FULL PAPER

BJC

British Journal of Cancer (2016) 115, 1048–1057 | doi: 10.1038/bjc.2016.302

Keywords: relapsed neuroblastoma; epidemiology; post-relapse progression-free survival; post-relapse overall survival; high risk; intermediate risk

Factors associated with recurrence and survival length following relapse in patients with neuroblastoma

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Background: Despite therapeutic advances, survival following relapse for neuroblastoma patients remains poor. We investigated clinical and biological factors associated with length of progression-free and overall survival following relapse in UK neuroblastoma patients.

Methods: All cases of relapsed neuroblastoma, diagnosed during 1990–2010, were identified from four Paediatric Oncology principal treatment centres. Kaplan–Meier and Cox regression analyses were used to calculate post-relapse overall survival (PROS), post-relapse progression-free survival (PRPFS) between relapse and further progression, and to investigate influencing factors.

Results: One hundred eighty-nine cases were identified from case notes, 159 (84.0%) high risk and 17 (9.0%), unresectable, *MYCN* non-amplified (non-MNA) intermediate risk (IR). For high-risk patients diagnosed >2000, median PROS was 8.4 months (interquartile range (IQR) = 3.0–17.4) and median PRPFS was 4.7 months (IQR = 2.1–7.1). For IR, unresectable non-MNA patients, median PROS was 11.8 months (IQR 9.0–51.6) and 5-year PROS was 24% (95% CI 7–45%). *MYCN* amplified (MNA) disease and bone marrow metastases at diagnosis were independently associated with worse PROS for high-risk cases. Eighty percent of high-risk relapses occurred within 2 years of diagnosis compared with 50% of unresectable non-MNA IR disease.

Conclusions: Patients with relapsed HR neuroblastomas should be treatment stratified according to *MYCN* status and PRPFS should be the primary endpoint in early phase clinical trials. The failure to salvage the majority of IR neuroblastoma is concerning, supporting investigation of intensification of upfront treatment regimens in this group to determine whether their use would diminish likelihood of relapse.

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Revised 11 July 2016; accepted 29 August 2016; published online 4 October 2016

Neuroblastoma, the second most common childhood solid tumour, accounts for 8% of all childhood (0–14 years) cancers in the United Kingdom (Stiller, 2007). It is one of the most difficult childhood cancers to cure with UK and Ireland 5-year survival of 64.7% for cases diagnosed during 2005–2007 (Gatta *et al*, 2014).

However, survival remains poor for children diagnosed with high-risk disease (50% of all neuroblastoma) (Cohn *et al*, 2009) defined as stage 4 > 1 year of age, or *MYCN* amplified (MNA) localised (stages 2 and 3) or MNA infant (< 12 month) disease, with relapse in > 50% of cases (Maris *et al*, 2007; Maris, 2010). Relapse also occurs in other risk groups including intermediate risk (around 20% of cases at diagnosis) defined as *MYCN* non-amplified (non-MNA), unresectable (stage 3) and non-MNA stage 4 < 12 months old cases. The presence of MNA is a well-established poor prognostic marker in patients with neuroblastoma with localized disease and infants < 12 months of age (Cohn and Tweddle, 2004; Maris *et al*, 2007; Canete *et al*, 2009). Survival from relapsed high-risk neuroblastoma is currently < 10% (London *et al*, 2011; Park *et al*, 2013). The disease control intervals and the patterns of recurrence are important for evaluation of new treatment strategies and early phase study designs, as they are increasingly being used to define alternative end-points to tumour response (Santana *et al*, 2008; Fox *et al*, 2014).

Some recent studies have reported clinical features of relapsed neuroblastoma (Garaventa *et al*, 2009; London *et al*, 2011; Simon *et al*, 2011), however few report length of post-relapse progression-free survival (PRPFS). The present study aimed to investigate factors associated with recurrence, survival length following relapse and length of progression-free survival in patients with neuroblastoma diagnosed and treated at four UK Paediatric Oncology principal treatment centres. This study included information from patient file review on PRPFS, which is essential for informing the design and judging the efficacy of new treatments tested in early phase clinical trials.

MATERIALS AND METHODS

Study patients. All cases of relapsed and refractory/progressive neuroblastoma diagnosed during 1990–2010 were identified from four UK Paediatric Oncology principal treatment centres (The Royal Victoria Infirmary, Newcastle, Leeds Teaching Hospitals NHS Trust, The Royal Manchester Children's Hospital and The Royal Marsden Hospital). Population-based data from three specialist registries were used: the Northern Region Young Persons' Malignant Disease Registry (NRYPMDR) (Cotterill *et al*, 2000), the Yorkshire Specialist Register of Cancer in Children and Young People (YSRCCYP) (Feltbower *et al*, 2004) and the Manchester Children's Tumour Registry (MCTR) (Birch, 1988), and a non-population database held at the Royal Marsden Hospital NHS Foundation Trust. All cases within the age ranges of the registries were included. Year 1990 was chosen as the start date as from this date onwards combination chemotherapy and high-dose myeloablative therapy were routinely used to treat high-risk neuroblastoma (Pritchard *et al*, 2005; Pearson *et al*, 2008). Nine patients who died within 7 days of relapse were excluded as their survival and treatment data at relapse would be limited.

Definition of relapse. The International Neuroblastoma Response Classification (INRC) criteria were used to define relapse as a new site of disease or 25% increase in tumour size following an initial response (including partial) to treatment, and refractory disease as tumours that did not respond to any first-, second- or third-line therapies and subsequently progressed (Brodeur *et al*, 1993).

Statistical analysis. Table 1 and Supplementary Table S1 show the demographic and clinical variables studied at diagnosis and relapse. Post-relapse overall survival time (PROS) was the primary end point, defined as time from first relapse/progression (including relapsed refractory disease) to death or date of last follow-up in survivors. Follow-up was censored at 31 March 2014. Kaplan–Meier methods were used to calculate estimates of PROS and PRPFS, which is the time between relapse and further relapse/progression (Kaplan and Meier, 1958). Log-rank tests were used to compare differences in survival estimates between variables. Cox proportional hazards regression analysis was used to investigate risk factors that may influence PROS (Cox, 1972). The associations between PROS and *MYCN* amplification, 1p deletion, time interval from diagnosis to relapse and age at diagnosis were analysed. Age at diagnosis was categorised into < 18 months, ⩾ 18 months and < 5 years, ⩾ 5 years, since 18 months is now used to stratify patients at diagnosis and there is evidence that older children have a more protracted relapse course (Cohn *et al*, 2009). Time interval from diagnosis to relapse was categorised as < 6 months, 6 to < 12 months, 12 to < 18 months, 18 to < 24 months and ⩾ 24 months to enable comparison with previously published studies (London *et al*, 2011; Simon *et al*, 2011). Two time periods of diagnosis, ⩽ 2000 and > 2000, were used to categorise patients as around this time treatment for high-risk neuroblastoma was intensified (Kohler *et al*, 2007; Pearson *et al*, 2008; Ladenstein *et al*, 2010; Kohler *et al*, 2013). Only models that met the Cox proportional hazard assumption are presented (Cox, 1972).

Subgroup analyses by risk group were carried out for high risk and intermediate risk (IR) patients using the International Neuroblastoma Risk Group (INRG) definitions and a comparison of survivors *vs* non-survivors (Cohn *et al*, 2009). Detailed analysis of unresectable, non-MNA cases was undertaken as this group has varied clinical behaviour (Park *et al*, 2009; Baker *et al*, 2010). Where the total number of cases was low, the 95% Wilson confidence intervals for binomial percentages were calculated. Stata version 12 was used for all analyses, with statistical significance taken to be $P < 0.05$ (StataCorp, 2011).

RESULTS

One hundred eighty-nine cases of relapsed neuroblastoma were identified in this study. A flow diagram showing numbers of patients by risk group is given in Figure 1. For all relapsed cases PROS significantly increased after 2000 ($P < 0.001$) (Supplementary Figure S1a and Supplementary Figure S1b).

High-risk group. The number of cases analysed for each variable differed depending on the completeness of available data (Table 1 and Supplementary Table S1). At diagnosis, 90% of cases were treated in a clinical trial or per clinical trial protocol (Supplementary Table S2). The overall response assessment according to the INRC criteria (Brodeur *et al*, 1993) at the end of first-line treatment was: partial response including very good partial response for 70 out of 139 (50%) cases and complete response for 30 out of 139 (22%) cases. At first relapse, 124 out of 159 (78%) of cases relapsed within 2 years of diagnosis and 13 out of 107 (12%) of patients relapsed at the primary site alone. In 60 patients (38%) in whom levels of urinary catecholamines were recorded at diagnosis and relapse, 48 (80%) had raised urinary catecholamines levels at both diagnosis and relapse.

The median PROS time for all high-risk cases was 4.5 months (interquartile range (IQR) 1.9–11.4), which was significantly increased for patients diagnosed after 2000 *vs* before ($P < 0.001$) (Figure 2A and B, Supplementary Figure S2a and Supplementary Figure S2b). Significantly more patients diagnosed after 2000, 60 out of 74 (81%), were treated actively at relapse compared with those who were given palliative radiotherapy or supportive care alone 14 out of 74 (19%); *vs* 22 out of 64 (44%) ⩽ 2000) ($P = 0.03$).

Table 1. Patient characteristics at relapse for all cases of relapsed neuroblastoma, high-risk cases, and intermediate risk, stage 3, unresectable, non-MNA cases

	All relapsed neuroblastoma *N* = 189 *N* (%, 95% CI)[b]	High-risk relapsed Neuroblastoma *N* = 159 (84.1%) *N* (%, 95% CI)[b]	Intermediate risk (unresectable MYCN non-amplified) *N* = 17 (9.0%) *N* (%, 95% CI)[c]	*P*-value[a] (high *vs* intermediate risk groups)
Time from diagnosis to relapse				
Mean	18.1 months	17.6 months	28.8 months	
Median	14.7 months	14.6 months	22.1 months	
Interquartile range	9.2–22.1 months	9.3–21.5 months	10.3–39.1 months	0.03
Range	1.3–96.2 months	1.3–96.2 months	4.0–68.5 months	
Time from diagnosis to relapse groups				
<6 months	32 (16.9, 11.9–23.1)	24 (15.1, 9.9–21.6)	1 (5.8, 1.0–27.0)	
6–12 months	38 (20.1, 14.6–26.5)	32 (20.1, 14.2–27.2)	4 (23.5, 9.6–47.3)	0.23
12–18 months	43 (22.8, 17.0–29.3)	40 (25.2, 18.6–32.6)	2 (11.8, 3.3–34.3)	
18–24 months	32 (16.9, 11.9–23.1)	28 (17.9, 12.0–24.4)	2 (11.8, 3.3–34.3)	
>24 months	44 (23.3, 17.5–30.0)	35 (22.0, 15.8–29.3)	8 (47.1, 26.2–69.0)	
Site of relapse	***N* = 132 (69.8)[d]**	***N* = 107 (67.3)[d]**	***N* = 13 (76.5)[d]**	
Primary	30 (22.7, 15.9–30.8)	13 (12.2, 6.6–19.9)	8 (61.5, 35.5–82.3)	
Metastases	102 (77.3, 69.2–84.1)	94 (87.8, 80.1–93.4)	5 (38.5, 17.7–64.5)	<0.001
Missing	57 (30.2)[e]	52 (32.7)[e]	4 (23.5)[e]	
Bone metastases at relapse	***N* = 170 (89.9)[d]**	***N* = 142 (75.1)[d]**	**15 (88.2)[d]**	
No	87 (51.2, 43.4–58.9)	70 (49.3, 40.8–57.8)	9 (60.0, 35.7–80.2)	
Yes	83 (48.8, 41.1–56.6)	72 (50.7, 42.2–59.2)	6 (40.0, 19.8–64.3)	0.43
Missing	19 (10.1)[e]	17 (24.9)[e]	2 (11.8)[e]	
Bone marrow metastasis at relapse	***N* = 140 (74.1)[d]**	***N* = 116 (73.0)[d]**	***N* = 13 (76.5)[d]**	
No	75 (53.6, 45.0–62.0)	58 (50.0, 40.6–59.4)	9 (69.2, 42.4–87.3)	
Yes	65 (46.4, 38.0–55.0)	58 (50.0, 40.6–59.4)	4 (30.8, 12.7–57.6)	0.24
Missing	49 (25.9)[e]	43 (27.0)[e]	4 (23.5)[e]	
Urinary catecholamine levels at relapse	***N* = 87 (46.0)[d]**	***N* = 73 (45.9)[d]**	***N* = 9 (52.9)[d]**	
Normal	20 (23.0, 14.6–33.2)	14 (19.2, 10.9–30.1)	6 (66.7, 35.4–87.9)	
Elevated	67 (77.0, 66.8–85.4)	59 (80.8, 55.1–77.7)	3 (33.3, 12.1–64–6)	0.006
Missing	102 (54.0)[e]	86 (54.1)[e]	8 (47.1)[e]	
Status				
Alive	16 (8.5, 4.9–13.4)	5 (3.1, 1.0–7.2)	4 (23.5, 9.6–47.3)	
Dead	166 (87.8, 82.3–92.1)	148 (93.1, 88.0–96.5)	13 (76.5, 52.7–90.4)	0.001
Lost to follow-up	7 (3.7, 1.5–7.5)	6 (3.8, 1.4–8.0)	–	

[a]Chi-squared test and the Fisher's exact test (where numbers were <5) were used to compare between high and intermediate risk group characteristics.
[b]95% CI were calculated for percentages using the asymptotic method.
[c]95% CI for proportions were calculated using the Wilson score method.
[d]Percent of the total number of cases.
[e]Percent with missing data.

Active treatments at relapse included second-line chemotherapy, mIBG therapy, or second-line chemotherapy followed by other treatments such as radiotherapy, or phase I or II clinical trials in 10 out of 138 (7%) cases (Table 2). Figure 3 shows that for cases diagnosed after 2000, 6 out of 76 (8%) patients treated by mIBG therapy had a median PROS of 12.2 months (IQR = 10.9–26.2 months), 5 out of 76 (7%) patients enrolled onto phase I or II trials had a median PROS of 13.5 months (IQR = 7.7–16.4) and 29 out of 76 (38%) treated by second-line chemotherapy had a median PROS of 6.6 months (IQR = 2.5–17.5) compared with 1.5 months (IQR = 0.6–2.9) for cases treated by supportive care ($P = 0.01$). In contrast, before 2000 only one patient had mIBG therapy. For 58 cases, the date of further relapse/progression was recorded and for those patients the median PRPFS was 4.5 months (IQR 2.2–8.7 months); it was 3.9 months (IQR 2.5–8.7 months) for 21 cases diagnosed before 2000 and 4.7 months (IQR 2.1–7.1 months) for 37 cases diagnosed after 2000 ($P = 0.54$). Treatments received at subsequent relapse/progression are given (Supplementary Table S3).

Cox univariable proportional hazards regression analyses for high-risk cases showed that PROS time was significantly worse for cases with MNA disease ($P < 0.0001$), both MNA and 1p deleted disease ($P = 0.02$), liver metastases at diagnosis ($P = 0.02$), and for cases who relapsed within 6 months of diagnosis compared with relapses >2 years ($P = 0.03$), while patients >5 years old at diagnosis had longer PROS ($P = 0.02$) (Supplementary Table S4). However, in multivariable analysis only MNA (adjusted HR = 2.06; 95% CI 1.22–3.46, $P = 0.007$) and bone marrow metastases at diagnosis (adjusted HR = 2.33; 95% CI 1.26–4.29, $P = 0.007$) were independently significantly associated with worse PROS (Table 3). Information on *MYCN* status was unknown for 30% of cases, so sensitivity analysis was carried out to include the *MYCN* unknown cases as an additional category in the model, which showed that it did not affect the results. MNA disease was significantly associated with worse PROS ($P < 0.001$) (Figure 2C and Supplementary Figure S2c). Similar results were obtained for PROS for cases diagnosed before ($P < 0.001$) (Figure 2D) or after 2000 ($P = 0.02$) (Figure 2E).

Intermediate risk, unresectable, *MYCN* non-amplified group. The number of cases analysed for each variable differed depending on the completeness of available data (Table 1 and Supplementary

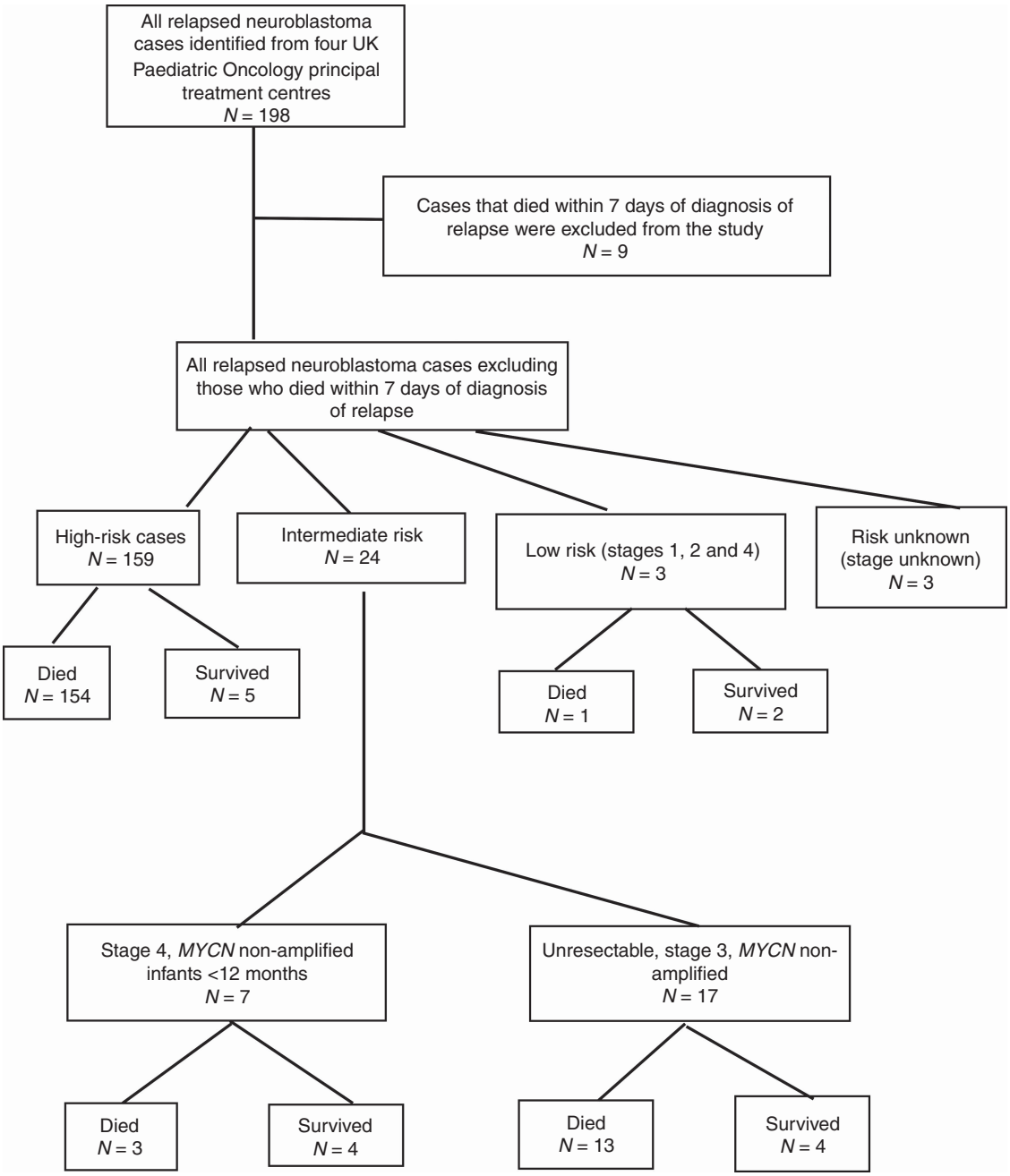

Figure 1. Flow diagram showing number of cases included in the study.

Table S1). The International Neuroblastoma Pathology Classification (INPC) histology (Shimada *et al*, 1999) was unfavourable in 13 out of 15 (87%; 95% CI 62–96%) cases and primary tumour site was abdominal in 14 out of 17 (82%; 95% CI 59–94%), 12 out of 17 (71%; 95% CI 47–87%) were treated in a clinical trial at diagnosis or per clinical trial protocol (four cases in European Neuroblastoma Study group Fifth study (ENSG5) (Pearson *et al*, 2008), two cases in European High Risk Neuroblastoma Study 1 of SIOP-Europe (HRNBL1) (Ladenstein *et al*, 2010), two in unresectable NB2009 (Kohler *et al*, 2013), four in ENSG9 (ClinicalTrials.gov identifier: NCT00276731; Supplementary Table S2). At the end of treatment 7 out of 15 (47%; 95% CI 25–70%) cases had achieved partial response and 6 out of 15 (40%; 95% CI 20–64%) complete response. The median PROS time for this group was 11.8 months (IQR = 9.0–51.6) (Supplementary Figure S3a and b).

Significantly more cases relapsed >2 years from diagnosis 8 out of 17 (47%; 95% CI 26–69%) compared with high-risk cases 35 out of 159 (22%; 95% CI 16–29; $P = 0.04$). Eight out of thirteen (62%; 95% CI 36–82%) relapsed at the primary site. In 9 out of 17 patients (53%, 31–74%) in whom levels of urinary catecholamines were recorded at diagnosis and relapse, 3 out of 9 (33%; 95% CI 12–65%) had raised urinary catecholamine levels at both diagnosis and relapse. Eleven out of sixteen patients (69%; 95% CI 44–86%) were treated with second-line high-risk type chemotherapy at first relapse (Table 2). A further relapse/progression date and treatment were recorded for eight cases, and for those the median PRPFS was 10.1 months (IQR = 7.8–12.3 months). Five out of eight (63%; 95% CI 31–86%) were treated with second line chemotherapy and the remainder treated by combined chemotherapy and surgery or radiotherapy including mIBG therapy or supportive care (Supplementary Table S3).

Survivors. Only 8% of all relapsed patients in our study survived to the end of the study period (Supplementary Table S5). Fifteen

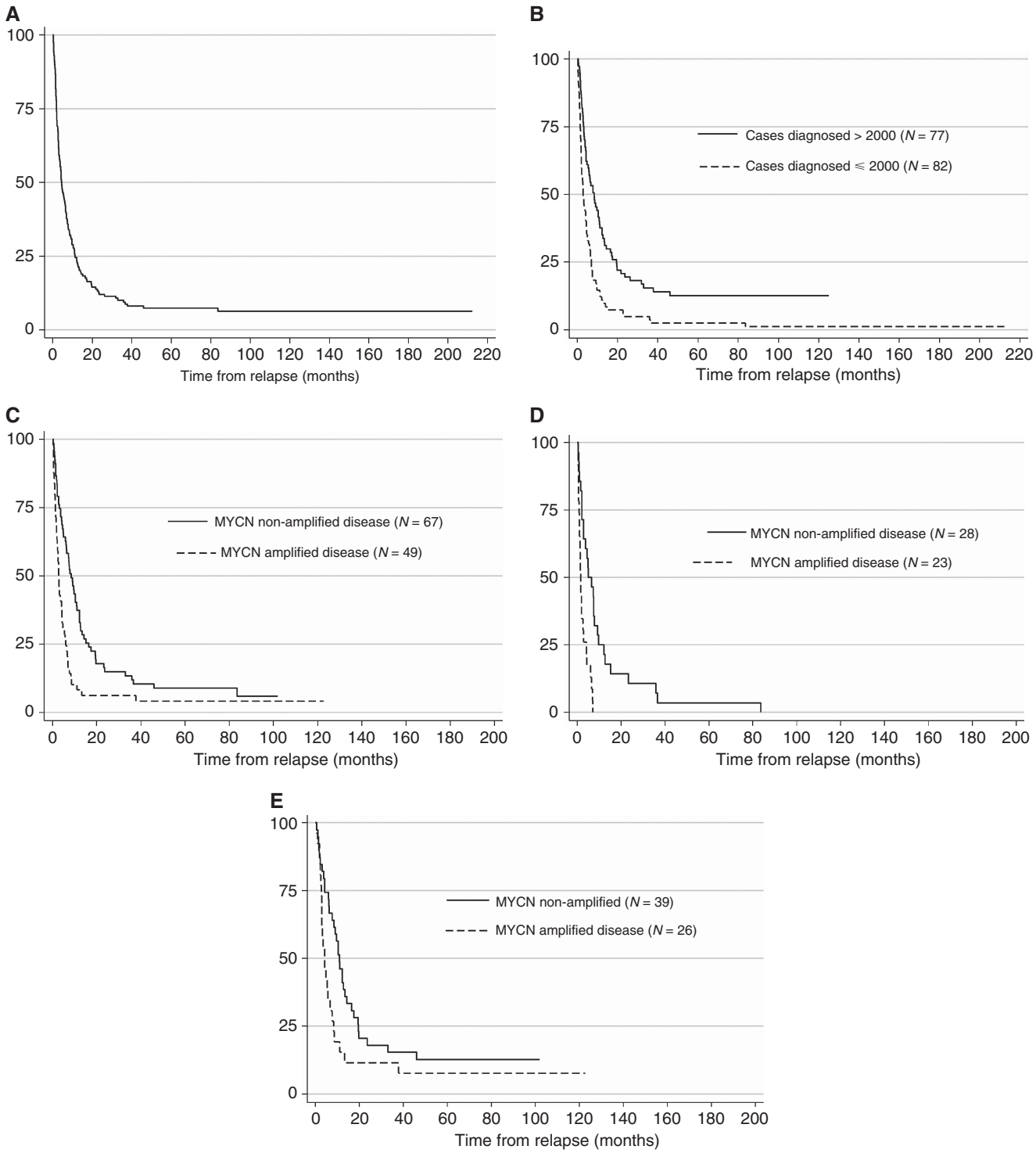

Figure 2. Kaplan–Meier graphs for post-relapse overall survival time for the high-risk group ($N = 159$). (**A**) Post relapse overall survival (PROS) time. Median PROS time for high-risk cases was 4.5 months (IQR = 1.9–11.4). Five-year PROS for high-risk cases was 7.4% (95% CI 4.0–12.1%). (**B**) Post-relapse overall survival by year of diagnosis. Median PROS time was 2.9 months (IQR 1.4–6.9) for cases diagnosed ⩽2000 *vs* 8.4 months (IQR 3.0–17.4) for cases diagnosed >2000 ($P < 0.001$). Five-year PROS for high-risk cases diagnosed ⩽2000 was 2.4% (95% CI 0.5–7.7%) *vs* 12.7% (95% CI 6.4–21.2%) for cases diagnosed >2000. (**C**) PROS by *MYCN* status. Median PROS time was 2.9 months (95% CI 2.0–4.3) for *MYCN* amplified *vs* 8.5 months for MYCN non-amplified (95% CI 5.9–11.1; $P < 0.001$). Five-year PROS for *MYCN* non-amplified cases was 9.0% (95% CI 3.7–17.2%) *vs* 4.1% (95% CI 0.8–12.3%) for *MYCN* amplified cases. (**D**) PROS for cases diagnosed ⩽2000 by *MYCN* status. For cases diagnosed ⩽2000, the median PROS for *MYCN* amplified disease was 1.5 months (95% CI 0.9–2.7) *vs* 5.1 months (95% CI 2.9–7.9) for *MYCN* non-amplified cases ($P < 0.001$). Five year PROS for *MYCN* non-amplified was 3.6% (95% CI 0.3–15.4%) *vs* 0% for *MYCN* amplified cases. (**E**) PROS for cases diagnosed >2000 by *MYCN* status. For cases diagnosed >2000, the median PROS for *MYCN* amplified disease was 4.3 months (95% CI 2.9–6.6) *vs* 10.9 months (95% CI 6.3–14.4) for *MYCN* non-amplified ($P = 0.02$). Five-year PROS for *MYCN* non-amplified cases was 12.8% (95% CI 4.7–25.2%) *vs* 7.7% (95% CI 1.3–21.7%) for *MYCN* amplified cases.

out of sixteen patients (8%; 95% CI 72–99%) were disease free 5 years from first relapse. Four out of five of the high-risk survivors and 1 out of 4 of the IR were diagnosed >2000. The median follow-up time from relapse for survivors was 11 years (IQR 6.6–16.5). Eight out of sixteen (50%; 95% CI 28–72%) patients were aged ⩽18 months at diagnosis, 5 out of 15 (33%; 95% CI 15–58%) had high-risk disease at diagnosis and 12 out of 13 (85%; 95% CI 58–96%) were non-MNA. At relapse, 8 out of 14 (57%; 95% CI 33–79%)

Table 2. Treatment received at first relapse for all relapsed neuroblastoma cases, high risk cases and intermediate risk, stage 3, unresectable, *non-MNA* neuroblastoma

	All relapsed cases *N* (%)			High Risk *N* (%)			Intermediate risk *N* (%)		
Treatment	**Diagnosed ⩽2000**	**Diagnosed >2000**	**Total**	**Diagnosed ⩽2000**	**Diagnosed >2000**	**Total**	**Diagnosed ⩽2000**	**Diagnosed >2000**	**Total**
Second-line Chemotherapy[a]	29 (36.3)	33 (38.4)	62 (37.4)	22 (34.4)	29 (39.2)	51 (37.0)	2 (22.2)	1 (14.3)	3 (18.8)
Combination of treatments[b]	15 (118.8)	17 (19.8)	32 (19.3)	10 (15.6)	12 (16.20	22 (15.9)	4 (44.4)	4 (57.1)	8 (50.0)
mIBG therapy	1 (1.3)	6 (7.0)	7 (4.2)	1 (1.6)	6 (8.1)	7 (5.1)	0 (0.0)	0 (0.0)	0 (0.0)
Other (e.g., surgery or radiotherapy)	7 (8.8)	10 (11.6)	17 (10.2)	4 (6.3)	8 (10.8)	12 (8.7)	2 (22.2)	2 (28.6)	4 (25.0)
Phase I or II trials	5 (6.3)	5 (5.8)	10 (6.0)	5 (7.8)	5 (6.8)	10 (7.3)	0 (0.0)	0 (0.0)	0 (0.0)
Palliative radiotherapy	10 (12.5)	6 (7.0)	16 (9.6)	10 (15.6)	6 (8.1)	16 (11.6)	0 (0.0)	0 (0.0)	0 (0.0)
Supportive care	13 (16.3)	9 (10.5)	22 (13.3)	12 (18.8)	8 (10.8)	20 (14.5)	1 (11.1)	0 (0.0)	1 (6.3)
Total	80 (100)	86 (100)	166 (100)	64 (100)	74 (100)	138 (100)	9 (100)	7 (100)	16 (100)

[a]Second-line chemotherapy includes vincristine, temozolamide, irinotecan, topotecan vincristine-doxorubicin or oral etoposide.
[b]Combination of chemotherapy, surgery, radiotherapy and miBG therapy.

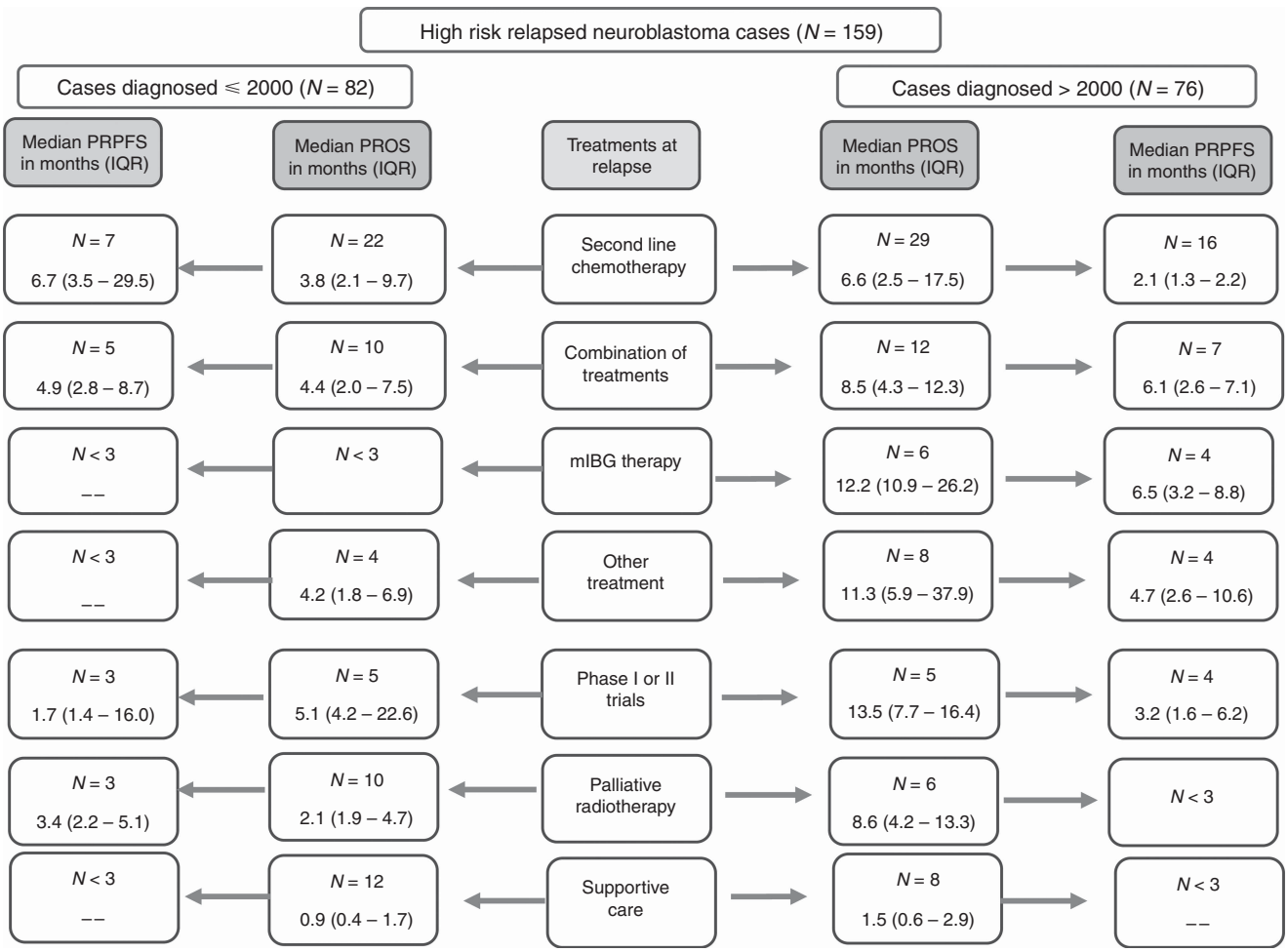

Figure 3. Flow diagram showing treatments that high-risk patients received at first relapse and its outcome. Abbreviations: PROS = post-relapse overall survival; IQR = interquartile range; PRPFS = post-relapse progression-free survival.

Table 3. Results of multivariable analysis for post-relapse overall survival for high-risk cases

Factor	*N* (%)	Hazard ratio (95% CI)	*P*-value
Age at diagnosis			
⩽5 years	126 (79.3)	1	
>5 years	33 (20.7)	0.77 (0.44–1.34)	0.35
Bone marrow metastases at diagnosis			
No	26 (17.8)	1	
Yes	120 (82.2)	2.33 (1.26–4.29)	0.007
Liver metastases at diagnosis			
No	122 (87.8)	1	
Yes	17 (12.2)	1.44 (0.73–2.83)	0.29
***MYCN* disease**			
Not amplified	67 (57.8)	1	
Amplified	49 (42.2)	2.06 (1.22–3.46)	0.007
Time from diagnosis to relapse			
>24 months	35 (22.0)	1	
18–24 months	28 (17.9)	1.18 (0.58–2.40)	0.65
12–18 months	40 (25.2)	1.21 (0.65–2.24)	0.54
6–12 months	32 (20.1)	1.22 (0.65–2.31)	0.54
<6 months	24 (15.1)	1.52 (0.76–3.01)	0.24

survivors were treated with second-line chemotherapy, and/or with radiotherapy and surgery and 1 out of 14 was treated with chemotherapy and autologous stem cell transplant.

Comparing survivors with non-survivors showed that 27% (95% CI 11–52%) of survivors had favourable INPC histology compared with 4% (95% CI 1–8%) of non-survivors ($P=0.006$), 93% (95% CI 70–99%) of survivors had complete or partial overall response at end of treatment compared with 71% of non-survivors (95% CI 64–79%; $P=0.04$), and 50% relapsed >2 years from diagnosis compared with 21% (95% CI 15–28%) of non-survivors ($P=0.01$) (Supplementary Table S6). Of the high-risk survivors, 3 out of 5 were ⩾18 months at diagnosis, 4 out of 5 relapsed after initial myeloablative therapy and 1 out of 5 relapsed after surgery.

DISCUSSION

This study was designed to gain knowledge about relapsed neuroblastoma from primary sources of information and to reflect the outcome of these patients, avoiding the selection bias of clinical trials. It is the first multi-institutional study to report on PRPFS which is a very useful parameter for judging the efficacy of early phase clinical trials. The observed interval between relapses is important for early phase trials design as it provides a baseline comparator for exploratory studies of new agents in relapsed neuroblastoma, where the time interval of PRPFS can be used as a primary endpoint when designing exploratory trials (Santana *et al*, 2008; Fox *et al*, 2014).

The current study reports the demographics of relapsed neuroblastoma cases diagnosed during 1990–2010 from four UK Paediatric Oncology principal treatment centres treating around 30% of all UK patients in total. PROS time remains very poor for both high risk and IR (unresectable, non-MNA) disease. The median PROS for high-risk cases was 4.5 months with only 7% of relapsed cases surviving more than 5 years. However, PROS has significantly increased for high-risk cases diagnosed after the year 2000 compared with before 2000 from 2.9 months to 8.4 months, achieving a 5-year survival of 12.7%. This significant improvement in PROS after 2000 may be due to more patients being treated actively at relapse with second line chemotherapy such as temozolomide, topotecan, irinotecan or oral etoposide. All these chemotherapy regimens increase the PRPFS, but long-term overall survival remains poor (Rubie *et al*, 2006; London *et al*, 2010; Bagatell *et al*, 2011; Fox *et al*, 2014).

High risk. In contrast to most previous epidemiological studies of relapsed neuroblastoma, our study reports second relapses. The duration from first to subsequent relapse is important particularly for early phase clinical trial design. For a sub-group of patients, the date of a second event was recorded and the median PRPFS time to second event was 4.5 months (IQR = 2.2–8.7). This is shorter than that reported by Santana and colleagues, who found a median disease progression-free interval of 7.2 months between first and second relapse (Santana *et al*, 2008). This may reflect a difference in treatments given, as very few patients in our cohort were treated on phase I or II clinical trials at first or subsequent relapse, partly due to a lack of available applicable trials before 2000 (Moreno *et al*, 2013). Recently trials for relapsed neuroblastoma have opened across Europe, such as the BEACON study, a randomised phase II trial exploring the use of bevacizumab and temozolomide or temozolomide-irinotecan (ClinicalTrials.gov identifier: NCT02308527). The results of our study demonstrating the very poor outcomes following relapse strongly argue for the inclusion of patients with high risk relapsed neuroblastoma in randomised clinical trials at relapse whenever possible. This study does not identify a specific group of patients who are less likely to benefit from further treatment at relapse.

Our results for PROS concur with those published using data from the INRG database (2226 relapsed cases), diagnosed during 1990–2002, where 5-year PROS for stage 4 cases, >18 months with non-MNA disease was 8%, and only 4% for stage 4 with MNA disease (London *et al*, 2011). Around 55 out of 189 (29%) cases from the present study may overlap with the INRG database. A study from the Italian neuroblastoma registry reported 10-year PROS for stage 4 disease at diagnosis was 1.5% following progression and 2% following relapse (Garaventa *et al*, 2009). Analysis of relapsed high-risk neuroblastoma patients from German trials diagnosed during1990–2007, treated with second-line chemotherapy at relapse, found 3-year PROS was 9.6% (Simon *et al*, 2011).

Several prognostic factors are used in risk stratification for neuroblastoma at diagnosis including age at diagnosis, MNA, presence of segmental chromosomal aberrations, metastatic disease and tumour histology (George *et al*, 2001). These factors may also be important in determining response to treatments at relapse. Age >5 years at

diagnosis had a longer PROS consistent with studies showing that older children have a more protracted relapse course (Cohn *et al*, 2009). We also found worse PROS in high-risk cases with *MYCN* amplification as previously reported (Lau *et al*, 2004; Garaventa *et al*, 2009; London *et al*, 2011; Simon *et al*, 2011), and with both MNA and chromosome 1p deletion at diagnosis. Chromosome 1p deletion has been shown to be associated with shorter median PROS (Lau *et al*, 2004; London *et al*, 2011), poorer event-free survival from diagnosis (EFS) (Maris *et al*, 2001), and to be strongly related to high-risk features (Attiyeh *et al*, 2005). Both 1p deletion and 11q loss were independently associated with shorter PRPFS in patients with low and IR disease (Attiyeh *et al*, 2005). However, in the present study insufficient data on 11q or other segmental chromosomal abnormalities precluded formal analysis. Interestingly, liver metastases at diagnosis were also associated with shorter PROS in our study. In previous studies, liver metastases were associated with MNA and had a worse prognosis in older children (DuBois *et al*, 1999), unlike infants with non-MNA disease (Kushner *et al*, 2006). Ninety percent of all relapsed cases we studied had primary tumours arising in abdominal sites and only 8% had thoracic or neck tumours. This is in-keeping with other studies reporting that adrenal tumours are associated with unfavourable clinical and biological characteristics and therefore worse EFS in contrast to thoracic tumours which are associated with favourable characteristics and better EFS (Sung *et al*, 2009; Vo *et al*, 2014). However, we acknowledge the small numbers of cases involved in this study and the missing data in some of the variables means some results should be interpreted with caution.

The duration from diagnosis to first relapse has been shown to be a prognostic factor for PROS (Lau *et al*, 2004; Garaventa *et al*, 2009; London *et al*, 2011). Around 80% of high-risk cases in our study relapsed within 2 years from diagnosis, and PROS was significantly shorter when relapse occurred within 6 months of diagnosis. Lau and colleagues found that patients who relapsed either within 6 months from diagnosis or 6 months from stem cell transplant had shorter median PROS (Lau *et al*, 2004). Other studies, however, reported recurrence within 6–24 months from diagnosis implied worse PROS (London *et al*, 2011; Simon *et al*, 2011). These differences may be due to patient heterogeneity; some studies included only high-risk cases (Simon *et al*, 2011), whereas others included all relapsed neuroblastoma (London *et al*, 2011), or different definitions of relapse.

The role of urinary catecholamines as a reliable marker for monitoring neuroblastoma relapse is controversial (Simon *et al*, 2003). They may be negative due to small tumour burden at relapse or decreased production in some previously treated neuroblastomas. In the present study, urinary catecholamines were raised at relapse in 80% of patients who had raised catecholamines at diagnosis, but were only measured in 46% of patients at relapse. The current study suggests that raised urinary catecholamines are helpful as a confirmatory indicator and a tumour marker of response (Kushner *et al*, 2009).

Intermediate risk. IR disease comprised 13% of relapsed neuroblastomas in our study, 4% who were IR stage 4, non-MNA <12 months old were not further analysed as this subgroup is usually salvageable at relapse (Maris *et al*, 2007; De Bernardi *et al*, 2009). However, 9% were IR, unresectable, non-MNA with mostly unfavourable INPC histology. The prognostic value of pathology in this group was confirmed in a recent SIOPEN study (Kohler *et al*, 2013). Analysis of stage 3 data from the INRG database showed that patients >18 months with undifferentiated INPC histology and elevated levels of serum ferritin had worse overall survival and EFS (Meany *et al*, 2014). In the absence of *MYCN* amplification other genetic markers in this group may be important risk factors, such as tumour cell ploidy (Bagatell *et al*, 2005; Baker *et al*, 2010), 11q aberrations (Attiyeh *et al*, 2005; Meany *et al*, 2014) or other segmental chromosomal abnormalities (Defferrari *et al*, 2015). The management of this group of patients remains controversial, those with unfavourable histological or biological features are treated with intensive multimodality therapy including myeloablative therapy in the United States (Baker *et al*, 2010), but not in the United Kingdom or Europe. However, in the present study 6 out of 17 patients (35%; 95% CI 17–59%) were treated in high-risk protocols (ENSG5 and HRNBL1) at the physician's discretion due to patient age and/or unknown *MYCN* status. Almost 50% of IR, unresectable, non-MNA neuroblastoma relapsed >2 years from diagnosis including 5 out of 17 (29%; 95% CI 13–53%) patients who had local radiotherapy as part of their frontline treatment at diagnosis. Salvage therapies at relapse failed in 75% of cases, suggesting the need for intensification of upfront treatment regimens for cases with unfavourable histology at diagnosis (Kohler *et al*, 2013), to determine whether their use would diminish the likelihood of relapse.

CONCLUSION

In conclusion, this study showed that the PROS for neuroblastoma patients has increased but long-term survival remains poor. Eighty percent of high-risk relapses occur within 2 years from diagnosis, in contrast to only 50% of IR unresectable, non-MNA neuroblastoma, and although this latter group comprise $<10\%$ of all relapsed neuroblastoma, the failure to salvage these patients in over 75% of cases, even when given high-risk-type treatment at relapse, is concerning. Since MNA disease has a worse OS as highlighted by previous studies, our study underscores the need for early phase clinical trial data to be analysed according to *MYCN* status. Finally, more patients need to be recruited to early phase studies where PRPFS should be considered as the primary endpoint in study design, especially if cytostatic novel therapies are given.

ACKNOWLEDGEMENTS

The study received ethical approval from the Newcastle and North Tyneside 2 Research Ethics Committee (REC reference number 10/H0907/40) dated 25 August 2010. We are very grateful to Cancer Research UK, grant number C8561/A12084 and the North of England Children's Cancer Research Fund (NECCRF) for providing financial support for NOB and PWJ, to the data managers at Manchester, Leeds, Newcastle and Marsden Hospitals, to Dr Danielle Ingham for data extraction and to Mr Richard Hardy, funded by the NECCRF, for providing IT support. We thank Cancer Research UK, the UK Department of Health and Neuroblastoma UK for funding the national neuroblastoma genetic reference centre. We also thank The Great North Children's Hospital Newcastle upon Tyne Hospitals NHS Foundation Trust, the Leeds Teaching Hospitals NHS Trust, the Royal Manchester Children's Hospital and The Royal Marsden NHS Foundation Trust. ADJP is funded through a Cancer Research UK Life Chair and Programme Grant included within a Cancer Research UK ICR Core Award (C347/A15403) and is supported from the NIHR RM/ICR Biomedical Research Centre. The Northern Region Young Persons Malignant Disease Registry is funded by the Newcastle upon Tyne Hospitals NHS Foundation Trust, The Yorkshire Specialist Register of Cancer in Children and Young People is funded by the Candlelighters Trust and The Manchester Children's Tumour Registry was funded by Cancer Research UK. We acknowledge the support of the National Institute for Health Research Clinical Research Network: Cancer (UKCRNID10308).

CONFLICT OF INTEREST

Dr Lucas Moreno discloses the following activities: consultancy/advisory board participation for Novartis, AstraZeneca, Roche-Genentech, Mundipharma and Bayer. The remaining authors declare no conflict of interest.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)