Recurrence patterns across medulloblastoma subgroups: an integrated clinical and molecular analysis


Summary

Background Recurrence medulloblastoma is a therapeutic challenge because it is almost always fatal. Studies have confirmed that medulloblastoma consists of at least four distinct subgroups. We sought to delineate subgroup-specific differences in medulloblastoma recurrence patterns.

Methods We retrospectively identified a discovery cohort of all recurrent medulloblastomas at the Hospital for Sick Children, Toronto, Canada; from 1994 to 2012 (cohort 1), and established molecular subgroups using a nanoString-based assay on formalin-fixed paraffin-embedded tissues or frozen tissue. The anatomical site of recurrence (local tumour bed or leptomeningeal metastasis), time to recurrence, and survival after recurrence were assessed in a subgroup-specific manner. Two independent, non-overlapping cohorts (cohort 2: samples from patients with recurrent medulloblastomas from 13 centres worldwide, obtained between 1991 and 2012; cohort 3: samples from patients with recurrent medulloblastoma obtained at the NN Burdenko Neurosurgical Institute [Moscow, Russia] between 1994 and 2011) were analysed to confirm and validate observations. When possible, molecular subgrouping was done on tissue obtained from both the initial surgery and at recurrence.

Results Cohort 1 consisted of 30 patients with recurrent medulloblastomas; nine with local recurrences, and 21 with metastatic recurrences. Cohort 2 consisted of 77 patients and cohort 3 of 96 patients with recurrent medulloblastoma. Subgroup affiliation remained stable at recurrence in all 34 cases with available matched primary and recurrent pairs (five pairs from cohort 1 and 29 pairs from cohort 2 [15 SHH, five group 3, 14 group 4]). This finding was validated in 17 pairs from cohort 3. When analysed in a subgroup-specific manner, local recurrences in cohort 1 were more frequent in SHH tumours (eight of nine [89%]) and metastatic recurrences were more common in group 3 and group 4 tumours (17 of 20 [85%] with one WNT, p=0.0014, local vs metastatic recurrence, SHH vs group 3 vs group 4). The subgroup-specific location of recurrence was confirmed in cohort 2 (p=0.0013 for local vs metastatic recurrence, SHH vs group 3 vs group 4), and cohort 3 (p=0.0015). Treatment with craniospinal irradiation at diagnosis was not significantly associated with the anatomical pattern of recurrence. Survival after recurrence was significantly longer in patients with group 4 tumours in cohort 1 (p=0.013) than with other subgroups, which was confirmed in cohort 2 (p=0.0075), but not cohort 3 (p=0.70).

Interpretation Medulloblastoma does not change subgroup at the time of recurrence, reinforcing the stability of the four main medulloblastoma subgroups. Significant differences in the location and timing of recurrence across medulloblastoma subgroups have potential treatment ramifications. Specifically, intensified local (posterior fossa) therapy should be tested in the initial treatment of patients with SHH tumours. Refinement of therapy for patients with group 3 or group 4 tumours should focus on metastases.

Introduction Medulloblastoma is the most common malignant brain tumour of childhood.1 With multimodal therapy, consisting of surgery, craniospinal irradiation, and adjuvant chemotherapy, 5-year overall survival approaches 85% for average-risk disease and 70% for high-risk disease.2-4 However, recurrent medulloblastoma is a great challenge, because it is almost always fatal in previously irradiated patients despite a multitude of therapies including re-resection, re-irradiation, high-dose chemotherapy with autologous stem-cell support, and enrolment in clinical trials.5 Integrative genomic studies have shown that medulloblastoma consists of at least four subgroups (WNT, SHH, group 3, and group 4) that are clinically, transcriptionally, and genetically distinct.5-8 Of these four subgroups, patients with WNT subgroup tumours have an excellent prognosis whereas patients with group 3 tumours have the worst prognosis and more commonly present with disseminated disease at diagnosis.5,8 Although these integrative genomic studies
have shown that there are significant differences in survival between the four subgroups, little is known with respect to subgroup-specific anatomical and temporal characteristics of recurrence.

In glioblastoma, molecular subgroup affiliation can change at recurrence, partly because of intratumoural heterogeneity based on geographical location.\(^{19,20}\) Although medulloblastoma subgroups have been shown to arise from distinct cells of origin, the stability of subgroup affiliation at recurrence remains unknown.\(^{21–23}\) Moreover, the clinical behaviour of the individual subgroups at recurrence has yet to be established. As such, an understanding of subgroup-specific temporal and spatial details could help to develop treatment of recurrent medulloblastoma, because the next generation of subgroup-specific clinical trials will probably be based initially in the context of recurrent medulloblastoma.

We aimed to characterise the subgroup-specific clinical patterns of recurrence in medulloblastoma.

**Methods**

**Patients**

We assembled a discovery cohort (cohort 1) and, to account for unobserved variables and potential bias due to different subgrouping methods, two non-overlapping validation cohorts (cohorts 2 and 3). Cohort 1 consisted of all patients with medulloblastoma with either frozen or formalin-fixed paraffin-embedded (FFPE) material along with clinical variables and survival data, treated between 1994 and 2012, at the Hospital for Sick Children (Toronto, ON, Canada).

Cohort 2 consisted of samples from patients with recurrent medulloblastomas from 13 centres, obtained between 1991 and 2012 (Munich University Hospital, Munich, Germany; Hospital de Santa Maria in Lisbon, Lisbon, Portugal; Duke University Medical Center, Durham, NC, USA; Lucille Packard Children’s Hospital, Palo Alto, CA, USA; Children’s National Medical Center, Washington, DC, USA; British Columbia Children’s Hospital, Vancouver, BC, Canada; A I duPont Hospital for Children, Wilmington, DE, Canada; Brain Tumour Bank of Canada, London, ON, Canada; Hospital Sant Joan de Deu de Barcelona, Barcelona, Spain; McGill University Health Centre, Montreal, QC, Canada; Children’s Hospital Boston, Boston, MA, USA; New York University Langone Medical Center, New York City, NY, USA; and Cincinnati Children’s Hospital, Cincinnati, OH, USA).

Cohort 3 consisted of samples from patients with recurrent medulloblastoma obtained at the NN Burdenko Neurosurgical Institute (Moscow, Russia) between 1994 and 2011.

Worldwide, only a minority of patients with relapsed medulloblastoma undergo a second surgery at recurrence. Therefore, most patients do not have tissue available at recurrence. We obtained matched samples from diagnosis and recurrence whenever possible, and assembled cohort 1 with the goal of acquiring as many of these paired samples as possible.

The research ethics boards at all participating centres approved the study and all samples and clinical information were obtained with consent in accordance with the research ethics board at the Hospital for Sick Children and collaborating centres.

**Procedures**

We extracted RNA from fresh frozen tissue using the guanidinium thiocyanate-phenol-chloroform extraction method (TRIzol, Invitrogen, Carlsbad, USA), according to the manufacturer’s instructions. RNA from FFPE samples (five to seven paraffin sections per sample or the

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1 (n=30)</th>
<th>Cohort 2 (n=77)</th>
<th>Cohort 3 (n=96)</th>
<th>p value</th>
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<tr>
<td>Male</td>
<td>20 (67%)</td>
<td>39 (59%)*</td>
<td>61 (64%)</td>
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<tr>
<td>Female</td>
<td>10 (33%)</td>
<td>17 (30%)*</td>
<td>35 (36%)</td>
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<td>Age (years)</td>
<td></td>
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<tr>
<td>Median</td>
<td>5.4 (3.6–8.8)</td>
<td>7.0 (3.9–11.6)</td>
<td>7.0 (4.0–11.0)</td>
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<tr>
<td>&lt;3</td>
<td>6 (20%)</td>
<td>10 (13%)</td>
<td>12 (13%)</td>
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</tr>
<tr>
<td>3–16</td>
<td>24 (80%)</td>
<td>53 (69%)</td>
<td>81 (84%)</td>
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</tr>
<tr>
<td>&gt;16</td>
<td>0</td>
<td>14 (18%)</td>
<td>3 (3%)</td>
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<tr>
<td>Histology</td>
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</tr>
<tr>
<td>LCA</td>
<td>6 (20%)</td>
<td>18 (30%)†</td>
<td>26 (27%)</td>
<td>---</td>
</tr>
<tr>
<td>Classic</td>
<td>21 (70%)</td>
<td>31 (52%)‡</td>
<td>63 (66%)</td>
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<tr>
<td>Desmoplastic or MBEN</td>
<td>3 (10%)</td>
<td>11 (18%)†</td>
<td>7 (7%)</td>
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</tr>
<tr>
<td>Metastases at diagnosis</td>
<td>8 (27%)</td>
<td>19 (20%)‡</td>
<td>44 (46%)</td>
<td>0.11</td>
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<tr>
<td>Extent of resection</td>
<td></td>
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<td></td>
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<tr>
<td>Gross-total</td>
<td>21 (73%)§</td>
<td>60 (62%)</td>
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<td>---</td>
</tr>
<tr>
<td>Sub-total</td>
<td>7 (25%)§</td>
<td>36 (38%)</td>
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<tr>
<td>Treatment</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CSI with or without chemotherapy</td>
<td>22 (80%)§</td>
<td>50 (70%)</td>
<td>75 (78%)</td>
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<tr>
<td>Chemotherapy only</td>
<td>6 (20%)§</td>
<td>21 (30%)‡</td>
<td>21 (22%)</td>
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<tr>
<td>Pattern of recurrence</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Tumour bed</td>
<td>9 (30%)</td>
<td>26 (34%)</td>
<td>24 (25%)</td>
<td>---</td>
</tr>
<tr>
<td>Metastatic</td>
<td>18 (60%)</td>
<td>42 (54%)</td>
<td>49 (51%)</td>
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<td>3 (10%)</td>
<td>9 (12%)</td>
<td>24 (25%)</td>
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<tr>
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<td>WNT</td>
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<td>---</td>
</tr>
<tr>
<td>SHH</td>
<td>11 (37%)</td>
<td>30 (39%)</td>
<td>21 (22%)</td>
<td>---</td>
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<tr>
<td>Group 3</td>
<td>9 (30%)</td>
<td>22 (29%)</td>
<td>37 (39%)</td>
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</tr>
<tr>
<td>Group 4</td>
<td>9 (30%)</td>
<td>25 (32%)</td>
<td>36 (38%)</td>
<td>---</td>
</tr>
<tr>
<td>Time to recurrence (months)</td>
<td>18.3 (11.4–44.8)</td>
<td>19.9 (10.9–32.6)</td>
<td>12.0 (8.0–21.9)</td>
<td>0.0038</td>
</tr>
<tr>
<td>Overall follow-up time (months)</td>
<td>32.9 (13.7–72.3)</td>
<td>33.2 (17.0–57.8)</td>
<td>30.0 (18.0–64.7)</td>
<td>0.91</td>
</tr>
<tr>
<td>Survival</td>
<td></td>
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<td>0.38</td>
</tr>
<tr>
<td>Alive</td>
<td>6 (20%)</td>
<td>24 (33%)</td>
<td>32 (33%)</td>
<td>---</td>
</tr>
<tr>
<td>Dead</td>
<td>24 (80%)</td>
<td>49 (67%)**</td>
<td>64 (67%)</td>
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</table>

Data are n (%) or median (IQR). Some percentages do not total 100 because of rounding. +values are from Fisher’s exact test for categorical variables, and Kruskal-Wallis test for continuous variables. LCA-large cell/anaplastic histology. MBEN-medulloblastoma with extensive nodularity. CSI-craniospinal irradiation. *Sex unavailable for 21 cases. †Histology unavailable for 17 cases in validation cohort 1. ‡Metastases at diagnosis unavailable in 18 cases. §Data missing for two patients. ¶Two patients in validation cohort 1 received radiation therapy to the posterior fossa only followed by chemotherapy. ||Data missing for six patients. **Data missing for four patients.

Table 1: Demographics of the three independent cohorts of recurrent medulloblastoma
Role of the funding source

The funding sources 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 all the data in the study and had final responsibility for the decision to submit for publication.

Results

Cohort 1 consisted of 131 patients, of whom 30 had recurrent medulloblastoma. Cohort 2 consisted of 77 patients and cohort 3 of 96 patients, all of whom had recurrent medulloblastoma. At first diagnosis, the WNT subgroup was rare, occurring in one (3%) of 30 patients in cohort 1, no patients in cohort 2, and two (2%) of 96 patients in cohort 3. Specimens from the remaining patients were distributed roughly equally between SHH, group 3, and group 4 in all cohorts (table 1). Of the 96 cases in cohort 3 subgrouped by immunohistochemistry, none were immunoreactive for more than one marker, and 39 were also subgrouped using whole genomic expression profiling or nanoString without any reclassifications of subgroup.

Table 1 shows the demographics of all three cohorts. The adult age group (>16 years) was over-represented in cohort 2, but median age did not differ between groups. Metastatic dissemination at diagnosis was higher in cohort 3 than in either of the other cohorts, although the difference was not significant. Median time to recurrence was significantly shorter in cohort 3 than in the other cohorts (table 2). Histological classification did not differ across the three cohorts. When available, treatment at diagnosis for all three cohorts are summarised in table 1 and the appendix (pp 8–9). Adjuvant chemotherapy regimens were all cisplatin-based across the three cohorts. Treatment in cohort 3 was uniform as per the German HIT protocols, as previously described.26

To establish whether subgroup affiliation remains stable at recurrence, 34 paired samples (five pairs from cohort 1, and 29 pairs from various centres as part of cohort 2) with FFPE tissue available from the initial surgery and recurrence were analysed by nanoString. At recurrence, tissue was obtained from the primary site in 16 tumours; 18 samples were from biopsies of leptomeningeal metastases. In all 34 paired samples, subgroup affiliation remained stable between diagnosis and the corresponding local or metastatic recurrence (15 SHH, five group 3, 14 group 4; figure 1; appendix p 10). One local recurrence was subgrouped as a group 4 at diagnosis and SHH at recurrence; however, upon review of the histological specimen by a senior neuropathologist (AK), the recurrence was established to be a radiation-induced secondary glioblastoma and this patient was therefore excluded from all other analyses (appendix p 12). To further confirm the finding that subgroup affiliation does not change, an orthogonal technique of subgroup determination using immunohistochemistry was done on 17 paired samples from the initial surgery and recurrence as part of cohort 3, using a four antibody method as previously described.27
In all 17 paired samples, the initial pattern of immunoreactivity remained stable at recurrence, with one WNT, six SHH, four group 3, and six group 4 patients, demonstrating the same subgroup affiliation at recurrence as at diagnosis (appendix p 11). Therefore, we conclude that medulloblastoma does not change subgroup at the time of recurrence.

Median time to recurrence for cohort 1 was 1·49 years (95% CI 1·09–1·90; table 2). When time to recurrence in the cohort 1 was analysed in a subgroup-specific manner, group 4 tumours recurred significantly later than both group 3 and SHH tumours (p=0·0080, generalised Wilcoxon; appendix p 13). Of the 30 recurrences in the cohort 1 was analysed in a subgroup-specific manner, patients with group 4 tumours had a significantly longer overall survival after recurrence than those with group 3 and SHH tumours (figure 2A; p=0·013, log-rank; table 2).

The two findings that patients with group 4 tumours recur later and have longer overall survival after recurrence than group 3 and SHH tumours were confirmed in cohort 2 but not in cohort 3 (figure 2B, 2C; table 2). For all subgroups, time to recurrence was significantly shorter for cohort 3 than for the cohort 1 or cohort 2 (table 2), suggesting that differences in therapy might account for this discrepancy; however, treatment regimens were much the same across cohorts (table 1). Therefore time to recurrence and overall survival after recurrence might still differ significantly between subgroups.

Survival data were available in 73 of 77 cases in cohort 2. Four patients were alive 5 years after recurrence: one
patient each with an SHH tumour and group 3 tumour and two patients with a group 4 tumour. Two patients in cohort 2, both with group 4 tumours, died of disease at 6·2 years and 7·4 years after the initial recurrence. Survival data were available for all 96 cases in cohort 3. Long-term survivors after recurrence were more common in this cohort, with 17 patients surviving more than 5 years after recurrence, of whom 15 received radiation at initial therapy. The subgroup distribution of these 17 cases was one WNT, four SHH, six group 3, and six group 4. Two recurrent WNT cases were also identified in cohort 3; one was a long-term survivor of 5·4 years after recurrence (time to recurrence 3·2 years), whereas the other died 0·67 years after recurrence (time to recurrence 1·25 years). When treatment information was available, we did not identify any consistent differences in treatment regimens in these long-term survivors compared with the remainder of the cohort.

To establish whether the location of recurrence differs between subgroups, we reviewed the location of first recurrence in cohort 1. The pattern of recurrence was available in all 30 recurrent cases. All patients were followed up with serial MRI of the craniospinal axis after completion of therapy as per treatment protocol. Recurrences were divided into local recurrences (tumour bed only without involvement of the cerebellar leptomeninges and when CSF examinations were available, no malignant cells in the CSF), metastatic recurrences (relapse at distant sites outside the tumour bed), and metastatic plus local recurrences (relapse at both distant sites and the tumour bed). We identified nine local recurrences and 21 metastatic recurrences. When we reanalysed these data in a subgroup-specific manner, eight of nine local recurrences were SHH tumours, and 17 of 20 metastatic recurrences were either group 3 or group 4, with one WNT metastatic recurrence (p=0·0014 Fisher’s exact test; local vs metastatic recurrence, SHH vs group 3 vs group 4; table 3, appendix p 15).

We also identified this pattern in the two independent, non-overlapping validation cohorts, and the results were significant (cohort 2 p=0·0013, cohort 3 p=0·0001, Fisher’s exact test; table 3, appendix p 15). We identified no significant difference in the pattern of relapse between group 3 and group 4 (appendix p 15).

In an exploratory analysis combining all three cohorts, incidence of local recurrences tended to be higher in group 4 in non-irradiated patients, suggesting that non-irradiated group 4 patients might recur locally (p=0·031; table 4, appendix p 16). When comparing the location of recurrence of patients given chemotherapy only versus craniospinal irradiation (with or without adjuvant chemotherapy), we noted no difference between SHH and group 3 in any of the three cohorts, and we noted no difference in these two treatment regimens when combining all three cohorts (SHH p=0·83, group 3 p=0·34; appendix p 16). CSF examinations in five of nine local recurrences in cohort 1 and in all local recurrences

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**Figure 2:** Subgroup-specific survival after recurrence across three non-overlapping cohorts of recurrent medulloblastoma

Kaplan-Meier survival estimates of overall survival after recurrence for (A) cohort 1, (B) cohort 2, and (C) cohort 3. p values were calculated using the log-rank test across the three subgroups.
in cohort 3 showed no metastatic dissemination. In cohort 2, CSF examinations were not available for all patients; however, in one local group 4 recurrence a CSF examination showed distant metastases (M1 disease). Two extraneural metastases occurred in cohort 2. The first case was an SHH tumour in an adult patient who received radiation and chemotherapy and the second occurred in a child given radiation only. Three WNT tumours recurred across all three cohorts: two recurred with distant metastases only and one recurred in the tumour bed only (appendix p 8). No significant differences were noted in either time to recurrence, or overall survival when comparing by location of recurrence in any of the three subgroups. SHH tumours recurred with metastases in 29% (18 of 62) of cases across all three cohorts. To work out whether age was a factor in disseminated relapse in SHH tumours we established the presence of metastatic recurrences by age across all three cohorts and noted no significant difference (appendix p 17).

Metastatic dissemination at diagnosis was associated with 21% (11 of 52) of SHH tumours across all three cohorts, and was not statistically significant when comparing local versus metastatic recurrences (p=0·25, appendix p 8). 39% (23 of 59) of group 3 patients and 58% (33 of 57) of group 4 patients with metastatic dissemination at relapse did not have distant metastases (ie, had M0 disease) at diagnosis (appendix p 8). We therefore conclude that SHH tumours more frequently have isolated tumour bed recurrences, and that group 3 and group 4 tumours usually recur with metastases.

Discussion
Our results show that medulloblastoma does not change subgroup at recurrence. This finding was not dependent on location of recurrence, because subgroup affiliation remained stable in both local tumour bed samples and metastatic samples at recurrence. We also identified significant differences across subgroups with respect to the anatomical and temporal patterns of recurrence, specifically SHH tumours recur mostly in the local tumour bed and group 3 and group 4 tumours recur almost exclusively with metastases. Patients with group 4 tumours also have an increased time to death after recurrence. These findings have important implications in the care of patients with recurrent medulloblastoma and provide insight into the planning of future clinical trials. As far as we are aware, this study represents the largest cohort of recurrent medulloblastoma assembled so far (appendix p 3) and the largest cohort of recurrent medulloblastoma with molecular correlation (panel).

Retention of subgroup provides further evidence supporting the notion that medulloblastoma arises from distinct cells of origin within the posterior fossa, the characteristics of which are carried forward from ontogeny into oncology. Indeed, this finding is in agreement with murine models in which WNT, SHH, and group 3 tumours have unique cells of origin, specifically the lower rhombic lip, the external granule layer, and postnatal cerebellar progenitor cells, respectively. Our findings suggest that group 4 tumours also arise from a yet to be identified cell of origin, distinct from the other three subgroups.20–23 Our findings contrast with some initial findings in glioblastoma that suggest that subgroup affiliation can change at recurrence.20–23 As in breast cancer and renal cell carcinoma, metastases from medulloblastoma have been shown to be highly genetically divergent from their matched primary tumour.6,21,22 Genetic divergence of medulloblastoma metastases from their primary tumour in the face of subgroup stability across both tumour types suggests that subgroup identity might be established in the cell of origin. Further investigation of paired samples from diagnosis and matched metastatic recurrences using next generation methods, such as RNA sequencing and

<table>
<thead>
<tr>
<th></th>
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<th>Metastatic</th>
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<tbody>
<tr>
<td><strong>Cohort 1 (p=0·0014)</strong></td>
<td></td>
<td></td>
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<tr>
<td>SHH (n=13)</td>
<td>8 (73%)</td>
<td>1 (9%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>Group 3 (n=9)</td>
<td>1 (11%)</td>
<td>1 (11%)</td>
<td>7 (78%)</td>
</tr>
<tr>
<td>Group 4 (n=9)</td>
<td>0</td>
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<td>8 (89%)</td>
</tr>
<tr>
<td><strong>Cohort 2 (p=0·0013)</strong></td>
<td></td>
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<tr>
<td>SHH (n=30)</td>
<td>18 (60%)</td>
<td>2 (7%)</td>
<td>10 (33%)</td>
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<td>Group 3 (n=22)</td>
<td>4 (18%)</td>
<td>5 (23%)</td>
<td>13 (59%)</td>
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<td>Group 4 (n=25)</td>
<td>4 (16%)</td>
<td>19 (76%)</td>
<td>2 (8%)</td>
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<td><strong>Cohort 3 (p&lt;0·0001)</strong></td>
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<td>SHH (n=21)</td>
<td>18 (86%)</td>
<td>2 (10%)</td>
<td>1 (5%)</td>
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<td>Group 3 (n=27)</td>
<td>1 (3%)</td>
<td>10 (27%)</td>
<td>26 (70%)</td>
</tr>
<tr>
<td>Group 4 (n=36)</td>
<td>3 (8%)</td>
<td>11 (31%)</td>
<td>22 (61%)</td>
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p values from Fisher’s exact test; comparison of local versus mixed versus metastatic pattern of recurrence. Two WNT recurrences in the discovery cohort and two WNT recurrences in validation cohort 2 are described in the Results.
recurrence. Our data for metastatic recurrence, in combination with previous reports affilia
tion, rather than treatment effect, seems to be the main driver of location of
independent cohorts, and was not dependent on therapy at diagnosis. As such, subgroup
showed that this subgroup-specific pattern of relapse was highly consistent across three
additional therapies aimed at the posterior fossa might be most benefi cial. Importantly, we
subgroup specifi c. SHH medulloblastomas almost always recur locally, suggesting that
As far as we are aware, our study is the largest single study of recurrent medulloblastoma
the anatomical pattern of recurrence in medulloblastoma, specifi cally
that recurrences can occur either in the tumour bed, along the leptomeninges, or both;
however, all these previous studies are limited by both small numbers and absence of
biological correlation. Several integrated genomic studies have identifi ed four subgroups of
medulloblastoma with distinct demographics, genetics, transcriptomes, and outcomes;
however, little is known with regards to the clinical implications of these subgroups at the
time of disease recurrence.

Interpretation
As far as we are aware, our study is the largest single study of recurrent medulloblastoma
and the fi rst to assess recurrent medulloblastoma in a subgroup-specifi c manner. Many
researchers in paediatric neuro-oncology have suggested that medulloblastomas might
recur as more aggressive subgroups (ie, an SHH or group 4 tumour becoming a
group 3 tumour). In this study we showed that at the time of recurrence, medulloblastomas
maintain their subgroup affi liation and that the anatomical pattern of recurrence is highly
subgroup specifi c. SHH medulloblastomas almost always recur locally, suggesting that
additional therapies aimed at the posterior fossa might be most benefi cial. Importantly, we
showed that almost all recurrences of group 3 and group 4 medulloblastoma are metastatic,
with recurrence in the posterior fossa in radiated patients being very rare. Moreover we
showed that this subgroup-specifi c pattern of relapse was highly consistent across three
independent cohorts, and was not dependent on therapy at diagnosis. As such, subgroup
affiliation, rather than treatment effect, seems to be the main driver of location of
recurrence. Our data for metastatic recurrence, in combination with previous reports
showing that metastases are clinically and genetically distinct from the primary tumour, suggest that the paediatric neuro-oncology community needs to radically shift the focus
away from the primary tumour towards metastases because patients with group 3 or
group 4 tumours are dying almost exclusively from metastatic disease.

Panel: Research in context
Systematic review
We searched PubMed and Google Scholar with the terms “medulloblastoma”, “recurrent
medulloblastoma”, “medulloblastoma recurrences”, and “medulloblastoma treatment”, for
reports in English, without any date restrictions. We identifi ed several studies over the past
30 years reporting the anatomical pattern of recurrence in medulloblastoma, specifi cally
that recurrences can occur either in the tumour bed, along the leptomeninges, or both;
however, all these previous studies are limited by both small numbers and absence of
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showed that this subgroup-specifi c pattern of relapse was highly consistent across three
independent cohorts, and was not dependent on therapy at diagnosis. As such, subgroup
affiliation, rather than treatment effect, seems to be the main driver of location of
recurrence. Our data for metastatic recurrence, in combination with previous reports
showing that metastases are clinically and genetically distinct from the primary tumour, suggest that the paediatric neuro-oncology community needs to radically shift the focus
away from the primary tumour towards metastases because patients with group 3 or
group 4 tumours are dying almost exclusively from metastatic disease.

whole genome sequencing, will be needed to establish the full spectrum of heterogeneity between primary and metastatic tumours.

Previous research has suggested that local recurrences are more common in younger children, and metastatic recurrences are more common in older children. Protocols for treatment of infants consisting of chemotherapy only have been associated with a higher propensity for local recurrence than those including irradiation. However, our data suggest a simple, proximate explanation: this outcome is probably driven by subgroup, because SHH tumours are more common in infants and group 4 tumours are more common in older children.

Overall, we noted no association between treatment regimen and site of recurrence, with the exception of a possible association between chemotherapy-only approaches and local recurrences in group 4 tumours. This finding suggests that in younger children who are not irradiated, local group 4 recurrences should not be unexpected; however, this conclusion is limited by a small sample size of non-irradiated patients with group 4 tumours (n=8) and warrants further investigation in a prospective trial. Treatment was uniform across cohorts and subgroups, with infants younger than 3 years given chemotherapy-only approaches and children older than 3 years given radiation followed by cisplatin-based adjuvant chemotherapy. Taken together, our data suggest that subgroup affi liation, rather than treatment effect, seems to be the primary driver of location of recurrence, particularly in patients with group 3 or SHH tumours.

A limitation of our study is the scarcity of knowledge with respect to assessments of distant metastases at relapse and absence of detailed treatment information at relapse. Future prospective studies will need to rigorously show that the CSF is free of metastatic disease in local recurrences, specifi cally in the rare situations in which local recurrences occur in group 3 and group 4 tumours, to exclude metastatic dissemination. Prospective multicentre longitudinal studies of recurrent medulloblastoma in a subgroup-specifi c manner are needed to establish whether present or future salvage therapies confer any benefi t. Our finding that the pattern of recurrence is highly subgroup specifi c needs prospective validation in a multicentre cooperative study of homogeneously treated patients.

Therefore, the worrying fi nding that most group 3 and group 4 recurrences are metastatic, in combination with the fact that metastases are genetically divergent from the primary tumour, and that medulloblastoma metastases are understudied, suggests that future basic science and clinical trials of group 3 and group 4 tumours should be more highly focused on metastases. Most patients with group 3 and group 4 tumours of average risk in our cohort were given high-dose craniospinal irradiation, further reinforcing the importance of generating novel approaches to therapy in metastatic disease. Moreover, our fi nding that most group 3 and group 4 metastatic relapses do not relapse in the primary site suggests that microscopic leptomeningeal metastases not visible by neuroimaging or CSF examination are resistant to existing therapy. As such, additional local therapies targeting the primary site in the posterior fossa are unlikely to increase the proportion of patients cured for patients with group 3 or group 4 medulloblastoma. Possible strategies aimed at the metastases include intrathecal consolidation regimens in addition to existing therapies, which achieve excellent local tumour bed control.

Currently clinical trials for previously irradiated relapsed metastatic medulloblastoma are very heterogeneous and
many are at phase 2 stage. Since any future subgroup-specific clinical trial will probably begin with relapsed patients, trials for relapsed group 3 or group 4 medulloblastoma are poised to fail if they are based on the biology of the primary tumour. Because SHH tumours predominantly recur in the posterior fossa (to treat local recurrence) while simultaneously reducing craniospinal doses (because leptomeningeal failure is rare) should be considered. Trials that are being planned will help to elucidate the effects of inhibitors of SMO on local tumour control for patients with SHH tumours.

Contributors
VR, MR, EB, SMP, AK, and MDT designed the study; MDT procured financial support. VR, MR, BL, CCF, SP, Y-JC, US, SG, RM, DB, MF, KLI, SLP, SD, JT, N, AF, DTWJ, MK, MAK, SLG, DZ, SN, JP, JM, EL, AWW, MR, OZ, EK, JA, SEC, JTDR, CH, UT, K-ETC, RJP, and AK collected data and provided study materials. VR, MR, EB, DJS, AMD, PAN, SMP, AK, and MD analysed and interpreted the data. VR, MR, EB, SMP, and MDT wrote the report. All authors approved the final report.

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MDT wrote the report. All authors approved the final report.

We declare that we have no conflicts of interest.

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We declare that we have no conflicts of interest.

References