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## Clinical Trial

# A randomised phase II trial of a trivalent ganglioside vaccine targeting GM2, GD2 and GD3 combined with immunological adjuvant OPT-821 versus OPT-821 alone in metastatic sarcoma patients rendered disease-free by surgery<sup>☆</sup>



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**Abstract Background:** Recurrence after resection of metastatic sarcoma is common. The gangliosides GM2, GD2 and GD3 are strongly expressed across sarcoma subtypes. We hypothesised that generation of anti-ganglioside antibodies would control micrometastases and improve outcomes in sarcoma patients who were disease-free after metastasectomy.

**Methods:** We conducted a randomised phase II trial of the immunological adjuvant OPT-821 with a KLH-conjugated ganglioside vaccine targeting GM2, GD2 and GD3, versus OPT-821 alone in patients with metastatic sarcoma following complete metastasectomy. Patients received 10 subcutaneous injections at Weeks 1, 2, 3, 8, 16, 28, 40, 52, 68 and 84 and were followed for evidence of recurrent disease. The primary end-point was relapse-free survival. Secondary end-points included overall survival and serologic response.

**Results:** A total of 136 patients were randomised, 68 to each arm. The mean age was 51.2, 52.2% were male, 90.4% had relapsed disease, 86.8% had high-grade tumours and 14% had  $\geq 4$  metastases resected. Histologies included leiomyosarcoma (33%), spindle cell sarcoma (14%), undifferentiated pleomorphic sarcoma (13%), osteosarcoma (10%), synovial sarcoma (9%), liposarcoma (9%) and others (12%). Most adverse events were Grade  $\leq 2$  (83.8% and 70.6% in the vaccine and adjuvant arms, respectively). The most common ( $\geq 20\%$  of patients) were injection site reaction (89.7%), fatigue (44.1%) and pyrexia (27.9%) on the vaccine arm, and injection site reaction (69.1%) on the adjuvant only arm. The 1-year relapse-free survival rate (34.5% and 34.8% in the vaccine and OPT-821 monotherapy arm, respectively) did not differ between arms ( $P = 0.725$ ). One-year overall survival rates were 93.1% and 91.5% in the vaccine and OPT-821 monotherapy arm, respectively ( $P = 0.578$ ). Serologic responses at week 9 were more frequent on the vaccine arm (96.5% of patients) than in the adjuvant arm (32.8%), and the difference between groups was durable.

**Conclusions:** A sustained serologic response to vaccination was induced with the vaccine, but no difference in recurrence-free or overall survival was observed between treatment arms.

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## 1. Introduction

Sarcomas are a heterogeneous group of mesenchymal malignancies representing less than 1% of adult cancers [1]. The prognosis of patients with metastatic sarcoma is poor, with a median overall survival (OS) of approximately 20 months from the time of metastasis in a recent study [2], although prognosis is often histology-specific [3]. The most frequent site of distant metastasis is the lung, followed by soft tissue, retroperitoneum and other distant sites [4]. Large retrospective studies have suggested that patients who undergo complete resection of distant metastases may have a higher survival rate than those with unresectable metastatic disease [5,6]. Nevertheless, more than 50% of patients recur after pulmonary metastasectomy with an expected 5-year survival rate of 34% [7].

Anti-cancer vaccination targeting tumour-associated antigens is an attractive strategy to activate the adaptive

immune system and eliminate minimal residual disease [8]. Gangliosides are a subclass of glycosphingolipids linked to sialic acids that are anchored to the cell membrane. They are often expressed in a range of sarcoma subtypes and are often immunogenic, triggering anti-ganglioside antibodies that are readily measurable in the peripheral blood [9–11]. Eliciting an immune response to multiple gangliosides may lead to greater anti-neoplastic activity compared to a monovalent vaccination strategy [12]. Further, conjugation of a ganglioside vaccine to keyhole limpet haemocyanin (KLH) induces a more potent T cell and antibody response [13,14].

This randomised phase II study was designed to evaluate the hypothesis that administration of a trivalent vaccine targeting the GM2, GD2 and GD3 gangliosides, in addition to the novel immunological adjuvant OPT-821, would improve survival in patients with metastatic

sarcoma rendered disease-free by surgery compared to treatment with OPT-821 alone. OPT-821 is a potent immunological adjuvant derived from the soapbark tree *Quillaja saponaria* that was found to be safe and immunogenic in the pre-clinical and clinical settings [15]. As subcutaneous injection of OPT-821 consistently induces local induration and erythema when administered alone or in combination, it was required in the control arm to maintain double-blind status.

## 2. Methods

This was a Phase II, randomised, double-blind, multi-centre study of a trivalent ganglioside vaccine plus the immunological adjuvant OPT-821 versus OPT-821 alone for patients with metastatic sarcoma who were rendered disease-free (NCT01141491). The study was completed according to the Declaration of Helsinki and the international standards of Good Clinical Practice. The independent ethics committee or institutional review board of each participating study centre approved the protocol and all amendments. All patients provided written informed consent.

### 2.1. Patient selection

Eligible patients were  $\geq 16$  years of age with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and pathologic confirmation of newly diagnosed metastatic or distant relapsed sarcoma at their respective sarcoma centre. Patients were required to be without radiological evidence of residual disease within 4 weeks of study initiation following surgery or multi-modality therapy. Non-surgical local ablative therapies, such as SRS or cryotherapy, were not acceptable as a replacement for surgical resection for the purpose of eligibility. Radiation or systemic chemotherapy must have been completed  $\geq 4$  weeks prior to treatment on this study and patients must have recovered from all adverse effects of treatment or have returned to baseline status. Adequate organ and marrow function at the time of enrolment was required.

Key exclusion criteria included: A diagnosis of Ewing sarcoma, embryonal rhabdomyosarcoma and gastrointestinal stromal tumour histology, based on limited data for ganglioside expression in these tumour types. Patients with brain or bone metastases, retroperitoneal and/or uterine sarcoma with recurrent disease only in the abdomen or pelvis, and those whose disease recurred within 6 months of preceding treatment were also excluded. Patients on continuous doses of anti-inflammatory medications or those treated with a drug that had not received regulatory approval, and patients with any immunodeficiency disorders or illness, serologic positivity for Hepatitis B or C virus, or HIV were excluded.

### 2.2. Randomisation and blinding

During screening, patients were entered into an electronic data management system and randomisation was accomplished by the method of random permuted blocks of four. Randomisation was stratified by disease state at the time of enrolment (relapsed versus de novo metastasis at diagnosis). Patients presenting in the relapsed setting were further stratified by most recent disease-free interval ( $< 12$  months versus  $\geq 12$  months) and number of relapses (1 versus  $> 1$ ).

### 2.3. Study procedures

Patients received a total of 10 injections, with one injection administered on visit weeks 1, 2, 3, 8, 16, 28, 40, 52, 68 and 84. Injections were administered subcutaneously into the upper arms, thighs or buttocks, rotating to different quadrants with each injection. Patients randomised to receive vaccine plus OPT-821 received a 1 mL subcutaneous injection containing 30 mcg of GM2, 30 mcg of GD2L and 30 mcg of GD3L, each conjugated to KLH, plus 150 mcg of OPT-821. Patients randomised to OPT-821 only received a 1 mL subcutaneous injection containing 150 mcg of OPT-821.

Patients were evaluated radiographically at Weeks 16, 28, 40, 52, 68 and 84, and every 12 weeks thereafter. Any clinical or radiographic evidence of recurrence was considered evidence of progression. If radiologic evidence of recurrence was found, pathologic confirmation was obtained whenever possible. If a biopsy was not possible and there were indeterminate radiographic findings, repeat imaging at a 4-week interval was permitted.

Adverse events (AEs) were assessed from the time of administration of the first vaccine through the end of the study (or death or recurrence of disease). Serology was collected at screening, prior to visit week 1, and at visit weeks 9, 17, 40, 52, 68, 96 and 120.

### 2.4. Statistical analyses

All statistical analysis utilised the intent-to-treat principle. All patients who received one or more injections of study treatment were included in the efficacy and safety analyses. In the efficacy analyses, patients were categorised by the arm to which they were randomised, regardless of the actual treatment received. For safety analyses, patients were categorised by the actual treatment received.

The primary end-point of this study was relapse-free survival (RFS) in patients with pre-treatment antibody titre levels of  $< 1:20$  against any one of the vaccine antigens (primary efficacy population). RFS was defined as time from randomisation until any evidence of tumour growth or appearance of disease recurrence anywhere in the body, or death from any cause as determined by the

Table 1  
Patient characteristics.

	Vaccine + OPT-821 (N = 68)	OPT-821 (N = 68)
<b>Age (years)</b>		
n	68	68
Mean (SD)	49.8 (13.25)	52.5 (15.24)
<b>Sex - n (%)</b>		
Male	39 (57.4)	32 (47.1)
Female	29 (42.6)	36 (52.9)
<b>Race - n (%)</b>		
American Indian or Alaska Native	1 (1.5)	0
Asian	4 (5.9)	3 (4.4)
Black or African American	1 (1.5)	3 (4.4)
White	62 (91.2)	61 (89.7)
Other	0	1 (1.5)
<b>Ethnicity - n (%)</b>		
Hispanic or Latino	3 (4.4)	2 (2.9)
Not Hispanic or Latino	65 (95.6)	66 (97.1)
<b>ECOG performance status— n (%)</b>		
0	57 (83.8)	48 (70.6)
1	11 (16.2)	20 (29.4)
<b>Number of metastases – n (%)</b>		
1 to 3	60 (88.2)	57 (83.8)
4 or more	8 (11.8)	11 (16.2)
<b>Primary tumour grade - n (%)</b>		
High	62 (91.2)	56 (82.4)
Intermediate	5 (7.4)	9 (13.2)
Low	1 (1.5)	3 (4.4)
<b>Primary site of original disease – n (%)</b>		
Soft tissue	35 (51.5%)	32 (47.1%)
Other	18 (26.5%)	20 (29.4%)
Bone	8 (11.8%)	6 (8.8%)
Abdomen	4 (5.9%)	7 (10.3%)
Lungs	1 (1.5%)	2 (2.9%)
Kidney	1 (1.5%)	1 (1.5%)
Head and Neck	1 (1.5%)	0
<b>Metastatic disease at time of original diagnosis – n (%)</b>		
Yes	13 (19.1%)	11 (16.2%)
No	55 (80.9%)	57 (83.8%)
<b>Most recent disease-free interval – n (%)</b>		
<12 months	34 (50.0%)	33 (48.5%)
≥ 12 months	28 (41.2%)	28 (41.2%)
<b>Number of relapses – n (%)</b>		
1	34 (50.0%)	36 (52.9%)
>1	28 (41.2%)	25 (36.8%)
<b>Prior radiotherapy - n (%)</b>		
Yes	35 (51.5%)	34 (50.0%)
No	33 (48.5%)	34 (50.0%)
<b>Prior chemotherapy – n (%)</b>		
Yes	35 (51.5%)	37 (54.4%)
No	33 (48.5%)	31 (45.6%)
<b>Tumour histology - n (%)</b>		
Leiomyosarcoma (n = 45)	17 (25%)	28 (41%)
Spindle cell sarcoma (n = 19)	12 (18%)	7 (10%)
Pleomorphic sarcoma (n = 18)	9 (13%)	9 (13%)
Osteosarcoma (n = 14)	7 (10%)	7 (10%)
Liposarcoma (n = 12)	6 (9%)	6 (9%)
Synovial sarcoma (n = 12)	5 (7%)	7 (10%)
Other (n = 16)	12 (18%)	4 (6%)

principal investigator at each study centre. The primary analysis was based upon a stratified log-rank test to compare the distributions of time to RFS. Patients

without evidence of disease progression at the last follow-up were censored at that time point. Patients lost to follow-up for reasons related to disease progression were treated as progressing as of their last follow-up. The null hypothesis was that the distributions of time to RFS for the two arms are the same. The significance level for testing the null hypothesis was 20% (2-sided), a significance level suggested for preliminary studies of cancer vaccines [16].

Secondary end-points included: OS, defined as time of survival from randomisation until death from any cause, efficacy analyses (RFS and OS) in the total population regardless of pre-treatment antibody levels, safety and tolerability of the trivalent ganglioside vaccine plus OPT-821, as determined by reported AEs and changes in physical examinations and laboratory tests. Translational end-points included evaluation of immune response to treatment, including correlating serologic response to vaccination with subsequent clinical outcome. In addition to the use of the log-rank test for comparing the distributions of time to event for RFS and OS, Kaplan–Meier curves characterised the event-free rates over time.

A total of 134 patients were planned for this study, with 67 patients entered per treatment arm. The sample size was calculated assuming that 5% of patients would be excluded from the primary efficacy population because of high baseline antibody titres, a two-sided type 1 error rate of 20%, log-rank test (underlying exponential distribution), vaccine plus OPT-821 arm would have a 1-year RFS rate of 50%, the OPT-821 monotherapy arm would have a 1-year RFS of 30%, accrual would take 18 months and a minimum of 12 months of follow-up per patient. With these assumptions and a sample size of 67 patients per treatment arm, the study would have 92% power to reject the null hypothesis. The primary analysis was scheduled when a total of 96 RFS events were recorded. The primary efficacy analysis was performed in August 2013, after 89 RFS events because of the unexpectedly slow event rate. Patients enrolled on the study were followed until November 2016, and the study closed in January 2017.

Continuous data were summarised with descriptive statistics of the mean, minimum, median, standard deviation and number of patients. Counts and percentages summarised all categorical data. All statistical analyses were performed with the SAS® system (version 9.1.3 or later). All disposition, demographic and baseline characteristics data were summarised and listed by treatment group and patient identifier.

The safety analysis included all patients enrolled in the study who received at least one injection of study treatment. Treatment emergent AEs were summarised by system organ class and preferred terms within a system organ class for each treatment group. AEs were coded using the MedDRA dictionary (version 11 or later).

### 3. Results

#### 3.1. Patient characteristics

Between June 2010 and December 2012, a total of 136 patients were randomised on this study, 68 to each treatment arm. Patient characteristics (Table 1) were generally well balanced between randomisation arms. Most study patients were <65 years of age (83.8% in the vaccine arm and 75% in the OPT-821 monotherapy arm). Approximately half of patients had a recent disease-free interval of <12 months and had received prior radiation therapy or chemotherapy. Most patients on the vaccine and adjuvant alone arms, respectively, had relapsed (n = 62 [91%] versus 61 [90%]) high-grade (62 [91%] versus 56 [82%]) soft tissue disease (35 [52%] versus 32 [47%]) that was localised at the time of diagnosis (55 [81%] versus 57 [84%]). Forty-one patients (31%) completed all 10 of the planned vaccine injections.

#### 3.2. Safety

Overall, study treatment was well tolerated across both arms. Sixty-six (97.1%) patients on the vaccine arm and 60 (88.2%) on the OPT-821 monotherapy arm experienced an AE. The most common AEs on the vaccine

arm were injection site reaction (89.7%), fatigue (44.1%), pyrexia (27.9%), injection site erythema (17.6%) and influenza-like illness (14.7%). On the adjuvant alone arm, the most frequent AEs were injection site reaction (69.1%), fatigue (19.1%), diarrhoea (13.2%) and injection site pain (11.8%) (Table 2). Most AEs were Grade  $\leq 2$  (83.8% and 70.6% in the vaccine and adjuvant arms, respectively). Grade  $\geq 3$  AEs occurred in nine patients (13.2%) in the vaccine arm and 12 patients (17.6%) on the adjuvant alone arm. There was one patient death on the vaccine arm (the cause of death was unknown) that was deemed possibly related to study treatment. Treatment emergent serious AEs occurred in four patients (5.9%) on the vaccine arm and seven (10.3%) in OPT-821 monotherapy arm (Supplemental Table S1). One patient on the adjuvant alone arm (generalised rash) and three on the vaccine arm (maculopapular rash, Grade 1–2 anaphylactoid reaction and death) discontinued treatment due to an adverse event.

#### 3.3. Efficacy

All patients enrolled and treated were included in the primary efficacy population. The mean (standard deviation) number of vaccine administrations was 6.2 (2.35) in the vaccine arm and 6.2 (2.23) in the adjuvant alone

Table 2  
Adverse events occurring in  $\geq 5\%$  of the study population.

AE by system organ class preferred term <sup>a</sup>	Vaccine plus OPT-821		OPT-821	
	Grade 1-2	Grade 3	Grade 1-2	Grade 3
<b>Gastrointestinal disorders</b>				
Abdominal pain	4 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)
Diarrhoea	2 (2.9)	0 (0.0)	9 (13.2)	0 (0.0)
Nausea	5 (7.4)	1 (1.5)	6 (8.8)	0 (0.0)
<b>General disorders and administration site conditions</b>				
Fatigue	30 (44.1)	0 (0.0)	13 (19.1)	0 (0.0)
Influenza like illness	10 (14.7)	0 (0.0)	3 (4.4)	0 (0.0)
Injection site erythema	12 (17.6)	0 (0.0)	5 (7.4)	0 (0.0)
Injection site oedema	8 (11.8)	0 (0.0)	1 (1.5)	0 (0.0)
Injection site pain	8 (11.8)	0 (0.0)	8 (11.8)	0 (0.0)
Injection site reaction	59 (86.8)	2 (2.9)	47 (69.1)	0 (0.0)
Malaise	5 (7.4)	0 (0.0)	3 (4.4)	0 (0.0)
Pain	5 (7.4)	0 (0.0)	3 (4.4)	0 (0.0)
Pyrexia	18 (26.5)	1 (1.5)	2 (2.9)	0 (0.0)
<b>Infections and infestations</b>				
Upper respiratory tract infection	8 (11.8)	0 (0.0)	7 (10.3)	0 (0.0)
<b>Musculoskeletal and connective tissue disorders</b>				
Arthralgia	4 (5.9)	0 (0.0)	5 (7.4)	0 (0.0)
Myalgia	4 (5.9)	0 (0.0)	4 (5.9)	0 (0.0)
Pain in extremity	4 (5.9)	0 (0.0)	6 (8.8)	0 (0.0)
<b>Nervous system disorders</b>				
Headache	6 (8.8)	0 (0.0)	6 (8.8)	1 (1.5)
<b>Psychiatric disorders</b>				
Anxiety	6 (8.8)	0 (0.0)	6 (8.8)	0 (0.0)
<b>Respiratory, thoracic and mediastinal disorders</b>				
Cough	8 (11.8)	0 (0.0)	3 (4.4)	0 (0.0)
<b>Skin and subcutaneous tissue disorders</b>				
Erythema	4 (5.9)	1 (1.5)	0 (0.0)	0 (0.0)
Rash	5 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)

<sup>a</sup> All numbers represent number of patients (%).

arm. The primary efficacy analysis was performed 8 months after enrolment of the last patient and 39 months after enrolment of the first patient. Progression events had occurred in 46 patients in the vaccine arm and 43 in the OPT-821 monotherapy arm. The RFS rate at one year was 34.5% in the vaccine arm and 34.8% in the OPT-821 monotherapy arm ( $P = 0.725$ ). The median RFS was 191 days in each arm (95% confidence interval [CI] 104–420 in the vaccine arm and 141–357 in the control) (Fig. 1A). Ten patients in the vaccine arm and 12 in the OPT-821 monotherapy arm died. The 1-year OS rates were 93.1% and 91.5% in the vaccine and OPT-821 monotherapy arm, respectively ( $P = 0.578$ ). Median OS was not reached in either treatment arm (Fig. 1B).

### 3.4. Serologic response

At baseline, 32% of patients (22 of 68) who received the vaccine and 22% (14 of 64) who received the adjuvant alone had a detectable serologic response (defined as any anti-ganglioside antibody titre of  $>1:20$ ) to one of the vaccine antigens. At week 9, serologic responses were more frequent in the vaccine arm (96.5%; 55 of 57) than in the adjuvant arm (32.8%; 21 of 64). On the adjuvant only arm, 9.4% of patients (6 of 64) had an increase of their titres with treatment compared to 93% (53 of 57) on the vaccine arm. At the last time point (weeks 40–68), the differences in serologic response remained apparent (89.3% [25/45] of patients on the vaccine arm versus 20.8% on the adjuvant only [4/44]) (Table 3).

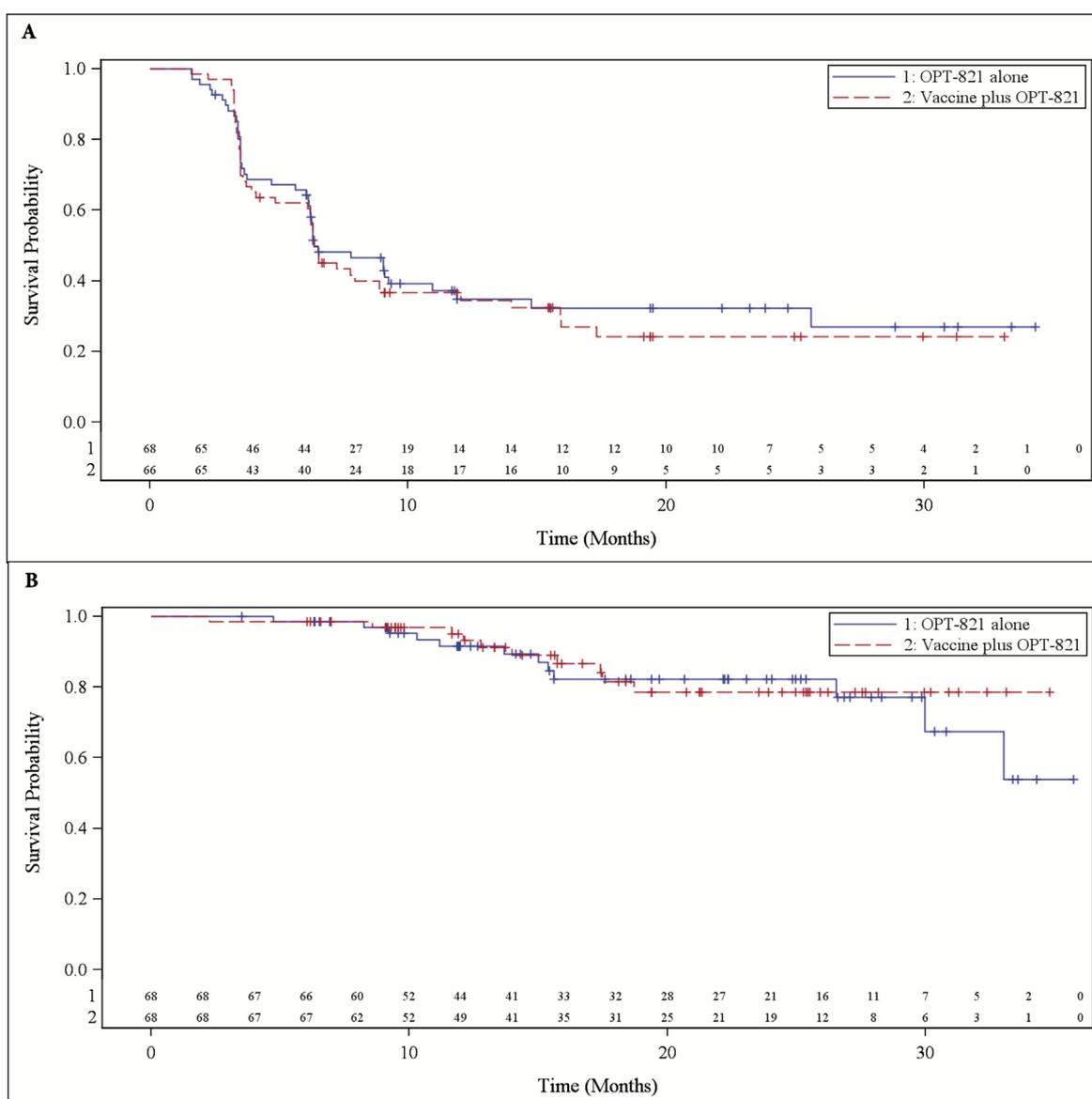


Fig. 1. Kaplan–Meier curves of (A) RFS (B) OS performed from the primary efficacy analysis data 3.2 years after trial initiation (8 months after accrual of final patient).

Table 3  
Overall serologic response to study treatment.

	Vaccine + OPT-821 (N = 68)	OPT-821 (N = 68)
Baseline - n (%)		
High	3 (4.4)	3 (4.7)
Low	19 (27.9)	11 (17.2)
Negative	46 (67.6)	50 (78.1)
<b>Total</b>	<b>68</b>	<b>64</b>
Week 9 - n (%)		
High	42 (73.7)	3 (4.7)
Low	13 (22.8)	18 (28.1)
Negative	2 (3.5)	43 (67.2)
<b>Total</b>	<b>57</b>	<b>64</b>
Week 17 - n (%)		
High	31 (68.9)	3 (6.8)
Low	13 (28.9)	12 (27.3)
Negative	1 (2.2)	29 (65.9)
<b>Total</b>	<b>45</b>	<b>44</b>
Week 40–68 - n (%)		
High	17 (58.6)	2 (8.3)
Low	10 (34.9)	4 (16.7)
Negative	2 (6.9)	18 (75.0)
<b>Total</b>	<b>29</b>	<b>24</b>

High titres: > 1:160, low: > 1:20–1:160, negative: ≤ 1:20.

#### 4. Discussion

To our knowledge, this randomised, double-blind, controlled trial is the largest vaccine trial of its kind in sarcoma patients. The study did not meet its primary end-point. No significant difference in RFS was seen between patients who received a trivalent KLH-conjugated ganglioside vaccine administered with the adjuvant OPT-821 compared to patients who received OPT-821 alone. There was a lack of efficacy in the vaccine arm above and beyond the control despite measurable and durable serologic responses seen across antibody class (IgM and IgG) and ganglioside target (GM2, GD2 and GD3). However, both the trivalent ganglioside vaccine and the adjuvant OPT-821 were safe and tolerable. AEs were generally mild and as expected for a subcutaneously injected vaccine, with injection site reactions or fatigue being the most common treatment related TEAEs on the vaccine arm.

A key strength of this study is its large size for a prospective interventional trial in a rare group of diseases. It sheds light on the natural history of sarcoma patients who underwent metastasectomy and were rendered free of disease. The time to progression in each treatment arm of this study was approximately 6 months, while the 1-year RFS rate was 35%. These data are in line with prior retrospective reports, in which the median time to recurrence after initial metastasectomy was 6.8 months [7]. Longer term survival data would be of interest, but is not available from the study sponsor MabVax Therapeutics, which is no longer in operation. Subgroup analysis by tumour histology was previously presented at the American Society of Clinical Oncology

Annual Meeting, showing no difference in RFS when stratified by histology [17].

The reason why the trivalent ganglioside vaccine did not prolong survival in this study population is not clear. Anti-GD2 monoclonal antibodies are now standard of care in the treatment of high-risk neuroblastoma in combination with interleukin-2 and/or granulocyte-macrophage colony-stimulating factor, serving as proof-of-principle in targeting these antigens with antibody therapy [18,19]. However, a recent phase II study of dinatuximab plus granulocyte-macrophage colony-stimulating factor in recurrent osteosarcoma rendered disease-free by surgery, a disease known to express high levels of GD2 [20], failed to reach its primary end-point of event-free survival [21]. These results suggest that anti-GD2 antibody monotherapy is ineffective in osteosarcoma. Similarly, the results of our study imply that dinatuximab alone would not be effective in other sarcoma subtypes, as anti-ganglioside antibody titres generated by the trivalent vaccine did not lead to improved clinical outcomes. However, a formal comparison between the quantity and quality of anti-GD2 antibodies in the dinatuximab studies and the vaccine-induced antibodies in our study is not possible. It is also notable that ganglioside expression was not measured in either the osteosarcoma-specific dinatuximab trial or in this study utilising a trivalent ganglioside vaccine. It is possible that ganglioside expression is lost in advanced sarcomas, in which case ganglioside-targeted therapies would be futile.

Even if ganglioside expression is retained in advanced sarcomas, anti-ganglioside antibodies titres generated by the vaccine may not have been high enough or had high enough affinity to elicit clinically meaningful activity, a possibility that could theoretically be addressed by utilising anti-ganglioside monoclonal antibodies with higher affinity. Alternatively, circulating antibodies alone may be insufficient to mount anti-tumour activity in sarcomas without activation of T cells. Other mechanisms may contribute to ganglioside vaccine resistance in sarcomas, such as upregulation of immunosuppressive myeloid-derived suppressor cells, heterogeneity of ganglioside expression in the tumour, downregulation of antigen-presentation machinery, or upregulation of inhibitor immune checkpoint molecules after vaccination [22–24]. Although this trial was initiated before the advent of anti-PD-(L)1 therapy across cancer types, there is increased interest today in combining immune checkpoint blockade with therapeutic vaccines to elicit a more robust immune response [25,26]. Recently reported data in high-risk neuroblastoma suggests that adding an oral yeast  $\beta$ -glucan as an adjuvant to a multivalent ganglioside vaccine conjugated to KLH can successfully boost antibody-dependent cell-mediated cytotoxicity [19,27]. GD2-directed chimeric antigen receptor (CAR) T cells have also demonstrated promise in very early clinical trials in both neuroblastoma and

H3K27M-mutated diffuse midline gliomas [28,29]. Thus, gangliosides such as GD2 are still promising targets in sarcoma, but novel strategies are likely needed to target them or to augment the anti-tumour activity of anti-ganglioside antibodies.

This trial has several limitations. It enrolled multiple histologic subtypes of sarcoma, which are now increasingly recognised as unique disease entities with their own natural histories and immune profile, which could make finding a signal of efficacy more difficult. Patients were not screened for or stratified by ganglioside expression, and GM2, GD2 and GD3 may not truly be ‘shared’ antigens across all tumours. Nevertheless, gangliosides are still attractive therapeutic targets. Future research should focus on confirming target engagement with an anti-ganglioside therapy and measuring immune activation or anti-tumour activity, above and beyond serological response.

#### Author contribution

**Evan Rosenbaum:** Data curation, Formal analysis, Writing – original draft. **Rashmi Chugh:** Investigation. **Christopher W. Ryan:** Investigation, Writing – review and editing. **Mark Agulnik:** Investigation, Writing – review and editing. **Mohammed M. Milhem:** Investigation. **Suzanne George:** Investigation. **Robin L. Jones:** Investigation, Writing – review and editing. **Bartosz Chmielowski:** Investigation. **Brian Andrew Van Tine:** Investigation, Writing – review and editing. **Hussein Tawbi:** Investigation. **Anthony D. Elias:** Investigation, Writing – review and editing. **William L. Read:** Investigation, Writing – review and editing. **G. Thomas Budd:** Investigation, Writing – review and editing. **Li-Xuan Qin:** Data curation, Formal analysis, Investigation, Writing – original draft. **Eve T. Rodler:** Investigation. **Joe Hirman:** Conceptualisation, Data curation, Formal analysis, Investigation. **Paul Weiden:** Conceptualisation, Data curation, Formal analysis, Investigation. **Cathryn M. Bennett:** Conceptualisation, Data curation, Formal analysis, Investigation, Writing – review and editing. **Philip O. Livingston:** Conceptualisation, Data curation, Formal analysis, Investigation, Writing – review and editing. **Govind Ragupathi:** Conceptualisation, Data curation, Formal analysis, Investigation, Writing – review and editing. **David Hansen:** Conceptualisation, Data curation, Formal analysis, Investigation, Writing – review and editing. **Sandra P. D’Angelo:** Investigation, Writing – review and editing. **William D. Tap:** Investigation, Writing – review and editing. **Gary K. Schwartz:** Investigation, Writing – review and editing. **Robert G. Maki:** Conceptualisation, Data curation, Formal analysis, Investigation, Writing – review and editing. **Richard D. Carvajal:** Conceptualisation, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing - original draft.

#### Conflict of interest statement

The authors declare the following financial interests/ personal relationships which may be considered as potential competing interests:

**Robin Jones:** MSD, GSK (grants/research support); Adaptimmune, Astex, Athenex, Bayer, Boehringer Ingelheim, Blueprint, Clinigen, Eisai, Epizyme, Daichii, Deciphera, Immunodesign, Immunicum, Karma Oncology, Lilly, Merck, Mundipharma, Pharmamar, Springworks, SynOx, Tracon, UptoDate (consultation fees). **Brian Van Tine:** Pfizer, Merck, Tracon, GSK (research grants); Accuronix Therapeutics (licences); Epizyme, ADRx, Ayala Pharmaceuticals, Cytokinetics, Inc., Bayer (consulting fees); Targeted Oncology, Adaptimmune, Bionest Partners, GSK, Intellisphere LLC (payments or honoraria); Hinshaw & Culbertson LLP, Rodney Law, CRICO Risk Management Foundation, Tracey & Fox Low Firm (expert testimony); Epizyme, GSK, Adaptimmune (travel support); Adaptimmune Limited, Apexigen Inc, Daiichi Sankyo, Deciphera, Epizyme, GSK, Novartis, Lilly, Epizyme, Bayer, PTC Therapeutics (DSMB or advisory board); Polaris (leadership or fiduciary role). **Robert Maki:** Bayer, Deciphera, Karyopharm, Peel Therapeutics, Tracon, American Board of Internal Medicine, American Society for Clinical Oncology, UptoDate (consulting fees/honoraria); Bayer, Tracon (travel support). **All others:** None declared.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejca.2022.09.003>.

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