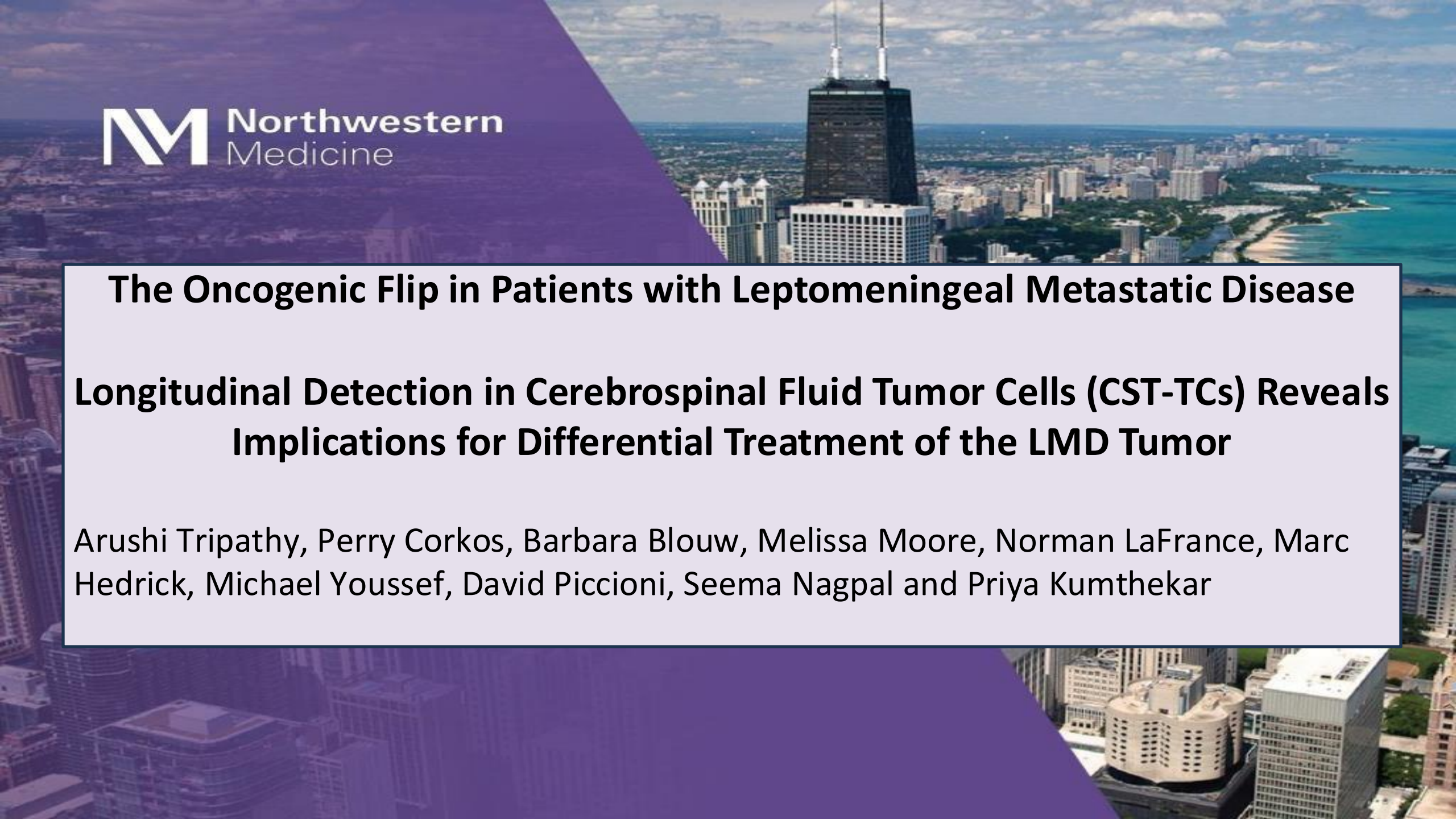


The Oncogenic Flip in Patients with Leptomeningeal Metastatic Disease

Longitudinal Detection in Cerebrospinal Fluid Tumor Cells (CST-TCs) Reveals Implications for Differential Treatment of the LMD Tumor

Arushi Tripathy, Perry Corkos, Barbara Blouw, Melissa Moore, Norman LaFrance, Marc Hedrick, Michael Youssef, David Piccioni, Seema Nagpal and Priya Kumthekar

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

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

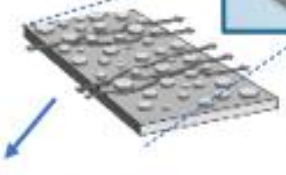
The CNSide Platform

Tumor Cell Detection Workflow

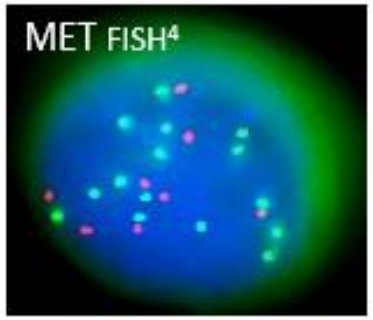
cfDNA Detection Workflow

Antibody Cocktail Tumor Cell Isolation[1,*]

Patented Microfluidic Channel [2,*]



Cytokeratin/DAPI ICC

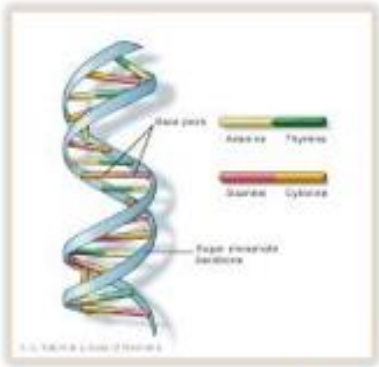


MET FISH[4]

Collection tube for ambient shipping up to 4 days

*Unique cell capture technology for FISH and protein expression assays

CSF cfDNA Isolation

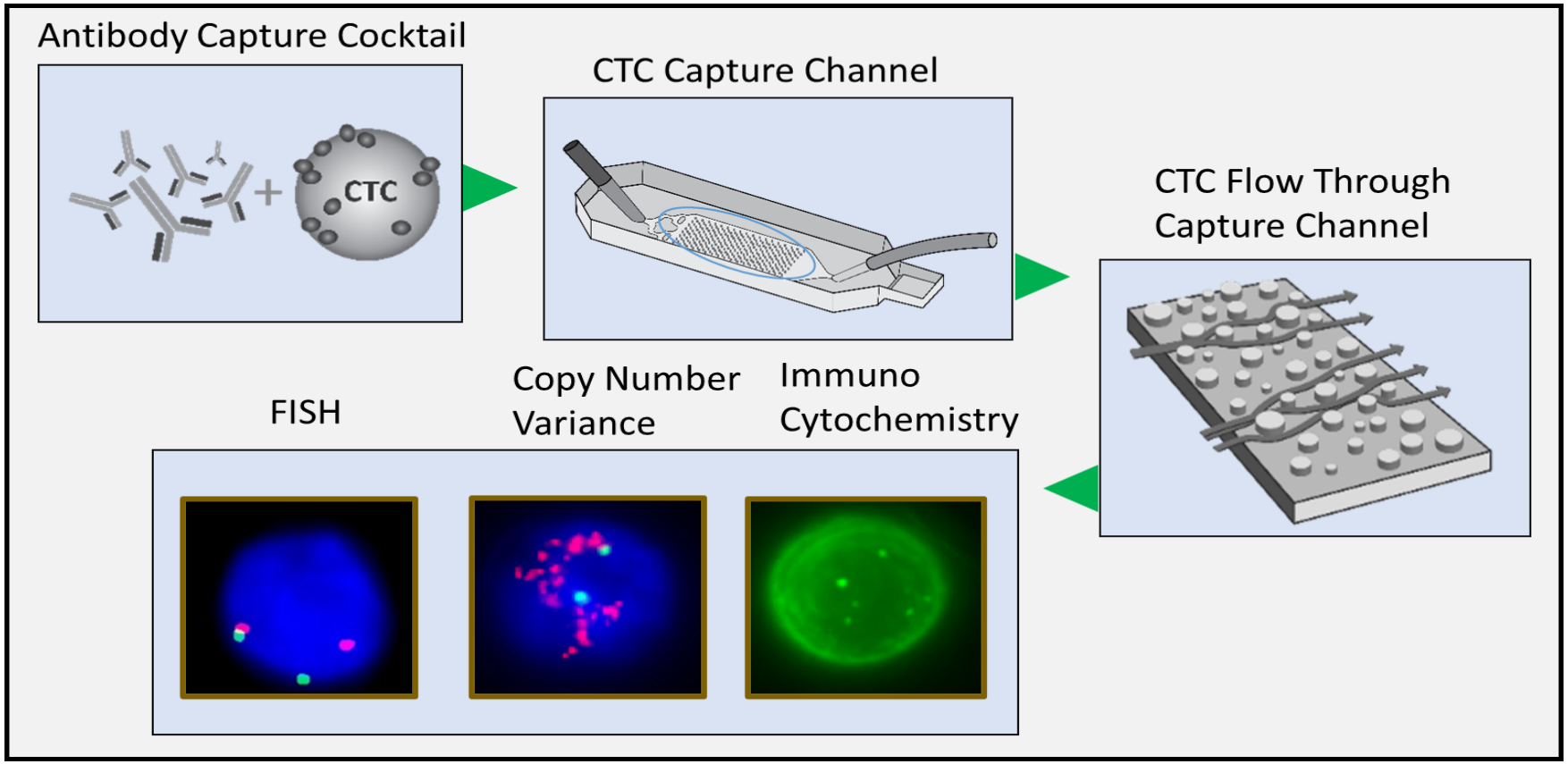


NGS on CSF cfDNA

[1] Mikolajzyk et al. JCO (2011), [2] Dickson et al. Microfluidics (2011)
[3] Pecot et al. Cancer Discovery (2011), [4] Mayer et al. Cancer Genetics (2011)

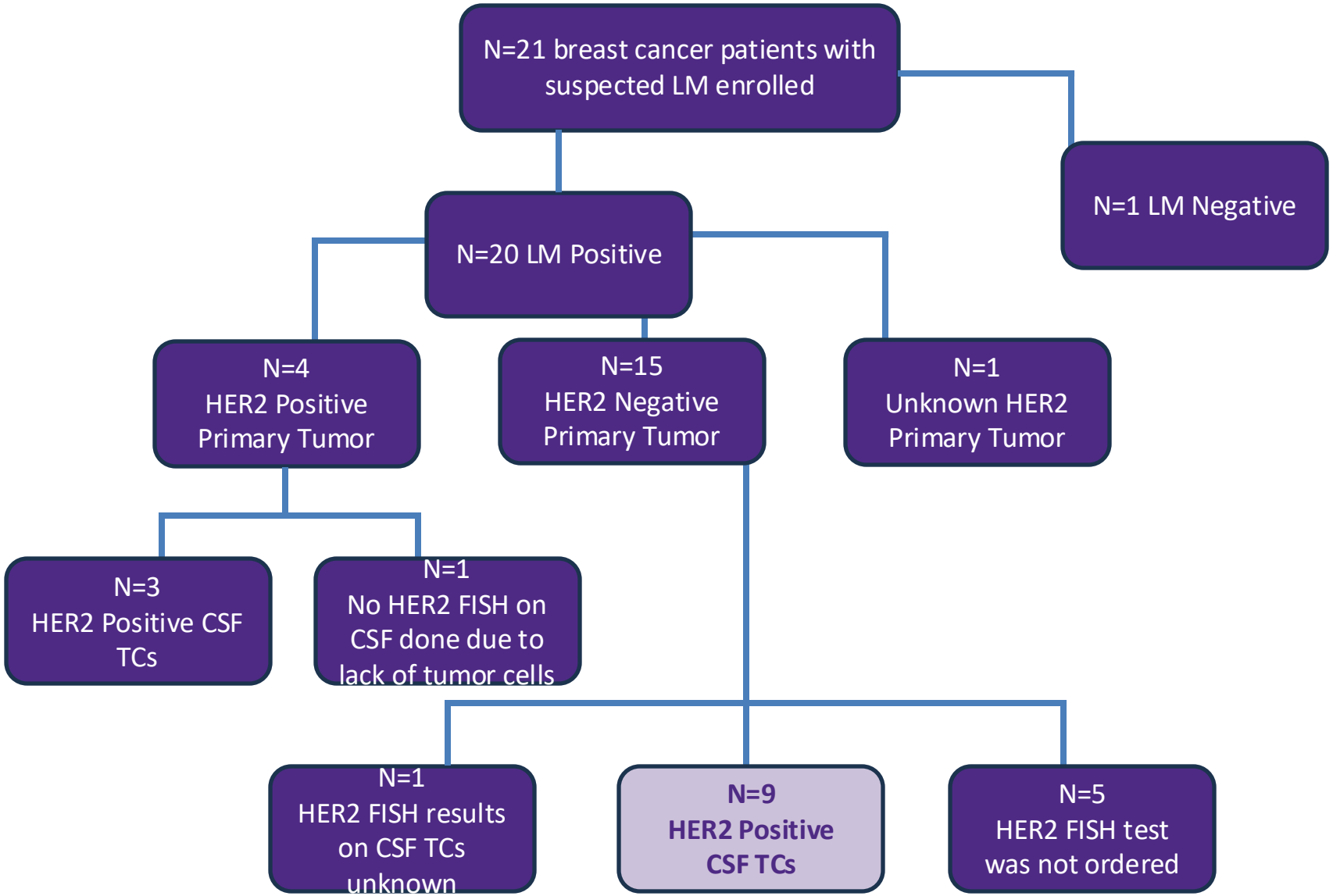
The CNSide Platform: Workflow and Prior Publications

- Uses a 10-antibody cocktail for cell capture
- Followed by biotinylation and passage through a microfluidic device
- Immobilization and FISH and ICC analysis
- HER2 FLIP phenomenon previously presented showing change in HER2 status



Puri et al (Neuro-Oncology Advances, 2023), Kumthekar et al (Frontiers in Oncology 2024)

Primary vs LM HER status: ('HER2 Flip')



Puri et al (Neuro-Oncology Advances, 2023), Kumthekar et al (Frontiers in Oncology 2024)

Does the CNSide Platform reveal other molecular “flips”?

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: Distribution by primary tumor type

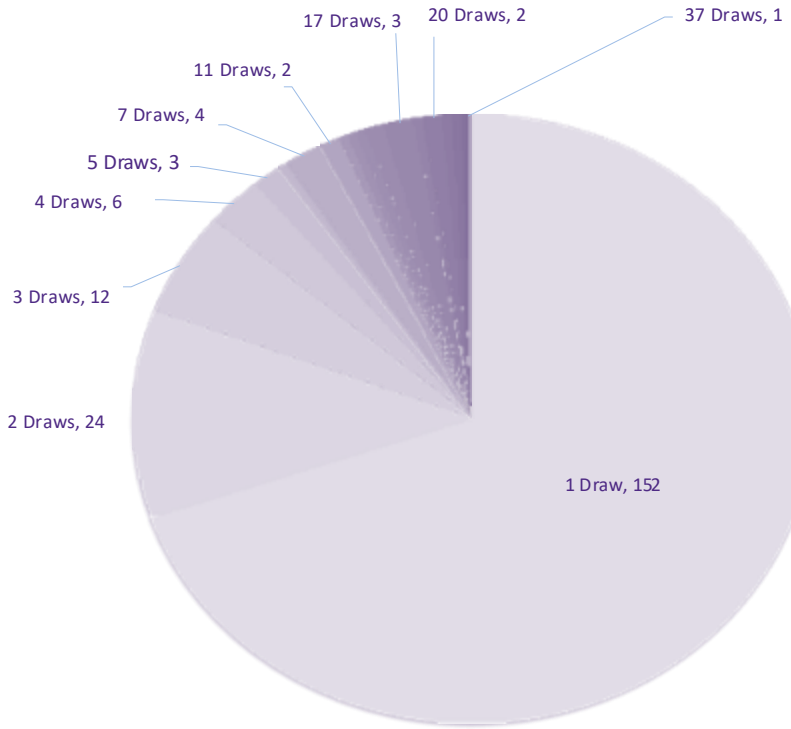
Primary Tumor Type	# of samples	# of Patients
Bladder	1	1
Breast	**294**	105
GI	24	10
Gynecologic	1	2
Head and Neck	6	4
Hepatic	7	1
Lung	**229**	65
Male GU	3	2
Miscellaneous	24	12
Neuroendocrine	3	3
Pancreatic	5	2
Renal	3	3
Skin	13	8
Total	**613**	**218**

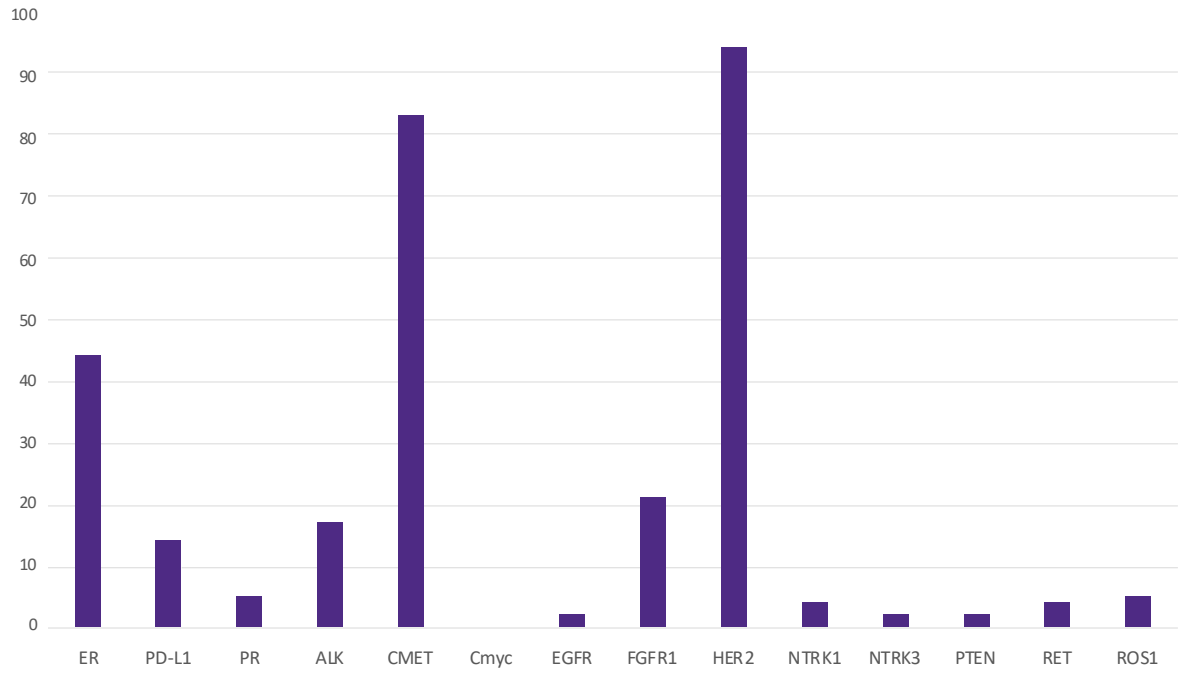
Results: Distribution of analyzed CSF by sampling method and primary vs subsequent detection of cells

	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**

Results

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.

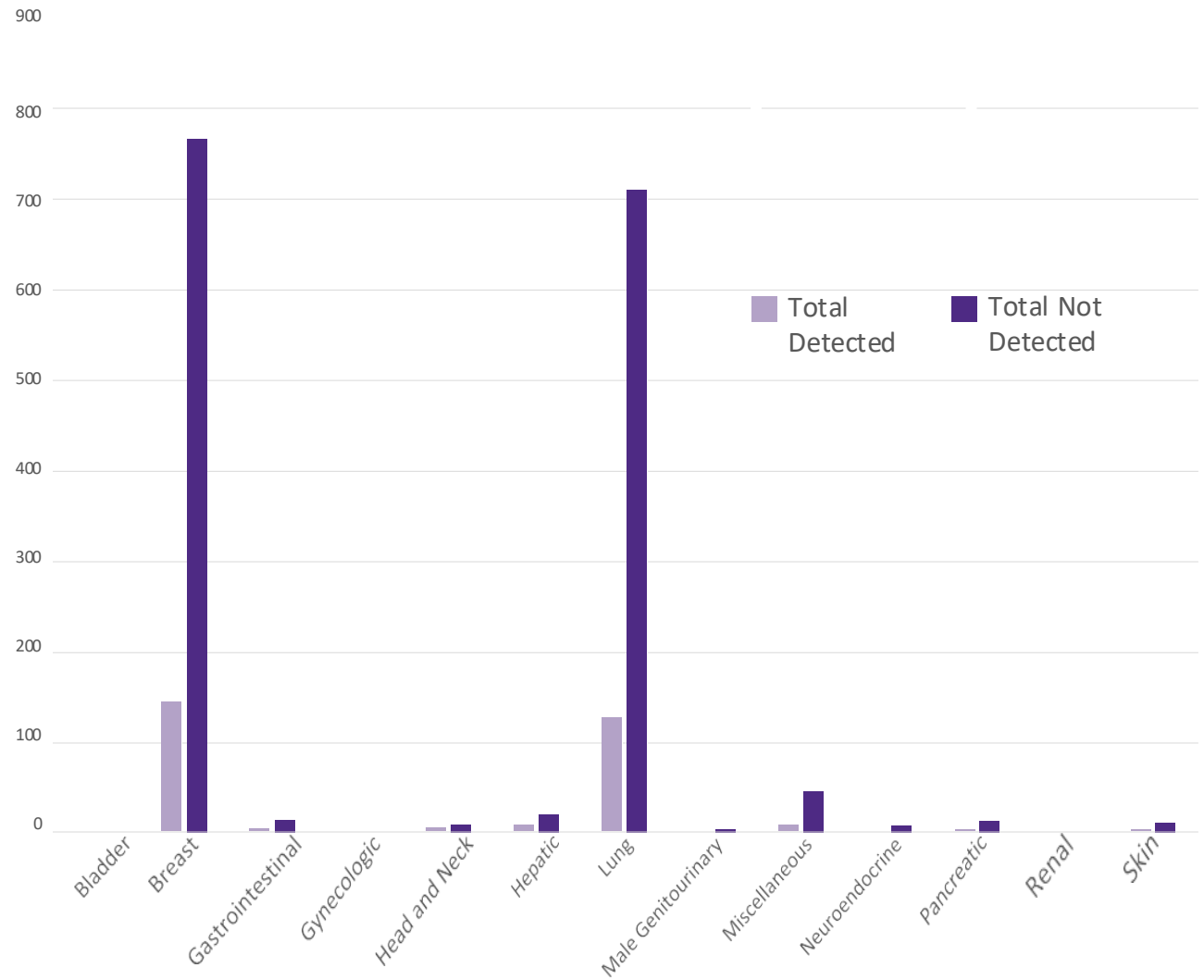




ICC and FISH Probe Detection: The total number of cases in which alterations were detected in each biomarker using ICC and FISH across all samples

Results

	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



Total biomarker detection by primary tumor type "Other" includes: bladder, gastrointestinal, gynecologic, head and neck, hepatic, male genitourinary, neuroendocrine, pancreatic, renal, skin, miscellaneous

Results: Oncogenic “flip” over time by biomarker type

			FLIPS	
Biomarker	# of samples analyzed of patients with >2 CNSide test performed	# 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

- 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)

* 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 can change 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

- Final analysis of the prospective FORESEE clinical trial to determine the impact of CNSide on clinical decisions is ongoing

Acknowledgements

- **Co-authors:**
- Arushi Tripathy, MD
- Perry Corkos
- Barbara Blouw, PhD
- Melissa Moore, PhD
- Norman LaFrance, MD
- Marc Hedrick, MD, MBA
- Michael Youssef, MD
- David Piccioni, MD, PhD
- Seema Nagpal, MD

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Appendix

Authors: Arushi Tripathy, Perry Corkos, Barbara Blouw, Melissa Moore, Norman LaFrance, Marc Hedrick, Michael Youssef, David Piccioni, Seema Nagpal and Priya Kumthekar

INTRODUCTION: Patients with LMD have poor prognosis and limited treatment options. Oncogene amplification of primary, metastatic, and CNS metastatic tumors can be heterogeneous. Therefore, patients with LMD may benefit from assessment of clinically relevant biomarkers in CSF, which may guide the choice of a targeted therapy specifically for the LMD tumor. CNSide is a CLIA-validated laboratory-developed test ordered commercially at the discretion of physicians for CSF-TC enumeration, immunocytochemical (ICC) and fluorescence in situ hybridization (FISH) analysis of oncogene amplification. We longitudinally analyze oncogenes in CSF-TCs in patients with LMD of various primary cancers. METHODS: CSF was collected from patients with suspected or confirmed LMD; 613 tests were ordered on 218 individual patients with breast (N=105 patients), lung (N=65), gastrointestinal (N=10), and other cancers. Using CNSide, CSF-TCs were isolated and tested via ICC (ER, PD-L1, and PR), and FISH (ALK, cMET, cMyc, EGFR, FGFR1, HER2, NTRK1, NTRK3, PTEN, RET, and ROS1). RESULTS: In patients with lung cancer, ALK was detected in 14% (17/118) of samples, CMET in 61% (78/128), HER2 in 73% (16/22), and RET in 4% (4/90). In patients with breast cancer, HER2 was detected in 39% (65/168) of samples, FGFR1 in 32% (19/60), ER in 26% (44/168) and PR in 4% (5/120). 66 patients underwent 2+ CSF draws; 24 underwent 5+. Among these, there were 13 ICC flips (7 acquired mutations) and 58 FISH probe detection flips (26 acquired mutations). 20/66 patients (30.3%) had at least one flip in their ordered biomarkers. CONCLUSION: CNSide can be used to detect oncogene amplification on CSF-TCs of patients with LMD, and mutational status of the LMD tumor may differ from the original tumor biopsy. CSF-TC analysis may provide therapeutic insights that vary from the original tumor and could open the door to additional treatment targets for patients struck with LMD.