The Oncogenic Flip in Patients with Leptomeningeal Metastatic Disease (LMD); Longitudinal Detection in Cerebrospinal Fluid Tumor Cells (CST-TCs) Reveals Implications for Differential Treatment of the LMD Tumor

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Background

+ Patients with LMD have poor prognosis and limited treatment options
+ Oncogene amplification of the primary, metastatic, and CNS metastatic tumors can be heterogeneous
+ Clinically relevant biomarkers in the CSF may help physicians choose a targeted therapy specifically for the LMD tumor

Introduction

+ CNSide is a CSF analysis platform employed in patients with solid tumors with suspected or confirmed LMD
+ Enumerates CSF-TCs with a sensitivity of 92% and a specificity of 95%[1]
+ Analyzes cells for oncogene amplification via fluorescence in-situ hybridization (FISH), immunocytochemical analysis (ICC), and next-generation sequencing (NGS)
+ Commercial assay in a CAP-accredited CLIA-certified laboratory and ordered at each physician's discretion
+ We aimed to assess its ability to detect clinically relevant biomarkers and assess change over time

Platform

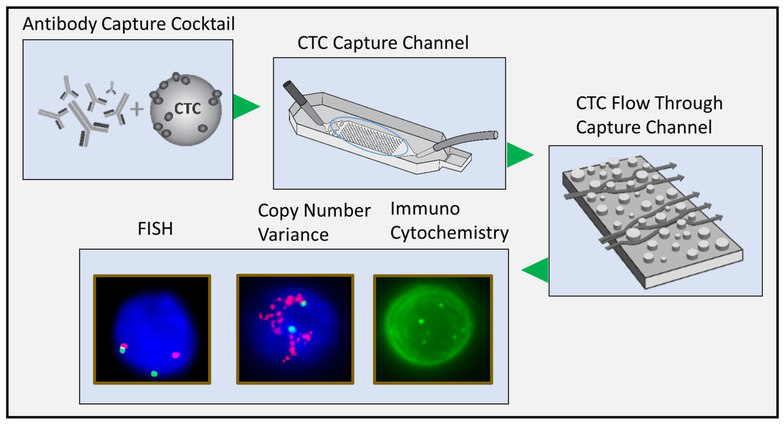


Figure 1. CNSide workflow demonstrating usage of a 10-antibody cocktail for cell capture, followed by biotinylation and passage through a microfluidic device for immobilization and FISH and ICC analysis.

Methods:

+ Retrospective analysis of test results between January 2020 and October 2022
+ 613 tests on 218 individual patients
+ Ordered by 19 physicians from 5 institutions under 2 health systems
+ To date, this is the largest cohort of patients in LMD literature evaluated using CSF-TC enumeration.

Results:

Demographics:

+ Sex: 74.3% female
+ Age: range 19-99 years, median 58
+ Most commonly analyzed: breast (n=105) and lung (n=65) cancers
+ CSF-TCs detected in 67% (412/613)
+ Lung cancer: ALK detected in 14% (17/118), CMET in 61% (78/128), HER2 in 73% (16/22), and RET in 4% (4/90)
+ Breast cancer: HER2 detected in 39% (65/168), FGFR1 in 32% (19/60), ER in 26% (44/168), PR in 4% (5/120)

Results

Table 1: Distribution of analyzed samples by primary tumor type.

Primary Tumor Type	# of samples	# of Patients
Bladder	1	1
Breast	294	105
GI	24	10
Gynecologic	1	2
Head and Neck	6	4
Hepatic	7	1
Lung	229	65
Male GU	3	2
Miscellaneous	24	12
Neuroendocrine	3	3
Pancreatic	5	2
Renal	3	3
Skin	13	8
Total	**613**	**218**

Table 2: Distribution of analyzed CSF by sampling method, divided by primary and subsequent CSF draw procedures, and by binary detection of CSF-TCs.

	Ommaya	Lumbar Puncture	Not Recorded	Total
Primary	23	123	72	218
Subsequent	256	33	106	395
Detected	207	73	122	402
Not Detected	71	83	55	209
NGS Only	1	0	1	2
Total	**279**	**156**	**178**	**613**

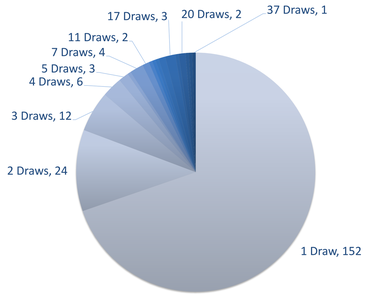


Figure 2: CSF draws per patient: Number of longitudinal CNSide tests ordered in a single patient. Clockwise from minimum (1) to maximum (37) CSF draws in a single patient.

Figure 3 (below): ICC and FISH Probe Detection: The total number of cases in which alterations were detected in each biomarker using ICC and FISH across all samples

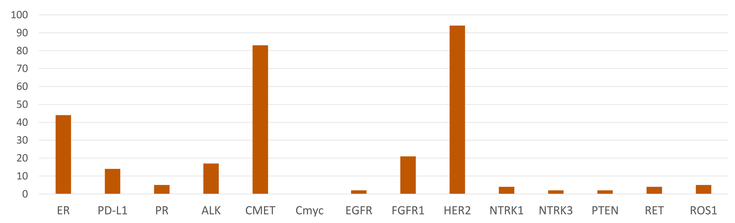
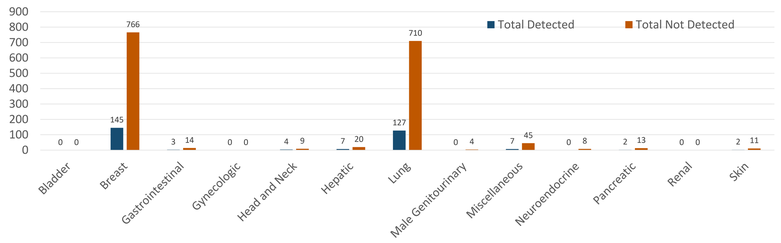


Table 3 and Figure 4 (top right): Total biomarker detection by primary tumor type. "Other" includes: bladder, gastrointestinal, gynecologic, head and neck, hepatic, male genitourinary, neuroendocrine, pancreatic, renal, skin, miscellaneous

	Lung		Breast		Other	
Biomarker	Detected	Not detected	Detected	Not detected	Detected	Not detected
ER	0	0	44	124	0	1
PR	0	2	5	115	0	0
PD-L1	7	157	7	157	0	39
ALK	17	101	0	1	0	11
EGFR	0	2	1	0	1	4
CMET	78	50	0	1	5	3
C-Myc	0	0	0	0	0	1
FGFR1	0	1	19	41	2	1
HER2	16	6	65	103	13	8
NTRK1	1	102	2	131	1	22
NTRK3	0	89	2	92	0	15
PTEN	0	1	0	0	2	4
RET	4	86	0	0	0	7
ROS1	4	113	0	1	1	8



+ 66 patients underwent 2 or more CSF draws; 24 underwent 5 or more
+ 20% (13/66) patients were found to have a flip in ICC detection (7 acquired mutations)
+ 88% (58/66) patients were found to have a flip in FISH probe detection (26 acquired mutations)

Table 5 (below): Oncogenic "flip" over time by biomarker type.

			FLIPS	
Biomarker	**Nr. of samples analyzed of patients with >2 CNSide test performed**	**Nr. of patients that had a flip in CNSide biomarker results**	**Flip in biomarker from: Not Detected -> Detected**	**Flip in biomarker from: Detected -> Not Detected**
ER	25	6	2	4
PD-L1	46	7	5	2
PR	16	0	0	0
ALK	14	5	2	3
CMET	17	8	4	4
Cmyc	0	0	0	0
EGFR	1	1	0	1
FGFR1	13	7	5	2
HER2	32	12	5	7
NTRK1	38	3	2	1
NTRK3	30	1	0	1
PTEN	1	1	1	0
RET	12	3	3	0
ROS1	13	4	4	0
Grand Total	**258**	**58**	**33**	**25**

* N=66 unique patients. The same CSF sample of some patients were assessed for multiple biomarkers simultaneously

Conclusions

+ CNSide can be used to detect gene amplification on CSF-TCs of patients with LMD
  + **CSF-TC analysis may provide therapeutic insights to specifically target the LMD tumor**
+ Mutational status of the LMD tumor frequently changes over time
  + **Longitudinal CSF-TC analysis may provide therapeutic insights to modify treatment of the LMD tumor over time**
+ Prospective studies are needed to evaluate long-term benefits (OS and PFS) of incorporating the CNSide assay into standard LMD diagnostic protocols
+ Analysis of the prospective FORESEE clinical trial to determine the impact of CNSide on clinical decisions is ongoing

References

1.Sweed NT, Hsiao HC, Blouw B, et al. A Microfluidic, Multi-Antibody Cell Capture Method to Evaluate Tumor Cells in Cerebrospinal Fluid in Patients With Suspected Leptomeningeal Metastases. Arch Pathol Lab Med. Published online May 27, 2024. doi:10.5858/arpa.2023-0295-OA