Molecular subgroups of atypical teratoid rhabdoid tumours in children: an integrated genomic and clinicopathological analysis

Jonathon Torchia, Daniel Picard*, Lucie Lafay-Cousin, Cynthia E Hawkins, Seung-Ki Kim, Louis Letourneau, Young-Shin Ra, King Ching Ho, Tiffany Sin Yu Chan, Patrick Sin-Chan, Christopher P Dunham, Stephen Yip, Ho-keung Ng, Jian-Qiang Lu, Steffen Albrecht, José Pimentel, Jennifer A Chan, Gino R Somers, Maria Zielenska, Claudia C Faria, Lucia Roque, Berivan Baskin, Diane Birks, Nick Foreman, Douglas Strother, Almos Klekner, Miklos Garami, Peter Hauser, Tibor Hortobágyi, Laszló Bognár, Beverly Wilson, Juliette Hukin, Anne-Sophie Carret, Timothy E Van Meter, Hideo Nakamura, Helen Toledano, Iris Fried, Daniel Fults, Takafumi Wataya, Chris Fryer, David D Eisenstat, Katrin Scheineman, Donna Johnston, Jean Michaud, Shayna Zelcer, Robert Hammond, David A Ramsay, Adam J Fleming, Rishi R Lulla, Jason R Fangusaro, Nongnuch Sirachainan, Noppadol Larbcharoensub, Suradej Hongeng, Muhammad Abrar Barakzai, Alexandre Montpetit, Derek Stephens, Richard G Grundy, Ulrich Schüller, Theodore Nicolaides, Tarik Tihan, Joanna Phillips, Michael D Taylor, James T Rutka, Peter Dirks, Gary D Bader, Monika Warmuth-Metz, Stefan Rutkowski, Torsten Pietsch, Alexander R Judkins, Nada Jabado†, Eric Bouffet†, Annie Huang†*

Summary

Background **Rhabdoid brain tumours, also called atypical teratoid rhabdoid tumours, are lethal childhood cancers with characteristic genetic alterations of *SMARCB1/hSNF5*. Lack of biological understanding of the substantial clinical heterogeneity of these tumours restricts therapeutic advances. We integrated genomic and clinicopathological analyses of a cohort of patients with atypical teratoid rhabdoid tumours to find out the molecular basis for clinical heterogeneity in these tumours.**

Methods **We obtained 259 rhabdoid tumours from 37 international institutions and assessed transcriptional profiles in 43 primary tumours and copy number profiles in 38 primary tumours to discover molecular subgroups of atypical teratoid rhabdoid tumours. We used gene and pathway enrichment analyses to discover group-specific molecular markers and did immunohistochemical analyses on 125 primary tumours to evaluate clinicopathological significance of molecular subgroup and ASCL1-NOTCH signalling.**

Findings **Transcriptional analyses identified two atypical teratoid rhabdoid tumour subgroups with differential enrichment of genetic pathways, and distinct clinicopathological and survival features. Expression of ASCL1, a regulator of NOTCH signalling, correlated with supratentorial location (p=0·004) and superior 5-year overall survival (35%, 95% CI 13–57, and 20%, 6–34, for ASCL1-positive and ASCL1-negative tumours, respectively; p=0·033) in 70 patients who received multimodal treatment. ASCL1 expression also correlated with superior 5-year overall survival (34%, 7–61, and 9%, 0–21, for ASCL1-positive and ASCL1-negative tumours, respectively; p=0·001) in 39 patients who received only chemotherapy without radiation. Cox hazard ratios for overall survival in patients with differential ASCL1 enrichment treated with chemotherapy with or without radiation were 2·02 (95% CI 1·04–3·85; p=0·038) and 3·98 (1·71–9·26; p=0·001). Integrated analyses of molecular subgroupings with clinical prognostic factors showed three distinct clinical risk groups of tumours with different therapeutic outcomes.**

Interpretation **An integration of clinical risk factors and tumour molecular groups can be used to identify patients who are likely to have improved long-term radiation-free survival and might help therapeutic stratification of patients with atypical teratoid rhabdoid tumours.**

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Introduction

CNS rhabdoid tumours, also called atypical teratoid rhabdoid tumours, are highly malignant neoplasms arising in very young children, with a median age of 18–22 months at diagnosis, and, until recently, were thought to be fatal.[1] Although the overall prognosis of patients with these tumours remains poor, with most patients living for less than 1 year from diagnosis, recent application of intensified multimodal therapy with whole craniospinal irradiation[2–4] or high-dose chemotherapy with stem-cell rescue[1,3,5–7] has improved survival. However, treatment intensification, particularly use of neuroaxis radiation, is associated with substantial acute and life-long physiological and neurological sequelae in these patients. Remarkably, long-term survival has been reported in some children with atypical teratoid rhabdoid tumours treated without neuroaxis radiation.[5,6,8] These findings draw attention to the substantial clinical heterogeneity of atypical teratoid rhabdoid tumours and the need to avoid radiation and its associated

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*Co-contributing authors

†Co-senior authors

Division of Hematology-Oncology (J Torchia MSc, D Picard MSc, T Sin Yu Chan PhD, P Sin-Chan MSc, Prof E Bouffet MD, A Huang MD, K C Ho MSc), **Arthur and Sonia Labatt Brain Tumour Research Centre, Hospital for Sick Children** (J Torchia, D Picard, T Sin Yu Chan, P Sin-Chan, A Huang, C E Hawkins PhD, K C Ho, M D Taylor PhD, J T Rutka MD, P Dirks PhD), **Department of Laboratory Medicine and Pathobiology** (J Torchia, D Picard, T Sin Yu Chan, P Sin-Chan, A Huang, C E Hawkins, G R Somers MBBS, M Zielenska PhD, M D Taylor, J T Rutka, P Dirks), **and Department of Pediatrics, University of Toronto, Toronto, ON, Canada** (J Torchia, D Picard, T Sin Yu Chan, P Sin-Chan, Prof E Bouffet, A Huang); **Alberta Children's Hospital, and Departments of Oncology and Pediatrics, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada** (L Lafay-Cousin MD, D Strother MD); **Department of Pathology, Hospital for Sick Children, Toronto, ON, Canada** (C E Hawkins); **Department of Pediatrics** (N Jabado PhD),

Department of Human Genetics (N Jabado), **and Genome Quebec Innovation Centre** (L Letourneau BSc, A Montpetit PhD), **McGill University, Montreal, QC, Canada; Department of Neurosurgery, Seoul National University Children's Hospital, Seoul, South Korea** (S-K Kim MD); **Division of Neurosurgery, Hospital for Sick Children, Toronto, ON, Canada** (M D Taylor, J T Rutka, P Dirks); **Department of Computer Science, Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, ON, Canada** (G D Bader PhD); **Department of Neurosurgery, Asan Medical Center, Songpa-gu, Seoul, South Korea** (Prof Y-S Ra MD); **Division of Anatomic Pathology, Children's and Women's Health Centre of British Columbia, Vancouver, BC, Canada** (C P Dunham MD); **Department of Neuropathology, Vancouver General Hospital, Vancouver, BC, Canada** (S Yip FRCPC); **Department of Anatomical and Cellular Pathology, Chinese University of Hong Kong, Hong Kong, China** (H-k Ng MD); **Department of Laboratory Medicine and Pathology, University of Alberta Hospital, Edmonton, AB, Canada** (J-Q Lu FRCPC); **Department of Pathology, Montreal Children's Hospital, McGill University Health Center Research Institute, Montreal, QC, Canada** (S Albrecht MD); **Department of Neurology, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Lisbon, Portugal** (J Pimentel PhD); **Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal** (C C Faria PhD); **Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, AB, Canada** (J A Chan MD); **Department of Paediatric Laboratory Medicine, Hospital for Sick Children, Toronto, ON, Canada** (G R Somers, M Zielenska); **Cytogenetic Laboratory, Centro de Investigação em Patobiologia Molecular, Portuguese Cancer Institute, Lisbon, Portugal** (L Roque PhD); **Department of Immunology, Genetics and Pathology, Uppsala University Hospital, Uppsala, Sweden**

neurotoxicity, without compromising survival. The biological basis for clinical heterogeneity in atypical teratoid rhabdoid tumours remains to be elucidated; this gap in knowledge has impeded development of therapeutic models and prospective treatment models.

Atypical teratoid rhabdoid tumours have histological features and hallmark alterations associated with the *SMARCB1* (*INI1/hSNF5/BAF47*) tumour suppressor locus on chromosome 22q11.23 that are also detected in rhabdoid tumours arising in other locations.[9,10] Up to 35% of patients with the CNS rhabdoid tumours have germline *SMARCB1* alterations and the rhabdoid predisposition syndrome characterised by development of several rhabdoid tumours.[11–13] The biological relation between atypical teratoid rhabdoid tumours and non-CNS rhabdoid tumours remains unclear; however, atypical teratoid rhabdoid tumours are thought to develop from *SMARCB1* loss in restricted, undefined, neural precursors. *SMARCB1* is a constitutive component of the ubiquitous SWI/SNF chromatin remodelling complex that has specific functions in neural development.[14] Results of animal studies show that loss of *Snf* is sufficient for the development of rhabdoid tumours, but intrinsic rhabdoid brain tumours have not been shown in *Snf5*–/– mice.[15–17] Although the findings of studies of small cohorts suggest molecular heterogeneity might underlie the clinical range of atypical teratoid rhabdoid tumours, cumulative genomic analyses including whole-exome sequencing studies have shown *SMARCB1* loss as the only recurrent genetic event in atypical teratoid rhabdoid tumours.[18,19] Reconciling clinical heterogeneity with tumour biology has been challenging for atypical teratoid rhabdoid tumours because it is a rare disease and there are only a few biological and clinical studies, thus determinants of survival and therapeutic responses remain poorly defined for patients. Furthermore, previous studies have been done on small, mixed cohorts of CNS and non-CNS rhabdoid tumours, and perhaps have lacked the power needed to define the genetic and molecular ranges of atypical teratoid rhabdoid tumours. In this study, we did integrated molecular and clinicopathological analyses of atypical teratoid rhabdoid tumours to define clinically relevant molecular classes of these tumours.

Methods

Patients and study design

This study was a retrospective analysis, with patients previously enrolled from 37 international institutions (appendix). The only inclusion criteria were a diagnosis of atypical teratoid rhabdoid tumours and centralised pathological review to confirm diagnosis. Tumour samples and clinical information were obtained with consent as per protocols approved by the hospital research ethics boards at participating institutions; all samples were reassessed centrally through a multistep histopathological and molecular evaluation to confirm the diagnosis of atypical teratoid rhabdoid tumours. All samples were retested to confirm absence of SMARCB1 protein expression with BAF47 immunostaining. BAF47-negative tumours were then further tested for genetic alterations of *SMARCB1* by use of multiplex ligation-dependent probe amplification (MLPA) and targeted sequencing of all nine exons of the *SMARCB1* locus with the Sanger method. Samples with confirmed negative BAF47/SMARCB1 protein immunostaining or *SMARCB1* biallelic genetic alterations or both (appendix), were reviewed for age, primary tumour occurrence, tumour location, and histopathological diagnostic features.[20] Germline *SMARCB1* information was not included in the current approval by the research ethics board and thus not reported in this study. For BAF47 immunohistochemistry, tumour tissue from a related embryonal brain tumour—primitive neural ectodermal tumour—was used as a positive control (appendix). Clinical information for a subset of patients was reported previously;[5,6,8,21] 143 (55%) of 259 patients in this study were new cases of atypical teratoid rhabdoid tumours. For clinicopathological and prognostic correlations, only cases with complete clinical and treatment information (appendix) were included. Four cases with concurrent diagnosis of atypical teratoid rhabdoid and non-CNS-rhabdoid tumours were excluded from prognostic analyses because they received individualised treatment. Ten cases in the discovery cohort were also subgrouped by ASCL1 immunohistochemistry and included in prognostic analyses.

Procedures

We did global genomic and transcriptional analyses only on samples from patients with confirmed diagnosis of atypical teratoid rhabdoid tumours, and who had been treated with upfront chemotherapy or combined chemotherapy-radiation regimens with curative intent. All patients who were treated would have received upfront surgery, and, if they were radiated, radiation was after upfront surgery or after post-operative chemotherapy. RNA or DNA, or both, were extracted from snap frozen samples of 49 primary atypical teratoid rhabdoid tumours with standard methods, and examined for gene expression (43 tumours; HT-12, version 4, Expression BeadChip Kit, Illumina, San Diego, CA, USA) and high resolution copy number (38 tumours, OmniQuad 2.5 SNP, Illumina microarrays); 32 samples were analysed by use of both gene expression and copy number. DNA and RNA hybridisations were done at the Centre of Applied Genomics Facility, Hospital for Sick Children, Toronto, ON, Canada, in accordance with the manufacturer's protocol. For gene expression, probes were collapsed into genes by taking the average value, quantile normalised with the Lumi R package (version 2.11), and batch corrected with ComBat (version 3.12.0).[22] Details of molecular analyses done on individual samples are shown in the appendix; all data are deposited in the UK Wellcome Trust, European Genome-Phenome Archive (accession number: EGAS00001000506).

Gene expression profiles were used to define molecular subgroups of tumours. Single nucleotide polymorphism array data were used to confirm and map the distinct chromosome 22q11.23 alterations associated with atypical teratoid rhabdoid tumours and to assess tumour purity and ploidy using allele-specific copy number analysis of tumours (appendix). We used two independent unsupervised clustering methods—hierarchical clustering and non-negative matrix factorisation analyses—on gene expression data to define molecular subgroups of atypical teratoid rhabdoid tumours and then applied a supervised *t* test to define genes and pathways that were most highly enriched in tumour subgroups. We confirmed the most highly differentially expressed genes by use of quantitative RT-PCR and then tested the suitability of a panel of candidate subgroup-specific markers for immunohistochemical analyses. For validation studies, molecular subgroups were confirmed with immunohistochemistry on formalin-fixed paraffin embedded material on tissue slides or microarrays.

For validation of array data, cDNAs were synthesised from 1 µg of tumour RNA (cDNA Reverse Transcription Kit, Life Technologies, Grand Island, NY, USA) with quantitative RT-PCR (qRT-PCR) using TaqMan and the TaqMan Gene Expression Master Mix (both Life Technologies). The gene probes and primers used for qRT-PCR are listed in the appendix. All assays were done in triplicate and the comparative cycle time method was used to calculate mRNA expression relative to mRNA actin.

Immunohistochemical analyses were done on two sets of tissue microarrays. A tissue microarray containing 55 atypical teratoid rhabdoid tumours obtained through the Canadian Paediatric Brain Consortium/C17 Research Network was constructed for this study. Previously, we assessed a second set of microarrays containing 33 atypical teratoid rhabdoid tumours and 14 non-CNS-rhabdoid tumours.[23] Immunohistochemical analyses were done on single slides for 100 atypical teratoid rhabdoid tumours and 13 non-CNS-rhabdoid tumours. For immunohistochemical analyses, all tissue sections were treated with heat-induced epitope retrieval and blocked for endogenous peroxidase and biotin. ASCL1 antibody (BD Biosciences, San Jose, CA, USA) and pSMAD1/5/8 (Cell Signaling, San Jose, CA, USA) reactions were visualised with a Biogenix detection kit (BioGenex Laboratories, San Ramon, CA, USA). Immunoreactivity for ASCL1 was scored manually on the basis of intensity (0=none, 1=low, 2=moderate, and 3=high) and distribution of stains (0=none, 1≤10%, 2=10–50%, and 3≥50%). A combined score of 4 or greater was regarded as positive. A score of less than 4 or absence of nuclear staining was regarded as negative for ASCL1. For tumours on microarray, we established immunoreactivity based on mean staining score in at least two tissue cores, whereas formalin-fixed paraffin embedded tumours were scored on the basis of the extent of staining in relation to the entire tumour section. For ASCL1, normal human lung and placenta tissues were used as positive and negative controls, respectively. For pSMAD1/5/8, normal human stomach and placenta, respectively, served as positive and negative controls. Samples processed in parallel without primary antibodies were also used as negative controls. All immunohistochemistry stains were scored by DP and KCH, who were masked to cancer status, and reviewed by AH and CEH.

Statistical analysis

We applied two orthogonal unsupervised consensus cluster methods to define the number of molecular subgroups of atypical teratoid rhabdoid tumours using gene expression data. The gene expression data were analysed by use of parallel unsupervised hierarchical clustering (Partek Genomics Suite, version 6.6) and non-negative matrix factorisation consensus cluster analyses. Genes were ranked on the basis of the coefficient of variation and we reiterated analyses using 200–2000 genes to show the optimal number of molecular classes over a range of 2–10 k classes with the highest cophenetic coefficient and optimal k-means class assignment. Hierarchical clustering analyses were also done with the same gene sets and concordance with non-negative matrix factorisation analyses was assessed with the Jaccard similarity coefficient. SigClust (version 1.1.0) was used to compute the significance of the clusters identified.[24] Subgroup-specific genes were identified with a supervised *t* test adjusted for multiple hypothesis testing using the false discovery rate method. To define regions of copy number alterations, we did partitioning-segmentation analyses on inferred copy number data with the Partek Suite with a single nucleotide polymorphism window of 150.

To define the clinical features of patients' molecular subgroups, sex, location, and individual loci differences between the molecular subgroups were analysed with a two-sided Fisher's exact test. Independent samples' median test was used to assess the significance of tumour subgroups in relation to age. To find out whether molecular subgroups had prognostic significance and to compare treatment effects, we only included patients who had been treated with upfront chemotherapy or combined chemotherapy-radiation regimens with curative intent. We applied a univariate Cox proportional hazard analysis to compare the significance of clinical prognostic factors and molecular subgrouping. Our cohort was underpowered for multivariate analyses; therefore, we used univariate analyses adjusted for ASCL1 status to assess the prognostic significance of molecular subgrouping relative to other individual clinical and treatment factors and to estimate the hazard ratios and 95% CIs. We combined clinical prognostic factors with molecular groups to define disease risk categories, and used the log-rank analysis with the

(B Baskin PhD); **Department of Pediatrics, University of Colorado Denver, Aurora, CO, USA** (D Birks MS, Prof N Foreman MD); **Department of Neurosurgery, University of Debrecen, Debrecen, Hungary** (A Klekner PhD, L Bognár PhD); **Second Department of Pediatrics, Semmelweis University, Budapest, Hungary** (M Garami PhD, P Hauser PhD); **Department of Histopathology, Faculty of Medicine, University of Szeged, Hungary** (T Hortobágyi PhD); **Division of Pediatric Hematology/Oncology, Department of Pediatrics, University of Alberta, Edmonton, AB, Canada** (B Wilson MD); **Division of Neurology and Oncology, Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada** (J Hukin FRCPC); **Division of Hematology-Oncology, Centre Hospitalier Universitaire Sainte-Justine, Université de Montréal, Montreal, QC, Canada** (A-S Carret MD); **Pediatric Hematology-Oncology, Department of Pediatrics, Virginia Commonwealth University School of Medicine, Richmond, VA, USA** (T E Van Meter PhD); **Department of Neurosurgery, Kumamoto University, Kumamoto, Japan** (H Nakamura MD); **Oncology Department, Schneider Hospital, Petach Tikva, Israel** (H Toledano MD); **Pediatric Hematology Oncology Department, Hadassah Hebrew University Hospital, Jerusalem, Israel** (I Fried MD); **Department of Neurosurgery, University of Utah, School of Medicine, Salt Lake City, UT, USA** (D Fults MD); **Department of Neurosurgery, Shizuoka Children's Hospital, Aoi-ku, Shizuoka, Japan** (T Wataya PhD); **Division of Hematology and Oncology, Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada** (C Fryer FRCPC); **Departments of Pediatrics and Medical Genetics, University of Alberta, Edmonton, AB, Canada** (D D Eisenstat MD); **Department of Pediatrics, McMaster University, Hamilton, ON, Canada** (Prof K Scheineman MD); **Department of Pediatrics**

, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada (D Johnston MD); Department of Pathology and Laboratory Medicine, Ottawa Hospital and Children's Hospital of Eastern Ontario, Ottawa, ON, Canada (J Michaud MD); Division of Children's Health and Therapeutics, Children's Health Research Institute, London, ON, Canada (S Zelcer MD); Department of Pathology, University of Western Ontario, London, ON, Canada (R Hammond MD); Department of Pathology, London Health Sciences Centre, London, ON, Canada (D A Ramsay MBChB); Division of Pediatric Hematology/Oncology, McMaster University, Hamilton, ON, Canada (A J Fleming MD); Division of Pediatrics-Hematology, Oncology and Stem Cell Transplantation, Ann and Robert H Lurie Children's Hospital of Chicago, Chicago, IL, USA (R R Lulla MD, J R Fangusaro MD); Division of Hematology and Oncology, Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (N Sirachainan MD, S Hongeng MD); Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (N Larbcharoensub MD); Department of Pathology and Microbiology, Aga Khan University Hospital, Karachi, Pakistan (M Abrar Barakzai MD); Department of Clinical Research Services, Hospital for Sick Children, Toronto, ON, Canada (D Stephens MSc); Children's Brain Tumour Research Centre, School of Clinical Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, UK (R G Grundy FRCPCH); Center for Neuropathology, Ludwig-Maximilians-University, Munich, Germany (U Schüller MD); Department of Pediatrics Hematology/Oncology (T Nicolaides MD), Department of Pathology and Laboratory Medicine (T Tihan PhD), and Department of Neurological Surgery (J Phillips PhD), University of California, San Francisco, CA, USA; Department of Molecular Genetics, University of

Kaplan-Meier method to compare survival times and χ^2 analyses to compare the proportion of survivors across tumour subgroups and disease risk categories, and effect of treatment on survival. Since this was a retrospective, discovery-based analysis, we did not do an a priori power analysis to define the adequate number of samples needed. Also, a centre-adjustment was not feasible because several institutions contributed single cases of tumours. All analyses were done in the R statistical environment (version 2.15.2) or with SPSS (version 22.0). A p value of less than 0·05 was regarded as significant for all analyses.

Role of funding source

The funders of this study had no role in the study design, data gathering, analysis, or interpretation, or writing of this report. JT, DP, NJ, EB, LL-C, and AH had full access to the data for this study and had final responsibility for the decision to submit for publication.

Results

We did unsupervised consensus cluster analyses of global gene expression profiles from 43 primary tumours using orthogonal bioinformatic methods to define molecular subgroups of atypical teratoid rhabdoid tumours. Hierarchical clustering and non-negative matrix factorisation analyses of 200–1000 genes showed the strongest cophenetic coefficient at k=2 (appendix), indicating two broad classes of atypical teratoid rhabdoid tumours (groups 1 and 2), with suggestion of further heterogeneity within group 2 tumours (appendix). Atypical teratoid rhabdoid tumours also segregated into two groups when compared with primitive neural ectodermal tumour and medulloblastoma, two closely related paediatric embryonal brain tumours (data not shown). The results of the SigClust analysis showed that the two clusters were significantly unique ($p<0·0001$; appendix); we did not use resampling methods because the small sample size made accurate interpretation of the results difficult. To establish the molecular features of each subgroup of atypical teratoid rhabdoid tumours, we identified genes most differentially enriched in each tumour subgroup using a supervised analysis of gene expression data and did functional and pathway enrichment analyses. Groups 1 and 2 tumours differed significantly in genes regulating cell lineage and developmental signalling (appendix). Specifically, group 1 atypical teratoid rhabdoid tumours were most highly enriched for genes involved in brain or neural development, and axonal guidance, and had upregulation of genes involved in the NOTCH developmental signalling pathway (appendix). Notably, *FABP7*[25] and *ASCL1*,[26] markers of primitive neural lineage, were among the most highly differentially upregulated genes with about 10–20 times greater expression in group 1 than in group 2 atypical teratoid rhabdoid tumours (appendix). The *HES5/6* and *DLL1/3* genes, which respectively encode targets and ligands of the NOTCH signalling pathway,[27] were also among the top enriched genes in group 1 tumours (appendix). By contrast, expression of neural lineage markers was significantly diminished in group 2 atypical teratoid rhabdoid tumours, which had the greatest enrichment of genes involved in mesenchymal differentiation and the bone morphogenetic protein (BMP) signalling pathway including the *BMP4*, *BAMBI*, *SOST*, *SERPINF1*, *FBN2*, and *MSX1* loci (appendix). Gene set enrichment analysis showed the MAPK signalling pathway and genes regulating cell adhesion and migration were significantly enriched in group 2 tumour transcriptomes (appendix). We confirmed the specific signalling and lineage-specific transcriptional features of group 1 and 2 atypical teratoid rhabdoid tumours with qRT-PCR analyses of the top-enriched individual genes (appendix).

To investigate the clinical relevance of the molecular subgroups of atypical teratoid rhabdoid tumours, we sought markers for tumour subgroups that could be reliably analysed with immunohistochemistry of formalin-fixed paraffin embedded tissues from a large cohort of patients with precise clinical diagnoses. We obtained 259 rhabdoid tumour samples (atypical teratoid rhabdoid tumours [diagnosis based on absence of SMARCB1 immunostaining] and non-CNS rhabdoid tumours) from 12 Canadian paediatric brain consortium centres and 25 international institutions (appendix) with a histopathological diagnosis of atypical teratoid rhabdoid tumours. Recurrent samples and samples with incomplete clinical information were excluded from all clinical-correlative analyses. Of the 259 rhabdoid tumours received, three (1%) with BAF47 immunopositivity and 27 (10%) non-CNS rhabdoid tumours were excluded after central review; they were not included in the genomic analyses and were assessed only for ASCL1 expression with immunohistochemistry (figure 1). Of the 229 tumours eligible for analysis, 51 (22%) had adequate materials for genomic analysis; the remaining 178 (78%) had formalin-fixed paraffin embedded material only and were analysed for validation—eight (4%) were excluded as secondary or concurrent atypical teratoid rhabdoid tumours and 26 (15%) because of inadequate material to give a validation cohort of 144 primary atypical teratoid rhabdoid tumours with subgroup established with ASCL1 immunohistochemistry, and these were included in clinical correlative analyses (figure 1). 70 tumours with intention-to-treat and location information were available for the assessment of survival outcome. We selected lineage-specific genes that were validated with qRT-PCR analyses and associated with enriched developmental signalling pathways in group 1 and 2 tumours for immunohistochemical testing. We tested commercially available FABP7 (Abcam, Toronto, ON, Canada), NOTCH1 (Santa Cruz, Dallas, TX, USA), and ASCL1 (appendix), a NOTCH pathway regulator,[27] as putative markers of group 1 tumours, and a proprietary NOTCH-

NICD antibody (Cleaved Notch 1, Cell Signaling). For group 2 markers, we tested commercial antibodies for CLDN1 (Invitrogen, Burlington, ON, Canada), BMP4 (Abcam), phospho-SMAD1/5 (Cell Signaling), and phosphor-SMAD1/5/8 (appendix), an effector of BMP signalling.[28] Despite extensive investigation with several putative group 1 and 2 markers, we identified a high degree of non-specificity in our samples in all but ASCL1, which showed robust immunostaining in the formalin-fixed paraffin-embedded tissues analysed, allowing for accurate distinction between *ASCL1*-positive and *ASCL1*-negative tumours (appendix). In a small subset of tumours with both gene expression and immunohistochemical analyses, we noted ASCL1 immunostaining correlated well with *ASCL1* gene expression levels in individual tumours and with tumour subgroup assignment based on gene expression data (appendix).

We assessed clinical features of tumour subgroups established by gene expression profiling in a molecular discovery cohort of 43 primary atypical teratoid rhabdoid tumours and for 41 (95%), 39 (91%), 43 (100%), and 29 (67%) cases, respectively, we had information about tumour location, patient's age, patient's sex, and metastatic status at diagnosis. Clinical features of the discovery cohort were compared with those of a distinct validation cohort of 144 primary atypical teratoid rhabdoid tumours, for which tumour grouping was established with ASCL1 immunohistochemistry. For 125 (86%), 127 (88%), 126 (88%), and 85 (59%) tumours in the validation cohort, information was available about tumour location, patient's age, patient's sex, and disease stage at diagnosis, respectively. The results of these comparative analyses showed that group 1 tumours were significantly associated with supratentorial location and group 2 tumours were significantly associated with infratentorial location in both the discovery and validation cohorts (table 1). Our analyses did not show significant differences in incidence of metastases or sex between children with group 1 or 2 atypical teratoid rhabdoid tumours (appendix). Median age at presentation was not significantly different between group 1 and 2 patients in our discovery cohort (appendix). Age-related information available for 127 (88%) of 144 patients in our validation cohort indicated 25 (63%) of 40 children with group 1 tumours and 50 (57%) of 87 children with group 2 tumours were older than and up to 18 months old at diagnosis, respectively (p=0·055; appendix).

Clinical features with prognostic significance for atypical teratoid rhabdoid tumours and the best treatment approach have been difficult to establish because small heterogeneous patient cohorts were assessed in most studies.[11,21,29–32] We therefore investigated whether atypical teratoid rhabdoid tumour molecular subgrouping had

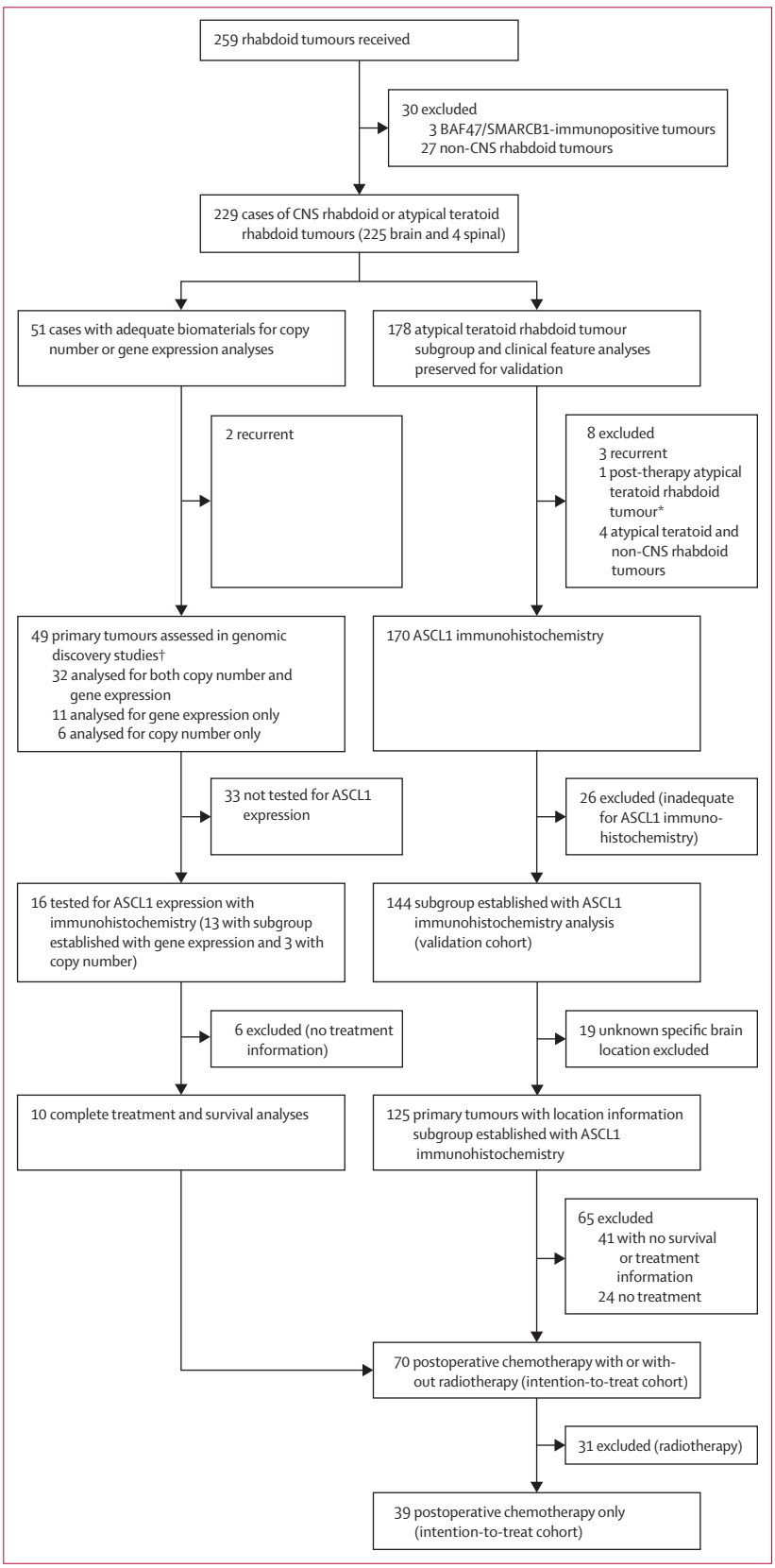

Figure 1: **Flow chart of sample analyses**
*Patient presented with brain and spinal tumours. †A total of 43 cases were analysed for gene expression and 38 for copy number.

	Molecular discovery cohort				Validation cohort			
	Total analysed	Group 1	Group 2	p value*	Total analysed	Group 1	Group 2	p value*
Number	43	21	22		144	45	99	
Location	41	20	21		125	39	86	
Supratentorial	17	13 (65%)	4 (19%)	0·004	55	25 (64%)	30 (35%)	0·003
Infratentorial	24	7 (35%)	17 (81%)		70	14 (36%)	56 (65%)	

Group 1 tumours were ASCL1 positive and group 2 were negative. *Fisher's exact test.

Table 1: **Difference in location between atypical teratoid rhabdoid tumour subgroups**

Toronto, ON, Canada (G D Bader); **Department of Neuroradiology, University of Wuerzburg, Wuerzburg, Germany** (Prof M Warmuth-Metz MD); **Department of Paediatric Haematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany** (Prof S Rutkowski MD); **Department of Neuropathology, University of Bonn Medical Center, Bonn, Germany** (Prof T Pietsch MD); **and Department of Pathology and Laboratory Medicine at Children's Hospital Los Angeles, Los Angeles, CA, USA** (A R Judkins MD)

Correspondence to: Dr Annie Huang, Division of Hematology-Oncology, Arthur and Sonia Labatt Brain Tumour Research Centre, Department of Pediatrics, Hospital for Sick Children, Toronto, ON, Canada **annie.huang@sickkids.ca**

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prognostic significance and how ASCL1 expression status or molecular grouping compared with reported clinical and treatment prognostic factors, including patient's age, tumour location, disease stage, extent of surgery, and receipt of high-dose chemotherapy or craniospinal radiation, or both.[1,3,5–7,21,29–32]

Without treatment, atypical teratoid rhabdoid tumours are fatal; we therefore only looked at samples taken from patients who had been treated with curative intent, and for which complete information about postsurgical chemotherapy or radiation, or both, was available. Analyses of ASCL1 expression and survival in 70 patients who received treatment with curative intent showed ASCL1 expression or tumour molecular subgrouping correlated with superior overall but not progression-free survival for all patients treated with chemotherapy with or without radiation (median progression-free survival 11·6 months, 95% CI 8·1–15·1, and 8·4 months, 6·7–10·1, for ASCL1-positive

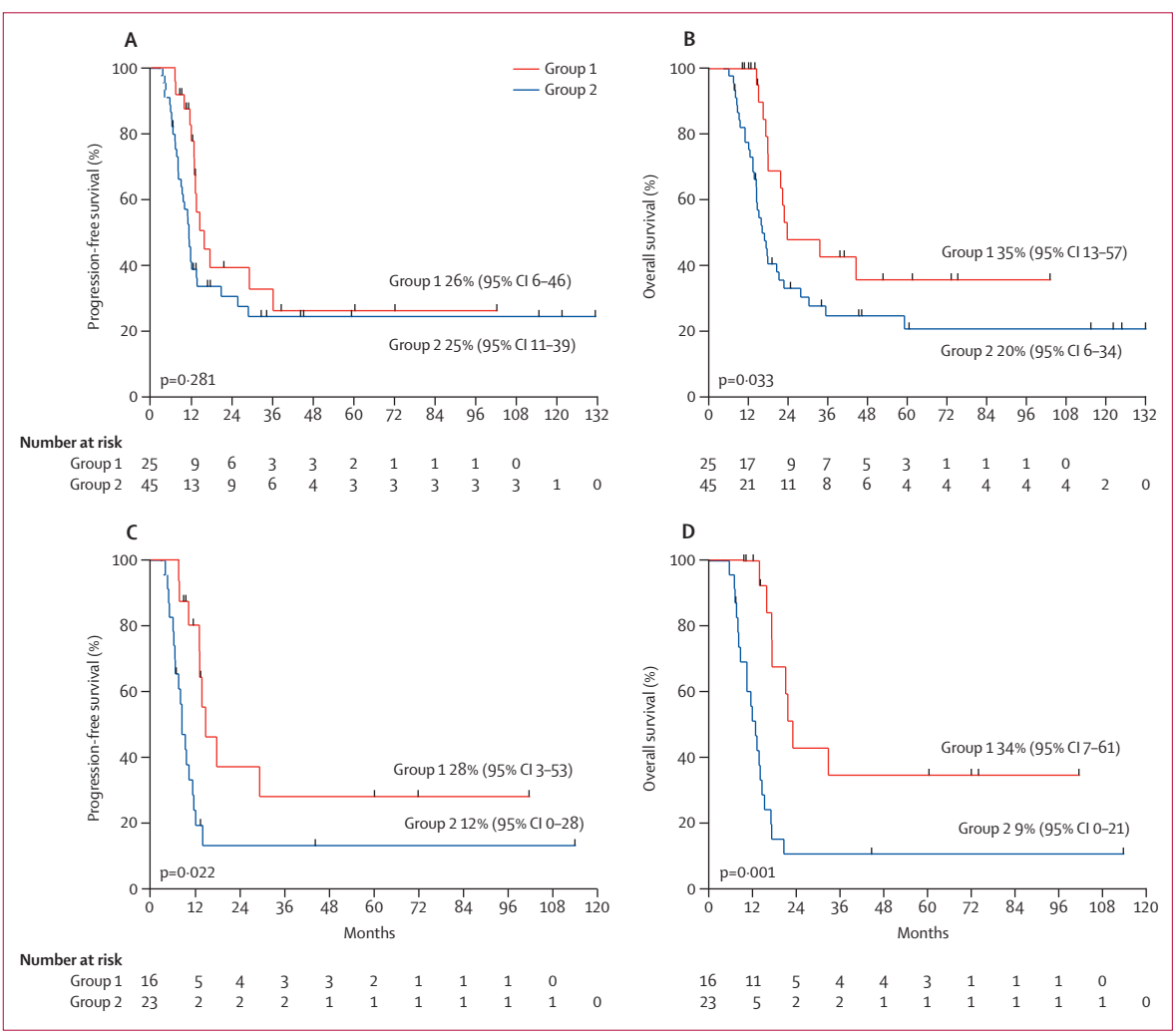

Figure 2: **Survival analyses of molecular subgroups of atypical teratoid rhabdoid tumours**

(A) Progression-free survival and (B) overall survival of 70 patients with atypical teratoid rhabdoid tumours treated with postoperative chemoradiotherapeutic regimens in relation to established tumour molecular subgroups. (C) Progression-free survival and (D) overall survival of 39 patients with atypical teratoid rhabdoid tumours treated with chemotherapy only without neuroaxis radiation regimens in relation to established tumour molecular subgroups. The Kaplan-Meier method and log-rank test were used to estimate and compare, respectively, progression-free survival and overall survival; 5-year survival estimates with 95% CIs are shown for each survival curve. Black lines indicate patients who were censored. The p values were calculated with log-rank analyses. Group 1 tumours were ASCL1 positive and group 2 were negative.

	Combined therapy				Radiation-free therapy			
	Total analysed	Group 1	Group 2	p value	Total analysed	Group 1	Group 2	p value
Number	70	25	45		39	16	23	
Age (months)								
Median (IQR)	70	21 (14·0–28·2)	15·2 (9·0–28·8)	0·134*	39	15·0 (13·0–22·0)	11·1 (6·6–13·8)	0·078*
≤12	21	3 (12%)	18 (40%)	0·016†	18	3 (19%)	15 (65%)	0·008†
>12	49	22 (88%)	27 (60%)		21	13 (81%)	8 (35%)	
≤18	37	9 (36%)	28 (62%)	0·047†	30	9 (56%)	21 (91%)	0·019†
>18	33	16 (64%)	17 (38%)		9	7 (44%)	2 (9%)	
Sex	70			0·32†	39			0·523†
Female	30	13 (52%)	17 (38%)		19	9 (56%)	10 (43%)	
Male	40	12 (48%)	28 (62%)		20	7 (44%)	13 (57%)	
Location	70			0·024†	39			0·043†
Supratentorial	29	15 (60%)	14 (31%)		14	9 (56%)	5 (22%)	
Infratentorial	41	10 (40%)	31 (69%)		25	7 (44%)	18 (78%)	
Stage	69			0·799†	38			0·001†
M0	42	16 (64%)	26 (59%)		22	11 (69%)	11 (50%)	
M1–M4	27	9 (36%)	18 (41%)		16	5 (31)	11 (50%)	
Extent of surgical resection	70			0·005†	39			0·001†
Complete	31	17 (68%)	14 (31%)		16	13 (81%)	3 (13%)	
Incomplete	39	8 (32%)	31 (69%)		23	3 (19%)	20 (87%)	
High-dose chemotherapy with stem-cell transplant	70			0·012†	39			0·001†
Yes	30	16 (64%)	14 (31%)		18	15 (94%)	3 (13%)	
No	40	9 (36%)	31 (69%)		21	1 (6%)	20 (87%)	
Radiotherapy	70			0·327†	..			
Yes	31	9 (36%)	22 (49%)		..	..	..	
No	39	16 (64%)	23 (51%)		..	..	..	
Relapse status	70			0·293†	39			0·146†
Relapse	46	14 (56%)	32 (71%)		28	9 (56%)	19 (83%)	
No relapse	24	11 (44%)	13 (29%)		11	7 (44%)	4 (17%)	
Survival status	70			0·041†	39			0·027†
Dead	45	12 (48%)	33 (73%)		28	8 (50%)	20 (87%)	
Alive	25	13 (52%)	12 (27%)		11	8 (50%)	3 (13%)	
Progression-free survival								
Median (months)		11·6 (8·1–15·1)	8·4 (6·7–10·1)	0·281		11·6 (9·0–14·2)	5·2 (3·3–7·1)	0·022
24 months‡		39% (17–61)	28% (14–42)			37% (10–64)	12% (0–28)	
60 months‡		26% (6–46)	25% (11–39)			28% (3–53)	12% (0–28)	
Overall survival								
Median (months)		29·2 (5·9–52·5)	11·7 (8·3–15·1)	0·033		19·3 (16·0– 22·6)	8·9 (5·7–12·1)	0·001
24 months‡		47% (25–64)	30% (16–44)			42% (15–69)	9% (0–21)	
60 months‡		35% (13–57)	20% (6–34)			34% (7–61)	9% (0–21)	

Data are number (%) or estimate (95% CI), unless otherwise indicated. Group 1 tumours were ASCL1 positive and group 2 were negative. *Independent-samples median test. †Fisher's exact test. ‡Log-rank (Mantel-Cox) test.

Table 2: **Summary of clinical features and treatment outcomes in atypical teratoid rhabdoid tumour molecular subgroups**

and ASCL1-negative tumours, respectively, p=0·281; median overall survival 29·2 months, 95% CI 5·9–52·5, and 11·7 months, 8·3–15·1 for ASCL1-positive and ASCL1-negative tumours, respectively, p=0·033; figure 2A, B; table 2). Because atypical teratoid rhabdoid tumour is predominantly a tumour of infancy, radiation-free approaches are often used for patients to minimise long-term neurocognitive sequelae. Although survivors of radiation-free approaches have been reported,[5] determinants of radiation-free survival remain unknown in children with atypical teratoid rhabdoid tumours. We therefore investigated how ASCL1 expression correlated with progression-free survival and overall survival in a subset of 39 patients who did not receive irradiation as part of their primary therapy. Remarkably, in patients treated with chemotherapy only, ASCL1-positive group 1 tumours

correlated significantly with higher 5-year progression-free survival (28%, 95% CI 3–53) and 5-year overall survival (34%, 95% CI 7–61) relative to ASCL1-negative group 2 tumours (5-year progression-free survival 12%, 95% CI 0–28, and 5-year overall survival 9%, 95% CI 0–21; table 2). Importantly, the results of the Cox proportional hazard analyses indicated ASCL1 expression associated with group 1 tumours as a significant factor for overall survival in all treated patients, and for progression-free survival in patients treated with only chemotherapy, although not for those patients treated with chemoradiotherapy (table 3). The significance of the molecular groupings of tumours in relation to various patients' characteristics and treatment could not be robustly assessed with multivariate analyses because complete clinical data were not available for all patients.

	Hazard ratio (95% CI)	p value	p value adjusted for ASCL1 expression
Combination therapy			
Progression-free survival (n=70)			
Age (≤18 months *vs* >18 months)	3·07 (1·66–5·65)	0·001	0·009
Location (infratentorial *vs* supratentorial)	1·37 (0·76–2·48)	0·30	0·12
Metastasis (yes *vs* no)	2·53 (1·40–4·57)	0·002	0·21
Surgical resection (gross total *vs* subtotal)	2·72 (1·44–5·15)	0·002	0·15
High-dose chemotherapy with stem-cell transplant (yes *vs* no)	2·40 (1·28–4·50)	0·006	0·029
Radiotherapy (yes *vs* no)	2·02 (1·11–3·66)	0·021	0·005
ASCL1 (positive *vs* negative)	1·68 (0·89–3·14)	0·11	..
Overall survival (n=70)			
Age (≤18 months *vs* >18 months)	3·91 (2·06–7·41)	0·001	0·006
Location (infratentorial *vs* supratentorial)	2·44 (1·31–4·59)	0·005	0·41
Metastasis (yes *vs* no)	1·47 (0·81–2·67)	0·21	0·15
Surgical resection (gross total *vs* subtotal)	3·06 (1·59–5·88)	0·001	0·054
High-dose chemotherapy with stem-cell transplant (yes *vs* no)	2·11 (1·12–3·97)	0·02	0·036
Radiotherapy (yes *vs* no)	2·15 (1·17–3·95)	0·013	0·001
ASCL1 (positive *vs* negative)	2·02 (1·04–3·85)	0·038	..
Radiation-free			
Progression-free survival (n=39)			
Age (≤18 months *vs* >18 months)	4·35 (1·30–14·49)	0·017	0·83
Location (infratentorial *vs* supratentorial)	1·49 (0·68–3·25)	0·32	0·18
Metastasis (yes *vs* no)	4·06 (1·72–9·60)	0·001	0·081
Surgical resection (gross total *vs* subtotal)	4·74 (1·84–12·2)	0·001	0·079
High-dose chemotherapy with stem-cell transplant (yes *vs* no)	4·18 (1·83–9·52)	0·001	0·10
ASCL1 (positive *vs* negative)	2·89 (1·28–6·49)	0·01	..
Overall survival (n=39)			
Age (≤18 months *vs* >18 months)	6·99 (1·64–29·41)	0·008	0·001
Location (supratentorial *vs* infratentorial)	2·22 (0·99–4·98)	0·053	0·26
Metastasis (yes *vs* no)	2·29 (1·06–4·93)	0·035	0·044
Surgical resection (gross total *vs* subtotal)	7·04 (2·58–19·23)	0·001	0·032
High-dose chemotherapy with stem-cell transplant (yes *vs* no)	6·17 (2·61–14·71)	0·001	0·047
ASCL1 (positive *vs* negative)	3·98 (1·71–9·26)	0·001	..

Table 3: **Cox proportional hazard analyses of molecular and clinical prognostic factors in atypical teratoid rhabdoid tumour molecular subgroups**

We therefore assessed the prognostic significance of ASCL1 expression in group 1 atypical teratoid rhabdoid tumours in relation to clinical and treatment variables. In children who received chemotherapy with or without radiotherapy, ASCL1 status retained prognostic significance for both progression-free survival and overall survival after adjustment for young age (<18 months), and receipt of intensified treatment with high-dose chemotherapy or radiation (table 3). These findings suggested that ASCL1 or tumour molecular grouping might have prognostic significance independent of treatment-related factors. ASCL1 expression did not correlate with progression-free survival in non-irradiated patients after adjustment for clinical and treatment factors; however, ASCL1 retained prognostic significance for overall survival relative to age, extent of surgical resection, and high-dose chemotherapy with stem-cell transplant (table 3). We noted a strong correlation between ASCL1 expression and supratentorial atypical teratoid rhabdoid tumours (data not shown); however, according to the results of univariate analyses, we identified ASCL1 and not tumour location as a significant prognostic factor for both progression-free survival and overall survival in non-irradiated children (table 3). A significantly higher percentage of children with ASCL1-positive tumours had complete tumour resection than did those with ASCL1-negative tumours irrespective of location (p=0·005 in children receiving combined therapy and p=0·001 in those receiving radiation-free therapy; table 2).

To investigate whether clinically relevant risk stratification of patients with atypical teratoid rhabdoid tumours might be achieved by integration of information about tumour biology and clinical prognostic factors, we investigated the survival features of patients stratified by a combination of ASCL1 status, tumour location, disease stage, and extent of tumour surgery, all of which correlated with prognostic significance in regression analyses. We excluded age in our analyses as a factor in our risk stratification schemes because treatment data available for 86 (40%) of 213 patients in our retrospective study cohort suggested a strong age-related bias against curative treatment (data not shown). Consistent with a historical bias against intensive treatment of very young children with brain tumours, we noted that 32 (46%) of 69 patients up to 18 months old and nine (21%) of 43 patients older than 18 months did not receive postoperative treatment (appendix). Although our retrospective patient cohort received heterogeneous treatments, regression analyses suggested that three risk categories of patients with distinct survival features can be identified with a combination of tumour molecular features and patients' clinical prognostic factors—average risk, high risk, and very high risk. Patients with completely resected ASCL1-positive non-metastatic supratentorial tumours (average risk) had the best 5-year progression-free survival (60%, 95% CI 17–100) and 5-year overall survival (60%, 95% CI 17–100; figure 3A, B; table 4). By contrast, children

with incompletely resected ASCL1-negative infratentorial tumours (very high risk) had poor 5-year progression-free survival (8%, 95% CI 0–22) and 5-year overall survival (6%, 95% CI 0–16), whereas those with ASCL1-positive or ASCL1-negative, metastatic or incompletely resected supratentorial or completely resected infratentorial tumours (high risk) had intermediate 5-year progression-free survival (29%, 95% CI 11–47) and 5-year overall survival (32%, 95% CI 14–50; figure 3A, B; table 4). Pairwise comparisons showed significant survival differences between patient risk categories except between average and high risk patients (table 4), which might be due to the small numbers of patients with average risk disease in our cohort (six [9%] of 69). Age was significantly different between risk categories: 19 (83%) of 23 patients at very high risk, 16 (40%) of 40 at high risk, and one (17%) of six at average risk were 18 months old or younger (p=0·001; table 4).

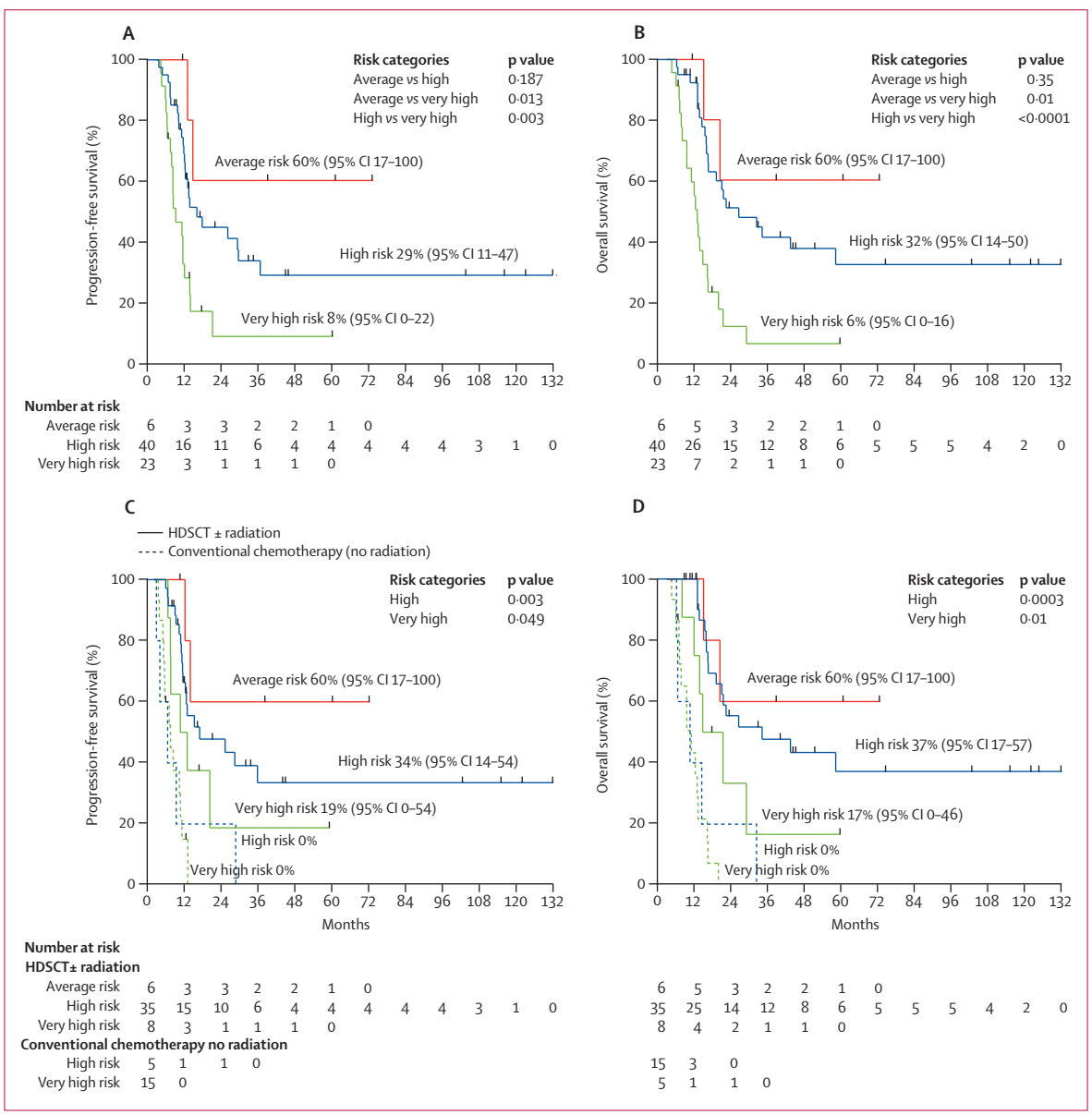

Figure 3: **Integrated molecular and clinical risk stratification of atypical teratoid rhabdoid tumours**
(A) Progression-free survival and (B) overall survival of 70 patients treated with curative intent in relation to disease risk categories. (C) Progression-free survival and (D) overall survival of 69 patients treated with HDSCT with or without neuroaxis radiation in relation to risk categories. The Kaplan-Meier method and log-rank test were used to estimate and compare, respectively, progression-free survival and overall survival; 5-year survival estimates with the 95% CIs are shown with the survival curves. Black lines indicate patients who were censored. The p values were calculated with log-rank analyses. The significance of treatment intensification could not be calculated for average risk patients because no patient received both radiation and high-dose chemotherapy. HDSCT=high-dose chemotherapy and stem-cell rescue. Group 1 tumours were ASCL1 positive and group 2 were negative.

There is continuing debate about the benefit of treatment intensification with high-dose chemotherapy with stem-cell transplant or irradiation for children with atypical teratoid rhabdoid tumours,[33,34] and these patients receive heterogeneous treatments. Since therapeutic studies have been done on biologically and clinically heterogeneous patient cohorts, we investigated whether effects of treatment intensification might differ for risk categories of patients. We investigated the survival of patients stratified by both disease risk features and receipt of treatment intensification with high-dose chemotherapy with stem-cell transplant or radiation, or both. Effect of treatment intensification in the average risk patients could not be assessed because all patients who received high-dose chemotherapy with stem-cell transplant did not receive radiation. Our analyses showed that treatment intensification was associated with significantly improved survival in patients with high risk

	Total analysed	Average risk	High risk	Very high risk	p value
Number	69	6	40	23	
Age (months)					
Median (IQR)	69	23·0 (20·25–26·88)	21·25 (13·18–33·33)	13·2 (6·95–17·45)	0·10*
≤12	20	0	9 (23%)	11 (48%)	0·027†
>12	49	6 (100%)	31 (78%)	12 (52%)	
≤18	36	1 (17%)	16 (40%)	19 (83%)	0·001†
>18	33	5 (83%)	24 (60%)	4 (17%)	
Sex	69				0·47†
Female	30	4 (67%)	17 (43%)	9 (39%)	
Male	39	2 (33%)	23 (58%)	14 (61%)	
Location	69				0·001†
Supratentorial	28	6 (100%)	22 (55%)	0	
Infratentorial	41	0	18 (45%)	23 (100%)	
Stage	69				0·063†
M0	42	6 (100%)	25 (63%)	11 (48%)	
M1–M4	27	0	15 (38%)	12 (52%)	
Extent of surgical resection	69				0·001†
Complete	31	6 (100%)	25 (63%)	0	
Incomplete	38	0	15 (38%)	23 (100%)	
High-dose chemotherapy with stem-cell transplant	69				0·011†
Yes	30	5 (83%)	20 (50%)	5 (22%)	
No	39	1 (17%)	20 (50%)	18 (78%)	
Radiotherapy	69				0·04†
Yes	31	1 (17%)	23 (58%)	7 (30%)	
No	38	5 (83%)	17 (43%)	16 (70%)	
Relapse status	69				0·044†
Relapse	45	2 (33%)	24 (60%)	19 (83%)	
No relapse	24	4 (67%)	16 (40%)	4 (17%)	
Survival status	69				0·011†
Dead	44	2 (33%)	22 (55%)	20 (87%)	
Alive	25	4 (67%)	18 (45%)	3 (13%)	
Progression-free survival					
Median (months)		..	12·9 (7·0–18·8)	6·1 (1·7–10·5)	0·187‡, 0·013¶, 0·003‖
24 months§		60% (17–100)	41% (23–59)	8% (0–22)	
60 months§		60% (17–100)	29% (11–47)	8% (0–22)	
Overall survival					
Median (months)		..	23·4 (6·3–40·6)	9·9 (7·4–12·4)	0·35‡, 0·01¶, 0·001‖
24 months§		60% (17–100)	48% (30–66)	11% (1–21)	
60 months§		60% (17–100)	32% (14–50)	6 (0–16)	

Data are number (%) or estimate (95% CI). *Independent-samples median test. †Fisher's exact test. ‡Average risk versus high risk. §Log-rank (Mantel-Cox) test. ¶Average risk versus very high risk. ‖High risk versus very high risk.

Table 4: **Clinicopathological and survival features of atypical teratoid rhabdoid tumour risk categories**

disease, but slight effects on survival of very high risk patients (figure 3C, D; table 4). Notably, these analyses also suggested a distinct, rapid progression of disease for very high risk patients and emphasise the substantial limitations of current treatments for these patients, who comprised a third of our cohort.

Discussion

Our results show that atypical teratoid rhabdoid tumours are a biologically heterogeneous disease that comprise at least two molecular subtypes with distinct clinicopathological associations. Group 1 tumours were predominantly supratentorial, whereas group 2 were predominantly infratentorial. Furthermore, the tumours could be stratified into average, high, and very high risk categories by integration of tumour molecular subgrouping through ASCL1 immunostaining with clinical prognostic factors. Importantly, we have identified biological and clinical risk features that are associated with long-term radiation-free survival in a small group of patients treated with chemotherapy alone. To our knowledge, this study is the first and largest integrated global molecular and clinicopathological analysis of childhood atypical teratoid rhabdoid tumours and our results provide an important first framework for molecular classification and treatment risk stratification of patients with this highly challenging disease of infancy and novel insights into therapeutic pathways (panel). Together with the enrichment of cell invasion and migration genes noted in group 2 tumours, our findings suggest that clinical prognostic factors, such as extent of resection and tumour location, might indicate inherent biological differences and treatment responsiveness between molecular classes of atypical teratoid rhabdoid tumours. Thus, outcomes might be determined by and dependent on a combination of tumour biology and clinical or treatment risk factors.

SMARCB1 alterations have been thoroughly validated and characterised in many atypical teratoid rhabdoid tumours.[10–13] However, global genomic and transcriptional features have only been reported for small cohorts.[18,19,35] Although animal models of non-CNS rhabdoid tumours[16,17] have been generated, modelling of atypical teratoid rhabdoid tumours has been unsuccessful and the results suggest that the tumours might arise from highly restricted cell types. Indeed, the results of our gene expression studies indicate at least two molecular classes of tumours with differential enrichment of neurogenic or forebrain markers (*LHX2*,[36] *MEIS2*,[37] *FABP7*,[25] and *ASCL1*[38]), hind-brain markers (*ZIC2*[39] and *OTX2*[40]), and mesenchymal lineage markers (*BMP4* and *MSX1*) with predominant locations in supratentorial and infratentorial brain compartments. In addition to cell lineage features, tumour subtypes differed in cellular processes and signalling features. In group 1 tumours, the proneural NOTCH signalling pathway and genes regulating neural differentiation were highly enriched, whereas in group 2 BMP signalling and cell adhesion or migration pathways were enriched. We noted that 12 (86%) of 14 non-CNS rhabdoid tumours also did not express ASCL1 (appendix), thus group 2 tumours and non-CNS rhabdoids in which BMP signalling is also implicated[41] might have similar pathogenic mechanisms. The distinct transcriptional signature and anatomical predilection of molecular subtypes of the atypical teratoid rhabdoid tumours suggest distinct cellular origins with lineage-specific *SMARCB1* loss and additional alterations of unknown modifier genes leading to the heterogeneous clinical phenotypes of the tumours. Although the clustering suggests two main molecular subgroups, our results will need to be corroborated in larger prospective studies. As shown by other investigators, the molecular classification of tumour samples is changeable and can be refined as additional samples become available; additional prognostic markers and subtypes might also emerge. Cluster analysis with gene expression is only one method for identification of putative prognostic loci and it will be crucial to corroborate our results in independent cohorts.

Our results show that the expression of neural differentiation features is an important novel prognostic factor for patients with atypical teratoid rhabdoid

Panel: Research in context

Systematic review

We searched PubMed and Google Scholar for molecular and clinical studies of atypical teratoid rhabdoid tumours between April 18, 1987, and Dec 31, 2014, using the search terms "ATRT", "AT/RT", "CNS-ATRT", and "atypical teratoid rhabdoid tumor" and the gene names "*SMARCB1*", "*hSNF5*", and "*INI1*". We considered clinical data for all cases with diagnosis of atypical teratoid rhabdoid tumours, according to pathological review of each respective institution. We restricted our search to English-language publications.

Interpretation

There is no consensus treatment strategy for atypical teratoid rhabdoid tumours because of their rare incidence; hence, clinical trials or studies have only been done on small, mixed cohorts of patients with CNS and non-CNS rhabdoid tumours. Treatment is empirical, based on the treatment of other embryonal brain tumours such as medulloblastoma for which whole brain and spine radiation are used for CNS prophylaxis. Although improved survival for patients with atypical teratoid rhabdoid tumours who are treated with such combined modality regimens has been reported, results from small series indicate that radiation therapy, which is detrimental to very young children, might not always be necessary and that there is biological heterogeneity in these tumours. Thus, whether treatment intensification with brain and spine radiation or the use of high-dose chemotherapy is justified for all patients remains debated because the biological basis for the different therapeutic profiles of patients remains unknown. The results of our current integrative genomic study build on our earlier findings of long-term survival of patients with atypical teratoid rhabdoid tumours treated with a chemotherapy-only, radiation-sparing approach with the intent to minimise neurocognitive sequelae in survivors. The results of our current study, which show molecular subtypes of tumours with distinct clincopathological and survival features, provide a powerful basis for a risk-stratified prospective clinical trial in which young patients might be spared unnecessary toxicity associated with the current intensive empirical treatment approaches without compromising their survival.

tumours. Specifically, we noted that the group 1 tumours characterised by high expression of the proneural ASCL1 marker correlated with superior long-term survival in all patients who received multimodal treatment with curative intent. Importantly, ASCL1 expression correlated with better overall survival, but not progression-free survival, in these patients. Since only complete information for primary treatment was available, it is possible that patients were given second-line therapy, which had additional beneficial effects in some patients. Patients with group 1 tumours had long-term overall survival of about 29 months (table 2), ranging from 5·4 to 98·8 months (appendix), whereas children in group 2, with nearly all infratentorial tumours, had an overall survival of only about 12 months (table 2), with most patients (32 [71%] of 45) progressing within 1 year after treatment (appendix). We noted that group 1 tumours were also associated with superior radiation-free survival; projected 24-month and 60-month overall survival were 42% and 34% for non-irradiated children with group 1 tumours compared with 9% for those with group 2 tumours (table 2). These findings suggest greater intrinsic chemosensitivity of the neurogenic group 1 tumours and importantly indicate that some very young children with favourable tumour biology could be spared radiation without compromising their survival.

Clinical features of the molecular subtypes of atypical teratoid rhabdoid tumours identified in this study largely corroborate many of the clinical prognostic factors reported in small studies (table 2).[11,21,29–32] Notably, infratentorial tumour location and BMP signalling, which are features enriched in the less favourable biology group 2 tumours, have been linked to worse outcomes previously.[35] We did not note overall subtype-specific differences in patients' ages (appendix), although age has been reported as an adverse prognostic factor in atypical teratoid rhabdoid tumours.[29] Comparisons of age categories showed a significantly greater percentage of children aged 12 months old and younger and aged 18 months old and younger with group 2 tumours than group 1 (table 2). Furthermore, we noted that age 18 months old and younger was significantly associated with poorer progression-free survival and overall survival (table 3). We noted a broader age distribution and more heterogeneity in gene expression profile for group 2 tumours than for group 1, suggesting that further age-associated group 2 subtypes might emerge with studies of much larger patient cohorts.

Importantly, we noted strong but not exclusive association of tumour molecular subgroups with location. Furthermore, previously reported adverse associations with germline *SMARCB1* status[12] and disease stage[7] were not evident in our study. Two of four long-term survivors in our cohort were infants at diagnosis and four non-radiated long-term survivors had germline *SMARCB1* alterations (data not shown). The overall incidence of metastases was not significantly different between group 1 and 2 tumours (appendix); however, we noted a significantly greater proportion of group 2 tumours with subtotal surgery (table 2) in keeping with functional enrichment analyses of group 2 expression signatures, suggesting more invasive and migratory cellular phenotypes than in group 1 tumours. In univariate analyses, the prognostic significance of tumour location, disease stage, and surgical resectability on progression-free survival and overall survival was lost or reduced when adjusted for tumour grouping based on ASCL1 expression (table 3). Together, these findings suggest that the prognostic significance of specific clinical factors might be dependent on the molecular subgrouping of the tumour.

We further defined risk categories of atypical teratoid rhabdoid tumours with distinct disease trajectories and therapeutic outcomes by integrating molecular features with clinical variables significantly associated with progression-free survival or overall survival including tumour location, disease stage, and extent of surgery. Our data suggest that children with localised supratentorial tumours with high ASCL1 expression and complete surgery represented a favourable risk category with a projected 5-year progression-free survival and overall survival of 60% and disease recurrence in only about a third of the patients. By contrast, children with metastatic or subtotally resected infratentorial tumours with no ASCL1 expression comprised the worst prognostic group. Nearly all patients in this very high risk category (>80%) died within 24 months of diagnosis, whereas the high risk category of patients with ASCL1-positive, localised but subtotally resected supratentorial or infratentorial tumours had intermediate outcome and progression (table 4). Although most children with atypical teratoid rhabdoid tumours are given intensive chemo-radiotherapeutic regimens used for high risk malignant brain tumours, it remains unclear whether high-dose chemotherapy or craniospinal radiation, or both, benefits or is necessary for all patients. Our findings suggest that different therapeutic approaches might be needed for the three risk categories of atypical teratoid rhabdoid tumours. Unlike the highest risk group in which early disease progression occurs despite interventions, the average risk patients who received only chemotherapy had the best survival with rare recurrences beyond 24 months after diagnosis. Despite the small number in our cohort, our data suggest that most patients with average risk disease might be cured with chemotherapy alone, whereas novel therapeutic agents are urgently needed for patients at very high risk. Our data suggest that conventional intensification of treatment with high-dose chemotherapy or craniospinal irradiation, or both, provides the greatest survival benefit for children in the high risk category with ASCL1-positive, localised but subtotally resected supratentorial or infratentorial tumours (figure 3). However, these patients had a more protracted disease course with adverse events up to

60 months after diagnosis (data not shown); thus, indicating that more prolonged treatment or maintenance regimens might provide additional survival benefits.

With improved survival of patients with atypical teratoid rhabdoid tumours,[3–8] it has become increasingly important to identify and assess prognostic factors to justify and stratify the use of highly aggressive and potentially toxic conventional interventions in very young patients[42] and to seek more specific therapeutic agents. Results of in-vitro studies have shown several promising biological agents for the treatment of atypical teratoid rhabdoid tumours;[43–47] however, their role in specific biological subtypes of tumours remains to be investigated. Our results suggest that inhibitors of NOTCH, BMP, and MAPK signalling and angiogenesis would be important novel, subgroup-specific therapeutic agents for atypical teratoid rhabdoid tumours. Studies to assess how such novel biological agents might be incorporated into conventional regimens will be important for reducing the substantial burden of current treatments in patients with atypical teratoid rhabdoid tumours.

Despite having a large cohort of patients with atypical teratoid rhabdoid tumours, our clinical findings need to be interpreted with the knowledge that complete clinical information was not available for all patients. Furthermore, as our retrospective study spanned decades it is also limited by biases and heterogeneity inherent in the treatment of rare diseases. Nonetheless, we believe that, our data are unique and valuable for informing and expediting biology-based trials and therapies for atypical teratoid rhabdoid tumours and other related cancers. The identification of ASCL1 as a marker for group 1 tumours is an important advance. However, further heterogeneity is likely to be shown in atypical teratoid rhabdoid tumours and additional markers will be needed to robustly distinguish all subtypes of these tumours. In view of our restricted success in establishing a multipanel immunohistochemical diagnostic panel, it is likely that additional diagnostic analyses will need to be combined with immunohistochemistry to establish robust clinical and diagnostic assays. Data from this study also provide novel information for developing disease models that capture the clinicopathological spectrum of atypical teratoid rhabdoid tumours. Most importantly, our data provide a crucial first framework for the development of prospective risk stratified clinical trials in patients with atypical teratoid rhabdoid tumours.

Contributors

AH, EB, LL-C, and NJ conceived the project ideas. JT and DP analysed the gene expression and clinical data with assistance from LL, AM, and GDB and supervision from AH. All validation experiments were done by KCH with assistance from TSYC and PS-C under the supervision of AH. LL-C, S-KK, Y-SR, CPD, SY, H-kN, J-QL, SA, JP, JAC, GRS, MZ, CCF, LR, BB, DB, NF, AK, MG, PH, TH, LB, BW, JH, A-SC, TEVM, HN, HT, IF, DF, TW, CF, DDE, KS, DJ, JM, SZ, RH, DAR, AJF, RRL, JRF, NS, NL, SH, MAB, RGG, US, TN, TT, JP, MDT, JTR, PD, MW-M, SR, TP, ARJ, NJ, and EB provided patient-related materials or clinical data used in this study. Statistical analyses were done by JT and DP in consultation with DSte. Histopathological analyses were done by CEH, ARJ, TT, JP, TP, and US. AH provided overall supervision for the project and wrote the manuscript with JT and DP and with input from EB, NJ, CEH, DStr, and LL-C.

Declaration of interests

We declare no competing interests.

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