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Co-chairs for 2019 Annual Meeting

Kyuson Yun, PhD ., Houston Methodist Research Institute
Craig Horbinski, MD, PhD., Northwestern University

American Brain Tumor Association®

ALUMNI RESEARCH NETWORK MEETING

AARN annual meeting 2018

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ABTA Grant Opportunities

Basic Research Fellowship

Two-year, $100,000 mentored grants supporting postdoctoral fellows who conduct laboratory or field-based research projects that focus on brain tumors.

Research Collaboration Grant

Two-year, $200,000 grants for multi-investigator and multi-institutional brain tumor collaborative research projects. Intended to promote team science, streamlining and accelerating research progress.

Discovery Grant

One-year, $50,000 grants for high risk, high impact research with the potential to change current diagnostic or treatment models.

Medical Student Summer Fellowship

Three-month, $3,000 grants awarded to medical students who wish to spend a summer conducting brain tumor research with esteemed scientist mentors.

Exchanging ideas and building relationships….

for better science, treatments, and care

how to read a scientific poster

Title, authors, and affiliations

TARGETED NEXT-GENERATION SEQUENCING PANEL (GLIOSEQ) PROVIDES COMPREHENSIVE GENETIC PROFILING OF CENTRAL NERVOUS SYSTEM TUMORS

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[1]University of Pittsburgh, [2]Northwestern University

brief background or abstract

how it was done (if you're interested)

results

BACKGROUND

•Identification of genetic changes in CNS tumors is important for the appropriate clinical management of patients.

•Targeted panel-based next-generation sequencing (NGS) can be used for detection of several types of genetic alterations, in a fast, cost-effective manner.

•Several general cancer-themed targeted NGS panels are commercially available, although none of them is designed to specifically target alterations important for pediatric and adult CNS tumors.

METHODS

•We designed the GlioSeq NGS panel using custom AmpliSeq primers (Life Technologies).

•The panel for DNA variant analysis consists of 396 amplicons in 2 primer pools. It covers 1147 brain-related hotspots and 7522 COSMIC hotspots. The panel detects mutations, gene fusions, and copy number changes among 30 high-yield genes in pediatric and adult brain tumors, including grade I-IV gliomas, medulloblastomas, and meningiomas

•NGS was performed using small amounts of DNA (20ng) and RNA (10ng) from FFPE resected tissue specimens or small snap-frozen brain biopsies.

•Standard library preparation and NGS techniques for the Ion Torrent Personal Genome Machine™ Sequencer or Ion Proton (Life Technologies) were used.

•The output data was analyzed with Torrent Suite Variant Caller (Life Technologies) and in-house developed bioinformatics pipelines. Alterations were confirmed using conventional techniques.

Mutations and Copy Number Variations					
AKT1	***ATRX***	*BRAF*	***CDK6***	***CDKN2A***	***CIC***
CTNNB1	***DDX3X***	***EGFR***	***FUBP1***	*H3F3A*	***HRAS***
IDH1	***IDH2***	*KLF4*	***KRAS***	***MET***	***MYC***
MYCN	***NF1***	***NF2***	***NRAS***	***PIK3CA***	***PTCH1***
PTEN	***RB1***	***SETD2***	***SMO***	*TERT*	***TP53***
Structural Alterations/Fusions					
EGFRvIII		*KIAA1549-BRAF*	*FAM131B-BRAF*		*FGFR3-TACC3*

Table 1. GlioSeq NGS panel design. It includes 30 genes (>1,360 CNS tumor related hot spots) analyzed for point mutations and small insertions and deletions, 24 genes (in bold) for copy number changes, 16 subtypes of *BRAF* and *FGFR3* gene fusions, and *EGFRvIII* structural alterations. In addition, it tests for the expression of three housekeeping genes (*GUSB*, *HPRT1*, *PGK*) for evaluation of RNA integrity.

Reagent Cost for GlioSeq vs. Conventional Techniques	
Test	**Cost**
GlioSeq NGS on PGM (Ion Torrent)	$472.61
Sanger Sequencing for detection of all GlioSeq mutations (396 amplicons)	$5,579.64
Copy Number SNP Array for detection of all GlioSeq CNVs	$714.70
Real-time RT-PCR for detection of all GlioSeq fusions (14 fusion types)	$1,050.00

Table 2. Cost comparison between GlioSeq and conventional techniques. Other costs, e.g. instruments and labor, are not included in the calculations.

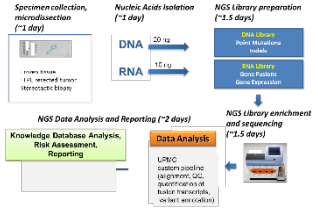

Figure 1. Workflow and turnaround time for GlioSeq.

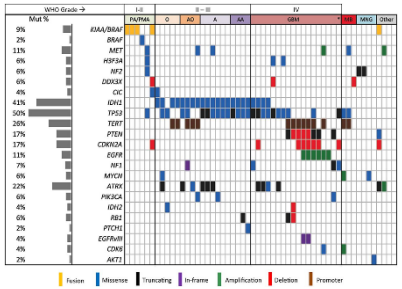

Figure 2. Genomic landscape of 54 CNS tumors profiled using the GlioSeq panel. Left pane indicates the mutation rate for each target across all the samples. Each vertical bar represents an individual case. A, astrocytoma (WHO grade II); AA, anaplastic astrocytoma (WHO grade III); AO, anaplastic oligodendroglioma (WHO grade III); GBM, glioblastoma multiforme (WHO grade IV); MB, medulloblastoma (WHO grade IV); MNG, meningioma; O, oligodendroglioma (WHO grade II); PA, pilocytic astrocytoma (WHO grade I); PMA, pilomyxoid astrocytoma (WHO grade II; *, gliosarcoma.

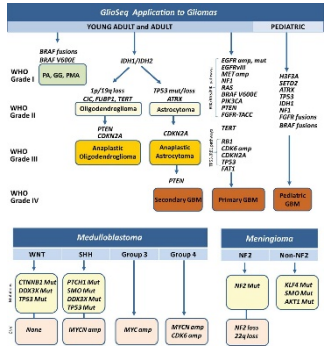

Figure 3. Application of GlioSeq panel for detection of genetic alterations relevant to different subtypes and grades of both adult and pediatric gliomas, medulloblastomas, and meningiomas.

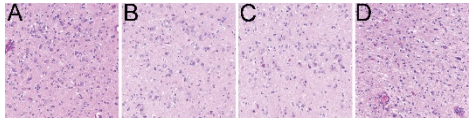

Figure 4. Example #1 of GlioSeq implementation in routine practice. A 45 year-old man had a lesion in the left hippocampus that had been originally signed out as "grade II diffuse astrocytoma" in 2010 at an OSH. However, because the patient had no clinical or MRI changes in 5 years (images not available), he came to Northwestern for a second opinion. H&E showed a mixture of neurons and glioma-like elements (A). GlioSeq detected a frameshift mutation in PTEN, but no other point mutations, copy number alterations, or fusions. In light of the clinical behavior, and the fact that isolated PTEN mutations are often seen in non-neoplastic malformations, this case was re-diagnosed as a "low-grade glioneuronal lesion," with periodic follow-up as the only recommendation.

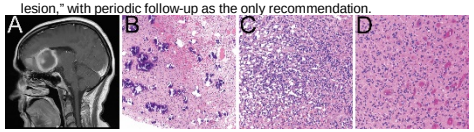

Figure 5: Example #2 of GlioSeq implementation in routine practice. A 56 year-old woman had a right frontal lobe mass (A) that was originally signed out as a "low grade astrocytoma" at an OSH, and came to Northwestern for a second opinion. H&E examination (B-D) showed a questionably infiltrative glioma with heavy calcification, but no mitoses, necrosis, or microvascular proliferation. GlioSeq detected mutations in the *TERT* promoter and TP53, deletions in *PTEN* and *NF2*, and an *FGFR3-TACC3* fusion. Based on the molecular data, the diagnosis was revised to "high-grade molecular signature, suggestive of glioblastoma," noting that an ongoing phase II clinical trial, NCT01975701, is evaluating the effects of the pan FGFR kinase inhibitor BGJ398 on gliomas with *FGFR1-TACC1* or *FGFR3-TACC3* fusions.

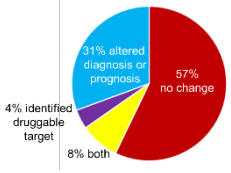

Figure 6. The impact of GlioSeq on patient management. A separate cohort of 49 gliomas or tissues suspected of containing a glioma was prospectively analyzed as part of routine patient care. Results were classified according to whether and how GlioSeq altered clinical management.

take-home points

CONCLUSIONS

•GlioSeq accurately and sensitively detects a wide range of genetic alterations in a single workflow

•NGS approaches often identify unexpected alterations that greatly impact the care of brain tumor patients.

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papers, funding, thanks

REFERENCE

•Targeted next-generation sequencing panel (GlioSeq) provides comprehensive genetic profiling of central nervous system tumors. Neuro Oncol. 2016 Mar;18(3):379-87.

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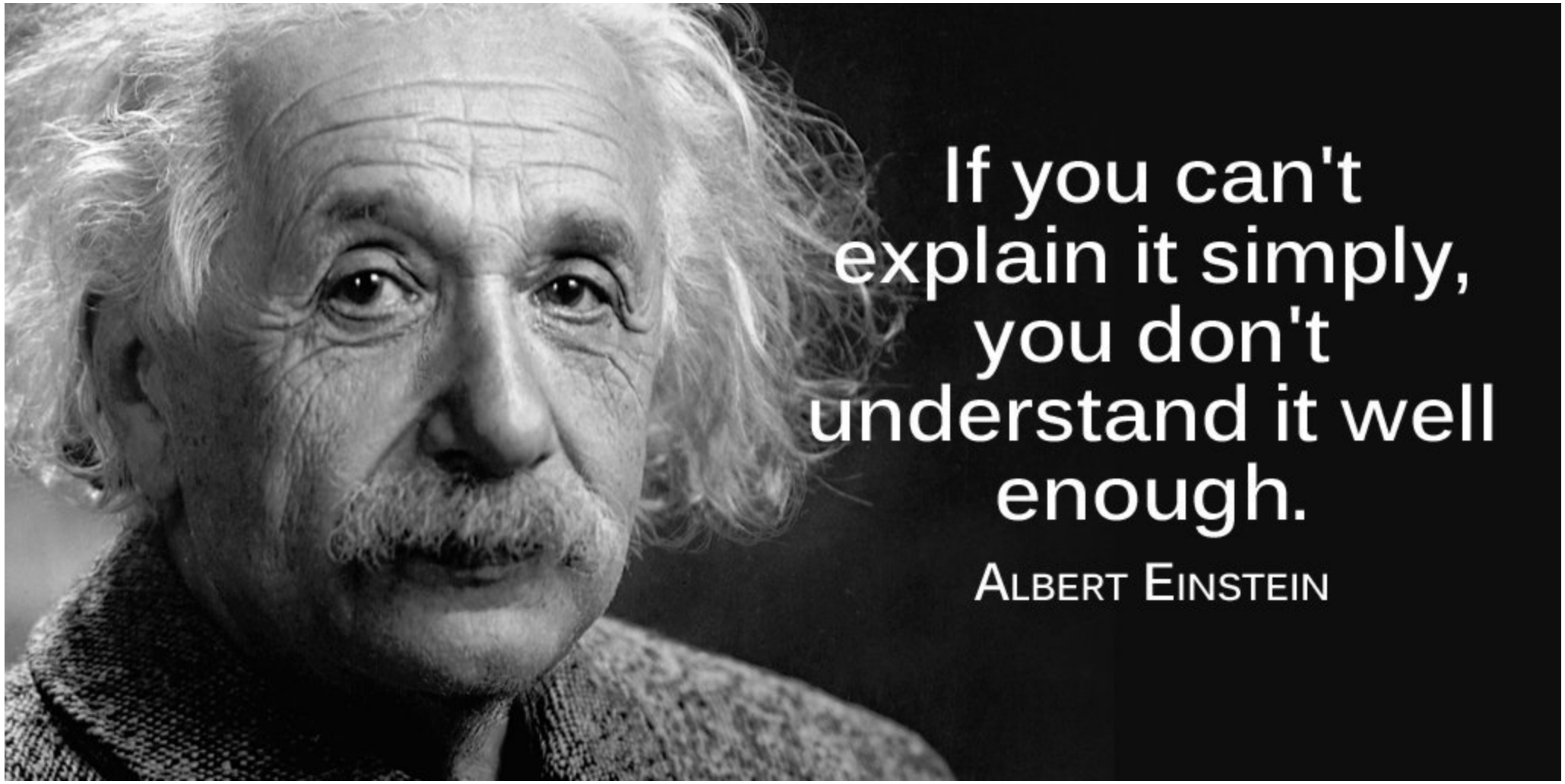
If you can't explain it simply, you don't understand it well enough.
ALBERT EINSTEIN