Impact of RNA-sequencing analysis in refining diagnosis in pediatric neuro-oncology

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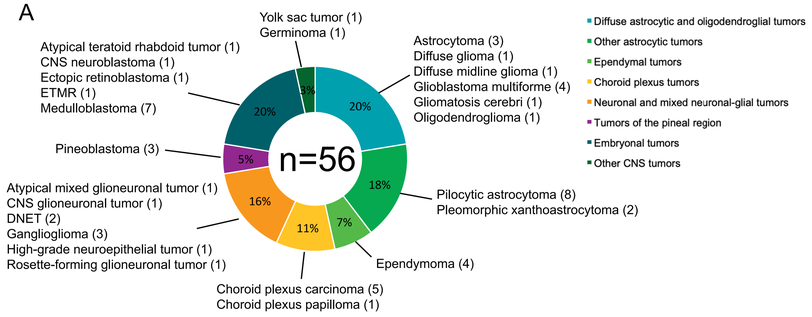
Background and demographics

The Nationwide Children's Hospital Institute for Genomic Medicine (IGM) translational research protocol enrolls pediatric patients with high-risk, relapsed, refractory, or difficult to classify tumors. *The goal of this protocol is to refine the patient diagnosis, identify targeted therapeutics relevant to the individual tumor biology and eliminate unsuitable treatments, and determine eligibility for clinical trials.* Findings are reported and new cases identified for enrollment at weekly multidisciplinary tumor boards, represented by oncology, surgery, pathology, radiology, radiation oncology, and IGM researchers. Medically actionable findings are CLIA validated to allow for return of results to the medical record.

To date, 56 unique pediatric patients with central nervous system (CNS) tumors have undergone comprehensive genomic and transcriptomic analysis (Figure 1A). A majority of sequenced patients are male (60%) and between the ages of 1-5 and 11-17 years-old (Figure 1B). Multiple tissue sections or time points were sequenced from some patients, thus a total of 70 unique tissues were evaluated, with 66% of tissues from the primary tumor (Figure 1C).



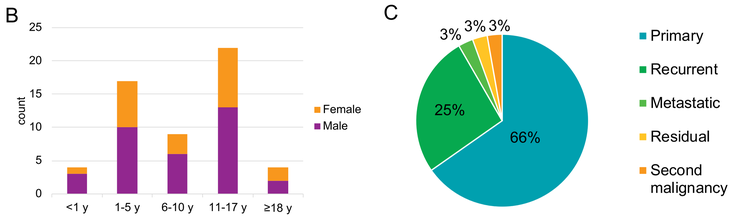


Figure 1: Demographics of the IGM translational research protocol CNS cohort. (A) Primary diagnoses from 56 unique pediatric patients with CNS tumors who underwent comprehensive genomic and transcriptomic sequencing on our IGM translational cancer protocol. Patient diagnosis is segregated by WHO classification of CNS tumors. ETMR: embryonal tumor with multilayered rosettes; DNET: dysembryoplastic neuroepithelial tumor. **(B)** Age and sex at primary tumor diagnosis. **(C)** Distribution of tumor occurrence for the sequenced tissue sections.

Cohort findings

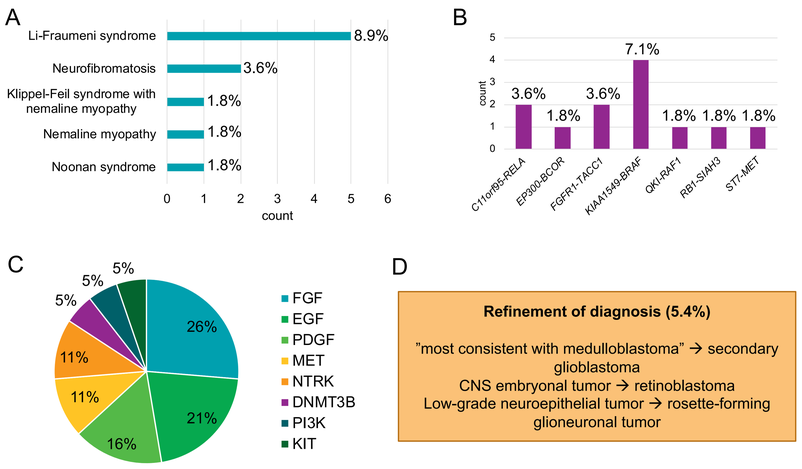


Figure 2: Summary cohort findings. (A) Constitutional disorders identified through genomic sequencing. **(B)** Medically actionable and biologically-relevant novel fusions identified through RNA-sequencing. **(C)** Potentially targetable gene overexpression. **(D)** Cases where genomic and transcriptomic sequencing helped to refine the patient diagnosis.

Methods

Transcriptomic analysis was performed from 70 tissue sections collected from 56 patients: snap frozen (n=48), formalin-fixed paraffin-embedded (FFPE) tissue (n=21), or disassociated cells (n=1). When possible, total RNA-sequencing was performed (n=60); however, when the sample was poor quality or low input, cDNA capture was utilized to enrich for exonic regions (n=8). RNA was unavailable for 2 patients. We aimed for a total of ≥80 million mapped reads per sample, with 4 samples falling below that threshold (average mapped reads: 197,334,031 ± 148,456,775 reads). The analysis pipeline is described in Figure 3.

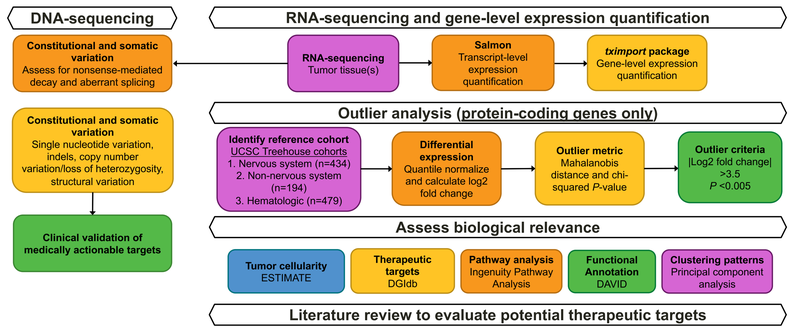


Figure 3: IGM translational cancer protocol comprehensive DNA/RNA sequencing analysis pipeline. RNA-sequencing is performed on tumors to aid in refining the patient diagnosis, identifying therapeutic targets, and confirming the transcriptional effects of constitutional and somatic DNA variation.

Case 1: Refine diagnosis

A 12-year-old was diagnosed with medulloblastoma, suggestive of Group 3 or Group 4, due to presence of isochromosome 17q. The patient recurred 3 years later. Six years after the initial diagnosis, the patient presented with a cerebellar lesion "most consistent with recurrent medulloblastoma", with comment recommending genomic studies to confirm the morphologic impression.

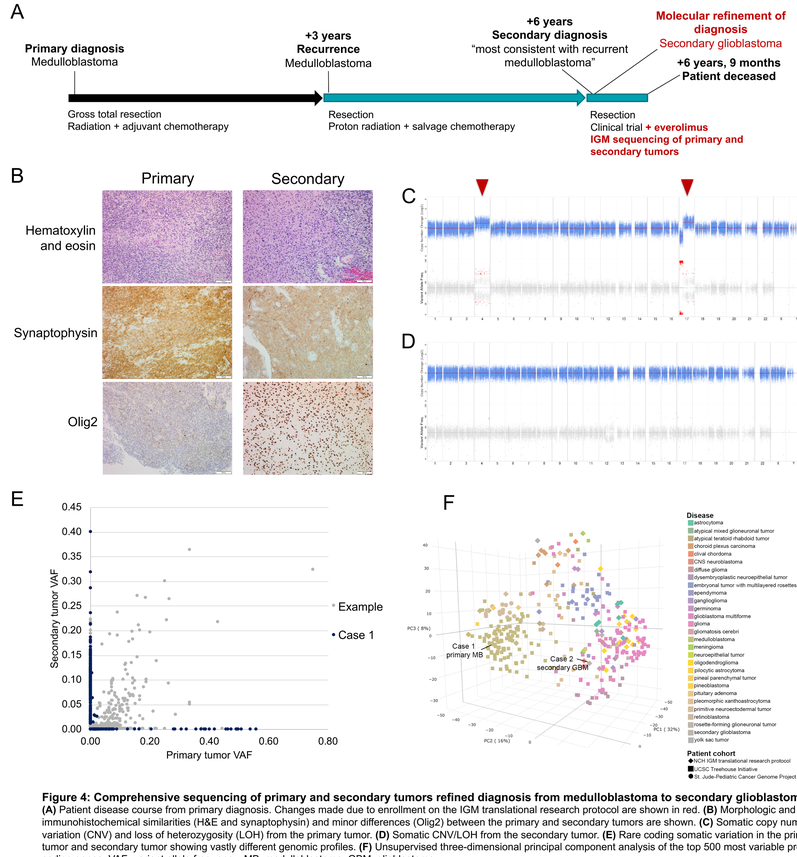


Figure 4: Comprehensive sequencing of primary and secondary tumors refined diagnosis from medulloblastoma to secondary glioblastoma. (A) Patient disease course from primary diagnosis. Changes made due to enrollment on the IGM translational research protocol are shown in red. **(B)** Morphologic and immunohistochemical similarities (H&E and synaptophysin) and minor differences (Olig2) between the primary and secondary tumors are shown. **(C)** Somatic copy number variation (CNV) and loss of heterozygosity (LOH) from the primary tumor. **(D)** Somatic CNV/LOH from the secondary tumor. **(E)** Rare coding somatic variation in the primary tumor and secondary tumor showing vastly different genomic profiles. **(F)** Unsupervised three-dimensional principal component analysis of the top 500 most variable protein coding genes. VAF: variant allele frequency; MB: medulloblastoma; GBM: glioblastoma.

Case 2: Refine diagnosis

A 10-month-old was diagnosed with a sella/suprasellar tumor, most consistent with a CNS embryonal tumor. No constitutional or somatic variations or copy number aberrations were noted. The tissue was also sent to an outside institution for the Infinium MethylationEPIC 850K array, reporting a classification of pineoblastoma group A/intracranial retinoblastoma (score=0.99).

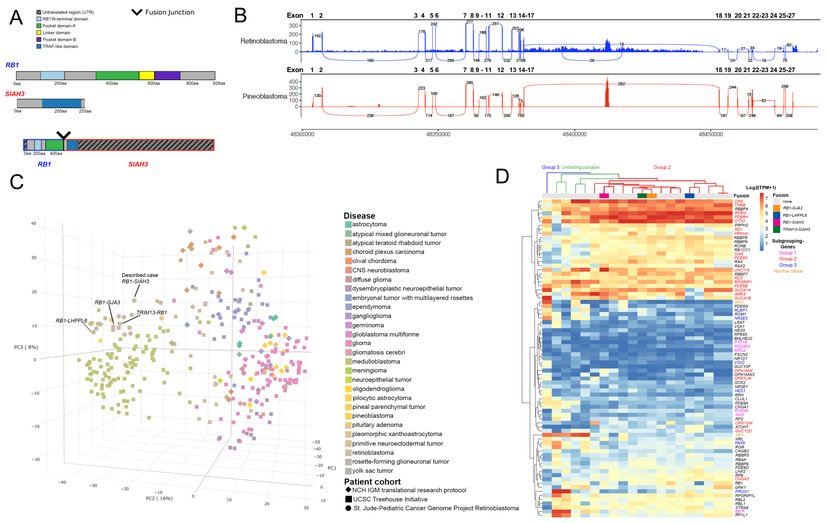


Figure 5: Refinement of a retinoblastoma diagnosis in the absence of constitutional *RB1* variation. (A) *RB1-SIAH3* fusion with fusion breakpoints at exon 17 of *RB1* and exon 2 of *SIAH3*. **(B)** Sashimi plot demonstrating a drastic reduction in spliced read depth following the fusion breakpoint at exon 17 of *RB1* in the described retinoblastoma case and consistent spliced read depth in a pineoblastoma patient with wild-type *RB1*. **(C)** Unsupervised three-dimensional principal component analysis of the top 500 most variable protein-coding genes. **(D)** Unsupervised hierarchical clustering of the St. Jude-Pediatric Cancer Genome Project retinoblastoma cases (n=19) and our described case using 79 retina-associated genes.

Case 3: Identify targeted therapy

A 1-year-old with choroid plexus carcinoma was treated with gross total resection and chemoradiation. Genomic analysis identified a somatic *TP53* mutation and 17p loss of heterozygosity. Comprehensive transcriptomic analysis revealed overexpression of multiple pathways (mTOR, FGF, and PDGF signaling) consistent with other IGM choroid plexus carcinoma cases and a 2017 case report (Cornelius *et al.* 2017 *Front. Pharmacol.*). Treatment on sunitinib (PDGF inhibitor), everolimus (mTOR inhibitor), and thalidomide (FGF inhibitor) was initiated.

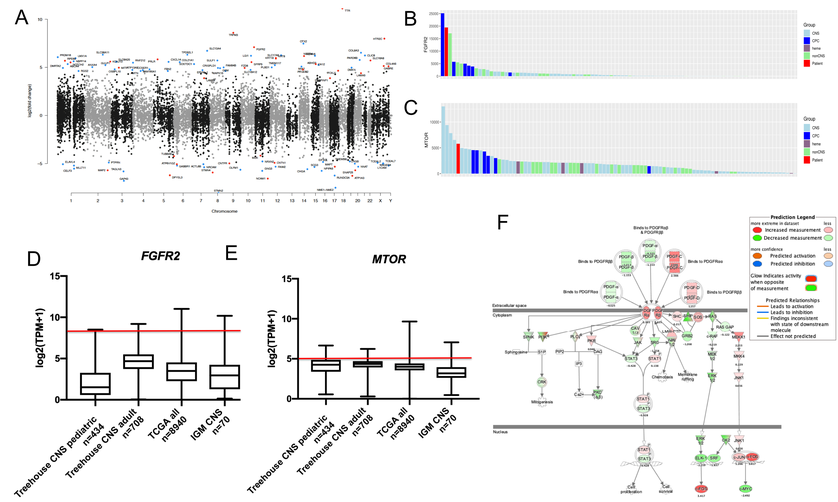


Figure 6: Transcriptomic analysis identifies therapeutically-relevant targets in a pediatric choroid plexus carcinoma patient. (A) Manhattan plot visualizing genome-wide differential expression relative to the UCSC Treehouse nervous system cohort (n=434). Outliers are shown as a blue dot. Outliers that are targetable, as determined by DGIdb, are shown as a red dot. **(B)** Distribution of DESeq2 normalized counts for *FGFR2* within the IGM translational research protocol cohort. **(C)** Same as (B) but for *MTOR*. **(D)** Boxplots showing the distribution of log2(TPM+1) values for *FGFR2* among varying cohorts. The described case is shown as a red line. **(E)** Same as (D) but for *MTOR*. **(F)** Overexpression of the PDGF signaling pathway, shown by Ingenuity Pathway Analysis.

We are grateful to the patients and their families for their participation.