Durvalumab and tremelimumab in patients with advanced rare cancer: a multi-centre, non-blinded, open-label phase II basket trial

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eClinicalMedicine
2025;79: 102991
Published Online xxx
https://doi.org/10.1016/j.eclinm.2024.102991

Summary

Background Dual inhibition of cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed death ligand 1 (PD-L1) has been shown to be an effective treatment strategy in many cancers. We sought to determine the objective response rate of combination durvalumab (D) plus tremelimumab (TM) in parallel cohorts of patients with carefully selected rare cancer types in which these agents had not previously been evaluated in phase II trials and for which there was clinical or biological rationale for dual immune checkpoint inhibitor therapy to be active.

Methods We designed a multi-centre, non-blinded, open-label phase II basket trial with each of the following 8 rare cancers considered a separate phase II trial: salivary carcinoma, carcinoma of unknown primary (CUP) with tumour infiltrating lymphocytes and/or expressing PD-L1, mucosal melanoma, acral melanoma, osteosarcoma, undifferentiated pleomorphic sarcoma, clear cell carcinoma of the ovary (CCCO) or squamous cell carcinoma of the anal canal (SCCA). The primary objective was to evaluate the response rate of the combination of D and TM, and the secondary objectives were to evaluate the tolerability and safety of D and TM combination. Eligible patients had advanced, metastatic or recurrent, or unresectable cancer with no known life-prolonging treatment option, age ≥16 years, ECOG performance status 0 or 1. Patients received D (1500 mg IV) + TM (75 mg IV) on Day 1 q4 weeks for 4 cycles followed by D q4 weeks until disease progression. This trial is registered with ClinicalTrials.gov, NCT02879162.

Findings From December 14th, 2016, to August 14, 2019, 140 patients enrolled into seven cohorts. The rare melanoma cohorts were closed due to lack of accrual. Of the 140 patients enrolled, 138 were eligible, 138 were evaluable for toxicity and 128 (91%) were evaluable for response. Durable responses were noted in all cohorts except for osteosarcoma. The overall response rate for eligible patients was 16% (95% CI: 10–23%). The response rates in each cancer cohort were undifferentiated pleomorphic sarcoma 15% (n = 3/20; 95% CI 3–38%), salivary carcinoma 20% (n = 4/20; 95% CI: 6–44%), CUP 17% (n = 3/18; 95% CI 4–41%), SCCA 10% (n = 2/20; 95% CI 12–32%) and CCCO 21% (n = 8/39; 95% CI 9–37%). Grade 3/4 adverse events were rare, where 4 patients experienced grade 4 related events and39 patients experienced grade 3 events.

Interpretation Durvalumab + tremelimumab treatment resulted in meaningful responses in salivary carcinoma and CCCO and deserves further exploration in front-line studies.

Funding AstraZeneca and Canadian Cancer Society.

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Keywords: Checkpoint inhibitors; Rare cancers; Ovarian carcinoma

Research in context

Evidence before this study

We searched PubMed using the search terms durvalumab, tremelimumab, nivolumab, ipilimumab, clinical trials, salivary carcinoma, carcinoma of unknown primary, mucosal melanoma, acral melanoma, osteosarcoma, undifferentiated pleomorphic sarcoma, clear cell carcinoma of the ovary (CCCO), squamous cell carcinoma of the anal canal for studies published between Jan 1, 2010, and Dec 31, 2023, without language restrictions. The response rates to nivolumab alone or in combination with ipilimumab for people with ovarian and other extra-renal clear cell carcinomas were 14.2 and 26.7%, respectively among whom the majority had CCCO (n = 24/30 patients). A phase 2 study in salivary CA reported response rate of 16% to combination of nivolumab + ipilimumab.

Added value of this study

Through this basket study, we were able to enrol patients with multiple rare cancers to test activity of durvalumab plus tremelimumab and demonstrated promising activity in CCO and salivary carcinoma.

Implications of all the available evidence

The findings from this study add further evidence that checkpoint inhibitors may play an important role in the treatment of patients with advanced CCCO and salivary carcinoma.

Introduction

Agents with inhibit cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and programmed death ligand 1 (PD-L1) have been shown to be an effective treatment for patients with many cancers. Durvalumab (D) is a human monoclonal antibody of the immunoglobulin G1 kappa subclass that binds to PD-L1 and blocks its interaction with PD-1, a co-inhibitory receptor known to be expressed on activated T cells.[1] Clinically, blockade of the PD-1 immune checkpoint pathway by inhibiting PD-L1/PD-1 engagement has been shown to induce tumour regression across many cancer types including melanoma, renal cell, colon, lung and bladder cancers.[2–5] Tremelimumab (TM) is a human monoclonal antibody of the immunoglobin G2 subclass that binds to human CTLA-4, a cell surface receptor expressed primarily on activated T cells.[6] The combination of anti-PD-L1 and anti-CTLA-4 is a promising approach against many cancer types because of non-redundant pathway blockade and synergy based on preclinical data as well as emergent clinical data.[7–9]

For this basket trial, we sought to explore parallel cohorts of relatively rare under-investigated cancers that had[1] biological features supporting their potential to respond to combination checkpoint inhibition D + TM such as expression of PD-L1, presence of immune cell infiltrates in tumour and/or high mutation burden,[2] clinical need for new systemic treatment options,[3] limited or no data on the value of checkpoint inhibitors and[4] feasibility to accrue sufficient patients to assess activity across participating Canadian centres. Based on the literature available at the time of study design, we included the following cancers: 1. salivary carcinoma[10] 2. carcinoma of unknown primary with tumour infiltrating lymphocytes (TILs) and/or expressing PD-L1 (CUP)[11] 3. mucosal melanoma[12] 4. acral melanoma[13] 5. osteosarcoma[14,15] 6. undifferentiated pleomorphic sarcoma[16] 7. clear cell carcinoma of the ovary (CCCO)[17,18] and 8. squamous cell carcinoma of the anal canal (SCCA).[19]

Methods

Study design and participants

This is a multi-centre, non-blinded, open-label single arm phase II basket trial of D + TM with 8 parallel independent cohorts of different histological subtypes of advanced rare cancers to assess objective response rate. The Canadian Cancer Trials Group conducted the trial and thirteen cancer centres participated across Canada. Eligible patients were ≥16 years of age with an ECOG performance status of 0 or 1 and had a confirmed diagnosis of 1. salivary carcinoma, 2. carcinoma of unknown primary with TILs and/or expressing PD-L1 (CUP), 3. mucosal melanoma, 4. acral melanoma, 5. osteosarcoma, 6. undifferentiated pleomorphic sarcoma, 7. clear cell carcinoma of the ovary (CCCO) or 8. squamous cell carcinoma of the anal canal (SCCA). Patients must have had cancer that was advanced, metastatic, or recurrent, or unresectable and for which there was no known life prolonging therapy. Patients were required to have tissue from primary or metastatic disease available, at least one measurable lesion as defined by RECIST 1.1 that had not been the site of the protocol mandated biopsy and have protocol-specified normal organ function.

Patients must not have systemic therapy within 2 weeks, five half-lives for investigational agents or standard cycle length of standard systemic therapies, nor

radiation therapy or major surgery within 28 days of registration. Notable exclusion criteria were a history of other malignancies, except adequately treated non-melanoma skin cancer, curatively treated in-situ cancer of the cervix, or other cancers curatively treated with no evidence of disease for ≥5 years; history of active or prior documented autoimmune or inflammatory disorders including inflammatory bowel disease or serious gastrointestinal chronic conditions associated with diarrhea, systemic lupus erythematosus, sarcoidosis, Wegener syndrome (granulomatosis with polyangiitis), rheumatoid arthritis, hypophysitis, uveitis, etc. within the past 3 years prior to the start of treatment with the following exceptions: alopecia, Grave's disease, vitiligo or psoriasis not requiring systemic treatment.

The protocol was amended to expand to twenty additional patients to the CCCO cohort (Amendment #2 dated January 25, 2019) and closed in December 2019 after the 42nd CCCO patient was registered. All remaining cohorts completed accrual as originally designed.

Ethics

The study was done in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and national policies for clinical trials and biological specimens. Each participating centre (University Health Network, Princess Margaret Cancer Centre, CAVA—BCCA, Ottawa Hospital Research Institute, CHUM-Centre Hospitalier de l'Universite de Montreal, Juravinski Cancer Centre at Hamilton Health Sciences, London Regional Cancer Program, CancerCare Manitoba, The Research Institute of the McGill University, BC Cancer and Molecular and Advanced Pathology Centre) obtained approval from their own institutional ethics review board. Written informed consent was obtained from all patients. Archival tumour tissue samples, pre-treatment biopsies, and radiographic scans were de-identified and sent for correlative studies and central review.

Procedures

Following registration, patients were treated with D at a dose of 1500 mg IV plus TM at 75 mg IV on Day 1 every 4 weeks for a total of four cycles followed by D q 4 weeks. Treatment continued until disease progression or unacceptable toxicity. Each drug was administered over 60 min. Patients were monitored for 1 h following the first infusion; if no reaction occurred, further monitoring was as per the investigator. Detailed toxicity monitoring and dose adjustments are listed in the protocol (Appendix A). Hematopoietic growth factors, supportive and palliative care treatments (i.e., pain medication, anti-emetics, and anti-diarrheal medications) were permitted on study. Cytokines, concurrent radiation, other anti-cancer drugs or investigational agents were not permitted. Corticosteroids at supra-physiological doses were not permitted except for the treatment of ≥ grade 3 infusion reaction, or treatment related toxicity. Topical applications (e.g., rash), inhaled sprays (e.g., obstructive airways diseases), eye drops or local injections (e.g., intra-articular) were allowed. Patients who were on low oral doses of prednisone (5 mg BID or dexamethasone equivalent) were required to discontinue prior to study entry unless medically contraindicated.

At baseline, physical exam, basic organ function blood work, assessment of left ventricular function, urinalysis and tumour evaluation were done. Thereafter, patients were assessed each cycle and tumour evaluation by imaging was conducted every 12 weeks. For the CUP cohort, expression of CD8+ TILs or PDL1 was required for enrolment. Archival tumour was evaluated for T cell infiltration on H&E slides, and PD-L1 expression was assessed by quantitative immunohistochemistry. For all other cohorts, archival tissue was retrospectively evaluated for PD-L1 expression and for lymphocyte infiltration including subtypes by immunohistochemistry (CD8). In addition, tumour mutation burden (TMB) was assessed in the CCCO cohort.

Pathology review

An H&E slide and block from each patient's archival tumour was requested on all patients for central pathology review and for biomarker studies. Central review of pathology specimens was conducted by pathologists with appropriate subspecialty expertise in head & neck, cutaneous, musculoskeletal, and gynecologic pathology. Pathologists had access to the scanned copy of the original pathology report and digital H&E image. In cases where this was insufficient to confirm the diagnosis, additional immunohistochemical and/or molecular tests were performed on the submitted block.

CD8 and PD-L1 expression

Pre-treatment formalin-fixed, paraffin embedded (FFPE) slides were stained for PD-L1 using the Ventana PD-L1 (SP263) immunohistochemistry assay (Ventana Medical Systems, Tucson, AZ) using a Ventana BenchMark XT immune autostainer. The percentage of membranous tumour cell staining (tumour proportion score, TPS) was assessed along with the percentage of PD-L1 positive immune cells (ICs, lymphocytes, and macrophages) and the ratio of total PD-L1 positive cells relative to all viable tumour cells (combined positive score, CPS). TPS and IC PD-L1 were scored semi-quantitatively as <1%, 1–10%, 10–24%, 24–49%, and ≥50%. CPS was scored as <1%, 1–10%, 10–24%, 24–49%, and ≥50%. Each slide was scored by two independent reviewers and non-concordant scores were re-reviewed by the same pathologists to determine consensus result.

Immune cell infiltration of tumour was assessed by CD8 immunohistochemistry performed using the Ventana Confirm anti-CD8 (SP57) rabbit monoclonal

antibody and Ultraview Universal DAB Detection Kit (#760–500), as per manufacturer's recommended protocol. Whole slides digital images were generated using the Olympus VS120 slide scanner at 20x magnification. The tumour boundary was annotated and CD8 positive cell densities within the tumour region were quantified using Indica Labs HALO image management system (version 3.5.3577).

Tumour mutation burden analysis

DNA from normal peripheral blood mononuclear cells and tumour was extracted and whole exome analysis to 100X was performed using standard protocols.[20] Fastq files were aligned to the reference genome hg38 using bwaMem. Alignment was followed by variant calling by mutect2 on a matched tumor and normal, mutect2 calls were then annotated by Variant Effect Predictor (VEP). TMB was estimated using custom scripts by calculating the proportion of the callable space, the region of the genome used for variant calling per Megabase, where protein altering mutations are identified as PASS by mutect2. Protein altering mutations includes the following VEP classes: Missense_Mutation, In_Frame_Ins, In_Frame_Del, Frame_Shift_Ins, Frame_Shift_Del, Splice_Site, Translation_Start_Site, Nonsense_Mutation, Nonstop_Mutation, Silent.

Statistics

The primary endpoint of this study was investigator reported objective response rate, defined as the proportion of response evaluable patients who had complete response (CR) or partial response (PR) as their best response as assessed by RECIST version 1.1 criteria (i.e., a 30% decrease in the sum of the longest diameters of the target lesions maintained for at least 4 weeks (PR), or complete disappearance of disease and cancer related symptoms, also maintained for at least 4 weeks (CR)). Early progression was defined as progressive disease at or prior to the first assessment. Progression free survival was defined as date of enrolment to death, censoring event or documented progressive disease. Overall survival was defined as date of enrolment to date of death or censoring event. Adverse events were categorized using NCI CTCAE version 4.

Each of the eight disease cohorts were a 2-stage phase II study. In stage 1, 10 response evaluable patients were entered. Using response hypotheses of H0 $\leq$ 5% and Ha $\geq$25%, the drug combination would be rejected at the end of the first stage if no responses were seen. Otherwise, an additional ten patients were accrued to the cohort. In stage 2 of accrual, the drug would be considered active if four or more responses are observed among the twenty patients. This tests the null hypothesis (H0) that the response rate is 5% versus alternating hypotheses (Ha) that the response rate is 25%. The significance level is $\alpha = 0.02$ and the power is 0.76. If the true response rate of an agent were 10%, it would be identified as ineffective with probability of 0.87. If the true response rate of the agent were 30%, it would be identified as effective with probability of 0.88. Since the eligibility was not initially limited to patients with tumour expression of PD-L1 and/or TILs (except for cohort 2), where feasible, a minimum of ten patients with tumours with TILs and/or PD-L1 expression was planned to be included in the response analysis. The Clopper-Pearson exact 95% confidence interval for the response rate was calculated.

Role of the funding source

The study was sponsored by the Canadian Cancer Trials Group, and CCTG investigators participated in the study design, data collection, data analysis, data interpretation, and the writing of the report. All authors had access to summary data and accepted final responsibility for publication. AstraZeneca supported the trial by providing the agents and funding.

Results

From December 14th, 2016 to August 14, 2019, a total of 140 patients were enrolled into seven cohorts. Of these, two patients are ineligible due to not having protocol required cancer both on the CCCO cohort (one patient had suspected metastatic recurrence subsequently found to be benign tumour and one patient had mixed clear cell histology), and two patients registered to the trial but did not go on to receive any study drugs; one on the CUP cohort (one patient had rapid decline in performance status and another on UPS cohort who had lipase/amylase elevation prior to starting treatment). Consort flow chart of participants is presented in Fig. 1. Patient characteristics are summarized in Table 1 (Additional patient characteristics by cohort are available in Supplementary Table S1). Accrual to cohorts proceeded as designed with the following exceptions: the acral and mucosal melanoma cohorts were closed early due to poor accrual as these patients became eligible to receive immune checkpoint inhibitors as standard of care. Following evidence of clinical activity among twenty patients with CCCO, the protocol was amended to expand that cohort for another twenty patients. Cohort accrual in summarized in Table 2.

Central pathology review

Central pathology review confirmed the original diagnosis of 99% of all cases (136/138). One patient entered on the CUP cohort was reviewed as representing a specific primary site diagnosis (cholangiocarcinoma) and one patient entered on the UPS cohort was diagnosed as an alternative specific sarcoma diagnosis (malignant peripheral nerve sheath tumor). The number of patients in each of the seven cohorts is shown in Table 1.

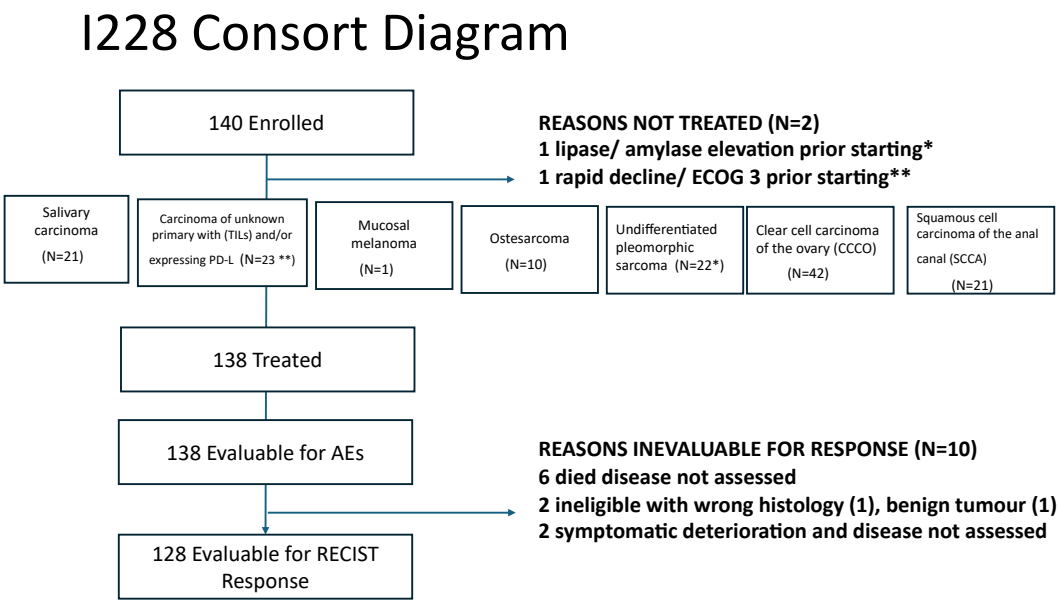

Fig. 1: CONSORT Flow chart of participants. Abbreviations: ECOG, eastern cooperative oncology group; AE, adverse events; RECIST, response evaluation criteria in solid tumors.

Efficacy of durvalumab + tremelimumab

Of the 136 eligible patients enrolled across all cohorts, 128 (94%) were evaluable for response. Reasons patients were inevaluable were as follows: progressed or died prior to planned 12-week imaging assessment (7 patients), never received treatment (2 patients) and imaging not performed when required.[1] The overall response rate for evaluable patients (n = 128) was 16% (95% CI: 10–23%) with durable responses in all cohorts except for the osteosarcoma. The response rates by cohort are listed in Table 2. Cohorts meeting the protocol defined criteria of success were salivary carcinoma with ORR of 20% (95% CI: 6–44%) and CCCO with ORR of 21% (95% CI 9–37%). The median time to progression (TTP) were highest on the salivary carcinoma cohort (5.3 months, 95% CI 2.6–7.9 months) followed by CUP (4.6 months, 95% CI 2.6–5.5 months), while the lowest was observed on the osteosarcoma cohort at median of 1.7 months (95% CI 0.7–2.6 months) (Table 3). PFS was also highest on the salivary cohort at 5.3 months (95% CI 2.6–7.9 months) while lowest was observed for osteosarcoma (1.7 months, 95% CI 0.7–2.6 months). Median overall survival was highest on the salivary cohort (20.7 months, 95% CI 10.0–37.5 months) followed by CCCO (11.0 months, 95% CI 7.0–24.1 months), osteosarcoma (10.0 months, 95% CI 1.9–14.6 months) and CUP (8.7 months, 95% CI 3.5–12.5 months), with the UPS cohort having the lowest median overall survival 5.0 months (95% CI 2.6–7.3 months). Kaplan Meier curves of PFS, TTP and OS by cohorts are shown in Supplementary Figures SA, SB, SC, respectively.

Treatment administration, safety and tolerability

A total of 961 cycles of D and 410 cycles of TM were administered, with a median of three cycles (IQR 2–4 cycles) for both agents. Actual dose intensity for D and TM were similar; with 83% of D (n = 138) and 86% (n = 138) of TM treated patients receiving ≥90% of the planned dose intensity. Doses were delayed in 33 (54%) patients due to toxicity: most treatment delays were due to diarrhea/colitis (n = 7), rash/pruritus (n = 4) and anemia (n = 6), elevated LFTs (n = 4) and neutropenia (n = 2). One patient had a cycle dose interrupted in cycle 3 for an infusion related reaction (grade 2) and another for paresthesia.

All AEs occurring in ≥10% of patients are listed in Table 4. Most non-hematologic, hematologic, and metabolic AEs were grade 1–2. The most common treatment related AEs were rash (n = 38, 28%), fatigue (n = 40, 29%), pruritus (n = 34, 25%), diarrhea (n = 36, 26%) and hypothyroidism (n = 25, 18%). Thirty-nine patients experienced grade 3 related adverse events and four patients experienced grade 4 related adverse events: seizure, sepsis, atrial fibrillation, and abdominal infection. Immune-related AEs of any grade attributed to D and TM occurred in 67% of patients (n = 93); and 18% (n = 25) were grade 3+. The most frequent immune-related AEs were colitis/diarrhea (n = 10); severe skin rashes (n = 5) and hepatitis (n = 3). Grade 3+ related events included diarrhea, pancreatitis, abdominal infection, pneumonitis, myositis, scleroderma, nephrotic syndrome and idiopathic pachymeningitis (Table 5). Laboratory AEs are summarized in Table 6. Only 1 pt (anal squamous carcinoma) was started on

	Category	N (%) Total N = 138
Age	Median (IQR)	59.4 (50.6–65.0)
Sex	Female	82 (59%)
	Male	56 (41%)
ECOG Performance Status	0	49 (36%)
	1	89 (64%)
Malignancy	Anal	21 (15%)
	Melanoma	1 (1%)
	Osteosarcoma	10 (7%)
	Clear Cell Ovary	42 (30%)
	Undifferentiated Pleomorphic Sarcoma	22 (21) 1 patient was not treated (15%)
	Salivary gland carcinoma	21 (15%)
	Carcinoma of Unknown Primary	23 (22) 1 patient was not treated (16%)
Prior therapy	Chemotherapy	109 (79%)
	Hormone Therapy	2 (1%)
	Immunotherapy	2 (1%)
	Radiotherapy	77 (56%)
	Other therapy	7 (5%)
Prior Chemotherapy Regimens	0	29 (21%)
	1	51 (37%)
	2	38 (28%)
	3	13 (9%)
	4	5 (4%)
	5	2 (1%)

Abbreviations: IQR, interquartile range; ECOG, eastern cooperative oncology group.

Table 1: **Patient characteristics.**

Pegfilgrastim soon after coming off study for PD following the completion of 3 cycles.

At the July 2022 data cut-off, four patients in the CCCO and one in the CUP cohort remained on D. A cut off date on 25 Oct 2023 was used for OS, PFS and TTP (Table 3). Ninety-three (70%) patients came off study due to objective PD and 9 (7%) for symptomatic progression. Fourteen (11%) patients came off study for adverse events (AE) related to protocol therapy. Fourteen (11%) patients died while receiving study treatment: three due to AEs related to study drugs (marantic endocarditis, renal failure, and pneumonitis), eight from disease progression, and three unrelated deaths (aspiration, pulmonary edema, and sudden death). Five patients refused further treatment (not related to adverse events), three others discontinued due to investigator decisions and one patient could not return to their home to resume study treatment due to COVID-19. Forty-six of 136 (34%) patients were able to complete all 4 TM cycles per protocol; 62 (47%) patients stopped sooner due to disease progression.

Biomarker analyses

Spearman correlations was calculated between levels of expression PD-L1 (using CPS, TPS and IC scores) and best tumour response. For the salivary cohort (number of patients with PD-L1 results n = 17), weak correlation has been observed (0.14 for CPS, –0.06 for TPS and 0.12 for IC); for CCCO cohort (n = 34), moderate non-significant correlation has been observed (0.30 for CPS (p = 0.062), 0.15 for TPS (p = 0.30) and 0.28 for IC (p = 0.091)).

Similarly, TMB was assessed in 30 patients with CCCO. Of these 30 patients, 4 had PR as the best response, 8 SD and 18 PD. The median TMB is 1.1 mutations/MB (IQR 0.9–1.7). Patients with PR had a slightly higher median TMB (1.34 mutations/MB) than patients with SD and PD (both groups with median 1.07 mutations/MB (Supplementary Figure SD). Using median TMB as cutoff, the median OS is 10.5 months (95% CI (6.7, 41.9)) for patients with TMB lower than the median and 14.9 months (95% CI (7, NA)) for patients with TMB higher than the median (Supplementary Figure SE).

Cohort	Patients (n)	Eligible (n)[a]	Evaluable for response	CR	PR	SD	PD	IN	RR (%)	95% CI(%)
Salivary gland carcinoma	21	21	20	0	4	8	8	1	20	6–44
Carcinoma unknown primary	23	22	18	0	3	8	7	4	17	4–41
Mucosal melanoma	1	1	1	0	0	0	1	0	0	0–0
Osteosarcoma	10	10	10	0	0	1	9	0	0	0–31
Undifferentiated pleomorphic sarcoma	22	21	20	0	3	0	17	1	15	3–38
Ovary (CCCO)	42	40	39	1	7	10	21	1	21	9–37
Anal (SCCA)	21	21	20	0	2	5	13	1	10	12–32
All	140	136	128	1	19	32	76	8	16	10–23

Abbreviations: CCCO, clear cell carcinoma of ovary; SCCA, squamous cell carcinoma of anal canal; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; IN, inevaluable for RECIST response; RR, response rate; CI, confidence interval (based on Clopper-Pearson exact method). [a]All eligible and treated patients.

Table 2: **Objective Responses, data cut off Oct 25, 2023.**

Cohort	Patients (n)[a]	Eligible (n)	Median PFS (months)	PFS 95% CI	Median TTP (months)	TTP 95% CI	Median OS (months)	OS 95% CI
Salivary gland	21	21	5.3	2.6–7.9	5.3	2.6–7.9	20.7	10.0–37.5
Carcinoma unknown primary	23	22	3.7	2.5–5.5	4.6	2.6–5.5	8.7	3.5–12.5
Mucosal melanoma	1	1	2.7	(–)	2.7	(–)	(–)	(–)
Osteosarcoma	10	10	1.7	0.7–2.6	1.7	0.7–2.6	10.0	1.0–14.6
Undifferentiated pleomorphic sarcoma	22	21	1.9	0.9–2.7	1.9	1.4–2.7	5.0	2.6–7.3
Ovary (CCCO)	42	40	2.8	2.6–4.6	2.8	2.6–5.3	11.0	7.0–24.1
Anal (SCCA)	21	21	2.7	2.0–5.2	2.7	2.0–5.6	9.1	4.9–13.4
All	140	136	2.7	2.6–2.8	2.8	2.6–2.9	10.1	7.6–11.0

Abbreviations: CCCO, clear cell carcinoma of ovary; SCCA, squamous cell carcinoma of anal canal; PFS, progression free survival; TTP, time to progression; OS, overall survival; CI, confidence interval; MO, months. [a]All eligible and treated patients.

Table 3: **Progression and Overall Survival, data cut off Oct 25, 2023.**

Discussion

Using a basket trial design, we showed the feasibility of assessing combination checkpoint inhibition in multiple cohorts of patients with rare cancers. Responses rates of interest to combined immune blockade occurred in patients with CUP which exhibit high TIL counts and/or positive PD-L1 expression, in CCCO and in salivary carcinomas. Treatment was well tolerated, with few treatment-emergent events and only a minority discontinuing therapy due to toxicity. However, this study was limited by the single arm design and lack of randomization, as well as overall small numbers of patients in each cohort.

CCCO is a rare and distinct subtype of ovarian cancer, seen in young women with poor prognosis independent of stage, rendering women incurable. CCCO is insensitive to traditional platinum-based chemotherapy regimens, however a few select case reports noted responses to immune checkpoint inhibitors in CCCO[21,22] stimulating the inclusion of this aggressive tumour type in the current trial. The response rate to combination D + TM in CCCO was independent of PD-L1 expression in the tumour. Microsatellite instability was not assessed in this study,[23] however, consistent with other studies, few patients with CCCO had high TMB and there was no significant correlation with response. In another phase II trial of pembrolizumab for recurrent ovarian cancer (KEYNOTE-100), the response rate of clear cell carcinoma (N = 19) was 15.8% compared to 8% for the entire cohort (N = 300) [21]. A phase II trial of durvalumab versus physician choice chemotherapy reported at response rate of 10.7%.[24] The response rates to nivolumab alone or in combination with ipilimumab (a similar regime) for people with ovarian and other extra-renal clear cell carcinomas were 14 and 26.7%, respectively.[25] The majority had CCC of the ovary (n = 24/30 patients).

Salivary carcinomas represents 5% of all head and neck cancers, with usual treatment paradigms involving complete surgical resection with or without radiation therapy.[26] Like all other cancers included in this study, patients with aggressive, advanced or recurrent disease uniformly fare poorly with minimal incremental benefit of standard systemic chemotherapy regimens.[27] In a large series of 167 patients, expression of PD-L1 (≥1% of the cells with PD-L1 positivity) was present in the salivary gland carcinomas from 17% of patients,

N = 138	All causality		Related to Durvalumab/ Tremelimumab	
Adverse event	All grades >10% N (%)	Gr 3-5 N (%)	All grade >10% N (%)	Gr 3-5 N (%)
Hypothyroidism	28 (20%)	1 (1%)	25 (18%)	1 (1%)
Abdominal pain	48 (35%)	2 (1%)		
Constipation	57 (41%)	1 (1%)		
Diarrhea	64 (46%)	8 (6%)	36 (26%)	7 (5%)
Dry mouth	19 (14%)	0 (0%)		
Nausea	61 (44%)	2 (1%)	20 (14%)	1 (1%)
Vomiting	38 (28%)	6 (4%)		
Edema limbs	28 (20%)	2 (1%)		
Fatigue	109 (79%)	7 (5%)	40 (29%)	4 (3%)
Fever	25 (18%)	0 (0%)		
Flu like symptoms	15 (11%)	0 (0%)		
Pain (general)	29 (21%)	1 (1%)		
Anorexia	59 (43%)	1 (1%)	16 (12%)	
Arthralgia	25 (18%)	1 (1%)		
Back pain	40 (29%)	4 (3%)		
Pain in extremity	31 (22%)	2 (1%)		
Dizziness	19 (14%)	0 (0%)		
Headache	29 (21%)	0 (0%)		
Peripheral sensory neuropathy	26 (19%)	1 (1%)		1 (1%)
Insomnia	27 (20%)	0 (0%)		
Cough	53 (38%)	1 (1%)		
Dyspnea	62 (45%)	10 (7%)		2 (1%)
Pruritus	43 (31%)	0 (0%)	34 (25%)	
Rash maculo-papular	42 (30%)	5 (4%)	38 (28%)	5 (4%)
Other skin & subcutaneous tissue	21 (15%)	0 (0%)		
Hot flashes	18 (13%)	0 (0%)		
Thromboembolic event	15 (11%)	6 (4%)		

Table 4: **All and treatment related adverse events occurring in >10% of patients.**

Adverse event	Related to Durvalumab/ Tremelimumab (N = 138)
	Grade 3–5 N (%)
Disseminated intravascular coagulation	1 (1%)
Atrial fibrillation	1 (1%)
Other cardiac disorders (Non-Bacterial thrombothic endocarditis)	1 (1%)
Hypothyroidism	1 (1%)
Colitis	6 (4%)
Diarrhea	7 (5%)
Nausea	1 (1%)
Pancreatitis	1 (1%)
Small intestinal perforation	1 (1%)
Vomiting	1 (1%)
Edema trunk	1 (1%)
Fatigue	4 (3%)
Infusion related reaction	1 (1%)
Other immune system disorders[a]	6 (4%)
Abdominal infection	1 (1%)
Sepsis	1 (1%)
CPK increased	1 (1%)
Hyperkalemia	2 (1%)
Myositis	1 (1%)
Depressed level of consciousness	1 (1%)
Peripheral sensory neuropathy	1 (1%)
Seizure	2 (1%)
Other nervous system disorders[b]	1 (1%)
Other renal and urinary disorders[c]	1 (1%)
Dyspnea	2 (1%)
Pneumonitis	2 (1%)
Rash maculo-papular	5 (4%)
Hypertension	1 (1%)

[a]Nephritis, nephrotic syndrome, hypophysitis, scleroderma, immune thrombocytopenia purpura, diabetes. [b]Idiopathic pachymeningitis and cauda equina syndrome. [c]Renal failure and pyelonephritis.

Table 5: **Grade 3-5 Adverse Events related to Durvalumab/Tremelimumab.**

	Evaluable Pts[a]	Grade[b]				
		0 N (%)	1 N (%)	2 N (%)	3 N (%)	4 N (%)
Anemia (Hemoglobin)	133	25 (19%)	51 (38%)	38 (29%)	19 (14%)	0 (0%)
Lymphocyte count	133	35 (26%)	33 (25%)	35 (26%)	26 (20%)	4 (3%)
Neutrophil count	134	121 (90%)	7 (5%)	4 (3%)	2 (2%)	0 (0%)
Platelet count	133	113 (85%)	16 (12%)	1 (1%)	1 (1%)	2 (2%)
White blood cell	134	110 (82%)	18 (13%)	4 (3%)	2 (2%)	0 (0%)
Creatinine	133	98 (74%)	24 (18%)	9 (7%)	0 (0%)	2 (2%)
Hypoalbuminemia	134	33 (25%)	48 (36%)	43 (32%)	10 (8%)	0 (0%)
Alkaline phosphatase	132	60 (45%)	46 (35%)	17 (13%)	8 (6%)	1 (1%)
ALT	132	75 (57%)	44 (33%)	9 (7%)	3 (2%)	1 (1%)
AST	132	73 (55%)	43 (33%)	8 (6%)	8 (6%)	0 (0%)
Blood bilirubin	132	118 (89%)	6 (5%)	2 (2%)	4 (3%)	2 (2%)
Serum amylase	109	92 (84%)	10 (9%)	4 (4%)	2 (2%)	1 (1%)
Lipase	130	107 (82%)	5 (4%)	6 (5%)	10 (8%)	2 (2%)

Abbreviations: ALT, aspartate transaminase; AST, aspartate transaminase; CTCAE, common terminology criteria for adverse events. [a]Includes all patients with at least one blood count done after day 1. [b]Adverse events graded according to CTCAE V4.0 using test values and normal limits.

Table 6: **Laboratory adverse events (worst per patient).**

correlating with tumor grade (p = 0.035) and significantly worse DFS and OS (p = 0.02 and p = 0.003, respectively).[28] A response rate of 12% with single agent pembrolizumab was reported,[29] 8.3% (2/24, 80% CI, 2.2–21%), with 2 partial responses in patients with salivary duct carcinoma (2/10, ORR: 20%) to single agent nivolumab[30] and 16% to combination of nivolumab + ipilimumab in patients with non-adenoid cystic histology,[31] which is consistent with our findings. Numbers of patients with specific histological subtypes of salivary carcinomas were limited in this study and activity within subtypes can be evaluated in future studies.

In contrast to its correlation with poor prognosis in salivary carcinoma patients, PD-L1 expression has been correlated with improved survival in patients with anal carcinoma, independent of HPV status.[32] A reported response rate of 11% to single agent pembrolizumab[33] is similar to the response rate of 9.5% with combination D + T in our study.

CUP is defined as a biopsy-proven metastatic carcinoma without a primary source evident after comprehensive clinical, imaging and pathology investigation. This entity represents approximately 2–5% of all adult cancers, is commonly of nonspecific adenocarcinoma histology, and is associated with a very short median PFS of 6 months.[34] CUP may express markers which correlate with response to immune checkpoint inhibitors such as PD-L1 or immune cell infiltration.[35] Because CUP arise from diverse sites of origin, TIL/PDL1 positivity was used to select patients. At the time of trial design, such markers were considered indicative of potential sensitivity to immune checkpoint inhibitors which have subsequently failed to preform well as predictive biomarkers for patients. Recent studies demonstrated activity of single agent nivolumab in patients with CUP (ORR 24%, 95% CI: 13–40%),[36] and single agent pembrolizumab of 20.0% (95% CI 6.8–41) comparable to our results[37] Further evaluation of activity to single agent versus combination immune checkpoint inhibitors, in less heavily treated patients, with further assessment of potential predictive biomarkers is warranted for CUP.

We found no evidence of benefit from combined checkpoint inhibitor therapies previously treated patients with osteosarcoma. However, there were three responders among patients with UPS and responses were durable (median 16.6 months, (range 5–27.3). UPS incorporates a group of biologically heterogeneous soft tissue sarcomas which limits evaluation of novel agents and the identification of biomarkers of activity. Nonetheless, our study adds to others[38,39] showing that there is a subset of UPS patients that respond to immune checkpoint inhibitors, warranting further investigation into potential underlying mechanisms determining immune responsiveness.

The tumour types in the study were selected following review of the literature for preclinical or early

clinical evidence sensitivity. Reports of responses in early phase studies permitting preclinical experiments suggesting sensitivity and studies assessing pathological samples for immune cell infiltrates and/or expression of PDL1 were identified. Subsequently, the ability to recruit patients with these cancers was assessed by survey of cooperative group sites. The basket design of this study enabled the enrolment of 140 patients with rare cancers in 3 years across 13 Canadian sites. We were able to confirm diagnosis of these rare cancers with expert subspecialist central pathology review and showed that all cohorts except osteosarcoma had patients with responses to dual checkpoint inhibition. The small numbers of patients within each cohort and lack of randomization to single agent versus combination precluded our ability to determine the contribution of each agent to activity. However, durvalumab + tremelimumab in CCCO and salivary carcinomas was associated with promising activity and acceptable toxicity and should continue to be investigated in patients with these cancers.

Contributors

AAG, MT, DT, JD and TN designed the study. JS, SZ and JD were responsible for conduct and data management. AT, DJ, RJ, HH, EW, QC, CK, RW, TA, AR, DP, JH, MK, CE, SH and AAG were responsible for patient enrolment. WT, SZ and JD analyzed the data. AAG, WT and JD wrote the manuscript. TN, MT, TC, JS, WT, AT, DJ, RJ, HH, EW, QC, CK, RW, TA, AR, DP, JH, MK, CE and SH reviewed the article. All authors had access to the Statistical Analysis Report. WT, SZ, JS, JD had access to and verified the underlying data. AAG and JD had responsibility for the decision to submit for publication. All authors have read and approved the final version of the manuscript.

Data sharing statement

CCTG has a formal data sharing policy and process found at www.ctg.queensu.ca/docs/public/policies/DataSharingandAccessPolicy.pdf. Protocol, analysis plan, and informed consent documents will be made available with publication.

Declaration of interests

AT–Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events: GSK, AZ, Eisai, Merck/Support for attending meetings and/or travel: GSK/Participation on a Data Safety Monitoring Board or Advisory Board: GSK, Eisai, Merck.

RJ–Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events: Pfizer/Participation on a Data Safety Monitoring Board or Advisory Board: BMS, Medison.

HH–Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events: Novocure Canada/Participation on a Data Safety Monitoring Board or Advisory Board: Novocure Canada.

EW–Grants or contracts: Merck, Roche/Genetech/Consulting fees: Merck, Roche, EMD Serono.

QC–Grants or contracts: AZ/Consulting fees: Abbvie, Amgen, AnHeart, Astellas, AZ, Boehringer Ingelheim, Bristol Meyer Squibb, Daichii Sankyo, Eli Lilly, GSK, Janssen, Merck, Novartis, Ocellaris, Pfizer, Roche, Takeda/Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events: Abbvie, Amgen, AnHeart, Astellas, Astra Zeneca, Boehringer Ingelheim, Bristol Meyer Squibb, Diachii Sankyo, Eli Lilly, GSK, Janssen, Merck, Novartis, Ocellaris, Pfizer, Roche, Takeda/Participation on a Data Safety Monitoring Board or Advisory Board: Merck, Serano/Leadership or fiduciary role in other board, society, committee or advocacy group, paid or unpaid: Lung Cancer Canada.

CK–Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events: Astellas, Bayer, BMS, Merck, Pfizer, Janssen, Eisai, Seagen, Ipsen, Abbvie, AstraZeneca/Support for attending meetings and/or travel: Ipsen, Novartis, Janssen, AstraZeneca/Participation on a Data Safety Monitoring Board or Advisory Board: Ipsen, BMS, Eisai, Novartis, Astellas, Bayer, AstraZeneca, Merck, Pfizer, BioTech, Seagen, Abbvie, TA–Consulting fees: BMS, Roche, AZ, Merck, Bayer/Support for attending meetings and/or Travel: Spring Works Therapeutics.

TN–All support for the present manuscript (e.g., funding, provision of study materials, medical writing, article processing charges, etc.): CCS.

DP–Grants or contracts: Exactis/Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events: GSK, AZ/Participation on a Data Safety Monitoring Board or Advisory Board: Exactis

JH–Consulting fees: BMS, AZ, Novartis, Eli-Lilly, Seattle Genetics, Gilead/Participation in data safety monitoring board or advisory board: BMS.

MK–All support for the present manuscript (e.g., funding, provision of study materials, medical writing, article processing charges, etc.): AZ/Grants or contracts: Eisai, Exelixis, Novartis, Eli Lilly/Consulting fees: Bayer/Participation on a Data Safety Monitoring Board or Advisory Board: Bayer, Ipsen.

SH–Consulting fees: BMS, Merck.

JD–Grants or contacts: AZ.

All other authors have no conflicts to declare.

Acknowledgements

This trial was funded by the Canadian Cancer Society grant 707213. Drug and partial funding support was provided by AstraZeneca. We would like to thank all patients who participated in IND228 as well as all their families and caregivers. We also thank AstraZeneca for their support of IND228.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102991.

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