Targeted next-generation sequencing (NGS) of temporally matched cerebrospinal fluid (CSF) and tumor tissue in patients with recurrent glioblastoma (GBM)

Aseel Abdalla[1], Jacob E. Till[1], Stephen J. Bagley[1], Stephanie S. Yee[1], Donald M. O'Rourke[1], Steven Brem[1], Nduka M. Amankulor[1], Han-Chiao Isaac Chen[1], Zev Binder[1], Arati S. Desai[1], Richard E. Phillips[1], Jasmin Hussain[1], Yolanda Kry[1], Michael Caldwell[2], Nike Beaubier[2], Erica L. Carpenter[1]

[1]University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States
[2]Tempus Labs, Chicago, Illinois, United States

Background

- Tissue acquisition in the brain is highly invasive and can be associated with substantial morbidity. Therefore, repeated attempts at tissue acquisition at times of tumor recurrence are often not possible.
- Additionally, sequencing of a single small specimen of tumor tissue may underrepresent the molecular heterogeneity of the tumor.
- Further, the blood-brain barrier limits the efficacy of blood-based liquid biopsy in brain tumor patients, preventing tumor materials, such as circulating tumor DNA (ctDNA), from entering systemic circulation.
- Cerebrospinal fluid (CSF)-based liquid biopsy is a promising alternative as collection is less invasive and may better represent the genomic profile of the tumor due to intimate contact between CSF and tumor lesions. However, datasets with contemporaneously collected CSF and tissue to support this claim have been lacking.
- To evaluate the performance of CSF next-generation sequencing (NGS), we conducted a pilot study in patients with GBM undergoing resection for suspected recurrence following first-line chemoradiotherapy.

Methods

- CSF and tissue were collected from 18 patients.
- CSF samples were collected directly from the ventricular space intraoperatively and either transferred to Streck Blood Collection Tubes and shipped to Tempus Labs, Inc. (Chicago, IL) or processed in-house by two step centrifugation and shipped at -80 °C.
- Paired CSF and tissue samples were sequenced using the Tempus xF and xT NGS assays:
  - Tissue, 648 genes; CSF, 105 genes
- Clinically meaningful genes* are those with variants that are potentially targetable using off-label or clinical trial options, exhibit prognostic value, or may predict response or resistance to specific treatments.

Results

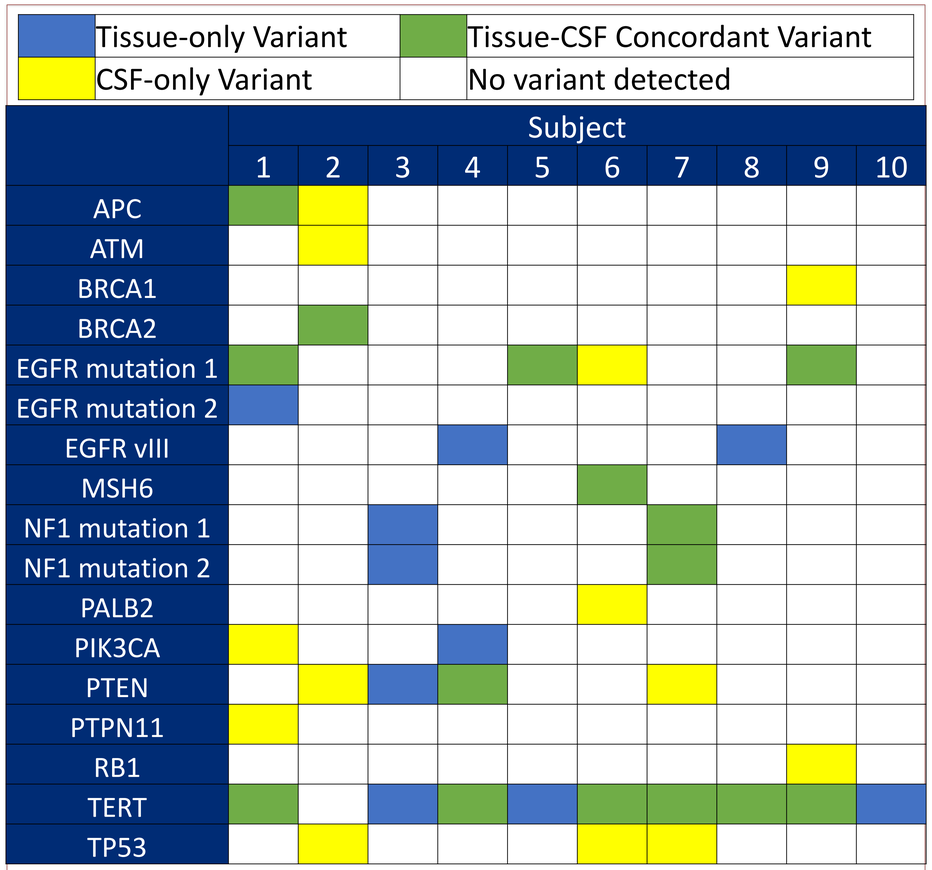

Figure 1. CSF/tissue pairs were sequenced successfully for 10 patients. Among 38 clinically meaningful genes*, 38 biologically relevant somatic variants were detected; 15 (39.5%) in both tissue and CSF, 13 (34.2%) solely in CSF and 10 solely in tissue (26.3%). Two different EGFR mutations were detected for subject 1, and 2 different NF1 mutations found for subjects 3 and 7.

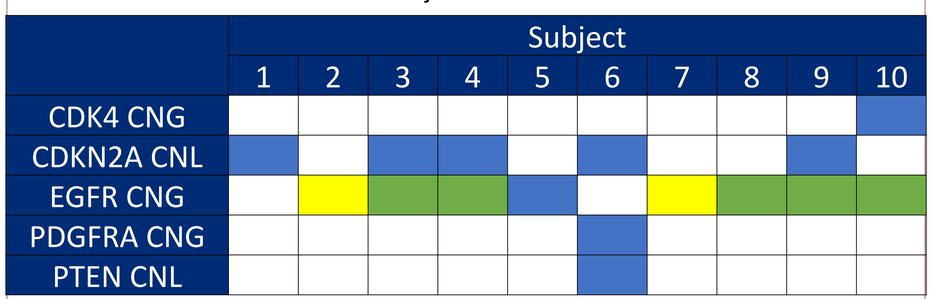

Figure 2. Among 38 clinically meaningful genes*, 16 copy number alterations were detected; 5 (31.3%) were detected in both tissue and CSF, 2 (12.5%) were detectable solely in CSF and 9 were detectable solely in tissue (56.3%).

***Clinically meaningful genes:** ATM, ATR, BRAF, BRCA1, BRCA2, CDK4, CDK6, CDKN2A, EGFR, FGFR1, FGFR2, FGFR3, FGFR4, IDH1, IDH2, MET, MLH1, MSH2, MSH3, MSH6, MTOR, MYC, MYCN, NF1, NF2, PALB2, PDGFRA, PDGFRB, PIK3CA, PIK3R1, PMS2, PTEN, PTPN11, RB1, RET, ROS1, TERT, TP53

	Concordant Variants	CSF VAF	Tissue VAF
Biologically Relevant Variant	MSH6 p.T1219I	34.3	25.9
	STK11 p.D176N	24.7	14.3
	TERT Promoter Mutation	36.1	31.5
VUS*	CCNE1 p.A386T	23.1	16.9
	KDR p.G23D	29.5	19.5
	KMT2A (MLL) p.A3615V	25.9	8.1
	MSH3 p.P18S	32.8	17.2
	NOTCH1 p.G2367S	34.9	18.6
	PDGFRA p.P345L	60.4	38.2
	PDGFRA p.S1054F	72.4	43.1
	RNF43 p.S2N	33.5	18.3
	RNF43 p.V480I	34.4	15.1
	STK11 p.A225V	21.8	12.6
	TSC2 p.H1135Y	29.1	17.9
	TSC2 p.R1745H	50.2	51.8

Figure 3. Somatic hypermutation was detected in one patient. Of the 82 variants detected in this patient, 15 (18.3%) were identified in both tissue and CSF, 15 (18.3%) were identified only in the tissue, and 52 (63.2%) were identified only in the CSF. *VUS=variant of uncertain significance

Conclusions

- CSF NGS detects clinically meaningful variants at a substantial rate and frequently identifies mutations not detected by matched tissue NGS.
- These results suggest that CSF may be a suitable source material for tumor profiling, overcoming the limitations of tissue, and may also provide a more comprehensive tumor profile.
- Future studies may aim to identify pre-analytical variables that influence the performance of CSF-based liquid biopsy.

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