Official Title: Pilot study evaluating the utility of OncoCEETM (Cell Enrichment and Extraction) technology, a novel immunocytochemical microfluidic device, in the diagnosis of leptomeningeal metastasis (LM) from solid tumors through identification of circulating tumor cells (CTCs) in cerebrospinal fluid (CSF)

Unique Protocol ID: AAAQ4761

Registration Identifier: NCT02948751

Document Date: July 17, 2018

Columbia University Medical Center
Herbert Irving Comprehensive Cancer Center

TITLE Pilot study evaluating the utility of OncoCEE™ (Cell Enrichment and Extraction) technology, a novel immunocytochemical microfluidic device, in the diagnosis of leptomeningeal metastasis (LM) from solid tumors through identification of circulating tumor cells (CTCs) in cerebrospinal fluid (CSF).

PRINCIPAL INVESTIGATOR

Kevin Kalinsky, MD, MS
Assistant Professor of Medicine
Columbia University Medical Center
Tel: 212-305-0178
E-mail: kk2693@columbia.edu

Teri Kreisl, MD
Assistant Professor of Neurology
Columbia University Medical Center
Tel: 212-342-0571
E-mail: tnk2109@columbia.edu

FUNDING AGENCY

NIH R03 (1 R03 CA208547-01)
Biocept, Inc

Protocol Synopsis

Title	Pilot study evaluating the utility of OncoCEE™ (Cell Enrichment and Extraction) technology, a novel immunocytochemical microfluidic device, in the diagnosis of leptomeningeal metastasis (LM) from solid tumors through identification of circulating tumor cells (CTCs) in cerebrospinal fluid (CSF).
Short Title	Microfluidic device to diagnose leptomeningeal metastasis in solid tumors
Study Duration	Approximately 24 months
Study Center(s)	Single center
Primary Objectives	Determine whether the microfluidic device will demonstrate a greater sensitivity for the detection of leptomeningeal metastasis (LM) in solid tumors as compared to standard cytopathologic analysis
Number of Subjects	46
Diagnosis and Main Inclusion Criteria	Patients with solid tumors who are undergoing lumbar puncture for suspicion of leptomeningeal metastasis
Statistical Methodology	We anticipate that using OncoCEE will result in a 25% improvement in the sensitivity to detect leptomeningeal metastasis vs. standard cytopathologic analysis on the first lumbar puncture (75% vs. 50%). We will have 80% power to detect this difference on the first lumbar puncture in 36 evaluable patients with unequivocal or suspicious findings (two-sided alpha, 0.05). In addition, we will accrue 10 patients with positive CSF fluid to compare OncoCEE in patients with definitive LM.

Contents

1. INTRODUCTION

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Columbia University Medical Center institutional research policies and procedures.

2. STUDY OBJECTIVES

2.1 Primary Objective

Determine whether OncoCEE^{TM} Cell Enrichment and Extraction technology, a novel immunocytochemical microfluidic device, will demonstrate improved sensitivity in the diagnosis of leptomeningeal metastasis (LM) from solid tumors through identification of circulating tumor cells (CTCs) in the cerebrospinal fluid (CSF) as compared to standard cytopathologic analysis.

2.2 Secondary Objective(s)

- Assess the feasibility of determining estrogen, progesterone and HER2 receptor status for breast cancer patients on the CSF CTCs collected by OncoCEE^{TM} technology
- Assess concordance between the receptor status of the primary and/or metastatic breast tumor, and that of the leptomeningeal cells collected by OncoCEE^{TM} technology
- Explore characteristics of the CTCs and cell free DNA collected from CSF and compare them to CTCs and cell free DNA collected simultaneously from peripheral blood. Examples include BRAF mutations in melanoma and EGFR mutations in non-small cell lung cancer.
- Explore the performance of OncoCEE^{TM} technology and standard cytopathology in diagnosing LM within 2 subgroups: patients with unequivocal MRI findings of the CNS and those with suspicious MRI findings of the CNS
- Explore the yield of checking for CSF CTCs and cell free DNA from CSF in individuals who have had an initial negative LP by standard cytopathologic and CTC analysis, for whom an additional LP is otherwise clinically warranted

3. BACKGROUND

Leptomeningeal Disease

Leptomeningeal metastasis (LM) is a condition in which cancer cells seed the meninges and may go on to invade the brain parenchyma, spinal cord, cranial nerves or peripheral nerves [1]. It is a devastating complication in solid tumors , and is often considered in the differential diagnosis when patients with

cancer present with new neurologic symptoms [2]. The incidence varies by tumor subytpes. For instance, it was previously thought to be a rare occurrence in breast cancer, but autopsy series have shown the true overall incidence to be up to 8% [3]. In fact, while the incidence of meningeal metastasis from other malignancies has decreased, the opposite is true of breast cancer, in which clinical evidence suggests an increasing incidence.

Diagnosing Leptomeningeal Metastasis

Diagnosing LM can be difficult, particularly at early stages. The diagnosis has traditionally been based on CSF cytologic analysis and more recently has incoprorated MRI findings upfront [4]. Brain and spine MRIs have been increasingly preferred for the initial evaluation of LM because of their noninvasive nature and convenience to patients. However, MRI findings can be equivocal, and unequivocal findings may only appear in late-stage disease. CSF cytopathologic analysis provides diagnostic confirmation of LM, but is associated with a relatively low sensitivity (approximately 50% on the first lumbar puncture) and is highly examiner-dependent [5, 6]. Repeat, multi-site and high volume lumbar punctures are often required [7], which may increase sensitivity up to 90%, but are associated with complications, treatment delays, and patient discomfort.

Peripheral Blood CTC's

Analysis of peripheral blood CTCs has been explored as a prognostic marker of disease and response to anticancer treatments in breast cancer [8]. Some studies have suggested that blood CTC enumeration may correlate with tumor burden and anticipate tumor progression [9]. Moreover, blood CTCs have been used to characterize genetic and immunophenotypic changes over time, with the ultimate goal of guiding the management of targeted, individualized therapies [10]. However, CTCs are extremely rare in the blood compared to normal (bystander) blood cells, and are found in ratios as low as one cell per one billion; therefore, isolating these cells has been a challenge [11]. The most successful isolation techniques have been immunocytochemical technologies that label CTCs for separation based on unique surface antigens that distinguish them from normal bystander cells.

OncoCEE^{TM} (Cell Enrichment and Extraction) microfluidic platform and CSF CTCs

OncoCEE^{TM} Cell Enrichment and Extraction is a novel technology that has been shown to more efficiently capture and detect CTCs utilizing biotin-tagged antibodies that bind selectively to CTCs. The antibodies are introduced into a suspension of blood cells intending that only CTCs will then display surface biotin molecules. Next, the cell suspension is passed through a microfluidic channel that contains about 9000 transverse, strategically placed streptavidin coated posts. A CTC making contact with a post has the opportunity to engage in a biotin-streptavidin reaction that immobilizes the cell, and creates an enriched sample. Standard marker and FISH analysis of the cells can be completed within the device. Bystander blood cells remain in suspension and pass through the channel. We hypothesize that such methodology can also be used to isolate CTCs in the CSF and diagnose LM in solid tumors with an increased sensitivity over standard cytopathologic analysis.. We are initiating a pilot study to evaluate the potential of this technology in this setting.

4. STUDY DESIGN

4.1 General Design

This study will prospectively enroll 36 evaluable subjects with solid tumors who are undergoing workup for clinical suspicion of leptomeningeal metastasis (LM) for unequivocal or suspicious findings by imaging or clinical determination. Neuroimaging consisting of MRI of the brain or total spine (or both, as clinically indicated) will be obtained in all patients. Patients will also undergo a lumbar puncture and standard CSF evaluation, which may consist of intracranial pressure measurement, CSF protein, glucose, white and red cell analysis, infectious cultures, as well as conventional cytopathologic analysis (cytocentrifuge). An additional CSF sample will be obtained for evaluation of CSF CTCs by OncoCEE^{TM} technology and cell-free DNA (recommended amount: 1 tube, 10 mL) at the time of lumbar puncture.

We will define LM as previously described [14]. Patients will be considered to have a definitive diagnosis of LM if they have a positive CSF cytology.. We will accrue 10 patients with positive cytology. Unequivocal MRI findings will be 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 will be considered suspicious but nondiagnostic. Patients with unequivocal or suspicious findings on imaging or clinical findings (without known CSF positivity) will be eligible for this study (n=36).

5. SUBJECT SELECTION AND WITHDRAWAL

5.1 Inclusion Criteria

- Adult (18 years or older) patients, with any solid tumor type, of all racial and ethnic origins
- Undergoing lumbar puncture for clinical or radiographic suspicion of leptomeningeal metastasis
- Provide study-specific informed consent
- Patients with unequivocal or suspicious MRI findings.
- Of those with a definitive diagnosis of LM (i.e. positive CSF cytology), 10 evaluable patients will be accrued.

5.2 Exclusion Criteria

- Prior CSF fluid which identified malignant cells after 10 evaluable patients with positive CSF are accrued.

5.3 Inclusion of Women and Minorities

Women of all races and ethnic groups are eligible for this trial.

5.4 Subject Recruitment

Patients with solid tumors who are undergoing lumbar puncture for suspicion of leptomeningeal metastasis will be screened by the treating clinical staff for eligibility. Upon providing informed consent, we will be notified so that preparations can be made for study inclusion. Clinical research coordinators

will be allowed to consent study patients.

At the time of obtaining consent, consent will be requested for blood tests to check peripheral blood CTC's and circulating DNA (2 tubes, 10 cc mL each), as well as consent for additional CSF from subsequent taps, to be performed if clinically indicated, usually in the setting of an initially negative tap.

Patient visits, lumbar punctures, CSF collection, clinical evaluation and data reporting will take place at Columbia University Medical Center. CSF collection from 46 eligible breast cancer subjects is estimated to take approximately 24 months.

Study Calendar

	Pre-Enrollment	Lumbar Puncture (LP) #1	LP #2 (if clinically indicated, i.e. previously negative tap)	LP #3 (if clinically indicated, i.e. previously negative tap)	Clinical Follow-up (2 and 6 month after LP #1)
Informed Consent	X				
Inclusion/Exclusion	X				
Standard Cytologic Assessment (CSF)		X	X	X	
OncoCEE (CSF)		X	X	X	
OncoCEE (peripheral blood)		X	X	X	
Assess Report of Metastatic Tumor (or Primary, if not available) – ER/PR/HER2, if breast cancer, as well as mutational profile for solid tumors		X			
Patient Status (unequivocal vs. suspicious for LM)	X				X[a]

[a] Patients will be followed at two additional time periods after LP #1: 2 (+/- 1 month) and 6 months (+/- 1 month) after LP #1. The goal of this is to determine LM status: unequivocal vs. suspicious and whether this changes pver time (i.e. suspicious to unequivocal). If the patient is not able to come in, communication with the patient over the phone is acceptable (but not ideal).

5.5 Early Withdrawal of Subjects

5.5.1 When and How to Withdraw Subjects

Patients may elect to withdraw from the study at any time by informing the Principal Investigator or Sub-Investigator.

5.5.2 Data Collection and Follow-up for Withdrawn Subjects

As this protocol does not involve any change in standard of care being delivered to the patients, no additional follow up is required for patients who elect to withdraw consent.

Each patient who consents to participate in the study will be assigned a unique study ID. The Principal Investigator or Sub-Investigator will collect pertinent data for each patient on Case Report Forms (CRFs). Information collected will include: demographic, pathologic, radiographic and specimen collection data.

6. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

6.1 Adverse events

This protocol does not require any additional tissue sample collection than that which would be standard of care, except for the additional tube of CSF collected during standard collection procedures during routine investigation of LM. Experimental data from this research will not influence the therapeutic decisions. The treating physician will not be informed of the result. The rate of adverse events is expected to be very low.

Risk will primarily be attributed to the additional CSF required for the study; withdrawing excess amounts of CSF can induce nausea, positional headache or light headedness.

6.1.1 Unanticipated Problem

An unanticipated problem is any incident, experience or outcome involving risks to subjects or others in any human subjects research that meets all of the following criteria:

- Unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are described in the IRB-approval protocol and informed consent document, and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in such research (e.g., there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in such research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic or social harm) than was previously known or recognized.

6.2 Recording of Adverse Events

As this trial is not an interventional/therapeutic study, we will not be recording adverse events. Only events qualifying as UPs will be reported to the CUMC IRB according to institutional policy/procedures.

7. DATA REPORTING / REGULATORY REQUIREMENTS

7.1 Data Collection

The Herbert Irving Comprehensive Cancer Center has an electronic clinical trials and data management system that will be used for data collection. CRFs for the study will be built into Velos for data entry. The system has full auditing capabilities which is web-based and housed on a server in a fully HIPAA compliant server room with restricted access and video camera monitoring. All users must login with their own application username and password. Users off campus must first access the Virtual Private

Network with their assigned campus username and password and then use their application credentials. Users are only able to see study information if they are indicated as study personnel in our electronic IRB system. Users are limited to access based on the role assigned in their corresponding protocol. Subject data is entered directly into the system, which (in the case of Columbia subjects) confirms the correct identity of patients via an interface with the electronic medical patient index. Staff with the appropriate IRB defined roles can run reports within the system for reporting purposes.

7.2 Quality Control and Quality Assurance

The PI and study team will perform routine quality reviews to ensure the protocol is being executed in compliance with the IRB approved procedures.

7.3 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (e.g., that the subject is alive) at the end of their scheduled study period.

The subject binders will be maintained with in the CPDM offices, a secured floor within the Herbert Irving Pavilion and only the investigator and study staff will have access to the file.

7.4 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

7.5 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is

left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A".

7.6 Records Retention

Records relating to a specific research activity, including research records collected by investigators, must be maintained for at least three years after completion of the research (45 CFR 46.115(b); 21 CFR 56.115(b); 21 CFR 312.62). This minimum retention period applies whether or not any subjects were enrolled in the study.

If the research is FDA regulated, records should be retained for at least two years after approval of the investigational agent by FDA; if it is not approved, records should be retained at least two years after the study is terminated and FDA is notified (note the additional requirement below for clinical research studies);
Clinical records, including consent forms that document clinical intervention or clinical diagnostic procedure research-related procedures, must be retained in medical records by the institution for at least seven years, per CUMC and NYP policy which is based on state law.

8. STATISTICAL CONSIDERATIONS

8.1 Study Design/Endpoints

CSF cytopathologic analysis is the gold standard in providing diagnostic confirmation of LM, but is associated with a relatively low sensitivity (approximately 50% on the first lumbar puncture) and is highly examiner-dependent. Repeat, multi-site and high volume lumbar punctures are often required, which may increase sensitivity up to 90% [7], but are associated with complications, treatment delays, and patient discomfort. We anticipate that using OncoCEE will result in a 25% improvement in the sensitivity to detect leptomeningeal metastasis vs. standard cytopathologic analysis on the first lumbar puncture (75% vs. 50%). We will have 80% power to detect this difference on the first lumbar puncture in 36 evaluable patients (two-sided alpha, 0.05) in patients with unequivocal or suspicious findings radiographically or clinically for LM. No false positives are assumed with this method, as seen with pilot data in peripheral blood (A.3). With this population, we anticipate that the prevalence of LM, defined as positive CSF cytopathologic analysis on lumbar puncture at any time point, will be approximately 90% (i.e. 32 out of 36 patients). In addition, we will accrue evaluable 10 patients with positive CSF fluid to compare OncoCEE in patients with definitive LM. Thus, the total sample size for the study witll be 46 patients.

8.2 Size/Accrual Rate

Based on an anticipated accrual rate of 1-2 subjects / month, we anticipate a 24 month enrollment period in order to accrue 46 subjects.

8.3 CSF Analysis (Mandatory)

Patients will undergo a lumbar puncture and standard CSF evaluation, which may consist of intracranial pressure measurement, CSF protein, glucose, white and red cell analysis, infectious cultures, as well as conventional cytopathology analysis (cytocentrifuge). Standard CSF evaluation will be performed at

Columbia University Medical Center. An additional 10 mL CSF sample will be obtained for evaluation of CSF CTCs in a CEE-Sure™ collection tube (Biocept Inc., San Diego, CA); this sample will be to delivered to Biocept's laboratory for processing, and it wll be evaluated for CTCs using OncoCEE™ microchannel technology. This CSF sample will also be tested for cell-free circulating tumor DNA (ctDNA). Depending on available technology, we may explore Next-Generation DNA sequencing to evaluate the sample for somatic mutations.

OncoCEE™ microchannels are manufactured at Biocept, Inc. (San Diego, CA). The cells will be run through the microchannel, captured using an antibody cocktail, and stained with a mixture of anti-cytokeratin antibodies labeled with AlexaFluor-488. Cells will be simultaneously stained with anti-CD45 labeled with AlexaFluor-594. ER/PR ICC will be performed using anti-ER (Abcam,Cambridge, MA) and anti-PR (Epitomics Inc, Burlingame, CA) monoclonal rabbit antibodies and secondary anti-Rabbit antibody labeled with AlexaFluor-546. The microchannels will undergo microscopic analysis for enumeration of CK+/CD45-/DAPI+ (CTC identification), CK-/CD45+/DAPI+ (background white blood cells), and all CK+ cells will be assessed for ER/ PR/HER2 positivity, if breast cancer. The microchannels will undergo immediate manual microscopic analysis for enumeration of CTCs and assessment of ER/PR followed by taking images and X/Y coordinates recorded using Olympus Bx51 fluorescent microscopes equipped with appropriate filters and the Metasystems imaging system v5.2 (Metasystems GmbH, Germany)[10, 15]. Depending on available technology, we will explore Next-Generation DNA sequencing to evaluate the CSF for somatic mutations. The samples will be stored at Biocept for potential future testing, which may include the assessment of somatic alterations. No germline testing will be performed.

8.4 Peripheral Blood Analysis (Mandatory)

Patients will also undergo venipuncture to withdraw two 10 mL vials of peripheral blood for analysis. These samples will be delivered to Biocept's laboratory for processing, and they wll be evaluated for CTCs using OncoCEE™ microchannel technology. These blood samples will also be tested for cell-free circulating tumor DNA (ctDNA). Depending on available technology, we may explore Next-Generation DNA sequencing to evaluate the samples for somatic mutations. The samples will be stored at Biocept for potential future testing, which may include the assessment of somatic alterations. No germline testing will be performed.

8.5 **Tumor Tissue (Optional)**

If Next-Generation DNA sequencing is performed on CSF and/or peripheral blood (ctDNA), we are asking patients for the option of assessing their tumor tissue (metastatic biopsy or primary tumor, if metastatic tissue not available) for somatic mutations. Germline testing will not be performed.

9. PROTECTION OF HUMAN SUBJECTS

This study is to be conducted in accordance with applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be obtained before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, as outlined in the IRB approved protocol, and the investigator-designated research professional obtaining the consent. All health information, cerebrospinal fluid, and blood samples will be de-identied.

10. CONSENT: LEGAL AUTHORIZED REPRESENTATIVE IF PATIENT WITHOUT CAPACITY

The disease studied in this protocol can cause physical or cognitive incapacity to consent using routine procedures. Therefore, if it appears to the study physician that a potential subject lacks cognitive capacity to understand the ICF, then a physician not associated with the study will be consulted. If the independent consultant agrees, then an appropriate legally authorized representative (LAR) will be identified in accordance with CUMC IRB Informed Consent Policy (CUIRB Policy, Informed Consent, 26Oct2013, Section 4.F) and New York State Law. The LAR will sign the IRB-approved ICF and HIPAA authorization in lieu of the subject. If the potential subject regains capacity during the study in the opinion of both the study physician and an independent consulting physician, then the study procedures performed to date will be discussed with the subject who will provide re-consent before continuing study related procedures per IRB policy. If the subject has cognitive capacity to consent but physical inability to provide a signature, then per CUMC IRB SOP V4.2-Nov.2,2012, section V.B.4.a7, the Investigator will conduct the informed consent discussion with the subject who will make his/her mark on current IRB-approved ICF and HIPAA, and such mark will be co-signed and dated by the LAR or an impartial witness. In all such circumstances, the consenting professional will document the consent process in the medical record, and co-sign and date the ICF.

11. RESOURCE SHARING PLAN

Data Sharing Plan: In order to fulfill the requirement of sharing the data obtained in the research program, we plan to create a website where the data acquired will be made available to the scientific community. Through this website, colleagues from other research centers will be able to read about the general information of the proposal, including its objectives and aims. The website will be updated on a regular basis (at least quarterly) to include the advances achieved during the research activities and new results obtained from data processing and statistical analysis. Hyperlinks pointing to other web pages with information related to the program will also be available on our website. These hyperlinks will be divided in those with information for patients and those with information for researchers.
The website section specifically made for researchers will include a list of scientific meetings where the

data was or will be presented as well as a list of published scientific manuscripts describing the results of the proposal. Researchers will be able to download abstracts presented and manuscript published that relate to our research program. Because of patient protection and confidentiality, all presented and published data, including breast optical imaging and clinicopathologic features, will be de-identified so as not to trace the data back to the patient.

12. STUDY FINANCES

12.1 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the Columbia University Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved prior to participation in this study. All CUMC investigators will follow the University conflict of interest policy.

12.2 Subject Stipends or Payments

There are no subject payments or stipends.

12.3 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor.

13. REFERENCES

1. DeAngelis, L.M., J.B. Posner, and J.B. Posner, *Neurologic complications of cancer*. 2nd ed. Contemporary neurology series. 2009, Oxford ; New York: Oxford University Press. xv, 634 p.
2. Taillibert, S., et al., *Leptomeningeal metastases from solid malignancy: a review.* J Neurooncol, 2005. **75**(1): p. 85-99.
3. Posner, J.B. and N.L. Chernik, *Intracranial metastases from systemic cancer.* Adv Neurol, 1978. **19**: p. 579-92.
4. Clarke, J.L., et al., *Leptomeningeal metastases in the MRI era.* Neurology, 2010. **74**(18): p. 1449-54.
5. Wasserstrom, W.R., J.P. Glass, and J.B. Posner, *Diagnosis and treatment of leptomeningeal metastases from solid tumors: experience with 90 patients.* Cancer, 1982. **49**(4): p. 759-72.
6. Glass, J.P., et al., *Malignant cells in cerebrospinal fluid (CSF): the meaning of a positive CSF cytology.* Neurology, 1979. **29**(10): p. 1369-75.
7. Glantz, M.J., et al., *Cerebrospinal fluid cytology in patients with cancer: minimizing false-negative results.* Cancer, 1998. **82**(4): p. 733-9.
8. Riethdorf, S., et al., *Detection of circulating tumor cells in peripheral blood of patients with*

metastatic breast cancer: a validation study of the CellSearch system. Clin Cancer Res, 2007. **13**(3): p. 920-8.
9. Cristofanilli, M., et al., *Circulating tumor cells, disease progression, and survival in metastatic breast cancer.* N Engl J Med, 2004. **351**(8): p. 781-91.
10. Kalinsky, K., et al., *Correlation of hormone receptor status between circulating tumor cells, primary tumor, and metastasis in breast cancer patients.* Clin Transl Oncol, 2015.
11. Allard, W.J., et al., *Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases.* Clin Cancer Res, 2004. **10**(20): p. 6897-904.
12. Park, I.H., et al., *Concordant HER2 status between metastatic breast cancer cells in CSF and primary breast cancer tissue.* Breast Cancer Res Treat, 2010. **123**(1): p. 125-8.
13. Magbanua, M.J., et al., *Molecular profiling of tumor cells in cerebrospinal fluid and matched primary tumors from metastatic breast cancer patients with leptomeningeal carcinomatosis.* Cancer Res, 2013. **73**(23): p. 7134-43.
14. Nayak, L., et al., *Rare cell capture technology for the diagnosis of leptomeningeal metastasis in solid tumors.* Neurology, 2013. **80**(17): p. 1598-605; discussion 1603.
15. Nora Dickson, M., et al., *Efficient capture of circulating tumor cells with a novel immunocytochemical microfluidic device.* Biomicrofluidics, 2011. **5**(3): p. 34119-3411915.