Nivolumab in children and young adults with relapsed or refractory solid tumours or lymphoma (ADVL1412): a multicentre, open-label, single-arm, phase 1–2 trial

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Summary

Background Immune checkpoint inhibitors targeting PD-1 have shown clinical benefit in adults with cancer, but data on these drugs in children are scarce. We did a phase 1–2 study of nivolumab, a PD-1 blocking monoclonal antibody, to determine its safety, pharmacokinetics, and antitumour activity in children and young adults with recurrent or refractory non-CNS solid tumours or lymphoma.

Methods We did a multicentre, open-label, single-arm, dose-confirmation and dose-expansion, phase 1–2 trial in 23 hospitals in the USA. Eligible patients for part A (dose-confirmation phase) of the study were aged 1–18 years with solid tumours with measurable or evaluable disease (by Response Evaluation Criteria in Solid Tumors [RECIST] version 1.1) regardless of histology. Eligible patients for part B (dose-expansion phase) were aged 1–30 years with measurable disease (by RECIST criteria) in the following disease cohorts: rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, neuroblastoma, Hodgkin lymphoma, non-Hodgkin lymphoma, and melanoma. Patients in part A and were given nivolumab 3 mg/kg intravenously over 60 min on days 1 and 15 of a 28-day cycle in a rolling 6 study design with de-escalation upon dose-limiting toxicities to establish the recommended phase 2 dose. Patients in part B were given the recommended phase 2 dose. The primary outcomes were the tolerability, systemic exposure, maximum tolerated dose, and the antitumour activity of nivolumab at the adult recommended dose in children and young adults. This trial is registered with ClinicalTrials.gov, NCT02304458, with follow-up ongoing and is closed to new participants.

Findings 85 patients were enrolled between Feb 22, 2015, and Dec 31, 2018, and 75 patients were fully evaluable for toxicity. Median follow-up was 30 days (IQR 27–83). In part A, 13 patients were enrolled and 12 were evaluable for toxicity. There were no dose de-escalations or dose-limiting toxicities and nivolumab 3 mg/kg was confirmed as the paediatric recommended phase 2. 72 patients were enrolled in part B and 63 were evaluable for toxicity. Five (7%) patients in part B had dose-limiting toxicities. The most common overall toxicity was anaemia (35 [47%] of 75 patients; five patients had grade 3 or grade 4) and non-haematological toxicity was fatigue (28 [37%] patients; none had grade 3 or grade 4). Responses were observed in patients with lymphoma (three [30%] of ten with Hodgkin lymphoma and one [10%] of ten with non-Hodgkin lymphoma; all responders had PD-L1 expression). Objective responses were not observed in other tumour types.

Interpretation Nivolumab was safe and well tolerated in children and young adults and showed clinical activity in lymphoma. Nivolumab showed no significant single-agent activity in the common paediatric solid tumours. This study defines the recommended phase 2 dose and establishes a favourable safety profile for nivolumab in children and young adults, which can serve as the basis for its potential study in combinatorial regimens for childhood cancer.

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Introduction

Outcomes for children and adolescents with cancer have improved over the past four decades.[1] However, patients with recurrent or refractory pediatric solid tumours have dismal survival rates.[2] Despite progress in identifying oncogenic drivers and encouraging results targeting such drivers in some rare disease subsets,[3–5] cytotoxic chemotherapy remains the most common treatment for most paediatric solid tumours. Immune therapies have shown promising activity, including chimeric antigen receptor modified T cells[6–8] and blinatumomab[9] in relapsed or refractory paediatric B-cell precursor acute lymphoblastic leukaemia and dinutuximab in high-risk neuroblastoma,[10] as well as in combination with chemotherapy in recurrent and refractory neuroblastoma.[11]

Immune checkpoint inhibitors block tumour-derived signals that inhibit immune responses, thus amplifying antitumour immunity. Immune checkpoint inhibitors

Research in context

Evidence before this study

We searched PubMed for data regarding the safety and efficacy of PD-1 and PD-L1 blockers for treatment of paediatric cancer between inception and July 17, 2019. The search contained key terms "checkpoint inhibitor", "nivolumab", "PD-L1", "PD-1", "children", and "pediatric" with specific attention, but not exclusive, to phase 1 and phase 2 trials. No language restrictions were applied. Our search highlighted the use of PD-1 or PD-L1 blockers, including nivolumab, in a wide range of adult tumours, often with substantial clinical benefit. Additionally, reports on the expression of PD-L1 in common childhood tumour histologies have been published. No phase 1 or phase 2 trials have been published that report on the safety or efficacy of PD-1 targeting in sporadic paediatric cancers.

Added value of this study

To our knowledge, our trial is the first to show safety and assess the antitumour activity of nivolumab in children and young adults with relapsed or refractory non-CNS paediatric solid tumours or lymphoma. The data show that nivolumab is well tolerated in the paediatric age group and that the drugs pharmacokinetics and pharmacodynamics are comparable to those observed in adult cohorts. In the expanded phase 2 cohorts, no objective responses were observed except for patients with recurrent lymphoma.

Implications of all the available evidence

Nivolumab has similar immune adverse events and pharmacodynamic profiles in children as it does in adult patients. Used alone, nivolumab has preliminary activity for children and young adults with lymphoma and further study of this drug is warranted in this group, but the drug is not active in the sporadic paediatric solid tumour histotypes evaluated in this trial.

have shown substantial benefit in several advanced cancers in adults[12] and can induce tumour regression in children with solid tumours associated with germline mismatch repair deficiency.[13,14] Nivolumab, a humanised IgG4 monoclonal PD-1 blocking antibody,[15] administered once every 2 weeks (240 mg or approximately 3 mg/kg) or once every 4 weeks (480 mg or approximately 6 mg/kg) is approved by the US Food and Drug Administration for adults and children (aged >12 years) with microsatellite instability-high or mismatch repair deficient metastatic colon cancer and as second-line therapy in adults with advanced melanoma, renal cell carcinoma, urothelial carcinoma, hepatocellular carcinoma, metastatic squamous cell non-small-cell lung cancer, head and neck cancer, small-cell lung cancer, and relapsed or progressed classical Hodgkin lymphoma. We did a phase 1–2 trial of nivolumab in children and young adults with recurrent or refractory solid tumours, excluding CNS tumours, or lymphoma, to characterise the toxicity and pharmacokinetics of nivolumab monotherapy in children, establish a recommended phase 2 dose, and evaluate clinical activity in common paediatric solid tumour and lymphoma cohorts.

Methods

Study design and participants

We did a multicentre, open-label, single-arm, dose-confirmation and dose-expansion, phase 1–2 trial in 23 hospitals in the USA (appendix p 10). Eligible patients for part A (dose-confirmation phase) were children aged 1–18 years with recurrent or refractory solid tumours with measurable or evaluable disease (by response evaluation criteria in solid tumours [RECIST] version 1.1). Eligible patients for part B (dose-expansion phase) were children and young adults aged 1–30 years with measurable disease (by RECIST criteria) in the following disease cohorts: rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, neuroblastoma, Hodgkin lymphoma, non-Hodgkin lymphoma, and melanoma. All study participants were required to have adequate organ function (neutrophils ≥0·75 mL, transfusion independent platelet count for 7 days ≥75 platelets/mL, bilirubin ≤1·5 times the upper limit of normal for age, alanine aminotransferase ≤135 000 U/mL, lipase equal to or lower than the upper limit of normal for age, no evidence of dyspnoea at rest, and pulse oximetry >92% on room air), recovered from the acute toxic effects of previous anticancer therapies, and adequate performance status (Karnofsky ≥50% or Lansky ≥60). At least 42 days must have elapsed between autologous stem-cell transplant, stem-cell infusion, or cellular therapy and study enrolment. Initially patients with allogenic stem-cell transplant were excluded; however, the protocol was amended on Sept 08, 2016 (version 4), to include patients who had an allogenic stem-cell transplant at least 100 days before study enrolment and had no evidence of graft-versus-host disease. Patients with known CNS metastases or CNS tumours, those requiring daily systemic corticosteroids, and those who had received systemic corticosteroids within 7 days before study enrolment were ineligible. All patients or guardians were required to and did sign written informed consent before enrolment and the study was approved the central institutional review board at each site. Additional detailed regarding pharmacokinetic evaluations, statistical analyses, populations assessed for primary and secondary outcomes and planned interim analyses are contained in appendix pp 1–4.

See Online for appendix

Procedures

Part A was a dose-confirmation to establish the recommended phase 2 dose. Six patients were planned to receive nivolumab 3 mg/kg intravenously over 60 min on

days 1 and 15 of a 28-day cycle. If two of six patients experienced a dose-limiting toxicity (appendix pp 4–8) during cycle 1, dose de-escalation was planned to 1 mg/kg. If fewer than two patients in part A experienced a dose-limiting toxicity, then 3 mg/kg would be considered the recommended phase 2 dose that would be tested in part B, as long as the pharmacokinetic analysis ensured that a minimum dose (C_{min}) of 10 µg/mL or more occurred in at least five patients in which C_{min} was defined as the cycle 1, day 15 pre-dose concentration, to confirm similar drug exposure to that observed in adults treated with the standard dose of 240 mg administered every 14 days. Accumulation was calculated as the ratio of the area under the concentration time curve ($AUC_{0–168h}$) for cycle 2 versus cycle 1 [A: Please state frequency and type of samples taken for PK analysis]. An additional six patients were planned to be enrolled in part A to complete a pharmacokinetic cohort.

Part B was done to test the recommended phase 2 dose determined in part A, identify signals of activity, and generate further information regarding toxicity of the drug in the following disease specific cohorts: rhabdomyosarcoma, Ewing sarcoma, osteosarcoma, neuroblastoma, Hodgkin lymphoma, and non-Hodgkin lymphoma. A cohort with non-measurable neuroblastoma detected only by metaiodobenzylguanidine scintigraphy was also enrolled. Because of the activity of nivolumab in adults with melanoma,[15] patients younger than 18 years with recurrent, measurable melanoma were eligible for enrolment in Part B as part of a non-statistically powered cohort.

Patients in part A and part B were monitored for toxicity weekly during the first cycle, and then at the beginning of subsequent cycles. For part B, occurrence of a toxicity that met the definition of a dose-limiting toxicity affected subsequent treatment for that patient, but did not inform selection of the recommended phase 2 dose. US National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0) was used for description and grading of all toxicities. Adverse events were deemed unrelated, unlikely, possibly, probably, or definitely related to nivolumab by the treating physician with central confirmation. Review of symptoms, physical examination, and laboratory assessments (electrolytes, complete blood count, liver enzymes, bilirubin, creatinine, and C-reactive protein) were done weekly during cycle 1, then before each cycle and as clinically indicated. Patients were able to continue on study for up to 2 years unless they showed disease progression (clinical or radiographic), experienced an adverse event requiring removal from therapy, refused further therapy, were non-compliant with therapy, or refused further participation. Dose interruptions were allowed for some adverse events on the basis of protocol definitions (appendix p 5 and protocol). Dose modifications were allowed for toxicities and are outlined in detail in the protocol (appendix). In general, protocol therapy was continued for grade 1 toxicities, with the exception of pneumonitis and cardiac toxicities in which case therapy was held for grade 1 toxicities. Grade 2 toxicities required holding all protocol therapy. If toxicity recovered to grade 1 or less within 7 days, protocol therapy could continue; if not, protocol therapy was discontinued. Protocol therapy was discontinued for all toxicities of grade 3 or 4.

Radiographic disease assessments were obtained after cycles 1 and 2, and then after every other cycle. Type of imaging (CT, MRI, PET, and meta-iodobenzylguanidine scan) was determined by the treating physician and tumour type. Patients with measurable disease at baseline were evaluable for response if they received at least one dose of study drug and had a disease re-evaluation done or clinical progression of disease was documented by the treating physician. Response was evaluated using RECIST (version 1.1), except in patients with neuroblastoma whose response could be evaluated only by metaiodobenzylguanidine scintigraphy, in which response was assessed using Curie scoring.[16,17] Due to the possibility of a delayed response following initial tumour progression, patients who experienced tumour growth greater than 20% but less than 40% were allowed to remain on the study for up to 12 weeks with more frequent disease monitoring as long as the patient showed no rapid disease progression or deterioration in performance status, had not experienced a disease-limiting toxicity, or were otherwise showing clinical benefit. Complete or partial responses required central radiographic review.

PD-L1 expression, assessed by immunohistochemistry on tumour tissue obtained at initial diagnosis or subsequent biopsy, was evaluated centrally using the Dako PD-L1 IHC 28–8 pharmDx assay (Agilent, Santa Clara, CA, USA) in formalin-fixed, paraffin-embedded tumour samples.[18] The percentage of tumour cells showing plasma membrane PD-L1 staining (score 0, 1+, 2+, or 3+; higher number indicates higher intensity) was quantified in a minimum of 100 evaluable tumour cells. The presence of tumour-associated immune cells, as well as the presence of PD-L1 membrane staining on associated lymphocytes or macrophages, were also assessed qualitatively.

Complete details on study design and execution are in the protocol. Amendments to the study protocol are detailed in the appendix (p 11).

Outcomes

Primary objectives were to determine the tolerability and describe the toxicities of nivolumab at the adult recommended dose of 3 mg/kg in children and young adults with relapsed or refractory solid tumours or lymphoma; to determine the systemic exposure of nivolumab in children compared to that in adults; to determine the maximum tolerated dose of nivolumab in children; to find out the antitumour activity of nivolumab

in selected childhood solid tumours or lymphoma in seven expansion cohorts.

Secondary objectives included exploring the presence of infiltrating lymphocytes and expression of PD-L1 in tumour specimens from patients. The study is ongoing and other prespecified analyses (immunogenicity of nivolumab, and nivolumab effects on circulating cytokines) will be reported separately.

Statistical analysis

Part A was a phase 1 dose-finding study using the rolling 6 study design. Part A evaluated a single dose of nivolumab 3 mg/kg. If out of six evaluable patients, one or none experienced dose-limiting toxicity and at least five achieved a C_{min} of at least 10 μg/mL, the 3 mg/kg nivolumab dose would be the recommended phase 2 dose because the dose is well tolerated and achieves the C_{min} associated with clinical activity in adults. If two or more of the six patients experienced dose-limiting toxicity at the 3 mg/kg dose, then the maximum treatment dose has been exceeded and the 1 mg/kg nivolumab dose would be evaluated. If one or none of six evaluable patients experienced a dose-limiting toxicity at the 1 mg/kg nivolumab dose and at least five patients achieve a C_{min} of at least 10 μg/mL, then this dose level would be the recommended phase 2 dose. The operating characteristics of the rolling 6 study design have been previously described.[19]

Part B used a Simon's two-stage design for each disease cohort separately. To establish whether nivolumab would be of sufficient interest for further evaluation in a disease category, part B defined a true response rate of 5% of insufficient interest for further evaluation and a response rate of 25% as sufficient for further evaluation. A Simon's optimal two-stage design for each cohort was used (type 1 error of 0·07 and 88% power to detect the alternative hypothesis) wherein each cohort would enrol ten patients in stage one. If no responses were observed, the stratum was terminated and nivolumab was deemed ineffective. If one or more patients showed objective response in stage one, the stratum would proceed to stage two and enrol an additional ten patients. If three or more responses were observed in 20 evaluable patients, the treatment would be considered sufficiently active to warrant further study.

For all parts of the study, any patient who received at least one dose of nivolumab and who experienced a dose-limiting toxicity (appendix p 5) during cycle 1 was considered evaluable for adverse events. In addition, for part A, during cycle 1, patients must have had the appropriate toxicity monitoring studies to be considered evaluable for dose-limiting toxicity. In part A, patients who did not have dose-limiting toxicity and did not receive at least 85% of the prescribed dose within the first 28 days (the observation period for dose-limiting toxicity) for reasons other than toxicities (eg, progressive disease) were not considered evaluable for toxicity and their data were not be included in the study report and an additional patient was enrolled at that dose to replace the inevaluable patient.

Any patient who was enrolled and met eligibility criteria for part B and received at least one dose of protocol therapy would be considered evaluable for response provided the patient showed progressive disease or death while on protocol therapy, the patient was observed on protocol therapy for at least one cycle and the tumour was not removed surgically before the time complete response or partial response was confirmed, or the patient showed a complete or partial response as confirmed according to protocol criteria. Two objective status determinations were required to confirm best response. All other patients were considered non-responders. All patients deemed to have a response (complete response or partial response) must have had imaging studies reviewed centrally. The central review was the final reviewed assessment of response. Data from patients not evaluable for response were not included in the study report and an additional patient was enrolled at that dose to replace the inevaluable patient.

The rolling 6 study design and Simon's two-stage study design were used for analysis of primary and secondary outcomes.[20,21] The rolling 6 study design uses interim analyses to determine whether to increase the dose level, decrease the dose level, or define the maximum tolerated dose or the recommended phase 2 dose. Interim analyses occur after a cohort of six (or three in some cases) evaluable patients are assessed. The Simon's two-stage design used interim analysis to determine whether or not to continue enrolling patients into the second stage of the study. Categorical variables are summarised by frequencies and continuous variables are summarised by medians (IQR). SAS (version 9.4) was used for all statistical analyses. This trial is registered with ClinicalTrials.gov, number NCT02304458.

Role of the funding source

The funders contributed to the study design, data collection, data analysis, data interpretation, and writing of the report in collaboration with the authors. All authors had full access to all of the data and the corresponding author had final responsibility for the decision to submit for publication.

Results

Enrolment occurred from Feb 22, 2015, to July 30, 2018, and data cutoff for analysis was Dec 31, 2018. 85 eligible patients were enrolled. Median follow-up was 30 days (IQR 27–83). Patient characteristics are shown in table 1. Two patients remained on protocol therapy at the data cutoff date.

Part A enrolled 13 patients with the following diagnoses: embryonal rhabdomyosarcoma (n=1), epithelioid sarcoma (n=2), Ewing sarcoma (n=1), neuroblastoma (n=4), osteosarcoma (n=3), undifferentiated sarcoma (n=1) and unspecified sarcoma (n=1), of whom 12 were evaluable for toxicity (one patient was removed

	All patients (n=85)
Age, years	14 (8–17)
Sex	
Male	51 (60%)
Female	34 (40%)
Race	
White	64 (75%)
Asian	5 (6%)
American Indian or Alaska Native	0 (0%)
Black or African American	8 (10%)
Mixed	2 (2%)
Unknown	6 (7%)
Ethnicity	
Non-Hispanic	70 (82%)
Hispanic	13 (15%)
Unknown	2 (2%)
Diagnosis	
Epithelioid sarcoma	2 (2%)
Ewing sarcoma	11 (13%)
Hodgkin lymphoma	12 (14%)
Non-Hodgkin lymphoma	
Diffuse large B cell	3 (4%)
Non-Hodgkin, NOS	1 (1%)
Burkitt lymphoma	3 (4%)
Mediastinal large B-cell lymphoma	3 (4%)
Melanoma	1 (1%)
Neuroblastoma, NOS	22 (6%)
Osteosarcoma, NOS	13 (15%)
Rhabdomyosarcoma, NOS	12 (14%)
Sarcoma, NOS	1 (1%)
Undifferentiated sarcoma	1 (1%)
Previous therapy	
Chemotherapy regimens*	3 (1–4)
Radiotherapy†	1 (1–2)

Data are median (IQR) or n (%). NOS=not otherwise specified. *Measured in 84 patients. †Measured in 56 patients.

Table 1: **Baseline characteristics**

	Grade 1–2	Grade 3	Grade 4
White blood cell decreased	21 (28%)	2 (3%)	1 (1%)
Neutrophil count decreased	18 (37%)	1 (1%)	3 (4%)
Anaemia	30 (40%)	5 (7%)	..
Lymphocyte count decreased	12 (16%)	7 (9%)	3 (4%)
Platelet count decreased	12 (16%)	0	2 (3%)
Investigations - Other, specify	20 (26%)	0	0
Aspartate aminotransferase increased	21 (28%)	1 (1%)	0
Fatigue	28 (37%)	0	0
Alanine aminotransferase increased	17 (23%)	1 (1%)	0
Nausea	14 (19%)	0	0
Anorexia	14 (19%)	0	0
Hypoalbuminemia	8 (11%)	1 (1%)	0
Fever	11 (15%)		0
Hypokalaemia	8 (11%)	1 (1%)	0
Vomiting	9 (12%)		0
Abdominal pain	7 (9%)	1 (1%)	0
Headache	11 (15%)	0	0
Hypothyroidism	10 (13%)	0	0
Hypocalcaemia	10 (13%)	0	0
Hyponatremia	10 (13%)	0	0
Hypophosphatemia	8 (11%)	0	0
Rash maculopapular	8 (11%)	0	0
Cough	8 (11%)	0	0
Sinus tachycardia	7 (9%)	0	0
Lipase increased	0	2 (3%)	1 (1%)
Febrile neutropenia	0	1 (1%)	1 (1%)
Pleural effusion	0	2 (3%)	0
Arthralgia	0	1 (1%)	0
Autoimmune disorder	0	1 (1%)	0
Dyspnoea	0	1 (1%)	0
Gastritis	0	1 (1%)	0
Mucositis oral	0	1 (1%)	0
Central nervous system dysfunction	0	1 (1%)	0
Stevens-Johnson syndrome	0	0	1 (1%)
Tumour pain	0	1 (1%)	0
Upper gastrointestinal haemorrhage	0	1 (1%)	0

Adverse events that were definitely, possibly, and probably related to study treatment are presented. Grade 1 and 2 adverse events occurring in ≥10% of patients, and all grade 3–4 events are listed. No deaths due to adverse events were reported.

Table 2: **Haematological and non-haematological adverse events**

from study before receiving study drug by the treating physician due to progressive disease) and none had a dose-limiting toxicity. 72 patients were enrolled into Part B. Before treatment with single-agent nivolumab, 71 (99%) patients were treated with a median of three (IQR 1–4) previous chemotherapy regimens, and 46 (64%) had received radiotherapy with a median of one (IQR 1–2) radiotherapy regimen. 63 (88%) were evaluable for toxicity. Seven (10%) patients did not receive the full dose of nivolumab (five due to disease progression and two due to patient or physician preference) and two (3%) patients did not have all required laboratory evaluations.

In part A, nivolumab 3 mg/kg was well tolerated and was confirmed as the paediatric recommended phase 2 dose; no dose de-escalation was required and no dose-limiting toxicities were observed. In Part B, five (7%) patients had protocol defined dose-limiting toxicities: grade 3 elevated lipase for more than 7 days (n=1), grade 4 neutropenia (n=1), grade 3 pain at tumour site (n=1), grade 3 upper gastrointestinal haemorrhage (n=1), and grade 2 enterocolitis infection (n=1; appendix p 6). Grade 4 increase in lipase not a protocol-defined dose-limiting toxcities. In part B, two (3%) patients required dose modifications, one due to grade 2 wheezing that resolved and the patient was able to

continue on therapy after one dose interruption and one due to grade 2 transaminitis that eventually necessitated coming off protocol. A complete listing of adverse events possibly, probably or definitely attributed to nivolumab is listed in the appendix (pp 8–19).

Grade 3 or 4 adverse events considered to be drug-related occurred in 27 (36%) of 75 patients. The most common toxicities attributable to therapy among all patients evaluable for toxicity were haematological: anaemia (35 [47%] of 75 patients, of whom five had grade ≥3), decreased white blood cells (24 [32%], three had grade ≥3), decreased lymphocytes (22 [29%], ten had grade ≥3), and decreased platelets (14 [19%], two had grade ≥3). The most common non-haematological toxicity was fatigue (28 [37%] of 75 patients; none were grade 3 or grade 4; table 2). The most common immune-related adverse event was hepatic toxicity (aspartate aminotransferase increase in 22 [29%] of 75 patients and alanine aminotransferase increase in 18 [24%]; table 3). Pleural or pericardial effusions were reported in 11 (15%) of 75 evaluable patients, three of whom developed both (we included all patients even if deemed unlikely or unrelated related to study therapy; only four patients were attributed to study therapy; table 4). Effusions developed during the first cycle of nivolumab in seven patients, during the second cycle in two patients, and after discontinuation of nivolumab in three patients; severity ranged from moderate (grade ≤2 in six patients) to severe (grade 3 in four) and life-threatening (grade 4 in one). Evaluation of the pleural fluid in one patient supported an inflammatory effusion because there was no evidence of malignant cells.

In total, seven (10%) of 72 patients in part B discontinued protocol therapy due to adverse events (two had prolonged elevation of liver enzymes, one had prolonged elevated lipase, one had prolonged fever, one had gastrointestinal bleeding, one had infection, and one had an autoimmune disorder, including thyroiditis, elevated creatine kinase, elevated creatinine). 37 (44%) of 85 patients died on study or during the follow-up period, none were attributable to study therapy.

12 patients in part A and 64 patients in part B were evaluable for pharmacokinetics (appendix p 7). An accumulation ratio of 1·7 (0·4) was consistent with a half-life of 13·2 days.

In this study, all patients with Hodgkin lymphoma had classical Hodgkin lymphoma and six were reported to be nodular sclerosing subtype. 12 patients with Hodgkin lymphoma were enrolled, but two patients did not start

	Grade 1–2	Grade 3	Grade 4
Aspartate aminotransferase increased	21 (28%)	1 (1%)	0
Alanine aminotransferase increased	17 (23%)	1 (1%)	0
Lipase increased	3 (4%)	2 (3%)	1 (1%)
Hypothyroidism	10 (13%)	0	0
Maculopapular rash	9 (12%)	0	0
Diarrhoea	6 (8%)	0	0
Hyperthyroidism	6 (8%)	0	0
Serum amylase increased	3 (4%)	0	0
Pleural effusion	2 (3%)	2 (3%)	0
Pericardial effusion	3 (4%)	0	0
Pancreatitis	2 (3%)	0	0
Rash acneiform	1 (1%)	0	0
Oesophagitis	1 (1%)	0	0
Autoimmune disorder	0	1 (1%)	0
Gastritis	0	1 (1%)	0

Adverse events that were definitely, possibly, and probably related to study treatment are presented. Grade 1 and 2 adverse events occurring in ≥10% of patients, and all grade 3–4 events are listed. No deaths due to adverse events were reported.

Table 3: **Immune-related haematological and non-haematological adverse events**

	Site	Cycle reported	Grade	Attribution to nivolumab	Received steroids	Intrathoracic disease at enrolment
Epithelioid sarcoma	Pleural	2	3	Possible	No	Paraoesophageal mass
Rhabdomyosarcoma, embryonal	Pleural	1	3	Unlikely	No	Pulmonary metastasis
Osteosarcoma	Pleural	1	3	Unrelated	No	Pulmonary metastasis
Neuroblastoma	Pleural	100-day follow-up	3	Possible	No	Pulmonary metastasis
Rhabdomyosarcoma, NOS	Pleural	100-day follow-up	2	Unrelated	No	None at enrolment
Rhabdomyosarcoma, alveolar	Pleural	1	1	Possible	Yes	Pulmonary metastasis
Ewing sarcoma	Pleural; pericardial	2; 2	2; 2	Unlikely; possible	Yes	Mediastinal and chest wall mass
Ewing sarcoma	Pleural; pericardial	1; 100-day follow-up	2; 2	Possible; possible	No	Pleural metastasis
Primary mediastinal large cell lymphoma	Pleural; pericardial	1; 1	2; 2	Unlikely; possible	Yes	Pleural and mediastinal metastasis
Ewing sarcoma	Pericardial	1	2	Unlikely	No	Pleural metastasis
Melanoma	Pleural	100 day follow-up	4	Unrelated	No	Pulmonary metastasis

NOS=not otherwise specified.

Table 4: **Incidence, timing, and attribution to nivolumab of pleural and pericardial effusions by patient diagnosis**

therapy due to changes in disease status following enrolment and therefore were not evaluable for response. In ten patients with Hodgkin lymphoma evaluable for response, one patient had a complete response, two had a partial response, five had stable disease (median 12·8 cycles, IQR 6–18), and two had a mixed response, with decrease in size of target lesions but appearance of new lesions during study. Two patients were removed from study due to physician preference. Patients with Hodgkin lymphoma completed a median of 4·5 cycles (IQR 2–8). Despite achieving the number of responses required to expand the Hodgkin lymphoma cohort in the planned Simon's two stage design, the Hodgkin lymphoma cohort was not expanded in this trial so that we could evaluate nivolumab in a combination regimen for patients with relapsed Hodgkin lymphoma in a separate trial (AHOD1712; NCT02927769).

Ten patients with non-Hodgkin lymphoma were enrolled and evaluable for response (table 1). One patient with mediastinal large B-cell lymphoma had a metabolic complete response by PET and partial response by RECIST (version 1.1) and remained on therapy for 11 cycles. The toxicity profile in patients with lymphoma was not different from that of other histologies (appendix pp 8–9). The non-Hodgkin lymphoma cohort was not expanded due to inadequate accrual to complete the expansion cohort.

As shown in the figure, no objective responses were observed in patients with solid tumours evaluated in this trial (74 patients were evaluable for response). Stable disease was reported as best response in 11 (33%) of 33 patients in the sarcoma cohorts and 5 (50%) of 10 patients in the neuroblastoma (measurable) cohort. Among patients enrolled in the neuroblastoma-metaiodobenzylguanidine cohort, four (40%) of ten were deemed to have stable disease (via central review) after two cycles of therapy. One patient with poorly differentiated melanoma (negative for $BRAF^{V600E}$) was enrolled and did not respond. This patient had metastatic disease at the time of enrolment and had progressed despite three surgical resections and previous therapy with interferon-α and dendritic cell therapy.

64 (75%) of 85 patients had archival tumour evaluated for PD-L1 expression on tumour cells and on tumour-infiltrating leukocytes by immunochemistry. All tissues tested were available before the start of anti-PD-1 therapy, although this was not an eligibility criterion. Using a conservative cutoff of 1% PD-L1 expression on tumour cells to define positivity, only 7–50% (seven of 47 total tested patients) of non lymphoma tumour cohorts expressed PD-L1 on their tumour cells (table 5). By comparison, in the Hodgkin lymphoma cohort, all nine patients tested had PD-L1 expression on their tumour cells, at levels ranging from 30% to 100% of cells (table 5). In the non-Hodgkin lymphoma cohort, PD-L1 expression was evaluated in eight (80%) of ten patients and ranged from 1% to 100% of tumour cells (table 5). The patient

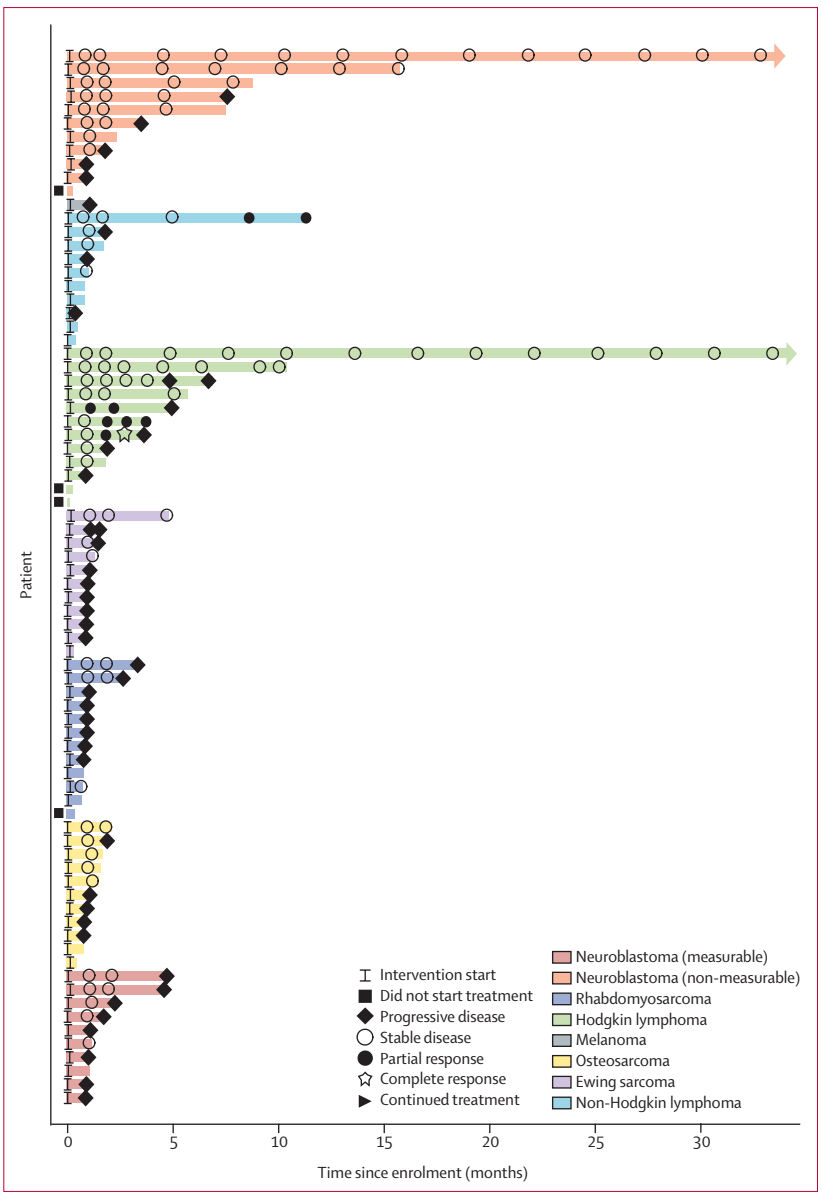

Figure: **Response to nivolumab**
Each bar represents one patient. For four patients who did not start treatment the bar represents the time from enrolment to withdrawal from study.

with 100% PD-L1 tumour staining had a partial response. A patient with diffuse large B-cell lymphoma showed 99% tumour positivity for PD-L1 and infiltrating lymphocytes expressing PD-L1, but did not show a response to nivolumab (table 5). 56 (88%) of 64 patients with available tissue showed infiltrating immune cells. PD-L1 membrane staining was most commonly observed on tumour-associated macrophages, although nine (16%) of 58 patients, across various histologies (three patients with non-Hodgkin lymphoma, two with neuroblastoma, and four with sarcoma), showed predominant infiltration of lymphocytes expressing PD-L1. Together, these results

	Patients who had tumour PD-L1 testing	PD-L1-positive tumour*	Range of PD-L1-positive tumour cells	Tumours with infiltrating immune cells	Predominant PD-L1-positive immune cell
Hodgkin lymphoma	9/12 (75%)	9/9 (100%)	30–100%	9/9 (100%)	Macrophages
Non-Hodgkin lymphoma	8/10 (80%)	7/8 (88%)	1–100%	8/8 (100%)	Macrophages or lymphocytes
Neuroblastoma	15/22 (68%)	1/15 (7%)	1%	14/15 (93%)	Macrophages or lymphocytes
Osteosarcoma	9/13 (69%)	2/9 (22%)	1–3%	7/9 (78%)	Macrophages or lymphocytes
Rhabdomyosarcoma	9/12 (75%)	1/9 (11%)	1%	8/9 (89%)	Macrophages or lymphocytes
Ewing sarcoma	10/11 (91%)	1/10 (10%)	1%	8/10 (80%)	Macrophages
Malignant melanoma	0	..	..	..	..
Other sarcoma†	4/4 (100%)	2/4 (50%)	5–70%	4/4 (100%)	Macrophages

Data are n/N (%) or %. *Positive tumours are those expressing PD-L1 on ≥1% of tumour cells. †Malignant spindle cell and epithelioid neoplasm with focally rhabdoid features (n=1), treated spindle and pleomorphic sarcoma (n=1), proximal type epithelial sarcoma (n=1), and metastatic epithelioid sarcoma (n=1).

Table 5: **PD-L1 expression on tumour cells and infiltrating immune cells**

show an overall paucity of PD-L1 expression on the common childhood solid tumours evaluated here, excluding lymphomas, and a predominance of macrophages expressing PD-L1 in the tumour microenvironment.

Discussion

In this phase 1–2 trial, we found that nivolumab was well tolerated in children and the pharmacokinetics of the drug in children were similar to those reported in adults receiving the standard dose of 240 mg every 14 days.[22] Similar to findings in adults,[23] no clinically relevant effects on nivolumab clearance were observed across the age spectrum of paediatric patients and across the wide range of diseases enrolled on this trial. Based on these data, the recommended phase 2 dose and schedule of nivolumab for paediatric patients is 3 mg/kg administered every 14 days. The frequency and type of adverse events attributed to nivolumab were consistent with those reported with PD-1 blockade in adults. We observed greater frequency of grade 3 or grade 4 haematological toxicity in our cohort compared with that reported in adults, which we suspect is due to our patient population having received multiple myelotoxic chemotherapy or radiotherapy regimens before enrolment. It was notable that pleural or pericardial effusions developed in 13 patients, 11 of which were evaluable for toxicity. Ten of these patients had neoplastic involvement of the lungs or chest at the time of treatment, suggesting that the effusions might be attributable to nivolumab-induced tumour associated inflammation. Although management of nivolumab associated effusions could not be systematically assessed in this study, several patients were treated with supportive care and corticosteroids and showed symptomatic improvement in the effusion.

Some patients with lymphoma experienced clinical benefit with nivolumab. Of the ten patients with Hodgkin lymphoma treated, three experienced an objective response (one complete response, two partial response) and five showed stable disease. Despite this encouraging activity, the response rate appears lower than the 66–87% overall response rate reported in adults with Hodgkin lymphoma.[24,25] The median age of patients with Hodgkin lymphoma is our cohort was lower than the median of 35 years in a study by Ansell and colleagues[24] and a mean of 39 years in a study by Younes and collagues[25] in which they reported on 80 adults with classical Hodgkin lymphoma.[25] Ansell and colleagues reported on 23 patients (median age 35 years) with Hodgkin lymphoma and found that 22 (96%) of 23 patients had a nodular sclerosing subtype of classical Hodgkin lymphoma.[24] Overexpression of PD-L1, associated with PD-L1 amplification, is believed to contribute to the high response rate of Hodgkin lymphoma to immune checkpoint inhibitors.[24,26] Although PD-L1 mutational status was not evaluated in this study, 100% of the Hodgkin lymphoma tumours analysed showed high levels of PD-L1 expression as previously reported in adult trials.[24,25] Thus, the differences in histological subtypes treated or PD-L1 expression levels, compared with previous reports in adults, do not seem to account for the lower response rate observed. In a phase 2 trial in primary mediastinal B-cell lymphoma, anti-PD1 monotherapy showed a 41% overall response rate.[27] In this study, of ten evaluable patients, we observed one partial response in a patient with primary mediatstinal B-cell lymphoma whose tumor showed PD-L1 positivity. Future work is needed to investigate whether children with Hodgkin lymphoma or primary mediastinal B-cell lymphoma experience a lower proportion of response compared with adults and whether this reflects age-associated distinctions in immunobiology associated with Hodgkin lymphoma or primary mediastinal B-cell lymphoma .

In this study, there was no evidence for single-agent activity of nivolumab in non-lymphoma paediatric solid tumours, although some paediatric tumour histologies such as Wilms tumour, malignant rhabdoid tumour, and germ-cell tumours were not evaluated in this study. Biological correlates used to predict response to immune checkpoint inhibitors are imperfect, although high

expression of PD-L1 on tumour cells or infiltrating T cells and high tumour mutational burden have been reported to correlate with response.[28–30] Our data, consistent with previously published reports,[31–33] show low PD-L1 expression on the histological tumour types enrolled in our study and a paucity of infiltrating T cells. Given the that response to PD-1 or PD-L1 blockade in non-lymphoma tumours is thought to reflect reinvigoration of T cells recognising neoantigens derived from tumour mutations, the absence of response might be indicative of the mutational burden in sporadic paediatric solid tumours.[28] Our study did not assess tumour mutational burden limiting assessment of its effect in the paediatric tumours studied here although previous studies have shown that approximately only 5% of sporadic paediatric solid tumours are classified as hypermutant, as defined by more than ten mutations per Mb,[28,29] a minimum level that has been suggested to be associated with response to immune checkpoint inhibitors. Of note, children with hypermutant tumours associated with germline mutations in mismatch repair genes show clinically significant responses to PD-1 blockade,[13,14] providing evidence that paediatric patients are capable of mounting antitumour effects induced by PD-1 in the presence of adequate antigens to drive T-cell responses. Future studies are needed to investigate whether selected cohorts of children with hypermutant sporadic solid tumours would show clinical benefit following PD-1 blockade.

In summary, our results show that nivolumab 3 mg/kg every 14 days is tolerable in children. Antitumour activity was observed in Hodgkin lymphoma and in one patient with non-Hodgkin lymphoma, but single agent activity was not observed for the common, non-lymphoma, non-CNS paediatric solid tumours studied here. Future studies evaluating nivolumab, or other PD-1 or PD-L1 blockers, alone or in combination with other immunomodulators, might be warranted in selected populations of patients with paediatric solid tumours whose tumours manifest higher mutational burden or other biomarkers that provide evidence for increased tumour immunogenicity.

Contributors

KLD, EF, MSM, BJW, CGM, and CLM contributed to the conception and design of the study. XL and CGM completed the statistical analyses. KLD, JMR, and RAK completed data analysis. All authors contributed to the data interpretation, drafting, and revision of the manuscript and final approval to submit the manuscript for publication.

Declaration of interests

CLM is a founder of Lyell Immunopharma; holds equity in Lyell Immunopharma and Apricity; receives consulting fees from Lyell Immunopharma; is an advisory board member for Neoimmune Tech, Nektar, PACT Pharma, Bryologyx, Allogene, and Apricity. MSM is employed by Epizyme and holds stock at AstraZeneca. All other authors declare no competing interests.

Data sharing

The Children's Oncology Group data sharing policy describes the release and use of Children's Oncology Group's individual subject data for use in research projects in accordance with National Clinical Trials Network Program and National Cancer Institute Community Oncology Research Program Guidelines. Only data expressly released from the oversight of the relevant Children's Oncology Group Data and Safety Monitoring Committee are available to be shared. Data from this study are available following the primary publication. An individual-level de-identified dataset containing the variables analysed in the primary results paper can be expected to be available upon request. Requests for access to Children's Oncology Group protocol research data should be sent to datarequest@childrensoncologygroup.org. Data are available to researchers whose proposed analysis is found by Children's Oncology Group to be feasible and of scientific merit and who agree to the terms and conditions of use.

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