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

Nermine O Basta¹, Gail C Halliday²,³, Guy Makin⁴,⁵, Jillian Birch⁴, Richard Feltbower⁶, Nick Bown⁷, Martin Elliott⁸, Lucas Moreno⁹, Giuseppe Barone⁹, Andrew DJ Pearson⁹, Peter W James¹, Deborah A Tweddle*²,³ and Richard JQ McNally*¹

¹Institute of Health & Society, Newcastle University, Newcastle upon Tyne, UK; ²Newcastle Cancer Centre at the Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK; ³Department of Paediatric Oncology, The Great North Children’s Hospital, Newcastle upon Tyne, UK; ⁴Institute of Cancer Sciences, Manchester Cancer Research Centre, Manchester Academic Health Sciences Centre, University of Manchester, Manchester, UK; ⁵Department of Paediatric Oncology, Royal Manchester Children’s Hospital, Manchester, UK; ⁶Division of Epidemiology and Biostatistics, School of Medicine, University of Leeds, Leeds, UK; ⁷Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK; ⁸Paediatric Oncology and Haematology Department, Leeds Teaching Hospitals NHS Trust, Leeds, UK and ⁹Children and Young People’s Unit, The Royal Marsden NHS Foundation Trust, Sutton, and Division of Clinical Studies and Cancer Therapeutics, The Institute of Cancer Research, Sutton, UK

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 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.

*Correspondence: Professor DA Tweddle; E-mail: deborah.tweddle@newcastle.ac.uk or Dr RJQ McNally; E-mail: richard.mcnally@ncl.ac.uk

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Survival in patients with relapsed neuroblastoma

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 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) (Cuttell et al., 2000), the Yorkshire Specialist Register of Cancer in Children and Young People (YSRCYP) (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 | High-risk relapsed Neuroblastoma | Intermediate risk (unresectable MYCN non-amplified) | P-value* |
|----------------------|-----------------------------|----------------------------------|----------------------------------------------------|----------|
|                      | N = 189 (N%, 95% CI)b        | N = 159 (84.1%) (N%, 95% CI)b    | N = 17 (9.0%) (N%, 95% CI)                        |          |
| Time from diagnosis to relapse |                               |                                   |                                                    |          |
| Mean                 | 18.1 months                 | 17.6 months                      | 28.8 months                                       | 0.03     |
| 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                                  |          |
| 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, 1–27.0)                                   | 0.6      |
| 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, 5.5–82.3)                                | <0.001   |
| Metastases           | 102 (77.3, 69.2–84.1)       | 94 (87.8, 80.1–93.4)             | 5 (38.5, 17.7–64.5)                               |          |
| Missing              | 57 (30.2)d                  | 52 (32.7)d                       | 4 (23.5)d                                        |          |
| Bone metastases at relapse | N = 170 (89.9)d             | N = 142 (75.1)d                  | N = 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)                               | 0.43     |
| Yes                  | 83 (48.8, 41.1–56.6)        | 72 (50.7, 42.2–59.2)             | 6 (40.0, 19.8–64.3)                               |          |
| Missing              | 19 (10.1)d                  | 17 (24.9)d                       | 2 (11.8)d                                        |          |
| 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, 46.0–59.4)             | 9 (69.2, 42.4–87.3)                               | 0.24     |
| Yes                  | 65 (44.4, 38.0–55.0)        | 58 (50.0, 46.0–59.4)             | 4 (30.8, 12.7–57.6)                               |          |
| Missing              | 49 (25.9)d                  | 43 (27.0)d                       | 4 (23.5)d                                        |          |
| 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)                               | 0.006    |
| Elevated             | 67 (77.0, 66.8–85.4)        | 59 (80.8, 55.1–77.7)             | 3 (33.3, 12.1–64.6)                               |          |
| Missing              | 102 (54.0)d                 | 86 (54.1)d                       | 8 (47.1)d                                        |          |
| Status               |                              |                                   |                                                    |          |
| Alive                | 16 (8.5, 4.9–13.4)          | 5 (3.1, 1.0–7.2)                 | 4 (23.5, 9.6–47.3)                                | 0.001    |
| Dead                 | 166 (87.8, 62.3–92.1)       | 148 (93.1, 88.0–96.5)            | 13 (76.5, 52.7–90.4)                              |          |
| Lost to follow-up    | 17 (8.7, 1.5–7.5)           | 6 (3.8, 1.4–8.0)                 |                                                    |          |

*Chi-squared test and the Fisher’s exact test (where numbers were <2) were used to compare between high and intermediate risk group characteristics.

b95% CI were calculated for percentages using the asymptotic method.

cPercent of the total number of cases.

dPercent 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
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).

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

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
Survival in patients with relapsed neuroblastoma

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%)

| Treatment | Diagnosed ≤ 2000 | Diagnosed > 2000 | Total | Diagnosed ≤ 2000 | Diagnosed > 2000 | Total | Diagnosed ≤ 2000 | Diagnosed > 2000 | Total |
|-----------|------------------|------------------|-------|------------------|------------------|-------|------------------|------------------|-------|
| Second-line Chemotherapy* | 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 treatmentsb | 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) |

*Second-line chemotherapy includes vincristine, temozolomide, irinotecan, topotecan vincristine-doxorubicin or oral etoposide.

| Abbreviations: PROS = post-relapse overall survival; IQR = interquartile range; PRPFS = post-relapse progression-free survival. |
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%; response at end of treatment compared with 71% of non-survivors 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%; response at end of treatment compared with 71% of non-survivors). 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: NCT032308527). 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 during 1990–2007, 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

| 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) | 0.77 (0.44–1.34) | 0.35 |
| >5 years | 33 (20.7) | | |
| Bone marrow metastases at diagnosis | | | |
| Yes | 26 (17.8) | 2.33 (1.26–4.29) | 0.007 |
| No | 120 (82.2) | | |
| Liver metastases at diagnosis | | | |
| Yes | 122 (87.8) | 1.44 (0.73–2.83) | 0.29 |
| No | 17 (12.2) | | |
| MYCN disease | | | |
| Amplified | 49 (42.2) | 2.06 (1.22–3.46) | 0.007 |
| Not amplified | 67 (57.8) | | |
| Time from diagnosis to relapse | | | |
| <6 months | 24 (15.1) | | |
| 6–12 months | 32 (20.1) | | |
| 12–18 months | 40 (25.2) | | |
| 18–24 months | 28 (17.9) | | |
| >24 months | 35 (22.0) | | |

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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, unresctable, 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 (Bagattelli 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, unresctable, 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 unresctable, 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.

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**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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