferred term n (%)	ONCOS-102 plus chemotherapy (N=20)			Chemotherapy alone (N=11)		
	All grade	Grade 3	Grade 4	All grade	Grade 3	Grade 4
Hematological events						
Anemia	15 (75.0)	3 (15.0)	0	10 (90.9)	3 (27.3)	0
Lymphopenia	4 (20.0)	1 (5.0)	0	1 (9.1)	0	0
Neutropenia	14 (70.0)	5 (25.0)	3 (15.0)	8 (72.7)	4 (36.4)	1 (9.1)*
Thrombocytopenia	6 (30.0)	3 (15.0)	2 (10.0)†	5 (45.5)	1 (9.1)	0
Non-hematological events						
Abdominal pain	3 (15.0)	0	0	0	0	0
Acute kidney injury	3 (15.0)	0	0	1 (9.1)	0	0
ALT increased	1 (5.0)	0	0	2 (18.2)	0	0
Asthenia	17 (85.0)	2 (10.0)	0	7 (63.6)	1 (9.1)	0
Chest pain	6 (30.0)	1 (5.0)	0	3 (27.3)	0	0
Constipation	5 (25.0)	0	0	4 (36.4)	0	0
Cough	6 (30.0)	0	0	1 (9.1)	0	0
C-reactive protein increased	7 (35.0)	0	0	4 (36.4)	0	0
Decreased appetite	7 (35.0)	1 (5.0)	0	6 (54.5)	0	0
Depressed mood	1 (5.0)	0	0	2 (18.2)	0	0
Diarrhea	5 (25.0)	0	0	3 (27.3)	0	0
Dyspnea	3 (15.0)	2 (10.0)	0	2 (18.2)	0	0
Fever	15 (75.0)	1 (5.0)	0	2 (18.2)	0	0
Injection site pain	4 (20.0)	0	0	0	0	0
Musculoskeletal chest pain	5 (25.0)	0	0	0	0	0
Nausea	15 (75.0)	1 (5.0)	0	5 (45.5)	0	0
Non-cardiac chest pain	0	0	0	2 (18.2)	0	0
Pain	4 (20.0)	0	0	0	0	0
Rash	1 (5.0)	0	0	2 (18.2)	0	0
Respiratory tract infection	3 (15.0)	2 (10.0)	0	0	0	0
Tinnitus	1 (5.0)	0	0	2 (18.2)	0	0
Vomiting	10 (50.0)	0	0	2 (18.2)	1 (9.1)	0

*One patient experienced two events of grade 4 neutropenia that were considered related to chemotherapy only.

†An event of grade 4 sepsis was also experienced by a patient with grade 4 thrombocytopenia.
ALT, alanine transaminase.

appetite (35.0% vs 54.5%) (table 2). Fever (75.0%) was the most frequent TEAE considered related to ONCOS-102 (alone or in combination with CPO). Other ONCOS-102-related TEAEs ($\geq 15\%$ of patients) included increased C-reactive protein (35.0%), vomiting (25.0%), asthenia (20.0%) and nausea (15.0%). All ONCOS-102-related AEs were mild/moderate (grade 1 or 2) in severity, except for one event (grade 3 fever).

Anemia (15.0% and 27.3%) and neutropenia (25.0% vs 36.4%) were the most frequent grade 3 TEAEs in the ONCOS-102 and chemotherapy-alone groups (table 2). Most grade 4 TEAEs were also hematological (ONCOS-102, neutropenia (three events),

thrombocytopenia (two events), sepsis (one event); chemotherapy-alone, n=1: neutropenia (two events)). All grade 4 AEs were considered related to chemotherapy except for one event of neutropenia (considered related to ONCOS-102 and chemotherapy).

Overall, 15 patients reported 21 serious AEs (SAEs). One SAE (grade 2 fever) was considered related to ONCOS-102, while across 4 patients and 2 patients in the ONCOS-102 and chemotherapy-alone groups, respectively, 9 SAEs (anemia, thrombocytopenia, vomiting (2 events, each), drug intolerance, sepsis, and acute kidney injury (1 event, each)) were considered related to chemotherapy. No patients discontinued ONCOS-102 due to TEAEs, and two patients

discontinued or switched chemotherapy due to TEAEs (grade 2 unspecified gastrointestinal disorder; general physical health deterioration) considered related to chemotherapy.

Twelve patients died during study treatment. Eleven deaths were due to disease progression (ONCOS-102 n=7, chemotherapy-alone n=4). One patient (with a history of cardiac disease) in the ONCOS-102 group died due to cardiac failure that was considered unrelated to ONCOS-102.

Efficacy

In the pooled population (n=31) 7 patients (ONCOS-102 n=5, chemotherapy alone n=2) were alive at 30-month follow-up, 30-month OS rate was 34.3% vs 18.2% for the ONCOS-102 versus chemotherapy-alone cohorts, median OS was 16.6 vs 18.3 months, respectively ($p>0.05$; [figure 3A](#)), and median PFS was 8.5 vs 8.3 months, respectively ($p>0.05$; [figure 3B](#)).

While the exploratory analyses were not statistically significant, there was a numerical trend for an impact of ONCOS-102 on survival in chemotherapy-naïve patients (n=17): 30-month OS rate was 34.1% vs 0%, median OS was 20.3 vs 13.5 months ([figure 3C](#)), and median PFS: 8.9 vs 7.6 months ([figure 3D](#)) in the ONCOS-102 versus chemotherapy-alone cohorts. More chemotherapy-naïve patients in the ONCOS-102 vs chemotherapy-alone cohort had OS exceeding 2 years (n=5 vs n=0), with PFS continuing beyond Month 46 (last recorded assessment) in one ONCOS-102-treated patient (online supplemental figure 1). In patients with epithelioid histology (n=24) OS (median (95% CI)) was 25.0 (10.4 to NA) vs 21.1 (3.0 to NA) months, and PFS (median (95% CI)) was 8.6 (2.0 to 13.4) vs 8.7 months (1.5, 15.2), in the ONCOS-102 versus chemotherapy-alone groups.

Four patients in the ONCOS-102 group (21.1%) and 5 patients in the chemotherapy-alone group (45.5%) had an objective response per RECIST V.1.1. Disease control rate (CR, PR, or SD) was 78.9% (n=15) and 90.9% (n=10) in the ONCOS-102 and chemotherapy-alone groups, respectively per RECIST V.1.1, and 84.2% (n=16) and 90.9% (n=10) per irRECIST. While ORR (RECIST V.1.1) was comparable in chemotherapy-naïve patients in the ONCOS-102 versus chemotherapy-alone cohorts (27.3 vs 33.3%), there was a numerical trend for lower ORR in the ONCOS-102 vs chemotherapy-alone arm for the subgroup of patients who were retreated with pemetrexed plus platinum chemotherapy (11.1% vs 60.0%).

Pharmacodynamic correlative analysis

Tumor infiltration of CD4+ ($p=0.021$), CD8+ ($p=0.022$), Granzyme B-expressing CD8+Tcells ($p=0.006$), CD8+regulatory T-cell (Treg) ratio ($p=0.003$), and M1:M2 macrophage polarization ($p=0.016$) increased from baseline to Day 36 in biopsies from the ONCOS-102 group (n=16), which was not observed in the chemotherapy-alone group (n=5, [figure 4A–E](#)). Differences in immunological pathway gene expression from baseline to day 36 between the treatment groups were less pronounced, except for elevated cytotoxicity

genes expression with ONCOS-102 ([figure 4F](#)). Elevated gene expression of *CD3E*, *CD4*, and *CD8A* at day 36 compared with baseline was also selectively seen in the ONCOS-102 cohort ([figure 4G](#)). Further expression analysis of selected genes at baseline to day 36 revealed minor differences, including elevations in some cytotoxicity genes with ONCOS-102 versus chemotherapy-alone (online supplemental figure 2). Overall, minimal changes in the prevalence of transcriptomes denoting CD4+ and CD8+ cell subtypes from baseline to day 36 were observed in the chemotherapy-alone group, while several patients in the ONCOS-102 group experienced marked elevations over time ([figure 4H](#)).

In the ONCOS-102 group, circulating GM-CSF at days 4 ($p<0.001$) and 8 ($p<0.01$) was significantly higher than on day 1, prior to ONCOS-102 treatment. Modest (non-significant) increase over time in circulating interferon-gamma was observed with ONCOS-102, along with a tendency for higher levels of interferon-alpha-2 on days 4 and 8, while cytokines levels generally were constant over time in the chemotherapy-alone group (online supplemental figure 3). Temporal modulation of other cytokine levels was not observed in either cohort.

Within the ONCOS-102 group, increased immune cell infiltration from baseline to day 36 was associated with survival at month 18. At day 36, patients who were alive at month 18 had greater tumor infiltration by CD4+ ($p=0.001$), CD8+ ($p=0.037$), Granzyme-B expressing CD8+Tcells ($p=0.003$), and increased CD8+regulatory T-cell (Treg) ratio ($p=0.029$) and increased macrophage polarization toward M1 phenotype ($p=0.024$), which was not observed in patients who had died by month 18 ([figure 5A–E](#)). Differential immunological pathways gene expression at day 36 relative to baseline was also seen in ONCOS-102-treated patients stratified by survival, including increased cytotoxicity gene expression in patients who were alive at month 18 ([figure 5F](#)). Nominal increased expression of other immunological pathways genes over time was also seen in month 18 survivors only, including checkpoint inhibitor genes and T-cell inflamed genes ([figure 5F](#)), *CD3E*, *CD4*, and *CD8A* ([figure 5G](#)). Expression analysis of other selected genes at baseline and day 36 revealed small differences in the ONCOS-102-treated patients stratified by survival at month 18, including elevations in some cytotoxicity genes, costimulatory genes, and checkpoint inhibitor genes in patients who remained alive (online supplemental figure 4). Marked increases from baseline to day 36 in transcriptomes denoting CD4+ and CD8+ cell subtypes were also observed in month 18 survivors only ([figure 5H](#)), as well as higher levels of GM-CSF on day 4 and day 8, following one and three doses of ONCOS-102, respectively ([figure 5I](#)).

Transcriptome analysis in the ONCOS-102 cohort, stratified by month 18 survival, showed marked differences in tumor gene expression at baseline (online supplemental figure 5A,B), including genes associated with complement activation (classical pathway), humoral immune response, and regulation of acute inflammatory response (online supplemental figure 5C). Differences in gene expression between survival groups were even more pronounced at day 36 (online supplemental figure 5D,E) and included adaptive

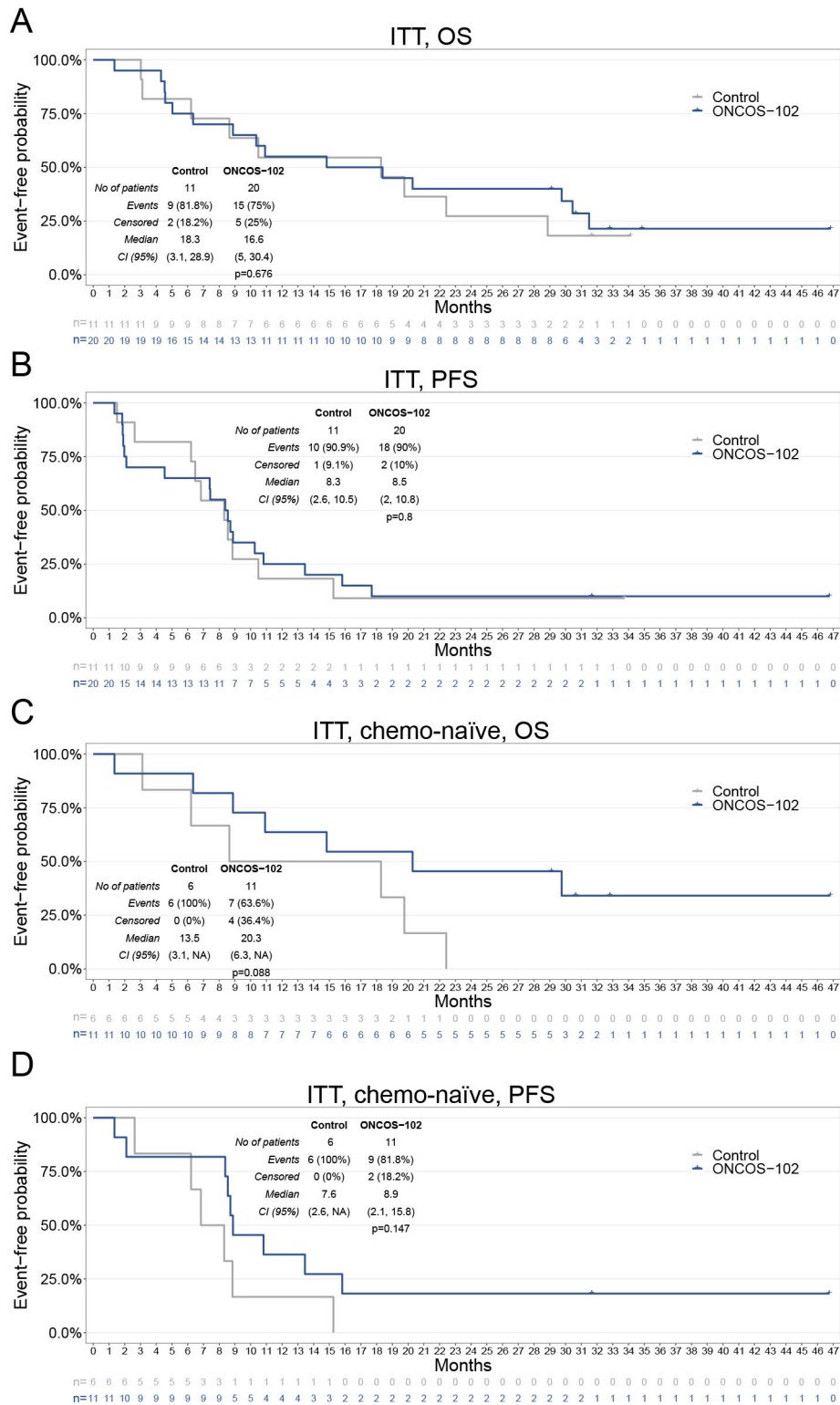


Figure 3 Overall survival and progression-free survival (ITT population). Kaplan-Meier probability curves of OS (A, C) and PFS (B, D) in relation to treatment regimen for the pooled population (n=31) and chemotherapy-naïve subgroup (n=17). Censored values (+) indicate the last follow-up time for patients who were alive after study completion. P values were obtained by log-rank tests. Control is pemetrexed (500 mg/m²) in combination with cisplatin (75 mg/m²) or carboplatin (AUC 5). ONCOS-102 is ONCOS-102 (3×10¹¹ virus particles in 2.5 mL) with pemetrexed (500 mg/m²) in combination with cisplatin (75 mg/m²) or carboplatin (AUC 5). ITT, intention to treat; NA, not assessable; PFS, progression-free survival; OS, overall survival.

immune response, T-cell activation, and leukocyte migration genes (online supplemental figure 5F). Using a previously described predictive inflammatory gene signature for

checkpoint inhibitor treatment in mesothelioma¹⁵ (online supplemental figure 6A), a binomial model assessing baseline transcriptome levels demonstrated 89% accuracy (16

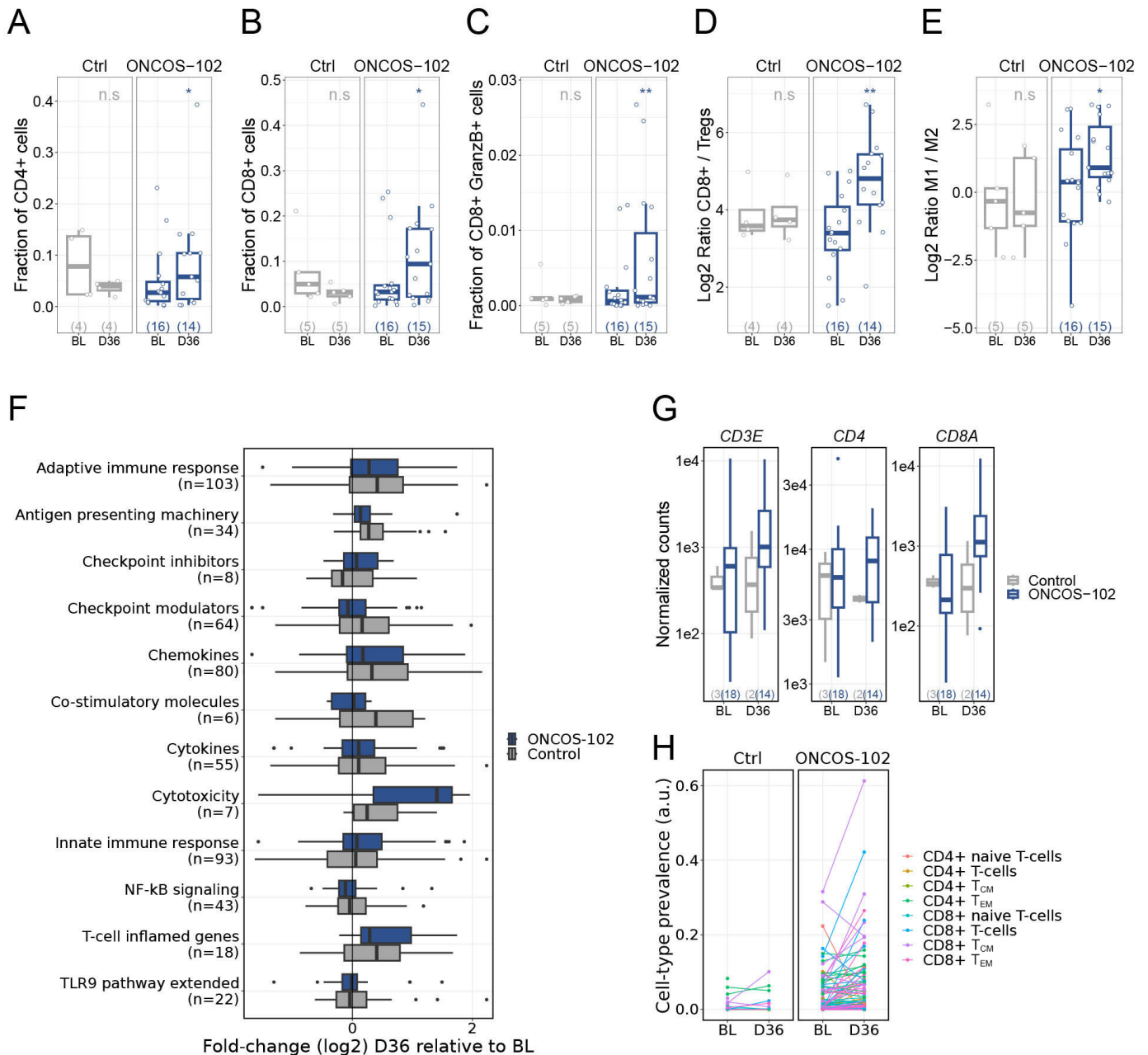


Figure 4 Change in tumor immune cell profile over time. Immunofluorescence histology for CD4+ (A), CD8+ (B), GranzymeB-positive CD8+ cells (C), CD8+:Treg ratio (D), and M1:M2 (E) for the chemotherapy-alone (left panel) and ONCOS-102 (right panel) groups at baseline and day 36. Boxes show median, lower, and upper quartiles, whiskers show minimum and maximum values within the 1.5 IQR, individual points denote outliers, and values in parentheses denote patients with available samples. P values are derived from quasi-binomial generalized linear model (A–C) or linear regression (D, E). (F) Change in gene expression in select immunological pathways (specified in online supplemental data) from day 36 relative to baseline, stratified by study treatment (number of genes in each pathway are denoted in parentheses). (G) Normalized (using DESeq2) expression of T-cell marker genes at baseline and day 36 stratified by study treatment (values in parentheses denote patients with available samples). (H) Deconvolution of cell-type abundance from transcriptome expression profiles for patients in the chemotherapy-alone (left panel) and ONCOS-102 (right panel) cohorts at baseline and day 36 (colors reflects subsets of CD4+ and CD8+ T-cells). Control is pemetrexed (500 mg/m²) in combination with cisplatin (75 mg/m²) or carboplatin (AUC 5). ONCOS-102 is ONCOS-102 (3×10¹¹ virus particles in 2.5 mL) with pemetrexed (500 mg/m²) in combination with cisplatin (75 mg/m²) or carboplatin (AUC 5). *p≤0.05; **p≤0.01. AUC, area under the concentration–time curve; BL, baseline; D, study day, GM-CSF, granulocyte-macrophage colony stimulating factor; IQR, interquartile range; M, macrophage; T_{CM}, central memory T-cells; T_{EM}, effector memory T-cells; TLR9, toll-like receptor 9; Treg, regulatory T-cells.

of 18 outcomes predicted, online supplemental figure 6B) and an AUC of 0.988 (online supplemental figure 6C) in predicting 18-month survival. Baseline expression of *STAT1*

and *LAG3* were positively associated with survival, while *PD-L1* expression was negatively associated with survival (online supplemental figure 6D).

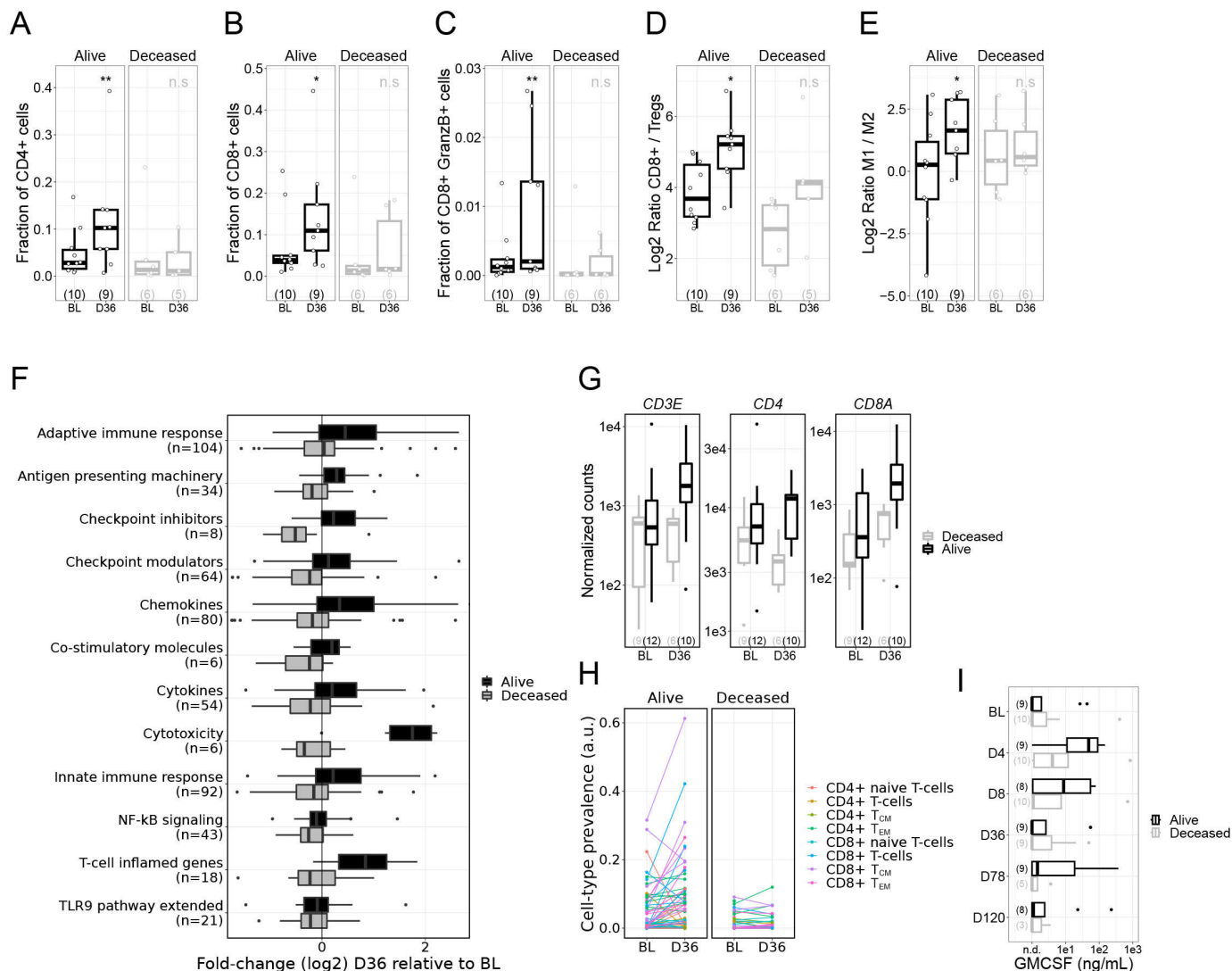


Figure 5 Tumor immune cell profile and gene expression, stratified by survival at 18 months (ONCOS-102 group^a). Immunofluorescence histology for CD4+ (A), CD8+ (B), GranzymeB-positive CD8+ cells (C), CD8+:Treg ratio (D), and M1:M2 (E) at baseline and day 36 for patients treated with ONCOS-102 who survived (left panel) and had died (right panel) at month 18. Boxes show median, lower, and upper quartiles, whiskers show minimum and maximum values within the 1.5 IQR, individual data points are shown, and values in parentheses denote number of patients with available samples. P values are derived from quasi-binomial generalized linear model (A–C) or linear regression (D, E). (F) Change in gene expression in select immunological pathways (specified in online supplemental data) from day 36 relative to baseline, stratified by survival at month 18 (number of genes in each pathway are denoted in parentheses). (G) Normalized (using DESeq2) expression of T-cell marker genes at baseline and day 36 stratified by survival at month 18 (values in parentheses denote patients with available samples). (H) Deconvolution of cell-type abundance from transcriptome expression profiles for patients at baseline and day 36 (colors reflect subsets of CD4+ and CD8+ T-cells) who were alive (left panel) and deceased (right panel) at month 18. (I) Serum GM-CSF levels at baseline and days 4, 8, 36, 78, and 120, stratified by 18-month survival. ^aONCOS-102 (3×10^{11} virus particles in 2.5 mL) with pemetrexed (500 mg/m²) in combination with cisplatin (75 mg/m²) or carboplatin (AUC 5). * $p \leq 0.05$; ** $p \leq 0.01$. AUC, area under the concentration–time curve; BL, baseline; D, study day, GM-CSF, granulocyte-macrophage colony-stimulating factor; IQR, interquartile range; M, macrophage; T_{CM}, central memory T-cells; T_{EM}, effector memory T-cells; TLR9, toll-like receptor 9; Treg, regulatory T-cells.

DISCUSSION

MPM is an aggressive tumor associated with a low 5-year survival rate ($\leq 10\%$).^{23, 24} This study was conducted prior to the recent approval of an immunotherapy regimen, and pemetrexed and platinum chemotherapy was a first-line standard of care for patients with unresectable MPM.²⁴ Several studies support rechallenge with a platinum plus pemetrexed regimen in patients with mesothelioma following an initial

favorable response.^{25–27} Furthermore, despite availability of immune checkpoint inhibitors, many patients with MPM are not eligible for this treatment or do not benefit due to primary or acquired resistance, and alternative therapies are needed. This study investigated ONCOS-102 in combination with pemetrexed plus cisplatin/carboplatin for unresectable MPM with or without prior chemotherapy. To our knowledge, this is the first phase 2 trial to evaluate intratumoral

therapy with an oncolytic virus in this setting. It demonstrated this strategy is feasible and can promote a proimmunogenic tumor microenvironment, and encouraging outcomes were observed.

The addition of ONCOS-102 to pemetrexed plus cisplatin/carboplatin was well tolerated. No patients discontinued ONCOS-102 due to TEAEs and no safety signals were identified that may impact further development of the treatment combination. The safety profile of ONCOS-102 plus chemotherapy broadly reflected that observed with chemotherapy alone. Addition of ONCOS-102 did not increase the hematologic toxicity observed with chemotherapy: severe (grade 3 or 4) neutropenia and anemia occurred at a broadly similar frequency in both study cohorts. Fever was the most frequent AE considered related to ONCOS-102 (reported by 75%) and was rarely severe (one grade 3 event (5%)). This observation is in line with findings with ONCOS-102 in patients with melanoma and reflects the anticipated safety profile of an oncolytic adenovirus.²⁸

Efficacy signals were not seen with ONCOS-102 when retreatment with pemetrexed plus cisplatin/carboplatin was attempted. In contrast, for patients receiving first-line therapy addition of ONCOS-102 to chemotherapy was associated with a trend for improved survival compared with chemotherapy alone (median OS 20.3 vs 13.5 months). Indeed, approximately half the chemotherapy-naïve patients who received ONCOS-102 plus chemotherapy (5 of 11) survived beyond 2 years, including one individual with progression-free disease at the last follow-up visit (~4 years). While these findings exceeded OS reported in CheckMate 743, the registrational study for nivolumab plus ipilimumab (18.1 months) in the first-line setting,¹³ efficacy data from our small, exploratory study should be interpreted with caution. Furthermore, baseline data on tumor burden indicated patients who received ONCOS-102 potentially had more severe disease than those randomized to chemotherapy alone. However, the morphology and growth patterns of MPM make assessment of disease burden particularly challenging, and modified RECIST V.1.1 criteria specific for MPM are now suggested for clinical trials.²⁹ Nevertheless, efficacy signals observed in our study are promising. This includes observations suggesting patients with epithelioid histology may benefit from ONCOS-102 plus chemotherapy. This is noteworthy, given data from CheckMate 743 indicated no obvious OS advantage with immunotherapy in epithelioid MPM, which comprises the majority of MPM cases.^{1,13} Data to inform the antitumor activity of ONCOS-102 in other, less frequent MPM histologic types are also needed.

MPM has a particularly immunosuppressive tumor microenvironment linked to asbestos-induced chronic inflammation and characterized by abundance of tumor-associated M2 macrophages along with paucity and decreased functionality of effector T-cells.^{24,30–32} To provide insight into the activity of intratumoral ONCOS-102, detailed immune profiling of biopsies obtained at

baseline and day 36 (during cycle 1 of chemotherapy (all patients) and following three priming dose of ONCOS-102 (ONCOS-102-treated patients, only)) was performed. Treatment with ONCOS-102 led to substantial tumor immune activation, including infiltration of CD4+, CD8+, and Granzyme-B+ T cells and increased expression of cytotoxicity genes that was not observed with chemotherapy alone. Within the ONCOS-102-treated group, elevated T-cell infiltration and differential expression of genes associated with multiple aspects of immune response at day 36 was seen in patients who survived to month 18 compared with those who had died by this time point. This observation supports the notion that clinical benefit with ONCOS-102 may be associated with immune activation, and is consistent with findings in two patients with MPM who received single-agent ONCOS-102 in a phase I dose-escalation trial.¹⁸ Tumor infiltration by CD8+ and CD4+ T cells and induction of PD-L1 expression was observed in response to ONCOS-102 in both patients, along with a 47% reduction in tumor metabolic activity in one individual.¹⁸ Given the need for biomarkers to inform treatment selection for patients with MPM, it is noteworthy that baseline expression of *STAT1*, *LAG3*, and *PD-L1* were strongly prognostic of survival at month 18 following treatment with ONCOS-102 plus chemotherapy in our study. Further validation of prognostic markers is required, including more in depth analyses of T-cell exhaustion markers. However, taken together the correlative data provide a mechanistic rationale for investigating whether ONCOS-102 can augment checkpoint inhibitor efficacy in patients with MPM, by triggering proinflammatory remodeling of the tumor microenvironment as was observed in patients with anti-PD-1-resistant advanced melanoma who received ONCOS-102 plus pembrolizumab.²⁸ This is timely given first-line pembrolizumab in combination with chemotherapy was recently found to extend OS in patients with MPM compared with chemotherapy alone.³³ It is also noteworthy that of the six patients who completed the study and received subsequent immunotherapy (n=3 had received ONCOS-102+chemotherapy and n=3 had received chemotherapy alone), subsequent immunotherapy did not appear to influence survival across the treatment groups. However, the duration of OS appeared longer in these patients from the ONCOS-102+chemotherapy cohort (30.4 and 31.5 months (one patient was alive at last survival follow-up)) compared with chemotherapy alone (3.0, 18.3, and 28.9 months).

Elevated levels of serum GM-CSF seen with ONCOS-102 on days 4 and 8 (3 days and 4 days after the first and second ONCOS-102 doses, respectively) were particularly marked in individuals who survived to month 18. This supports clinical benefit being associated with effective virus infection and replication followed by an early virus-induced immune activating response. Timings of blood sampling, which differed in the ONCOS-102 and chemotherapy-alone arms, limit further interpretation, and the potential contribution of endogenous cytokine

cannot be ruled out. However, elevated serum GM-CSF is consistent with viral persistence, indicated by expression of ONCOS-102-encoded GM-CSF mRNA, in melanoma biopsies obtained from patients with disease control following ONCOS-102 plus pembrolizumab in a recent pilot study.²⁸

In summary, this study demonstrated that ONCOS-102 co-administered with pemetrexed plus cisplatin/carboplatin was feasible and well tolerated and may provide a survival advantage in treatment-naïve patients with MPM. ONCOS-102 induced remodeling of the tumor microenvironment, evidenced by increased T-cell infiltration and proinflammatory gene expression compared with pemetrexed plus cisplatin/carboplatin alone. This immune activation was associated with survival at month 18. Similar immunomodulatory effects of ONCOS-102 in the tumor microenvironment in MPM and melanoma,²⁸ two pathogenetically and histologically distinct tumor types, suggests the potential usefulness of this virus across diverse malignancies, and supports further evaluation of ONCOS-102-containing treatment combinations in larger studies.

Author affiliations

¹Department of Medical Oncology, Hospital Universitario 12 de Octubre, Madrid, Spain

²Department of Medical Oncology, Vall d'Hebron University Hospital, Barcelona, Spain

³Vall d'Hebron Institute of Oncology, Barcelona, Spain

⁴Department of Pulmonology, Centre Hospitalier Universitaire de Rennes, Rennes, France

⁵Centre Georges-François Leclerc, Dijon, France

⁶Department of Medical Oncology, Instituto Oncológico Rosell, Grupo Quironsalud, Hospital Universitario Dexeus, Barcelona, Spain

⁷Department of Radiology, Centre Hospitalier Universitaire de Rennes, Rennes, France

⁸H120-CNIO Lung Cancer Research Unit, Madrid, Spain

⁹Department of Medicine, Complutense University of Madrid, Madrid, Spain

¹⁰Research and Development, Circio Holding ASA, Oslo, Norway

Present affiliations The present affiliation of Santiago Ponce is: Gustave Roussy, Paris, France; Nicolas Isambert is: Oncology Hospitalier Universitaire de Poitiers, Poitiers, France and Santiago Viteri is: UOMi Cancer Center, Clínica Mi Tres Torres, Barcelona, Spain.

Twitter Thomas Birkballe Hansen @ncrnalab

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Ethics approval The study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization Guidelines for Good Clinical Practice, and local regulatory requirements. The study protocol was approved by the independent ethics committees at participating study sites (Hospital 12 de Octubre Clinical Research Ethics Committee (opinion number 16/010) and Comité de Protection des Personnes Sud-Ouest and Outre-Mer IV (opinion CPP17-067a/2015-005143-13)). All patients provided informed, written consent.

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ORCID iD

Thomas Birkballe Hansen <http://orcid.org/0000-0002-7573-9657>

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1 **Supplementary figure 1. Swimmer plot of overall survival and progression-free survival**

2 (A) ITT population (n = 31); (B) chemotherapy-naïve patients (n = 17)

3 Control = pemetrexed (500 mg/m²) in combination with cisplatin (75 mg/m²) or carboplatin (AUC 5)

4 ONCOS-102 = ONCOS-102 (3 × 10¹¹ virus particles in 2.5 mL) with pemetrexed (500 mg/m²) in

5 combination with cisplatin (75 mg/m²) or carboplatin (AUC 5)

6 > denotes censoring

7 PFS, progression-free survival; OS, overall survival

8

9 **Supplementary figure 2. Selected gene expression profiles stratified by study treatment**

10 Normalized (using DESeq2) expression of genes classified as cytotoxicity (A), co-stimulatory (B), or
11 checkpoint inhibitor (C) genes in tumour biopsies obtained at baseline and Day 36 stratified by study
12 treatment. Values in parenthesis reflect the number of samples for each group at each timepoint.

13 Control = pemetrexed (500 mg/m²) in combination with cisplatin (75 mg/m²) or carboplatin (AUC 5)

14 ONCOS-102 = ONCOS-102 (3 × 10¹¹ virus particles in 2.5 mL) with pemetrexed (500 mg/m²) in

15 combination with cisplatin (75 mg/m²) or carboplatin (AUC 5)

16

17 **Supplementary figure 3. Serum cytokine concentrations**

18 Circulating concentrations of GM-CSF (A), IFNα2 (B) and IFN-gamma (C) were assessed prior to study
19 medication on Days 1, 43, 85, and 127 in the chemotherapy-alone cohort (upper panels), and on
20 Days 1 Days 4, 8, 36, 78, and 120 in the ONCOS-102 cohort. P-values were calculated by linear
21 regression.

22 Control = pemetrexed (500 mg/m²) in combination with cisplatin (75 mg/m²) or carboplatin (AUC 5)

23 ONCOS-102 = ONCOS-102 (3×10^{11} virus particles in 2.5 mL) with pemetrexed (500 mg/m^2) in
24 combination with cisplatin (75 mg/m^2) or carboplatin (AUC 5)

25 ** $P < 0.01$; *** $P < 0.001$

26 D, study day, GM-CSF, granulocyte-macrophage colony stimulating factor; IFN, interferon; IL2,
27 interleukin 2

28

29 **Supplementary figure 4. Selected gene expression profiles stratified by survival at Month 18**

30 **(ONCOS-102 group^a)**

31 Normalized (using DESeq2) expression of selected cytotoxicity genes (A), co-stimulatory genes (B), or
32 checkpoint inhibitor genes (C) in tumour biopsies obtained at baseline and Day 36 stratified by 18-
33 month survival. Numbers in parenthesis reflect the number of samples for each group at each
34 timepoint.

35 ^aONCOS-102 (3×10^{11} virus particles in 2.5 mL) with pemetrexed (500 mg/m^2) in combination with
36 cisplatin (75 mg/m^2) or carboplatin (AUC 5).

37

38 **Supplementary figure 5. Tumour transcriptome analysis stratified by survival at Month 18**

39 **(ONCOS-102 group^a)**

40 Heatmaps of the top 100 most differentially expressed genes in patients stratified by survival (alive
41 or deceased at Month 18) in baseline (A) and Day 36 (D) tumour biopsies from patients who
42 received ONCOS-102. Volcano plot depicting change in all tumour-expressed genes at baseline (B)
43 and Day 36 (E) in patients (alive or deceased at Month 18) (Benjamini-Hochberg adjusted P -values).
44 Gene ontology analysis of tumour genes with significant differential expression at baseline (C) and
45 Day 36 (F) in patients who were alive versus deceased at Month 18.

46 ^aONCOS-102 (3×10^{11} virus particles in 2.5 mL) with pemetrexed (500 mg/m²) in combination with
47 cisplatin (75 mg/m²) or carboplatin (AUC 5).

48

49 **Supplementary figure 6. Predictors of survival at Month 18 following (ONCOS-102 cohort^a)**

50 (A) Baseline tumour transcriptome levels of *PD-L1*, *CD8A*, *LAG3*, and *STAT1* stratified by survival at
51 Month 18. (B) 2x2 contingency table comparing the binomial GLM classifier build on transcriptome
52 data shown in (A) with ground-truth survival. (C) ROC-analysis of model prediction. (D) GLM
53 coefficients reflecting the predictive value for each gene on survival.

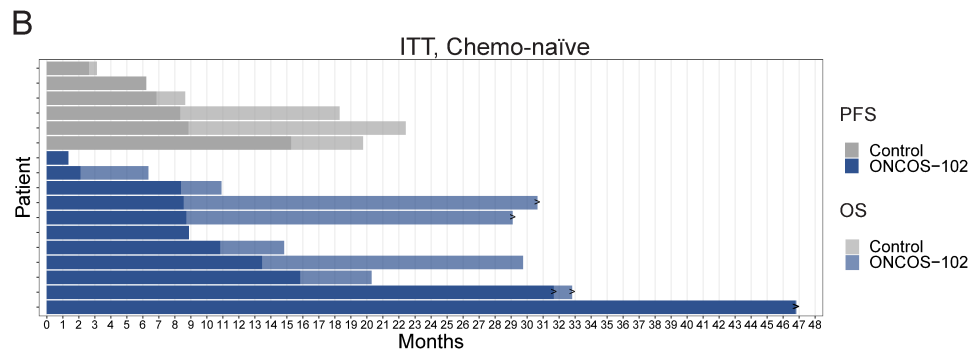
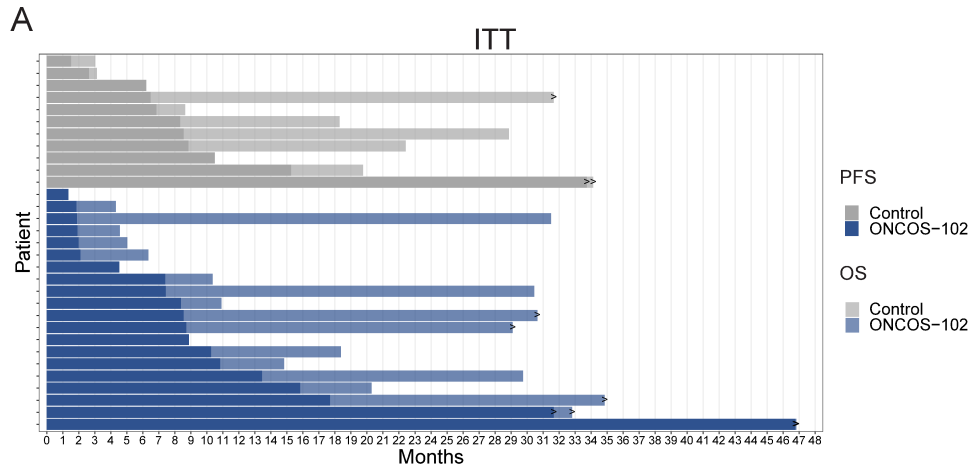
54 ^aONCOS-102 (3×10^{11} virus particles in 2.5 mL) with pemetrexed (500 mg/m²) in combination with
55 cisplatin (75 mg/m²) or carboplatin (AUC 5).

56 BL, baseline; D, day; GLM, generalized linear model; ROC, receiver operating characteristics

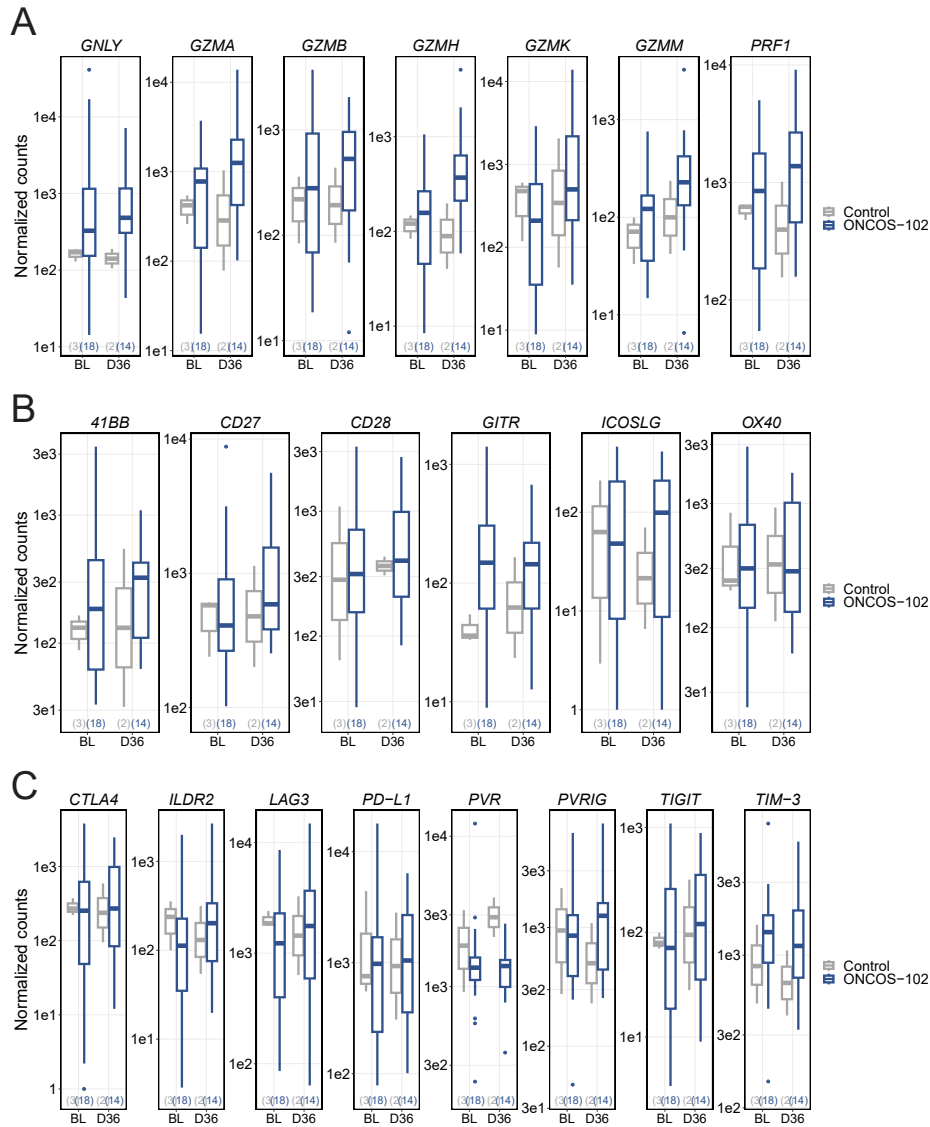
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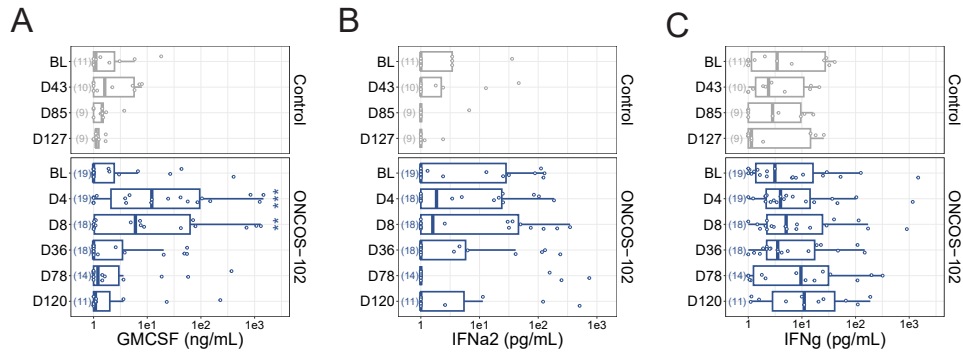
Supplementary Figure 1. Swimmer plot of overall survival and progression-free survival



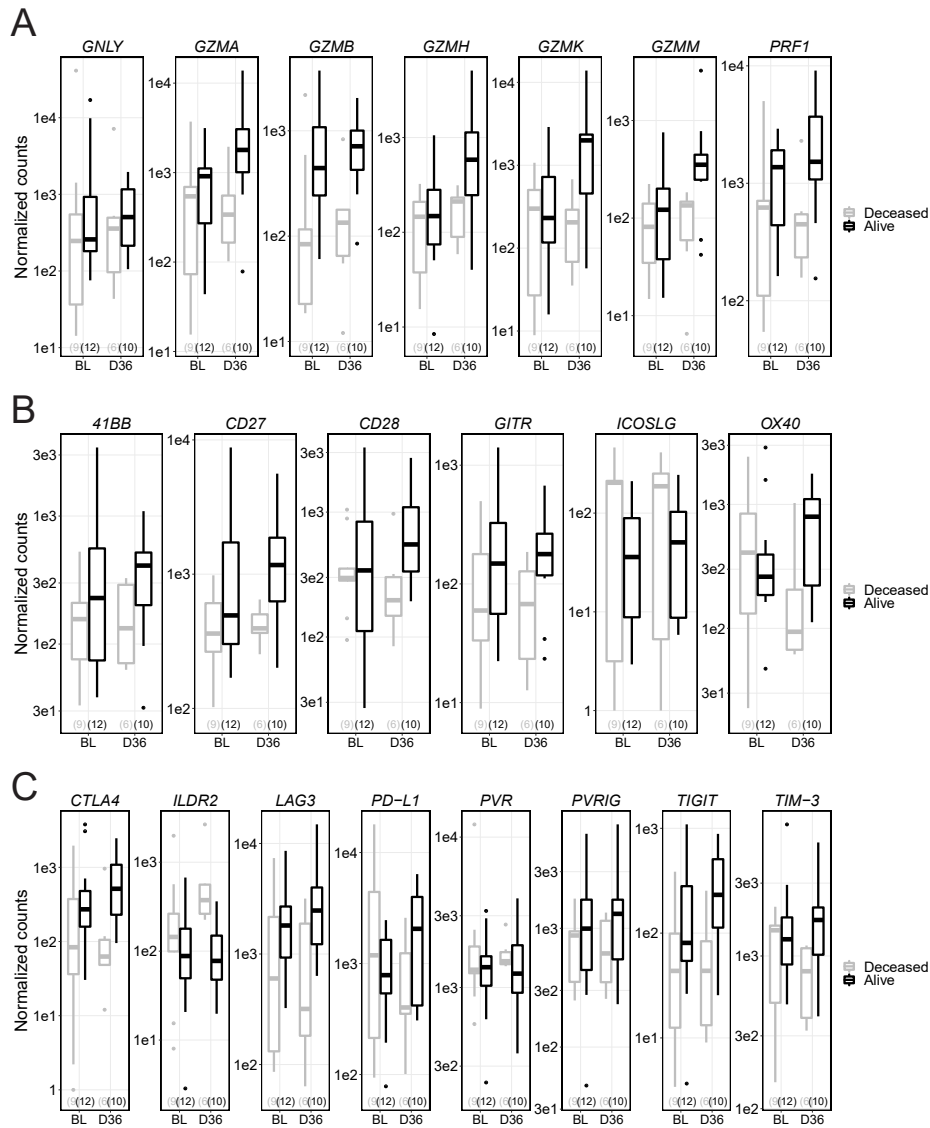
Supplementary Figure 2. Selected gene expression profiles stratified by study treatment

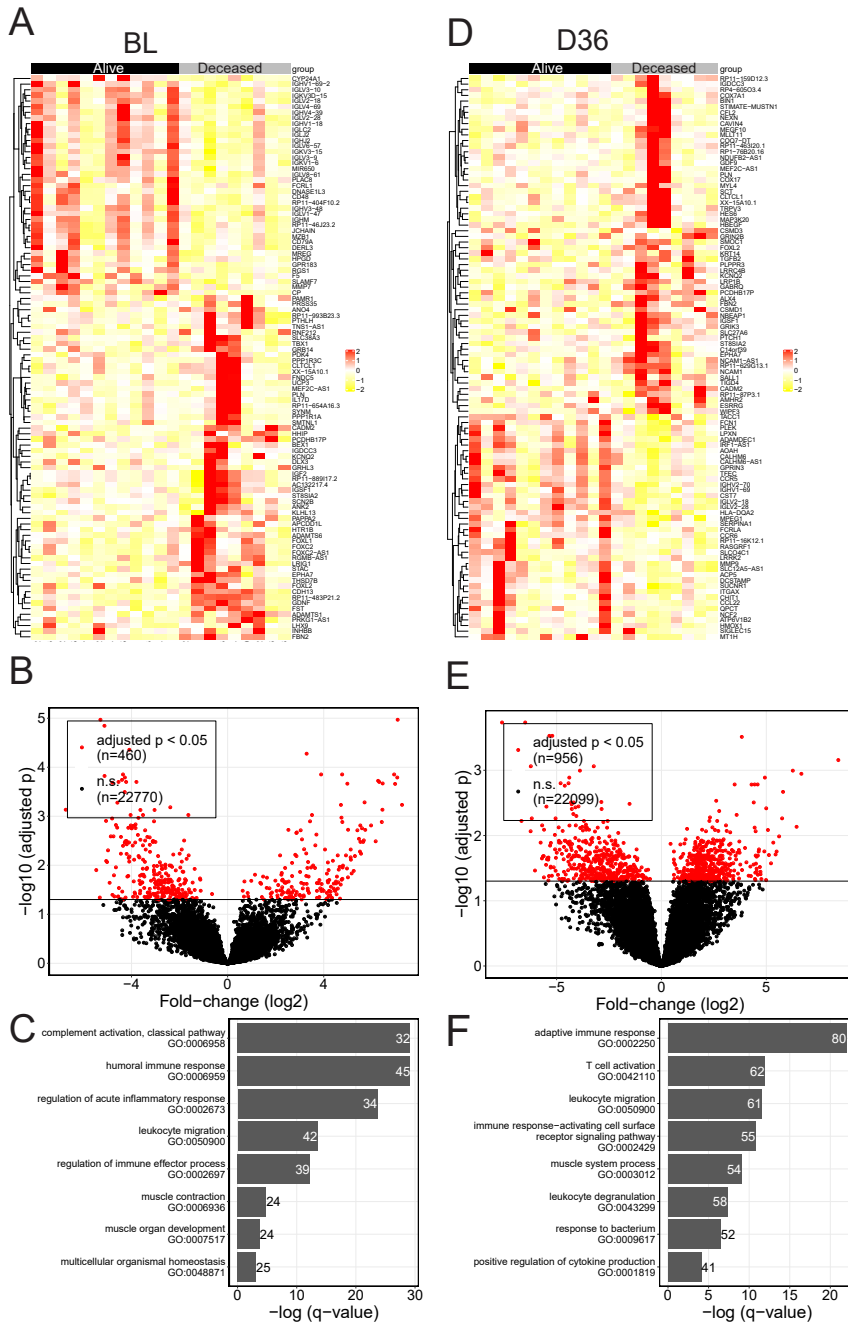


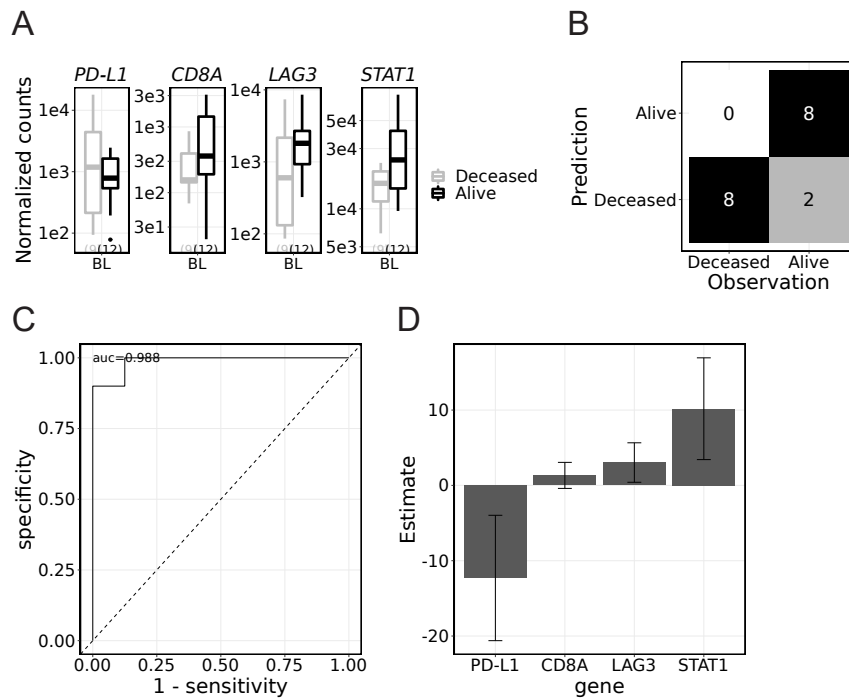
Supplementary Figure 3. Serum cytokine concentrations



Supplementary Figure 4. Selected gene expression profiles stratified by survival at Month 18 (ONCOS-102 group^a)



Supplementary Figure 5. Tumour transcriptome analysis stratified by survival at Month 18 (ONCOS-102 group^a)

Supplementary Figure 6. Predictors of survival at Month 18 following (ONCOS-102 cohort^a)

Supplementary Methods

Immunofluorescence histology

Detection and quantification of various immune cell infiltrates was carried out using a multiplexed approach with a combination of fluorescence and chromogenic chemistry. This allowed staining of up to six different antibodies and two different nuclear stains from the same FFPE section, and analysis of the different channels independently or together as overlays. For analysis of different immune cell infiltrates on biopsy samples, 3.5 µm sections were cut from FFPE tissues and processed for staining. The following monoclonal primary antibodies were used: mouse anti-CD8+ (cat. number: M7103, Clone C8/144B), rabbit anti-CD4+ (cat. number: 133616, Clone EPR6855), and rabbit anti-Granzyme B (cat. number: ab4059, polyclonal). Detection utilized either TSA 647 (for CD8+, Granzyme B), or TSA 750 (for CD4+). The stained biopsy samples were imaged with an automated whole-slide scanner (Panoramic 250 FLASH, 3DHISTECH Ltd or similar) using a Plan-Apochromat 20 × objective (numerical aperture 0.80). For quantitation, a color information-based image processing methodology was applied as developed at Institute for Molecular Medicine (Helsinki, Finland). Nuclei were visualized by staining with DAPI.

Next-generation sequencing analyses

DNA and RNA from tumor biopsies were extracted (Qiagen AllPrep DNA/RNA kit) and underwent next generation sequencing (Illumina NovaSeq 6000 Systems at Personalis Inc., CA, USA). For RNA sequencing analysis, the paired-end reads were initially trimmed using trim_galore (RRID:SCR_011847, version 0.6.5) with adaptor sequence 'AGATCGGAAGAGC'. Reads were mapped to the human genome (hg38) with the ONCOS-102 genome appended using STAR (RRID:SCR_015899, version 2.7.3a) and gene expression was quantified by featureCounts (RRID:SCR_012919, version 2.0.0) with gencode annotation (version 38). Differential gene expression analyses were performed with DESeq2 (RRID:SCR_015687) using default parameters. Coefficients were fitted for survival at Month 18, and patient id to allow a full-rank model matrix, and contrasts between deceased or live at Month 18 coefficients were extracted. DNA sequencing analysis was performed by Personalis Inc. (CA, USA) using their Immunoid platform. Briefly, HaplotypeCaller (GATK) was used to generate the core set of singlenucleotide variant (SNV) calls and their accompanying quality metrics. The pipeline then used GATK's variant quality score recalibration module, which stratified SNVs by their likelihood of representing false-positive calls, and in-house SNV accuracy software, which incorporated both genomic context and sequence alignment information into a model that corrected miscalled variants. All calls were made on BAM files recalibrated by GATK's BAM processing tools. MuTect (RRID:SCR_000559) was used to call somatic SNVs and indels, with Vardict used for calling small somatic insertions or deletions (<50 bp). Somatic SNV and indel calls were combined and analyzed through a comprehensively tested set of filters based on (i) alignment metrics such as sequence coverage and read quality, (ii) positional features such as proximity to a gap region, and (iii) likelihood of presence in normal tissue.