Editorial

The impact of molecular analysis on the survival of children with embryonal tumors

Nicole L. Drezner, Roger J. Packer

Brain Tumor Institute, Children's National Health System, Children's National Medical Center, Washington, DC, USA

Correspondence to: Roger J. Packer, MD. Senior Vice President, Center for Neuroscience and Behavioral Medicine, Gilbert Endowed Distinguished Professor in Neurofibromatosis, Director, Gilbert Family Neurofibromatosis Institute, Director, Brain Tumor Institute, Children's National Health System, 111 Michigan Avenue NW, Washington, DC 20010, USA. Email: RPacker@childrensnational.org.

Abstract: Embryonal tumors represent a heterogeneous group of malignancies characterized by poorly differentiated cells and generally aggressive behavior. Although advances in survival rates have been made in several of these tumor types, including Wilms' tumor, retinoblastoma, and medulloblastoma, survival of patients with central nervous system (CNS) embryonal tumors, including primitive neuro-ectodermal tumors (PNETs) and atypical teratoid rhabdoid tumors (AT/RT), are particularly poor. Advancing molecular analysis techniques and the development of gene expression profiles has led to the formulation of different subdivisions within many of the umbrella CNS tumor groups with clinical and prognostic implications. Some subgroups have been identified as having improved survivorships, likely not captured by large scale population data given their small numbers and relatively recent characterization. Importantly, identification of differing molecular pathways has begun to result in targeted therapies which may pave the way for even more surviving patients in the coming years.

Keywords: Embryonal tumors; pediatrics; molecular profiling

Submitted Nov 10, 2015. Accepted for publication Nov 10, 2015.
doi: 10.3978/j.issn.2224-4336.2015.11.01
View this article at: http://dx.doi.org/10.3978/j.issn.2224-4336.2015.11.01

Embryonal tumors represent a heterogeneous group of malignancies generally composed of undifferentiated cells that resemble the organ system of the embryo from which they arise (1-3). Pathologic exam of these tumors typically reveals undifferentiated or poorly differentiated cells with a high mitotic index (3,4) that may reflect early embryonal precursor cells (5). Given their lack of differentiation and elevated growth potential, this group of tumors generally acts aggressively and modern treatment algorithms have had variable success depending on tumor type. Embryonal tumors can be divided by location of origin into primary non-central nervous system (CNS) and CNS tumors and these are represented by the following groups within the International Classification of Childhood Cancer, Third Edition (ICCC-3) cohort: IIIc. Intracranial and intraspinal embryonal tumors, IV. Neuroblastoma and other peripheral nervous cell tumors, V. Retinoblastoma, VIa. Nephroblastoma and other nonepithelial renal tumors, VIIa. Hepatoblastoma, and XII. Other unspecified malignant neoplasms (for the purpose of this review, pulmonary blastoma and pleuropulmonary blastoma) (6).

Tulla *et al.*'s comprehensive study on pediatric embryonal tumors registered in the German Childhood Cancer Registry (GCCR) from 1991 to 2012, evaluating incidence rates, trends, and survival probabilities, provides interesting information (1). A total of 8,337 cases of embryonal tumors (ICCC-3 groups as listed above) in patients under the age of 15 years and living in Germany were included. AT/RT was only recognized as a clinical entity in 1996, so its incidence and trends were analyzed from the year 2000 onwards; prior to 2000 these tumors were classified as either medulloblastoma or PNET. In concordance with prior known incidence rates, the largest tumor group was neuroblastoma, representing 35.9% of the cases, followed by Wilms' tumor at 25.9% and medulloblastoma at 17% of cases (1). Aside from medulloblastomas (median age

6 years, 9 months) and ganglioneuroblastomas (median age 4 years), the highest incidence rates for all tumors was in the <1 year old age group. Incidence rates of most tumors remained static over time except for hepatoblastomas, with a statistically significant increasing average annual percentage change (AAPC) of 4.6% and AT/RT, with a statistically significant increasing AAPC of 6.1%. In concordance with the increasing AAPC of AT/RT, PNETs demonstrated a significantly decreasing AAPC of –4.3% (1), likely due to the recognition of AT/RT as a distinct clinical entity in 1996. Male gender was found to be a risk factor for medulloblastomas, PNETs, AT/RTs, and neuroblastomas (1).

The reported incidence rates in this study were in keeping with those reported amongst other countries within the European Union and in the United States. Five- and 10-year survival rates amongst patients with medulloblastoma were intermediate between patients in the UK and in the United States during the same time periods and AT/RT survival rates were similarly dismal in the US and in France (1). The increase in survival amongst PNET patients between the years 2003–2006 and 2007–2010 may be due in part to the recognition and increase in diagnosis of AT/RT as a distinct entity from PNET with a poorer survival probability and its removal from the PNET subgroup. Due to their overlapping histological features consisting of poorly differentiated neuroepithelial cells and complex immunophenotypes (3,7,8), embryonal tumors have often been grouped under the same umbrella, with AT/RT frequently misdiagnosed as PNET, medulloblastoma, or less commonly, as choroid plexus carcinoma or germ cell tumor. It is now known that deletions or mutations of the *SMARCB1* (*INI1/hSNf5/BAF47*) tumor suppressor locus on chromosome band 22q11.2 are characteristic of CNS (and other) rhabdoid tumors (7). The inactivation of *INI1* in AT/RT is represented by a negative INI1 immunohistochemical assay (8). This expression pattern of *INI1* loss is now a major source of diagnostic clarity and has led to increased precision in otherwise difficult to distinguish CNS embryonal tumors. Nevertheless, the increase in knowledge behind a potential driver mutation in the development of AT/RT tumors is just beginning to result in molecularly targeted therapy, and clinical risk factors (with younger age, metastatic disease, infratentorial location, and less than complete remission after chemotherapy being negatively influencing factors) may allow for the selection of a group of patients with improved survival (7,9). In a recent multicenter analysis of 259 rhabdoid tumors from 37 institutions, genome and pathway analysis revealed the clustering of at least two distinct subgroups of patients, with average, high or very high risk designations, in part based on ASCL1 expression. Through its interaction with the Notch receptor, the protein Asc, coded for by the *ASCL1* gene, is involved in early neuroblast differentiation. Group 1 tumors tended to be supratentorial whereas group 2 tumors were infratentorial; group 1 tumors were also characterized by high *ASCL1* expression which in turn correlated with increased long term survival (29 *vs*. 12 months) (7).

Survival probabilities amongst embryonal tumors overall were determined only for those cases diagnosed prior to 2010 and those with maximum follow-up through 2010, corresponding to 7,629 and 7,307 cases respectively. Overall, CNS tumors showed the poorest survival probabilities with 5-year survivals for medulloblastoma, PNET, and AT/RT being 69%, 38% and 32%. The highest survival probabilities were seen in retinoblastoma (20 year survival 95%) and Wilms' tumor (20 year survival 90%) (1). In terms of survival by age, patients <5 with medulloblastoma had a significantly inferior 5 year survival than those aged 5–14 years (<50% for patients <1, 54% for 1–4 year olds, and 77% for 5–14 year olds). Similarly, patients with PNET had 5 year survival rates of 33% in the <1 year old group, 31% amongst 1–4 year olds, and 48% in 5–14 year olds (1). Finally, an increase in survival in medulloblastoma patients took place between the years 1995–1998 and 1999–2000 (from 64% to 80%). PNET patients also saw an increase in survival between the years 2003–2006 and 2007–2010 (from 44% to 61%) (1).

Treatment advances in some of these tumor types have led to significant gains in survival, allowing for risk stratification and reduction or intensification of therapy depending on stage (4,10,11). For example, treatment of localized Wilms' tumor now results in a >90% cure rate, with metastatic disease still resulting in >75% overall survival (11). Wilms' tumor staging is based upon the extent of surgical resection, tumor extension beyond the kidney, and histology (favorable *vs*. unfavorable) (11). Likewise, hepatoblastoma staging relies heavily on the extent of resection and involvement of the liver (12). Neuroblastoma staging encompasses a more complex group of characteristics involving clinical and biological factors including the International Neuroblastoma Staging System stage (INSS, describing localization and presence of lymph node involvement and metastases), age, MYCN status, DNA index or ploidy and International Neuroblastoma Pathology Classification (INPC) histology. Furthermore, allelic losses (e.g., chromosome 1p loss of heterozygosity), segmental chromosome aberrations, somatic gene mutations such as those in the *ALK* gene,

along with molecular expression signatures, are now known to play a role in neuroblastoma behavior and have prognostic implications (4). This emerging reliance on molecular phenotype is characteristic of the advances in our understanding of tumor biology in general and the way in which it impacts tumor behavior and, ultimately, patient survival.

This age of discovery has been particularly robust within the field of CNS embryonal tumors, which make up the largest group of malignant pediatric brain tumors and include medulloblastoma, atypical teratoid rhabdoid tumor (AT/RT), central nervous system primitive neuro-ectodermal tumors (CNS PNETs), and a rarer group of neoplasms most recently categorized by some as embryonal tumors with multilayered rosettes (ETMR) (13). Medulloblastoma has been found to represent four molecularly distinct subtypes characterized by genome-wide DNA copy number and mRNA expression data: WNT, Sonic hedgehog (SHH), group 3 and group 4 (14,15). These subgroups have clinical and prognostic implications and offer opportunities for targeted agents to be used as alternate treatment strategies, for example, the use of smoothened receptor (SMO) antagonists which target the Hedgehog signaling pathway. Knowledge of the different clinical behavior of each subgroup, with treatment of WNT tumors resulting in >90% cure rates and patients with MYCN amplified group 3 tumors faring more poorly (cure rates <50%), has resulted in reduction of the historically used chemotherapy and radiation therapy doses in the most recent clinical trials for patients with WNT tumors and intensification of therapy for those with higher risk tumors (16). Clinical trials for patients with medulloblastoma have historically categorized patient risk groups by age, histology (classic, desmoplastic, large cell/anaplastic), extent of surgical resection and presence of metastases; with infants studied separately. Molecular genetic findings are now just being incorporated into risk stratification on an international level to guide therapy.

Although CNS PNETs resemble medulloblastoma histologically and were once grouped under the same umbrella (2,3), patients with CNS PNET have significantly worse clinical outcomes even when treated with intensified protocols designed for patients with metastatic medulloblastoma. Medulloblastomas were often classified as infratentorial PNETs whereas CNS PNETs are supratentorial and predominantly hemispheric (3). Due to their relative rarity, knowledge about their biologic makeup has lagged behind the advances made within the field of medulloblastoma, and is only beginning to result in a stratification schema that may separate outcome data according to risk category. A recent multi-institutional, international collaboration allowed for the most substantial analysis to date of primary CNS-PNETs and included gene expression, copy number, and immunohistochemical analyses to classify 123 tumors and led to the creation of three molecular subgroups (groups 1, 2, and 3) with distinct gene expression signatures and, importantly, divergent clinical features (2). In this schema, group 1 tumors are aggressive, arise in younger children and have frequent amplification of the oncogenic microRNA (miRNA) cluster *C19MC* and differential expression of the *LIN28* gene. Histologically, these are primitive neural tumors, and contrast with oligoneural group 2 tumors which are localized, arise in older children, and have high OLIG2 expression, and group 3 mesenchymal tumors which arise in all ages and are associated with frequent metastases and lack either LIN28 or OLIG2 expression by immunohistochemistry (2). PTEN signaling pathway genes and IGF2 were also upregulated in group 3 tumors yielding a possible therapeutic target.

It is possible that future treatment of PNETs may involve intensification of therapy for patients with more aggressive group 1 tumors and the use of molecularly targeted agents for those with group 3 tumors. Furthermore, the use of immunohistochemistry to detect the presence of LIN28 and OLIG2 may be useful clinical tools to rapidly identify a patient's subgroup, allowing for treatment stratification. The authors observed the poorest survival amongst patients with group 1 tumors, with no significant difference in survival between patients with group 2 and group 3 tumors. However, there was a trend toward improved survival in children >4 years with group 3 tumors versus group 2 tumors (52% *vs.* 26% respectively) (2). Interestingly, an analysis of the molecular signature of ETMR tumors has also demonstrated LIN28A positivity and amplification of the *C19MC* cluster, along with frequent trisomy 2 (13), indicating that this historically highly aggressive and therapy-resistant tumor group is distinct within the group of CNS PNETs. This emerging data underscores the need to separate outcome data amongst patients with PNETs and ETMRs and would not have been represented in the population wide studies performed by Tulla *et al.*, as their survival probabilities only included patients with follow up through 2010. It is likely that the blanket survival rate of all patients with CNS-PNET does not reflect the different outcomes of the three subgroups or the much smaller group of ETMR.

Although more precise molecular subclassification of medulloblastomas, CNS PNETs and even AT/RTs provides

desperately needed biologic information and enables the discovery of targetable molecular pathways, it also results in challenges which were previously not encountered. As the tumor types are further subdivided, the number of children in each subgroup becomes smaller and conventional clinical trials become more difficult to carry out and interpret. Some clustering of different subgroups of patients will likely be required or there must be a shift in translational research to much smaller, molecularly driven trials with employment of robust molecularly-informed biomarkers to assess "success." Unless therapeutic interventions are extremely effective, resulting in high levels of long term survival, therapeutic benefits may be difficult to prove, especially if they are incremental.

The report of improving survival rates amongst patients with embryonal tumors reported by Tulla *et al*. is encouraging. The work being done to risk stratify patient groups by their individual molecular signatures and to identify and target different genes and signaling pathways will hopefully lead to better therapeutic options, fewer late effects as a result of less intensive therapy, and even greater survival in the coming years.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Tulla M, Berthold F, Graf N, et al. Incidence, Trends, and Survival of Children with Embryonal Tumors. Pediatrics 2015;136:e623-32.
2. Picard D, Miller S, Hawkins CE, et al. Markers of survival and metastatic potential in childhood CNS primitive neuro-ectodermal brain tumours: an integrative genomic analysis. Lancet Oncol 2012;13:838-48.
3. Fischer PG. Embryonal Tumors. In: Gupta N, Banerjee A, Haas-Kogan D, editors. Pediatric CNS Tumors. Springer, Berlin, 2004:83-105.
4. Irwin MS, Park JR. Neuroblastoma: paradigm for precision medicine. Pediatr Clin North Am 2015;62:225-56.
5. Badve S, Logdberg L, Lal A, et al. Small cells in hepatoblastoma lack "oval" cell phenotype. Mod Pathol 2003;16:930-6.
6. Stellarova-Foucher E, Stiller C, Lacour B, et al. International Classification of Childhood Cancer, third edition. Cancer 2005;103:1457-67.
7. Torchia J, Picard D, Lafay-Cousin L, et al. Molecular subgroups of atypical teratoid rhabdoid tumours in children: an integrated genomic and clinicopathologic analysis. Lancet Oncol 2015;16:569-82.
8. Judkins AR, Burger PC, Hamilton RL. INI1 protein expression distinguishes atypical teratoid/rhabdoid tumor from choroid plexus carcinoma. J Neuropathol Exp Neurol 2005;64:391-7.
9. von Hoff K, Hinkes B, Dannenmann-Stern E, et al. Frequency, risk-factors and survival of children with atypical teratoid rhabdoid tumors (AT/RT) of the CNS diagnosed between 1988 and 2004, and registered to the German HIT database. Pediatr Blood Cancer 2011;57:978-85.
10. Pietras W. Advances and changes in the treatment of children with nephroblastoma. Adv Clin Exp Med 2012;21:809-20.
11. Szychot E, Apps J, Pritchard-Jones K. Wilms' tumor: biology, diagnosis and treatment. Transl Pediatr 2014;3:12-24.
12. Aronson DC, Czauderna P, Maibach R, et al. The treatment of hepatoblastoma: its evolution and the current status as per the SIOPEL trials. J Indian Assoc Pediatr Surg 2014;19:201-7.
13. Korshunov A, Sturm D, Ryzhova M, et al. Embryonal tumor with abundant neuropil and true rosettes (ETANTR), ependymoblastoma, and medulloepithelioma share molecular similarity and comprise a single clinicopathological entity. Acta Neuropathol 2014;128:279-89.
14. Northcott PA, Korshunov A, Witt H, et al. Medulloblastoma comprises four distinct molecular variants. J Clin Oncol 2011;29:1408-14.
15. Northcott PA, Shih DJ, Peacock J, et al. Subgroup-specific structural variation across 1,000 medulloblastoma genomes. Nature 2012;488:49-56.
16. Gajjar A. A clinical and molecular risk-directed therapy for newly diagnosed medulloblastoma (SJMB12). Available online: https://clinicaltrials.gov/ct2/show/NCT01878617

Cite this article as: Drezner NL, Packer RJ. The impact of molecular analysis on the survival of children with embryonal tumors. Transl Pediatr 2016;5(1):5-8. doi: 10.3978/j.issn.2224-4336.2015.11.01

