Irinotecan–temozolomide with temsirolimus or dinutuximab in children with refractory or relapsed neuroblastoma (COG ANBL1221): an open-label, randomised, phase 2 trial


Summary
Background Outcomes for children with relapsed and refractory neuroblastoma are dismal. The combination of irinotecan and temozolomide has activity in these patients, and its acceptable toxicity profile makes it an excellent backbone for study of new agents. We aimed to test the addition of temsirolimus or dinutuximab to irinotecan–temozolomide in patients with relapsed or refractory neuroblastoma.

Methods For this open-label, randomised, phase 2 selection design trial of the Children’s Oncology Group (COG; ANBL1221), patients had to have histological verification of neuroblastoma or ganglioneuroblastoma at diagnosis or have tumour cells in bone marrow with increased urinary catecholamine concentrations at diagnosis. Patients of any age were eligible at first designation of relapse or progression, or first designation of refractory disease, provided organ function requirements were met. Patients previously treated for refractory or relapsed disease were ineligible. Computer-based randomisation with sequence generation defined by permuted block randomisation (block size two) was used to randomly assign patients (1:1) to irinotecan and temozolomide plus either temsirolimus or dinutuximab, stratified by disease category, previous exposure to anti-GD2 antibody therapy, and tumour MYCN amplification status. Patients in both groups received oral temozolomide (100 mg/m² per dose) and intravenous irinotecan (50 mg/m³ per dose) on days 1–5 of 21-day cycles. Patients in the temsirolimus group also received intravenous temsirolimus (35 mg/m² per dose) on days 1 and 8, whereas those in the dinutuximab group received intravenous dinutuximab (17.5 mg/m² per day or 25 mg/m² per day) on days 2–5 plus granulocyte macrophage colonystimulating factor (250 μg/m³ per dose) subcutaneously on days 6–12. Patients were given up to a maximum of 17 cycles of treatment. The primary endpoint was the proportion of patients achieving an objective (complete or partial) response by central review after six cycles of treatment, analysed by intention to treat. Patients, families, and those administering treatment were aware of group assignment. This study is registered with ClinicalTrials.gov, number NCT01767194, and follow-up of the initial cohort is ongoing.

Findings Between Feb 22, 2013, and March 23, 2015, 36 patients from 27 COG member institutions were enrolled on this groupwide study. One patient was ineligible (alanine aminotransferase concentration was above the required range). Of the remaining 35 patients, 18 were randomly assigned to irinotecan–temozolomide–temsirolimus and 17 to irinotecan–temozolomide–dinutuximab. Median follow-up was 1–26 years (IQR 0–68–1–61) among all eligible participants. Of the 18 patients assigned to irinotecan–temozolomide–temsirolimus, one patient (6%; 95% CI 0–16–1) achieved a partial response. Of the 17 patients assigned to irinotecan–temozolomide–dinutuximab, nine (53%; 95% CI 29–76–7) had objective responses, including four partial responses and five complete responses. The most common grade 3 or worse adverse events in the temsirolimus group were neutropenia (eight [44%] of 18 patients), anaemia (six [33%]), thrombocytopenia (five [28%]), increased alanine aminotransferase (five [28%]), and hypokalaemia (four [22%]). One of the 17 patients assigned to the dinutuximab group refused treatment after randomisation; the most common grade 3 or worse adverse events in the remaining 16 patients evaluated for safety were pain (seven [44%] of 16), hypokalaemia (six [38%]), neutropenia (four [25%]), thrombocytopenia (four [25%]), anaemia (four [25%]), fever and infection (four [25%]), and hypoxia (four [25%]); one patient had grade 4 hypoxia related to therapy that met protocol-defined criteria for unacceptable toxicity. No deaths attributed to protocol therapy occurred.

Interpretation Irinotecan–temozolomide–dinutuximab met protocol-defined criteria for selection as the combination meriting further study whereas irinotecan–temozolomide–temsirolimus did not. Irinotecan–temozolomide–dinutuximab shows notable anti-tumour activity in patients with relapsed or refractory neuroblastoma. Further evaluation of biomarkers in a larger cohort of patients might identify those most likely to respond to this chemoimmunotherapeutic regimen.

Funding National Cancer Institute.
**Introduction**

Despite the use of maximally intensive treatment, survival for children with newly diagnosed high-risk neuroblastoma remains about 50%.[1] Molecularly targeted therapies are being studied, and the combination of targeted agents with chemotherapy could be advantageous. Irinotecan and temozolomide can be safely administered to patients with relapsed or refractory neuroblastoma, providing a backbone onto which targeted agents with chemotherapy could be integrated.2,3 Temsirolimus inhibits mTOR, which has a role in regulation of protein synthesis and cell proliferation.4 Neuroblastoma cells are sensitive to mTOR inhibitors in vitro and in vivo.5 Although single-agent activity was modest in some preclinical studies,6 data suggest that mTOR inhibitors might be effective in subsets of neuroblastoma tumours.7 Additionally, mTOR inhibitors have synergistic or additive effects when combined with chemotherapeutics.8,9 Previous studies10–13 provided information about temsirolimus dosing, and a Children's Oncology Group (COG) trial14 showed that irinotecan–temozolomide–dinutuximab was encouraging.

Dinutuximab, a chimeric antibody targeting the disialoganglioside GD2, was also combined with irinotecan–temozolomide during our trial. GD2 is expressed on neuroblastoma cells, but expression in healthy tissues is restricted to cerebellar neurons, skin melanocytes, and peripheral pain fibres.15–17 Because of this expression pattern, anti-GD2 antibodies have been studied as targeted immunotherapy for neuroblastoma.8 Dinutuximab became a standard component of high-risk therapy after a randomised COG trial showed an improvement in event-free survival for patients assigned to receive dinutuximab with granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin 2 following myeloablative therapy.18 GM-CSF was selected for use in this study rather than interleukin 2 because interleukin 2 has been associated with more substantial capillary leak syndrome and more frequent renal dysfunction when given in combination with dinutuximab.19 Because monoclonal antibodies in combination with chemotherapy were shown to be effective beyond the setting of minimal residual disease in adults,20–22 the combination of irinotecan–temozolomide–dinutuximab with GM-CSF merits evaluation.

This COG trial (ANBL1221) was designed to study responses to irinotecan–temozolomide with either...
temsirelimus or dinutuximab. The primary objective was to determine whether temsirelimus or dinutuximab merits testing in a front-line trial for children with high-risk neuroblastoma.

Methods

Study design and participants

COG ANBL1221 was an open-label, randomised, phase 2 trial with a so-called pick-the-winner selection design.28–30 open to all member institutions in the COG, which includes more than 200 hospitals, universities, and cancer centres across North America, Australia, New Zealand, Europe, and in Saudi Arabia (appendix p 6). Within each treatment regimen, a Simon’s two-stage activity design was used to ascertain whether a given regimen did not meet the minimum required clinical activity and would be eliminated. This activity was defined as four or more responders out of 17 participants or seven or more responders out of 25 participants randomly assigned to a given regimen. If both regimens met the minimum activity, the selection design would be applied. In the selection design, the winner would be the regimen with three or more responders above the number of responders to the other regimen. If the winner could not be identified by these criteria, other criteria (toxicity, feasibility, and progression-free survival) were to be used to select the winner.

To be eligible for this trial, patients had to have histological verification of neuroblastoma or ganglioneuroblastoma, or have tumour cells in bone marrow with increased urinary catecholamine concentrations (ie, more than two-times the upper limit of normal [ULN]) at diagnosis. Patients had to have disease that was measurable by MRI or CT or disease that was evaluable by ¹³¹I-metaiodobenzylguanidine (MIBG) scan. Patients whose only site of disease was bone marrow were not eligible. Patients were eligible at first designation of relapse (defined as recurrence after response to treatment) or progression, or first designation of refractory disease (defined as inadequate response to treatment that included at least four cycles of two or more chemotherapeutic agents, including an alkylator and a platinum-containing compound).

Patients of any age were eligible for this trial; adults could also enrol. Patients had to have Karnofsky or Lansky performance status scores of 50% or higher. Other requirements included recovery from acute toxic effects of previous therapies, negative pregnancy test for women of childbearing potential, and adequate organ function (serum creatinine ≤ULN based on age and sex or glomerular filtration rate of ≥70 mL/min per 1·73 m², alanine aminotransferase ≤five×ULN for age, bilirubin ≤1·5×ULN, prothrombin time ≤1·2×ULN, serum triglycerides ≤3·39 mmol/L, serum cholesterol ≤7·77 mmol/L, shortening fraction ≥27% by ECHO, and no symptoms of pulmonary dysfunction). Patients had to have an absolute neutrophil count of at least 0·75×10⁹ cells per L and an unsupported platelet count of at least 75×10⁹ per L. Patients who had undergone stem-cell transplantation or MIBG therapy during first-line treatment were eligible 6 weeks or longer after these therapies if other criteria were met. Patients previously treated with anti-GD2 antibodies were eligible unless they had progressive disease during anti-GD2 therapy.

Patients previously treated for refractory or relapsed disease were ineligible (including those previously treated with irinotecan–temozolomide), as were patients with bone marrow as the only site of disease. Chemotherapy was not permitted within 2 weeks of enrolment. Biological therapeutics (including anti-GD2 antibodies), retinoids, or growth factors were not allowed within 7 days of enrolment. At least 4 weeks had to have elapsed since radiotherapy to target lesions; progression in such lesions was required. Palliative radiotherapy to non-target lesions was allowed without timing restrictions. Patients with diarrhoea or uncontrolled illnesses and those taking enzyme-inducing anti-convulsants were ineligible. Patients with a history of significant allergic reactions to anti-GD2 antibodies or compounds similar to temsirelimus were ineligible, as were those who had previously received an mTOR inhibitor with chemotherapy.

The study was approved by the Pediatric Central Institutional Review Board of the National Cancer Institute (NCI) and a local institutional review board. Written informed consent was obtained from parents or guardians of minor participants (according to definition of minor for each participating country).

Randomisation and masking

Patients enrolled by the treating investigator were randomly assigned (1:1) to irinotecan–temozolomide plus either temsirelimus or dinutuximab using computer-based randomisation with sequence generation defined by permuted block randomisation, with a block size of two. Randomisation was stratified to ensure equal distribution of disease category (measurable vs evaluable), previous exposure to anti-GD2 antibody therapy (previous dinotuximab vs no previous dinotuximab), and tumour MYCN amplification status (amplified vs non-amplified). The COG RandoNode web service (integrated with the NCI OPEN system) assigned treatment such that the allocation sequence was not known at the site when treatment assignment was done. Participants and families and those administering assigned therapy were aware of the treatment assignment. However, radiology central review was done without information about group assignment.

Procedures

All patients received intravenous irinotecan (50 mg/m² per dose given over 90 min) and oral temozolomide (100 mg/m² per dose) on days 1–5 of 21-day cycles. Patients in the irinotecan–temozolomide–temsirelimus
group received intravenous temsirolimus (35 mg/m² per dose) over 30 min on days 1 and 8. Patients in the irinotecan–temozolomide–dinutuximab group initially received intravenous dinutuximab (25 mg/m² per day over 10 h) on days 2–5. The infusion could be extended to 20 h if patients had pain, fever, tachycardia, tachypnoea, or hypotension unresponsive to supportive measures. A change in manufacturing of dinutuximab and use of a calculated rather than theoretical extinction coefficient led to revision of the prescribed dose to 17·5 mg/m² per day (protocol amendment on Jan 21, 2014, approved by the National Cancer Institute Cancer Therapy Evaluation Program, Pediatric Central Institutional Review Board, and local institutional review boards). Patients in the irinotecan–temozolomide–dinutuximab group also received GM-CSF (250 µg/m² per dose) over 10 h) on days 2–5. The infusion could be extended to 20 h if patients had pain, fever, tachycardia, tachypnoea, or hypotension unresponsive to supportive measures.

The primary endpoint was the proportion of patients achieving a best overall response of complete or partial response, based on the results of CT or MRI imaging, MIBG scans, and bone marrow aspirates or biopsies, determined after completion of six cycles of protocol therapy. Responders were defined as those with best overall response of complete response or partial response. Patients with overall complete response had no evidence of tumour and normal urinary catecholamine concentration. Patients with partial response in soft tissue disease (per RECIST) had to have at least a 50% reduction in tumour and normal urinary catecholamine concentration. Patients with partial response in bone marrow disease had at least a 20% or more increase in longest dimension of a soft tissue mass. For a given patient, the response endpoint was binary (ie, responder or non-responder). Prespecified exploratory endpoints of the trial included progression-free survival, defined as the time from enrolment until a progression-free survival event (relapse, disease progression, or death attributable to tumour or treatment), overall survival (time from enrolment to death from any cause), toxicity, feasibility, and proportion of patients achieving an objective response using the existing INRC and the proposed modified INRC. Progression-free survival, overall survival, and toxicity and feasibility data are included in this report. Findings related to the comparison of response using the existing and the proposed modified INRC will be reported separately.

Outcomes

For patients who had neutropenia or thrombocytopenia causing a delay of 14 days or more between treatment cycles, temozolomide doses were reduced by 25% for subsequent cycles. If a patient had grade 4 treatment-associated diarrhoea despite maximal use of anti-diarrhoeal medications and appropriate use of prophylactic antibiotics, irinotecan doses were reduced by 25% for subsequent cycles. Dinutuximab treatment was to be held in patients with hypotension or capillary leak syndrome unresponsive to standard interventions, those with severe allergic reactions to dinutuximab, and those with persistent increases in creatinine concentrations to 2 times or more of the ULN for age and sex persisting despite optimised fluid management. Dinutuximab was also to be held for patients with pain unresponsive to narcotics and those with grade 4 neurotoxicity.

Pre-treatment disease evaluations were done within 3 weeks of study enrolment, and response was assessed after cycle two, cycle four, and cycle six, and every four cycles thereafter. Response Evaluation Criteria in Solid Tumors (RECIST) criteria (version 1.1) were used for response assessment in patients with disease measurable by CT or MRI. Responses based on anatomic imaging were centrally reviewed. For patients with MIBG-avid lesions, Curie scoring determined response during central review. Bone marrow involvement was assessed with use of routine staining; bilateral assessments were required. Modified International Neuroblastoma Response Criteria (INRC) were used to integrate the results of disease evaluation procedures and permit assessment of overall response.
All eligible, randomly assigned patients were considered evaluable for the intention-to-treat analysis of response. If a second stage in the activity design were to be required, 25 patients were to be assigned to each treatment. This optimal two-stage design has a 91.1% power to detect a 25% difference (15% difference under the null hypothesis and 40% difference under the alternative hypothesis) in response with a type 1 error of 0.064.

All eligible patients who received at least one dose of temsirolimus or dinutuximab were considered evaluable for toxicity. The protocol included a three-stage stopping rule for unacceptable toxicity. Age was compared with a two-sided Wilcoxon rank sum test. A 95% Wald CI was placed on the proportion of responding patients for each regimen.

We constructed Kaplan-Meier survival curves, with standard errors according to Peto. We analysed survival on an intention-to-treat basis; all eligible patients were considered evaluable for survival endpoints. We compared survival curves using a two-sided log-rank test. For progression-free survival, we calculated time to event from enrolment to first occurrence of relapse, progressive disease, or death related to disease or its treatment, or time of last patient contact if no event occurred. We censored patients at time of death not due to disease. For overall survival, time to event was time from enrolment until death from any cause, or time to last contact if the patient was still alive. We presented progression-free survival and overall survival as 1-year point estimates with standard errors. We derived hazard ratios (HRs) for the difference between treatment groups from Cox proportional hazards models. We considered p values less than 0.05 as significant.

Data were entered at COG treating centres and aggregated at the COG Statistics and Data Centre (Gainesville, FL, USA). We used SAS version 9.4 for our data analyses, and created survival curves using R.

This study is registered with ClinicalTrials.gov, number NCT01767194.

Role of the funding source
The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to

Figure 1: Trial profile
ALT=alanine aminotransferase. *Did not meet protocol-defined criteria for unacceptable toxicity.
all data in the study and had final responsibility for the decision to submit for publication.

Results
Between Feb 22, 2013, and March 23, 2015, 36 patients were enrolled (figure 1). One patient’s alanine aminotransferase concentration was above the required range for eligibility and was therefore ineligible. Of the remaining 35 patients, 18 were randomly assigned to irinotecan–temozolomide–temsirolimus and 17 to irinotecan–temozolomide–dinutuximab. One patient randomly assigned to the dinutuximab group withdrew before receiving any treatment; this patient was included in the intention-to-treat analyses but not in the safety population.

Age at enrolment ranged from 2·1 to 16·2 years (median 5·7 years [IQR 4·5–9·1]; table 1, appendix pp 1–4). Time from diagnosis of high-risk disease to enrolment ranged from 3·3 months to 60·4 months (median 9·8 months [IQR 6·1–32·1]). 34 (97%) of 35 patients had stage 4 disease of the International Neuroblastoma Staging System (INSS) at diagnosis, only one (3%) patient had relapsed INSS stage 3 disease. MYCN status was known for 32 patients; eight (25%) had MYCN amplified tumours. 22 (63%) of 35 patients had measurable disease, of which 12 were randomly assigned to irinotecan–temozolomide–temsirolimus and ten to irinotecan–temozolomide–dinutuximab. 13 (37%) patients had evaluable disease, of which six were randomly assigned to irinotecan–temozolomide–temsirolimus and seven to irinotecan–temozolomide–dinutuximab. 19 (54%) of 35 patients had a first episode of relapsed neuroblastoma and 16 (46%) had disease that was refractory to initial therapy. Previous treatment included high-dose chemotherapy with autologous stem-cell transplantation in 19 (54%) patients, and previous anti-GD2 antibody therapy in ten (29%). The treatment groups were well balanced with respect to patient characteristics (table 1). Among all eligible study participants, median follow-up was 1·26 years (IQR 0·68–1·61). Among all eligible patients still alive at the data freeze on June 30, 2016 (n=21), median follow-up was 1·36 years (1·15–1·61). Among all eligible study participants, median follow-up was 1·26 years (IQR 0·68–1·61). Among all eligible patients still alive at the data freeze on June 30, 2016 (n=21), median follow-up was 1·36 years (1·15–1·61).

The 18 patients assigned to irinotecan–temozolomide–temsirolimus received 98 total courses of treatment (median 3 [IQR 2–10]); the 17 patients assigned to irinotecan–temozolomide–dinutuximab received 148 courses (median 6 [IQR 3–17]). Objective responses (ie, complete or partial response; table 2) were recorded in one patient (6%, 95% CI 0·0–16·1) randomly assigned to irinotecan–temozolomide–temsirolimus and nine patients (53%, 29·2–76·7) randomly assigned to irinotecan–temozolomide–dinutuximab. In the activity design, irinotecan–temozolomide–temsirolimus did not meet the minimum activity requirement (ie, at least four of 17 responders) whereas irinotecan–temozolomide–dinutuximab exceeded the requirement (table 2). Having eliminated irinotecan–temozolomide–temsirolimus, application of the selection design was unnecessary. Dinutuximab met the criteria to be designated the agent meriting further study.

Because the stopping boundary at stage 1 had been met for irinotecan–temozolomide–temsirolimus, accrual to stage 2 was not required for either group. In patients assigned to the irinotecan–temozolomide–dinutuximab group, four of eight with refractory disease at baseline had objective responses, as did five of nine with relapsed disease (table 3, appendix pp 3, 4). Objective responses were recorded both in patients with measurable disease (three of ten) and evaluable disease (six of seven), and in those whose tumours were MYCN amplified (two of three) and non-amplified (six of 12). Seven of ten who had previously had stem-cell transplantation responded to irinotecan–temozolomide–dinutuximab, as did four of six who had received previous anti-GD2 antibody therapy.

Data are n (%), unless otherwise stated. INRG=International Neuroblastoma Risk Group. MMT=multimodality induction therapy. MIBG=metaiodobenzylguanidine. ASCT=autologous stem-cell transplantation. *Several patients had multiple sites of disease. †Two patients had soft tissue at both primary and metastatic sites.
Ten of 18 patients from the irinotecan–temozolomide–temsirolimus arm and four of 17 patients from the irinotecan–temozolomide–dinutuximab arm had stable disease as best response (table 2). A total of 11 (61%) of 18 patients in the temsirolimus group and 13 (76%) of 17 patients in the dinutuximab group therefore had stable disease or better. In patients who had progressive disease on therapy, none had progressive disease in bone marrow only.

We evaluated progression-free survival and overall survival as prespecified exploratory objectives of this trial. 15 progression events occurred in the 18 patients assigned to irinotecan–temozolomide–temsirolimus and six events occurred in the 17 patients assigned to irinotecan–temozolomide–dinutuximab. 1-year progression-free survival in the irinotecan–temozolomide–temsirolimus group was 24.7% (95% CI 0.4–49.0), whereas in the irinotecan–temozolomide–dinutuximab group it was 76.5% (56.3–96.7; figure 2). There were ten deaths in the 18 patients assigned to irinotecan–temozolomide–temsirolimus and four deaths in the 17 patients assigned to irinotecan–temozolomide–dinutuximab. Deaths in the patients assigned to irinotecan–temozolomide–temsirolimus occurred between 92 days and 2.47 years after study enrolment. The median time to death from study enrolment was 0.83 years (IQR 0.29–1.75). 1-year overall survival was 64.7% (40.8–88.6) in the irinotecan–temozolomide–temsirolimus group, and 88.2% (72.3–100) in the irinotecan–temozolomide–dinutuximab group (figure 3).

Grade 3 or worse adverse events related to protocol therapy are shown in table 4. There were no substantial differences in grade 3 or worse diarrhoea (two [11%] of 18 patients in the irinotecan–temozolomide–temsirolimus group vs one [6%] of 16 in the irinotecan–temozolomide–dinutuximab group), neutropenia (eight [44%] vs four [25%]), or thrombocytopenia (five [28%] vs four [25%]) between the groups. More patients in the dinutuximab group than in the temsirolimus group experienced grade 3 or worse pain with treatment (seven [44%] vs one [6%]). Four (25%) of 16 patients had grade 3 or worse hypoxia during irinotecan–temozolomide–dinutuximab therapy (three grade 3 and one grade 4; appendix p 5) compared with no patients in the temsirolimus group. One patient in the irinotecan–temozolomide–dinutuximab group had grade 3 peripheral motor neuropathy beginning on day 6 of cycle six. This patient had bilateral lower extremity weakness with inability to ambulate independently for 4 weeks. No additional irinotecan–temozolomide–dinutuximab was administered and motor function returned to baseline within 6 weeks of onset of neuropathy.

Five patients in the irinotecan–temozolomide–temsirolimus group and ten patients in the irinotecan–temozolomide–dinutuximab group required dose modifications. The temsirolimus dose was modified in four patients; the dose reductions were due to haematological toxicity (n=3; neutropenia and thrombocytopenia) and an infusion reaction (n=1). The temozolomide dose was reduced along with the temsirolimus dose in two patients with haematological toxicity. The irinotecan dose was also modified in one of those two patients. Four patients in the irinotecan–temozolomide–dinutuximab group required temozolomide dose modifications; these were due to a formulation issue (n=1), emesis (n=1), and haematological toxicity (n=2; neutropenia and thrombocytopenia). An additional six patients required dinutuximab dose modifications; these were due to hypoxia (n=1), bronchospasm (n=1), pain (n=2), infection (n=1), and hypotension (n=1). In all patients requiring dose modifications, only those with hypoxia and bronchospasm required discontinuation of protocol therapy because of toxicity.

Two deaths occurred during protocol therapy. One patient in the irinotecan–temozolomide–dinutuximab group had progressive disease in the chest and died from respiratory failure during cycle two, and one patient in the same group, who achieved a complete response after cycle six, died unexpectedly after 14 cycles of treatment. The cause of death in this case was not determined despite a full autopsy, and the association of death to protocol
therapy is unclear. No other deaths were reported during protocol therapy and no deaths were directly attributed to treatment. One patient in the irinotecan–temozolomide–dinutuximab group developed grade 4 hypoxia possibly related to therapy (on day 5 of cycle one) and met protocol-defined criteria for unacceptable toxicity. No other events met this definition. The stopping rule for unacceptable toxicity was not met for either regimen.

**Discussion**

The results of our study show that the chemoimmunotherapy combination irinotecan–temozolomide–dinutuximab has substantial activity in patients with relapsed or refractory neuroblastoma, with a manageable toxicity profile. Nine of 17 patients randomly assigned to irinotecan–temozolomide–dinutuximab had objective responses whereas only one of 18 patients assigned to...
Table 4: Treatment-related adverse events

<table>
<thead>
<tr>
<th></th>
<th>Irinotecan-temozolomide-temsirolimus (n=18)</th>
<th>Irinotecan-temozolomide-dinutuximab (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 3</td>
<td>Grade 4</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>6 (33%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2 (11%)</td>
<td>3 (17%)</td>
</tr>
<tr>
<td>Anaemia</td>
<td>6 (33%)</td>
<td>0</td>
</tr>
<tr>
<td>Fever and infection</td>
<td>2* (11%)</td>
<td>0</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>3 (17%)</td>
<td>0</td>
</tr>
<tr>
<td>Capillary leak syndrome</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypotension</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pain</td>
<td>1 (6%)</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>2 (11%)</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2 (11%)</td>
<td>0</td>
</tr>
<tr>
<td>Mucositis</td>
<td>2 (11%)</td>
<td>0</td>
</tr>
<tr>
<td>Dehydration</td>
<td>3 (17%)</td>
<td>0</td>
</tr>
<tr>
<td>Elevated creatinine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Increased bilirubin</td>
<td>1 (6%)</td>
<td>0</td>
</tr>
<tr>
<td>Hyponatraemia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypokalaemia</td>
<td>3 (17%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Increased alanine aminotransferase</td>
<td>5 (28%)</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral motor neuropathy</td>
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<td>0</td>
</tr>
</tbody>
</table>

Data are n (%) for all patients who received treatment. No treatment-related deaths occurred. As per the protocol, data for grade 1–2 events were not collected unless they resulted in hospitalisation within 24 h (which did not occur in any patients). *Two of these patients had grade 3 or worse fever during the time period in which the patient was receiving antibody. The other two had documented infections including bacteremia and urinary tract infection.

Objective responses of the magnitude observed in our study have been reported previously, but these were studies of topotoc- containing regimens in patients never previously treated with topotecan. A widely used induction regimen now includes topotecan; response to topotecan-based therapy is expected to be lower in previously exposed patients than in those not previously exposed. Similarly, although nine of 17 patients in first relapse responded to high-dose ifosfamide–carboplatin–etoposide in one study, a lower response could be expected in patients previously treated with high-dose carboplatin and etoposide used in North American consolidation regimens. In a study of a GD2-directed antibody (3F8) with GM-CSF without chemotherapy, ten (38%) of 26 patients with non-progressing, primary refractory, evaluable (non-irradiated) osteomedullary disease responded to treatment. The objective response of 53% recorded in our study requires confirmation, but suggests that addition of irinotecan–temozolomide to dinutuximab and GM-CSF might result in activity in a broader group of patients. Patients receiving irinotecan–temozolomide–dinutuximab had adverse events known to accompany dinutuximab therapy (including pain, fever, and electrolyte abnormalities); however, hematological toxicity was relatively modest. Monitoring for peripheral motor neuropathy will be important going forward. No second malignancies in patients treated in our trial have been reported so far, although follow-up was relatively short.

Because the primary objective of ANBL1221 was met after accrual to stage 1 of the activity design, the number of patients treated with irinotecan–temozolomide–dinutuximab was small. This sample size is an important limitation of the study. Therefore, treatment of additional patients is required to verify the encouraging response and better define the toxicity profile of this treatment combination. Expansion of the patient population might also permit subgroup analyses. Another limitation is that this trial included only patients with a first episode of relapsed or refractory disease; the role of irinotecan–temozolomide–dinutuximab in other settings is unknown. This study was not specifically powered to evaluate survival endpoints, and survival data should be interpreted in light of the small sample size and the fact that event-free survival in children with neuroblastoma can vary considerably given the clinical heterogeneity of this disease. Results of overall survival analyses should also be interpreted with caution, because survival for patients in both groups might have been affected by therapy received following study treatment.
The 6% objective response for irinotecan–temozolomide–temsirolimus is not substantially lower than the response for irinotecan–temozolomide alone (8–15%) reported in other studies. Additionally, ten patients who were given irinotecan–temozolomide–temsirolimus had stable disease in this study. However, this study was not designed to compare clinical benefit following irinotecan–temozolomide–temsirolimus with the benefit following irinotecan–temozolomide alone.

To more rigorously compare these therapies, a much larger clinical trial would be necessary.

This study used the same modified version of the 1993 INRC criteria used in previous COG phase 2 trials. Modification was necessary because the 1993 criteria do not include MIBG scans as an assessment modality and because the use of tumour volume for assessment of measurable disease (as in the 1993 criteria) does not permit comparison with modern-era studies that use RECIST-style approaches. A new version of the INRC has been developed that includes parameters for MIBG assessment and includes RECIST-style guidance for evaluation of soft-tissue disease. However, these consensus criteria had not yet been agreed upon at the time of the development of this trial. The use of the COG modification of the 1993 INRC limits comparisons with studies done by other groups, however this limitation will be overcome in future trials when the new INRC are incorporated worldwide.

Potential mechanisms of response to this combination merit consideration. Chemotherapy-induced capillary modification could increase antibody dispersion and improve access to tumour cells. Irinotecan–temozolomide might also potentiate the effects of immunotherapy by changing the tumour microenvironment, producing responses that exceed those observed following GD2-directed antibody therapy alone or with cytokines but without irinotecan–temozolomide.

Evaluation of cytokines in peripheral blood and assessment of tumour-infiltrating leukocytes and macrophages might reveal the basis for responses. Assessment of expression of immune checkpoint proteins affecting activity of macrophages and natural killer cells might help in the development of predictive biomarkers. Natural killer cell-mediated cytotoxicity can be diminished because of inhibitory interactions between killer immunoglobulin-like receptors and their ligands, the genotypes of which might correlate with response to anti-GD2 therapy and should therefore also be assessed as potential biomarkers. Fc receptor polymorphisms influence response to antibody therapy in adults with lymphoma and might affect response to GD2-directed therapy in children, further assessment of Fc receptor genotypes might be warranted. Additional studies to elucidate the specific contributions of irinotecan, temozolomide, and GM-CSF in augmenting dinutuximab activity might also be helpful. During this study, GM-CSF was administered following completion of irinotecan–temozolomide–dinutuximab.

Alternative GM-CSF schedules could not be studied in this initial trial, but response following concurrent administration of GM-CSF and irinotecan–temozolomide–dinutuximab could be assessed in a future study. Response following irinotecan–temozolomide–dinutuximab with GM-CSF compared with response following dinutuximab and GM-CSF alone could also be assessed in the future.

This trial was designed to identify an agent meriting further study in combination with chemotherapy in the front-line setting. A GD2-directed antibody has been administered with a multi-agent induction regimen in a single institution study. No unexpected toxicities were observed in that trial, however further study of dinutuximab in combination with chemotherapy agents other than irinotecan–temozolomide is needed. A multi-institution pilot study to evaluate the safety of dinutuximab in combination with induction chemotherapy is being developed.

Contributors
RM, ALY, WBL, and RB did the literature search. RM, AN, CVR, ALY, WBL, BLS, MTP, S-E-NS, PMS, JGB, JMM, JRP, and RB designed the study. RM, AN, CVR, WBL, BLS, MTP, S-E-NS, MB, JGB, JMM, JRP, and RB did the data collection. RM, AN, CVR, WBL, BLS, MTP, and RB designed the figures. All authors did the data analysis and data interpretation, and contributed to the writing of this manuscript.

Declaration of interests
BLS is a member of the Data Safety Monitoring Board for a study of Lymphoseek (Nav 3-18). JGB has received support for clinical trials from Merck, Amgen, Lilly, Pfizer, Bristol-Myers Squibb, Celgene, Eisai, Novartis, and Ignyta, all outside the submitted work. All other authors declare no competing interests.

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