

# Diagnosis of leptomeningeal metastasis through identification of circulating tumor cells in cerebrospinal fluid using the TargetSelector™ assay

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## Abstract

**Introduction:** Diagnosis of leptomeningeal metastasis (LM) is limited by low sensitivity of cerebrospinal fluid (CSF) cytopathology. Detecting circulating tumor cells (CTCs) in CSF might be more sensitive. We evaluated if TargetSelector™ (TS), a novel assay that captures CTCs in peripheral blood, detects CTCs in CSF.

**Methods:** We enrolled adults with metastatic solid tumors or primary CNS malignancies and clinical suspicion for LM to undergo lumbar puncture (LP) for CSF cytopathology and TS. TS captured CTCs using a primary 10-antibody mixture, streptavidin-coated microfluidic channel, and biotinylated secondary antibodies. CTCs from patients with metastatic breast cancer (MBC) were assessed for estrogen receptor (ER) expression by fluorescent antibody and HER2 amplification by fluorescent *in situ* hybridization (FISH). CSF cell-free DNA (cfDNA) was extracted for next-generation sequencing (NGS).

**Results:** Fourteen patients, median age 56 years (range, 32–75), underwent diagnostic LP. Primary malignancies were breast (n=10), lung (n=1), colon (n=1), CNS lymphoma (n=1), and glioma (n=1). Among thirteen patients who underwent CSF evaluation by TS, TS had sensitivity of 83% (95% Confidence Interval [CI], 33–100%) and specificity of 86% (95% CI, 42–100%) for LM, defined as positive CSF cytology and/or unequivocal MRI findings. Among MBC patients, concordance of ER and HER2 status between CTCs and metastatic biopsy were 60% and 75%, respectively. NGS of CSF cfDNA identified somatic mutations in three MBC patients, including one with *PIK3CA*p.H1047L in blood and CSF.

**Conclusions:** TargetSelector™ is a viable platform to detect CSF CTCs, with potential use as a diagnostic tool for LM. Additional, larger studies are warranted.

## Introduction

Despite advances in systemic and radiation therapies, LM is associated with a poor prognosis, with median overall survival less than one year [1–3]. The reported incidence of LM among patients with advanced malignancies is as high as 8%, and the most common primary tumors associated with LM are breast, lung, melanoma, non-Hodgkin's lymphoma, and primary CNS malignancies [4, 5]. The incidence of LM is thought to be increasing because of improvement in imaging modalities, longer survival among patients with metastatic malignancies, and poor CNS penetration of many systemic therapies. Recent clinical trials have evaluated the role for intravenous and intrathecal chemotherapy with whole brain radiation therapy [6–8]; however, there is currently no consensus regarding the treatment of LM [9, 10].

Diagnosis of LM has traditionally been based on cytopathologic analysis of cerebrospinal fluid (CSF), with the presence of malignant cells in CSF considered the gold standard. However, CSF cytopathologic analysis has low sensitivity, approximately 50–75% on the first lumbar puncture, and is highly examiner-dependent [11–16]. Repeat, multi-site and high-volume lumbar punctures are often required, which may increase sensitivity up to 80–90%, but are associated with potential complications, treatment delays, and patient discomfort [12, 17]. While brain and spinal magnetic resonance imaging (MRI) has been incorporated into the initial diagnostic evaluation for LM, MRI findings can be equivocal, and unequivocal findings may only appear in late-stage disease [5, 18]. There is therefore an unmet need for a sensitive diagnostic tool for LM that can allow for accurate diagnosis without repeated lumbar punctures.

Novel methods to quantify and characterize circulating tumor cells (CTCs) in the CSF have been evaluated as potential diagnostic modalities for LM. The TargetSelector™ (TS) Cell Enrichment and Extraction microfluidic platform is a novel technology that has been shown to capture and detect CTCs in the peripheral blood, independent of expression of epithelial surface markers such as the epithelial cell adhesion molecule (EpCAM). TS uses a biotin-tagged antibody cocktail against a variety of antigens, including both EpCAM and non-EpCAM epithelial and mesenchymal antigens, and has been shown to more efficiently detect CTCs in the blood than assays utilizing antibodies against EpCAM alone [19–

22]. TS also allows for more detailed phenotypic and genomic characterization of CTCs through use of immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) [20, 23].

In this study, we evaluated the potential application of the TS technology to diagnose LM. We analyzed the CSF samples of patients with metastatic solid tumors or primary CNS malignancies and clinical suspicion for LM using the TS technology, with the hypothesis that TS can detect CTCs in the CSF and is a sensitive tool for the diagnosis of LM. In additional analyses, we performed next-generation sequencing (NGS) cell-free DNA (cfDNA) in the CSF with comparison to NGS on peripheral blood CTCs, and assessed the feasibility of determining hormone receptor and HER2 status on CSF CTCs in patients with metastatic breast cancer using the TS technology.

## Materials And Methods

### Study Cohort

We conducted a prospective study among patients with clinically suspected or confirmed leptomeningeal disease at Columbia University Irving Medical Center (CUIMC) in New York, NY. Patients aged 18 years or older with a histologically proven metastatic solid tumor or primary central nervous system (CNS) malignancy were eligible for enrollment if they were planned for a diagnostic lumbar puncture (LP) for clinical suspicion of LM and/or unequivocal or suspicious findings for LM on magnetic resonance imaging (MRI). Patients were considered to have a definitive diagnosis of LM if they had malignant cells detected by CSF cytopathologic analysis (positive cytology) and/or unequivocal MRI findings. Unequivocal MRI findings were defined as leptomeningeal enhancement with subarachnoid nodules, enhancement in basal cisterns, or enhancement/clumping of nerve roots. Findings such as multiple superficial brain metastases, intraventricular masses, dural enhancement associated with epidural metastasis, or new hydrocephalus was considered suspicious but non-diagnostic.

The study was originally designed to enroll only patients with metastatic breast cancer with an anticipated sample size of 36; however, the trial was amended in July 2018 to allow for patients with any solid tumor or primary CNS histology, due to limited accrual. There were no limitations on the number of systemic therapies received prior to enrollment. Patients who had undergone an LP with malignant cells identified in CSF prior to enrollment were excluded. Patients were recruited from the CUIMC medical oncology and neuro-oncology clinics. The study was approved by the CUIMC Institutional Review Board (AAAQ4761) and written informed consent was obtained from each participant.

For enrolled patients, we collected clinicopathologic data included patient age, sex, primary tumor histology, and prior systemic therapies. Among those patients with metastatic breast cancer, we also reviewed the electronic health record and collected information about the most recent biopsy of a metastatic site for estrogen receptor (ER), progesterone receptor (PR), and HER2 status.

### CSF and Peripheral Blood Collection

Study participants underwent LP and standard CSF assessment at CUIMC consisting of intracranial pressure measurement, CSF protein, glucose, white and red cell analysis, infectious cultures, as well as conventional cytopathology analysis (cytocentrifuge). In addition to standard CSF collection, 10 mL CSF sample was obtained in CEE-Sure™ collection tubes (Biocept Inc., San Diego, CA) for CTC assessment, along with two 10 mL vials of peripheral blood for evaluation of serum CTCs and cfDNA in CEE-Sure™ collection tubes. Samples were delivered to Biocept's laboratory for processing and evaluated for CTCs using TS microchannel technology.

### CSF and Peripheral Blood Analysis

All samples were collected in CEE-Sure™ collection tubes. CSF samples were spun down and approximately 1mL containing the cell pellet was processed for CTC testing and the supernatant used for molecular testing. Following

centrifugation of whole blood, plasma was removed for molecular testing and the buffy coat layer was isolated for CTC testing using a percoll density gradient. CTC testing was performed by incubating with a primary 10-antibody mixture that targets cell surface epitopes, followed by incubation with biotinylated secondary antibody. The primary capture antibody mixture contained anti-EpCAM, tumor-associated calcium signal transducer 2, anti-c-MET, anti-Folate-binding protein receptor, anti-N-Cadherin, anti-CD318, and anti-mesenchymal stem cell antigen. Labeled CTCs are then captured in a streptavidin coated microfluidic channel and stained with a mixture of anti-cytokeratin 17, 18, 19, pan-cytokeratin, CD45, streptavidin and DAPI. CTC enumeration analysis was undertaken and classified either as cytokeratin positive (CK+/CD45-/DAPI+) or cytokeratin negative (SA+/CD45-/DAPI+). CTCs were assessed for ER expression by fluorescent antibody and for HER2 amplification evaluated by FISH. The supernatant from CSF and plasma from peripheral blood was used to extract cell free DNA and RNA. Amplicon-based NGS was performed using the 12 gene TS Breast NGS Panel powered by Oncomine ThermoFisher. Libraries were prepared, templated on the Ion Chef and sequenced using the Ion S5 XL system. Data analysis was performed using the Ion Reporter software (version 5.10).

## Data Analysis

The study was designed to enroll 36 evaluable patients to have 80% power to detect a 25% improvement in the sensitivity to detect leptomeningeal metastasis using CTCs in the CSF by TS vs. standard cytopathologic analysis on the first lumbar puncture (75% vs. 50%) with a two-sided test at a significance level of 0.05. The sample size justification assumed that 90% of patients (N = 32) would have LM. However, given low accrual, the study was ultimately stopped early. Thus, the analyses are descriptive in nature.

Sensitivity and specificity of TS and cytopathology for LM was calculated among patients with CSF samples evaluable for cytopathologic and TS analyses and are reported with respective 95% exact binomial confidence intervals. Patient baseline characteristics were summarized and reported using median and range for continuous variables and frequencies (percent) for categorical variables. For patients with MBC, we evaluated characteristics of CTCs and cfDNA collected from CSF and compared results to CTCs and cfDNA assays collected simultaneously from peripheral blood. These results were reported by patient and summarized among patients. In addition, for those patients with metastatic breast cancer, we assessed and reported concordance of the primary and/or metastatic tumor with receptor ER and HER2 on CTCs and CSF using TS technology.

## Results

Between January 2017 and December 2019, fourteen patients with clinical or radiologic suspicion for LM disease were enrolled and underwent a lumbar puncture (Table 1). Median age at enrollment was 56 years (range, 32–75), and thirteen (93.0%) of the patients were female. Nearly all patients (n = 12, 85.7%) had non-CNS primary tumors, and the majority of patients (n = 10, 71.4%) had metastatic breast cancer (MBC). The patients with non-breast primaries had colon cancer (n = 1), lung cancer (n = 1), CNS lymphoma (n = 1), and glioma (n = 1). Patients had received a median of 2.5 (range 0–8) prior lines of systemic therapy in the advanced/metastatic setting. While all patients had at least clinical concern for leptomeningeal metastases, only six (42.9%) had definitive LM by either CSF cytology only (n = 3), MRI findings only (n = 2), or both (n = 1).

**Table 1**  
**Baseline clinicopathologic characteristics of enrolled patients, n = 14.**

<b>Characteristic</b>	<b>No. of patients (%)</b>
Median age, in years (range)	56 (32–75)
Female sex	13 (93.0%)
Primary tumor	10 (71.0%)
Breast	1 (7.0%)
Lung	1 (7.0%)
Colon	1 (7.0%)
CNS lymphoma	1 (7.0%)
Glioma	
Median number of prior lines of systemic therapy for advanced/metastatic disease (range)	2.5 (0–8)
Definitive leptomeningeal disease	6 (42.9%)
CSF cytology	3 (21.4%)
MRI	2 (14.3%)
Both	1 (7.1%)
CSF evaluable for cytology	13 (93.0%)

The results of CSF analysis by cytopathology and TS are summarized in Table 2. Out of the 14 patients, 6 had definitive LM and 13 underwent CSF analysis using TS. Among the six patients with definitive LM, five had CTCs detected by TS; the one patient with definitive LM but no CTCs detected also had negative cytology in the CSF sample collected on the same date. The sensitivity and specificity of TS for LM were 83% (95% Confidence Interval [CI], 36–100%) and 86% (95% CI, 42–100%), respectively. Among the thirteen patients with evaluable CSF for cytopathologic analysis, the sensitivity and specificity of cytopathology for LM were 60% (95% CI, 15–95%) and 100% (95% CI, 59–100%), respectively. Six patients had CTCs detected in CSF samples by TS, among whom five (83.3%) had MBC and one (16.7%) had CNS lymphoma. Among these six patients, three (50%) had positive CSF cytology, all with MBC; two patients (one with MBC, one with CNS lymphoma) had negative cytology, and one patient (with MBC) did not have evaluable CSF cytology. Of note, one patient with CTCs detected by TS in the CSF did not carry a definitive diagnosis of LM by either cytopathology or MRI.

Table 2  
Patient-level results of cerebrospinal fluid analysis by standard cytopathologic analysis and TargetSelector™, n = 14.

ID	Primary Tumor	CSF cytology on date of TS	TS CSF CTCs	Definitive LM
1	Breast	+	+	Y
2	Breast	Not performed	+	Y
3	Breast	-	-	N
4	Breast	-	-	N
5	Breast	+	+	Y
6	Breast	+	+	Y
7	Breast	-	-	N
8	Breast	-	-	N
9	Breast	-	-	N
10	Breast	-	+	N
11	Lung	-	-	Y
12	CNS lymphoma	-	+	Y
13	Glioma	-	-	N
14	Colon	-	Not performed	N

CSF = cerebrospinal fluid, LM = leptomeningeal metastasis, defined by positive CSF cytology (after enrollment) and/or unequivocal MRI findings

+ = positive for malignant cells or CTCs; - = negative for malignant cells or CTCs

Y = yes, N = no

The results of CSF analysis, including cytology, CTC detection, receptor status, and genomic alterations in cfDNA, for the ten patients with MBC are presented in Table 3. Among the nine MBC patients who underwent both CSF cytology and TS analysis from samples collected at the same time, TS and standard cytopathologic analysis had agreement in eight (88.9%) patients; the one patient in whom there was discordance had negative cytology but CTCs detected. Concordance of receptor status between metastatic biopsy and CSF CTCs was also assessed. Among the five MBC patients with CTCs detected in CSF, ER status was concordant with metastatic tumor tissue in three (60.0%). HER2 status was not able to be assessed in one patient, but was found to be concordant in three of the four evaluable patients (75.0%). Of note, a repeat metastatic biopsy was not required as part of this study; however, most metastatic biopsies used for comparison to CTCs were performed within one year of CSF analysis for this study.

Table 3  
Phenotypic and genomic characterization of CTCs using TargetSelector™ among patients with MBC, n = 10.

ID	CSF cytology at TS collection	TS CSF CTCs	Receptor status on metastatic biopsy (ER/HER2)	TS CSF receptor status (ER/HER2)	ER Status Concordant with TS?	HER2 Status Concordant with TS?	Genomic Alteration in CSF	Genomic Alteration in blood (TS)
1	+	+	-/-	+/-	N	Y	None detected	None detected
2	Not performed	+	-/-	-/+	Y	N	<i>TP53</i> p.K132R	None detected
3	-	-	+/-	N/A	N/A	N/A	None detected	<i>ESR1</i> p.E380Q <i>ESR1</i> p.Y537D <i>TP53</i> p.R273H
4	-	-	unk	N/A	N/A	N/A	None detected	None
5	+	+	-/-	-/-	Y	Y	None detected	<i>TP53</i> p.V272M <i>TP53</i> p.C238Y
6	+	+	+/-	-/-	N	Y	<i>PIK3CA</i> p.H1047L	<i>PIK3CA</i> p.H1047L
7	-	-	unk	N/A	N/A	N/A	None detected	None detected
8	-	-	-/-	N/A	N/A	N/A	Not performed	Not performed
9	-	-	unk	N/A	N/A	N/A	<i>CCND1</i> deletion	<i>PIK3CA</i> p.E542K <i>PIK3CA</i> p.E545K <i>PIK3CA</i> p.E726K
10	-	+	-/-	-/unk	Y	N/A	None detected	None detected

TS = TargetSelector™, CSF = cerebrospinal fluid, ER = estrogen receptor

Y = yes, N = no, + = positive, - = negative, Unk = unknown, N/A = not available

Patients with MBC also underwent analysis for genomic alterations in cfDNA in the CSF and plasma (Table 3). Eight patients had evaluable cfDNA from the CSF for detection of genomic alterations, and analysis identified genomic alterations in cfDNA from three patients, with alterations detected in *TP53*, *PIK3CA*, and *CCND1*. Of note, the patient with *CCND1* deletion detected in the CSF had negative CSF cytology and no CTCs detected in the CSF. Analysis of the cfDNA from plasma in nine patients identified genomic alterations in four, with multiple alterations noted in three patients. Affected genes included *ESR1*, *TP53*, and *PIK3CA*. Notably, a concordant *PIK3CA*p.H1047L mutation were detected in the

CSF and serum of one patient. The remaining patients with alterations noted in the CSF and/or serum did not have concordance.

## Discussion

Our findings support TS as a viable platform for the detection of CTCs in the CSF, with potential use as a sensitive diagnostic tool for LM. Among fourteen patients with metastatic solid tumors or CNS primaries and a clinical and/or radiologic suspicion of LM, TS analysis had a sensitivity of 83% (95% CI, 36–100%) and specificity of 86% (95% CI, 42–100%) for LM. In addition, among patients with MBC and CTCs present in the CSF, ER and HER2 status were concordant between CTCs and primary or metastatic tissue in 60 and 75 percent, respectively. TS was also able to identify genomic alterations in genes including *PIK3CA* in the CSF cfDNA of patients with MBC.

Our findings are in agreement with recent data that an EpCAM-based assay had a sensitivity of 94% and specificity of 100% for LM among patients with metastatic epithelial malignancies, compared with a sensitivity of 76% for standard cytopathologic analysis [24]. Epithelial cell adhesion molecule (EpCAM) is a surface antigen on epithelial malignancies, and EpCAM assays such as CellSearch have been developed to detect CTCs in the CSF with reported sensitivities of 76–100% [14–17, 20–22]. However, the use of EpCAM assays is limited to metastatic epithelial malignancies, and also carries a theoretical risk of false-positive results from CSF contamination with skin epithelial cells during lumbar puncture [21, 22]. In addition, loss of epithelial markers can occur during epithelial-mesenchymal transition, which is thought to be a key step in the development of metastasis and could result in decreased detection of CTCs [23]. The potential advantage of TS over EpCAM-based assays is its incorporation of non-epithelial antigens, which could expand its application to non-epithelial malignancies, which our data with TS CTC detection in the CSF of a patient with primary CNS lymphoma supports. Future larger, prospective studies are therefore warranted to further compare the performance of TS to cytopathologic analysis in the diagnosis of LM, including among patients with non-epithelial malignancies.

Among the patients with MBC, we also evaluated whether ER expression and HER2 status could be determined from CSF CTCs. We found that ER and HER2 status were concordant between CTCs and metastatic biopsy in 60 and 75 percent of patients with CTCs detected, respectively. Among those with discordant ER and HER2 status, possible explanations include that the metastatic biopsy used for comparison was not consistently performed at the time of CSF sampling and also that receptor conversion due to clonal evolution might have occurred during the course of metastatic disease. Receptor conversion for hormone receptors and HER2 occurs frequently in the course of disease progression in breast cancer, particularly in the CNS [25]; approximately 36% of patients with MBC to the CNS have discordance in receptor status between brain metastases and primary tumor, with the majority of this subset also showing discordance between brain and extracranial metastases [26]. We demonstrated that ER and HER2 status could be determined from CSF CTCs collected using TS with high concordance with other metastatic sites, similar to our previous findings using TS to detect CTCs in the peripheral blood of patients with MBC [23], and supporting further evaluation of its potential application in patients with MBC and confirmed or suspected LM. Characterization of hormone receptor and HER2 status in the CSF in these patients could guide treatment decisions. For example, a patient with MBC confined to the leptomeninges without another site accessible to biopsy, the ability to evaluate these markers would be critical to guide systemic therapies.

Lastly, we also demonstrated that NGS of cfDNA in the CSF using TS can identify potentially actionable mutations among patients with MBC, including one patient with the same *PIK3CA* sequencing variant identified in CSF and blood. Given that the identification of variants in *PIK3CA* and other genes can have treatment implications in MBC [27], TS could have clinical utility among patients with CNS metastatic disease. Similarly, previous studies using EpCAM-based assays and NGS have identified actionable mutations in the CSF of patients with metastatic malignancies, including *EGFR* mutations in non-small cell lung cancer [14, 24]. Further studies using TS should evaluate its ability to detect genomic variants in the CSF of patients with non-breast malignancies.

Limitations of our study include its small number of enrolled patients at a single academic institution, which might limit its generalizability as well as statistical comparison of the sensitivity and specificity of TS to standard cytopathologic analysis for LM. Our cohort also predominantly included patients with metastatic breast cancer, and so requires further investigation in patients with other cancers.

In conclusion, TS is a viable platform for the detection of CTCs in the CSF with high sensitivity for LM, and among patients with MBC, TS allowed for phenotypic and genomic characterization through evaluation of ER and HER2 status as well as NGS for identification of potential actionable mutations in the CSF. Future larger prospective studies are warranted to investigate TS as a diagnostic tool for LM among patients with metastatic solid tumor and primary CNS malignancies.

## Declarations

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**Conflict of Interest:** MW, JEM, KF, LEF, SL, DC, MKA, MST, DLH, KDC, FI, MRW, AH declare no conflict of interest. KK declares the following potential conflicts of interest: Medical Advisor - Immunomedics, Pfizer, Novartis, Eisai, Eli-Lilly, Amgen, Immunomedics, Merck, Seattle Genetics, and Astra Zeneca; Institutional Support - Immunomedics, Novartis, Incyte, Genentech/Roche, Eli-Lilly, Pfizer, Calithera Biosciences, Acetylon, Seattle Genetics, Amgen, Zentalis Pharmaceuticals, and CytomX Therapeutics; Speakers Bureau – Eli-Lilly; Spouse - Array Biopharma, Pfizer, Grail. TK declares the following potential conflicts of interest: employment by Novartis. ABL declares the following potential conflicts of interest: personal fees and non-financial support from Bioclinica, Sapience, Novocure, Forma, NW Biotherapeutics, WebMD, Celgene, prIME Oncology, Physicians' Education Resource, Abbott Molecular; grants, personal fees and non-financial support from Karyopharm, QED, Bayer, Orbus, Agios, AbbVie;; grants and non-financial support from Kadmon, VBI, Beigene, Oncoceutics, Pfizer, Genentech/Roche, Millenium, Celldex, Novartis, BMS; non-financial support from Tocagen, Aeterna Zentaris. RDS, LH, ES, DF, JAM report the following disclosure: current employment by Biocept. VS reports the following disclosure: former employment by Biocept.

**Ethics approval:** The study was approved by the CUIMC Institutional Review Board (AAAQ4761)

**Consent to participate:** Written informed consent was obtained from each participant prior to enrollment.

**Consent for publication:** Patients signed informed consent regarding publishing their data

**Availability of data and material:** Authors can confirm that all relevant data are included in the article and/or its supplementary information files.

**Authorship statement:** Study design and conceptualization: KK, SL. Acquisition of data: KK, DC, TK, MW, and JEM. Analysis and Interpretation of the data: MW, JEM, KF, LF, SL. The first draft of the manuscript was performed by MW, JEM, and VMS. All authors critically revised subsequent versions of the manuscript. MW and JEM were responsible for compiling the final draft. All authors read and approved the final draft.

**Previous Presentations:** Data in this manuscript have been presented at the annual American Society of Clinical Oncology (ASCO) virtual meeting, May 29-31, 2020.

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