

Cerebrospinal Fluid Cell-Free DNA in Patients with CNS Metastases

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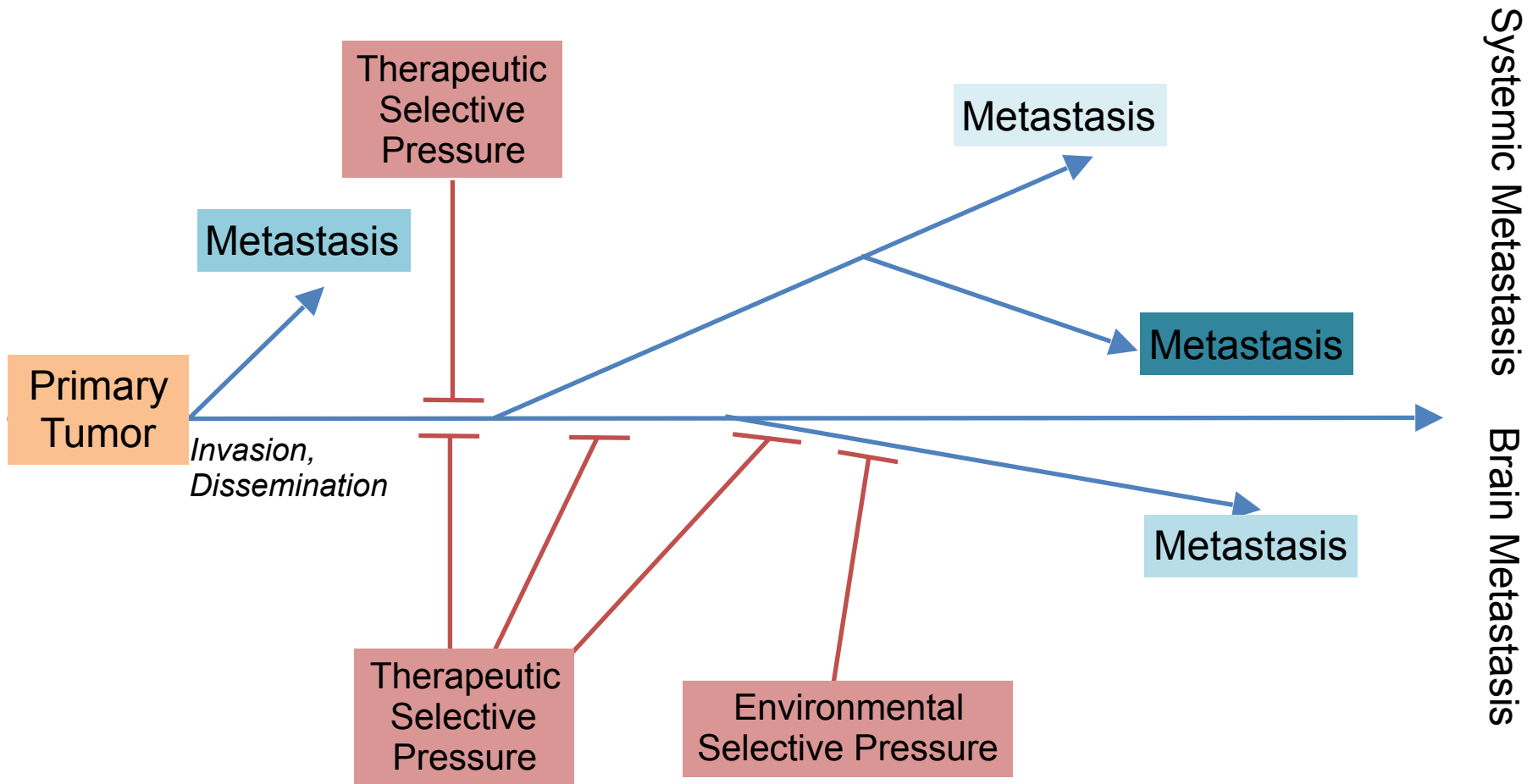
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September 21, 2018

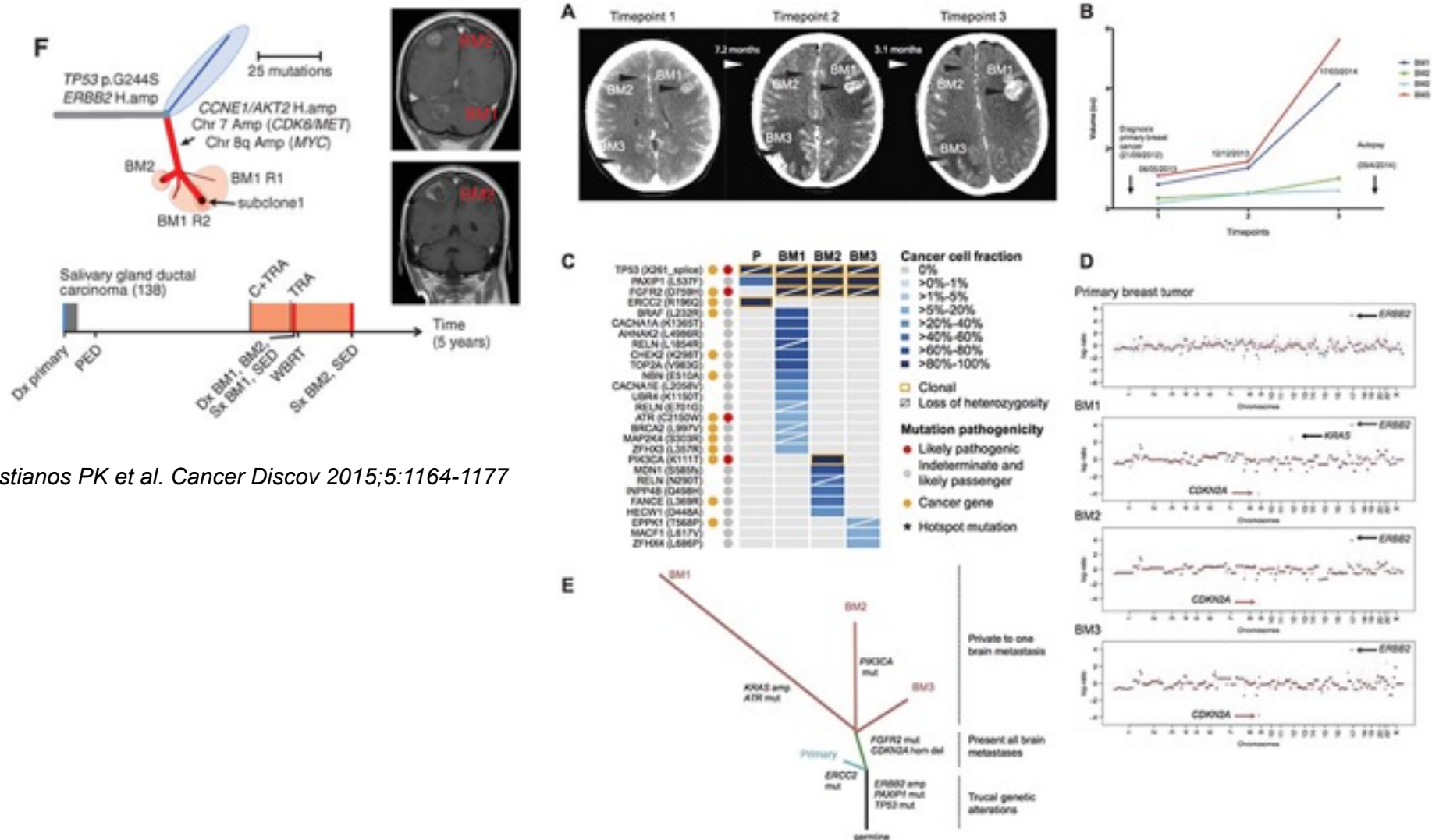


Memorial Sloan Kettering
Cancer Center

Metastasis: An Evolutionary Problem



Evidence of Divergent Evolution in Brain Metastasis



Brastianos PK et al. *Cancer Discov* 2015;5:1164-1177

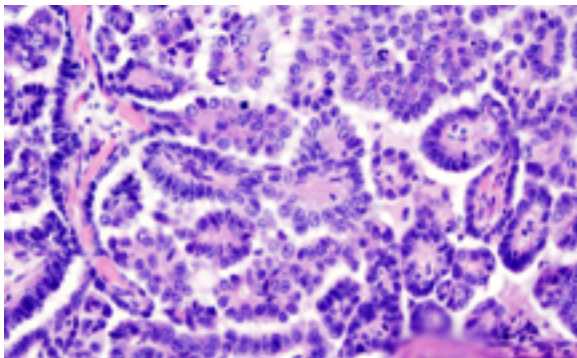
De Mattos-Arruda L et al. 2018 *Oncotarget* 9(29):20617

How to clinically cope with this dynamic genetic heterogeneity?

Gold Standard:

Sequencing of Resected Tissue

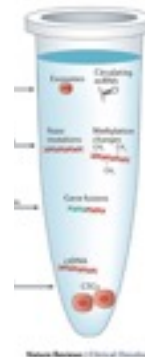
- Not all lesions are accessible
- One metastasis at a time
- Invasive procedures require recovery prior to subsequent treatments
- Each procedure adds potential additional morbidity



New Approach:

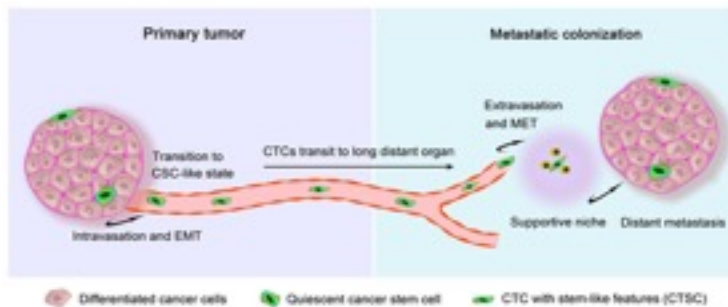
Liquid Biopsy

- No need for surgical approach
- Ensemble read of all metastases
- No delay in treatment schedule
- Serial "biopsies" possible
- Not a replacement for Pathologic diagnosis

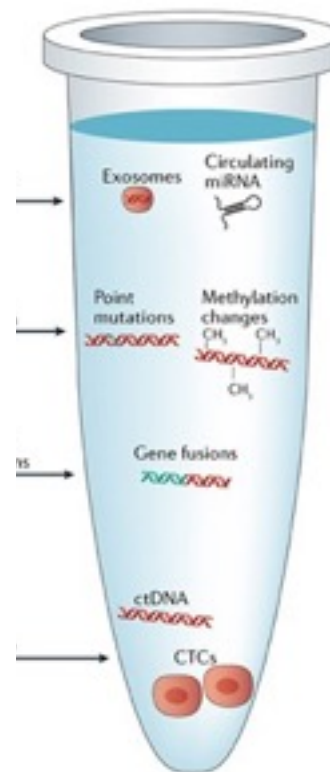


“Liquid Biopsy”

Analysis of a biological fluid to gain knowledge of cancer’s stage or molecular composition.



Front. Pharmacol., 01 February 2017



Nature Reviews | Clinical Oncology

Low-Speed Centrifugation

High-Speed Centrifugation

Silica Column Purification

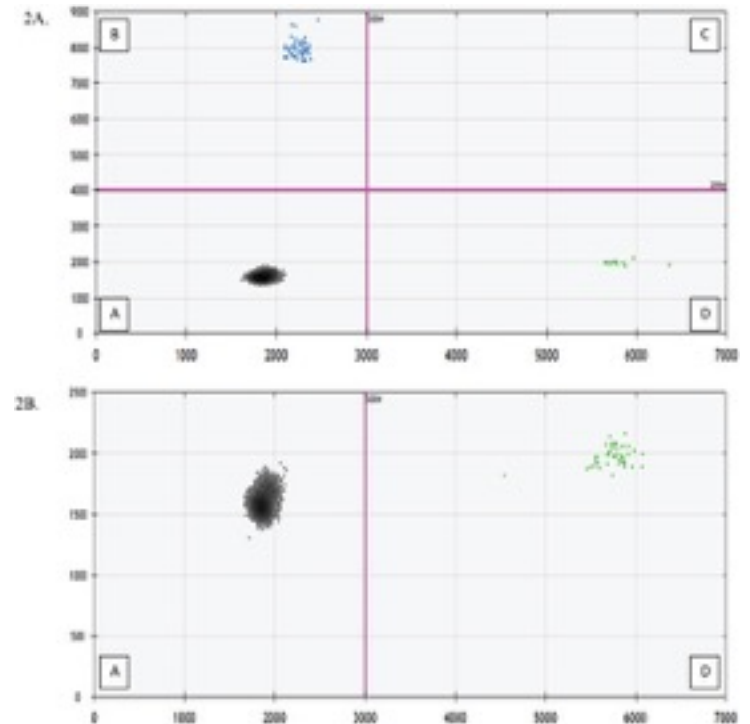
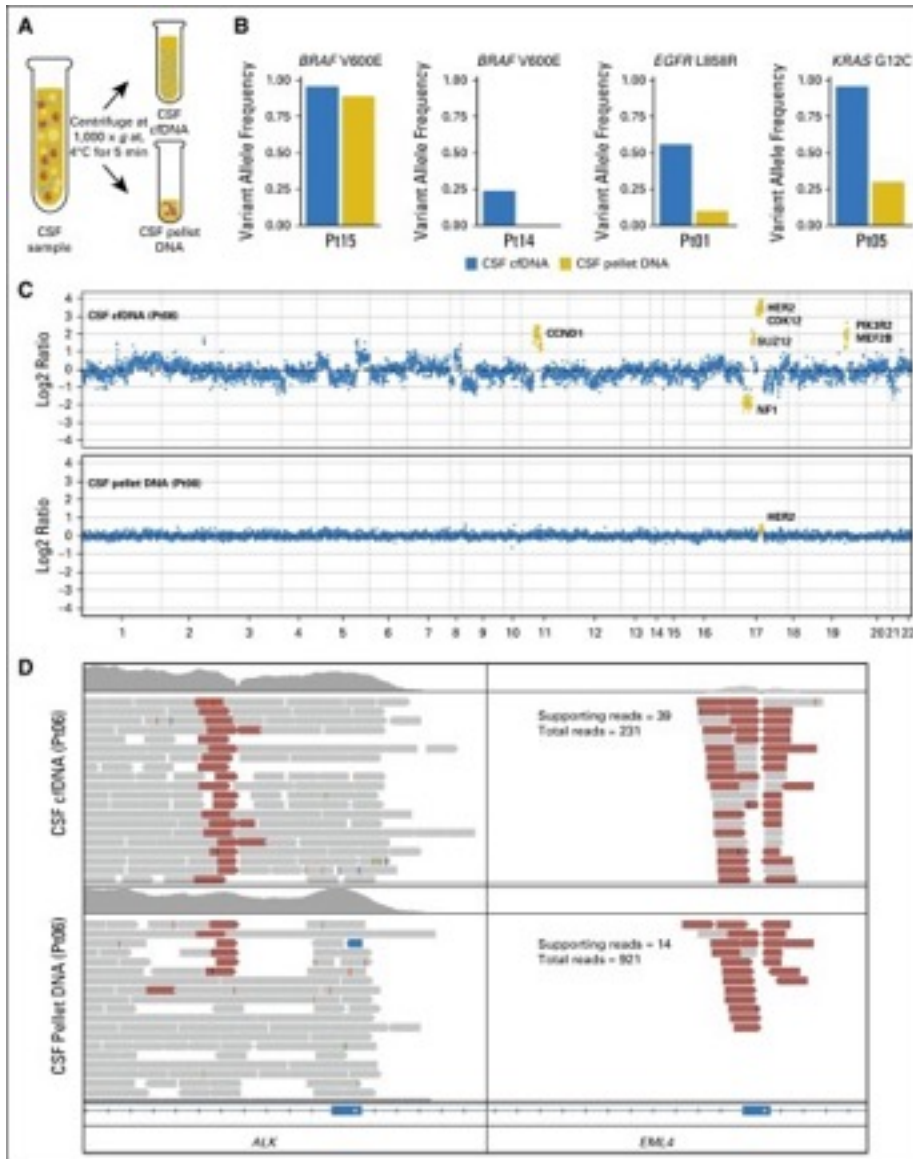
Quantify total cfDNA

Library Prep

Sequencing

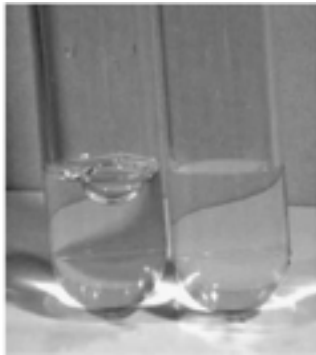
Analysis

ctDNA Technology: Cells vs. cfDNA

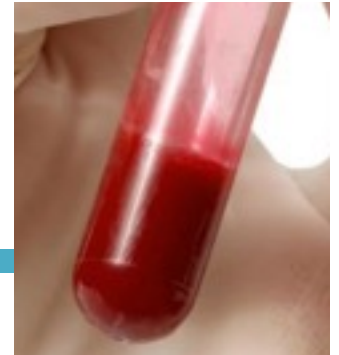


Momtaz P et al 2016 Oncotarget 7(51):85430

Pentsova EI et al 2016 JCO 34(20):2404



Spinal Fluid vs. Blood



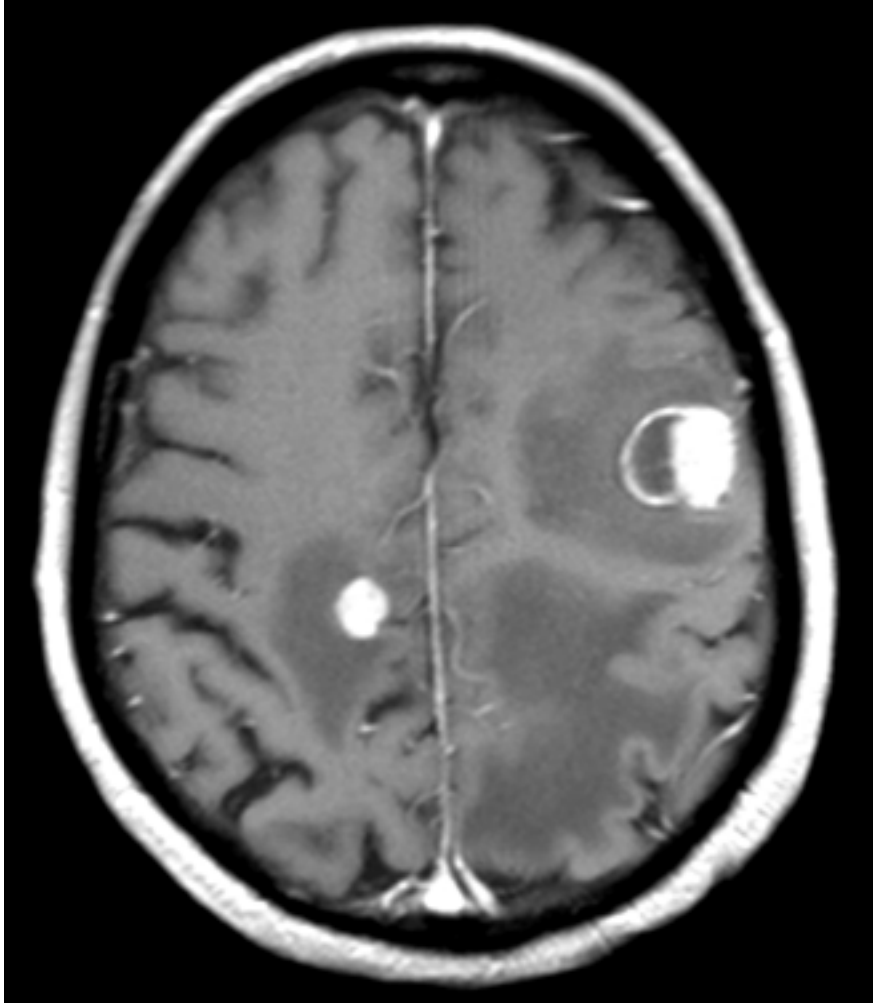
| Component | Spinal Fluid | Blood |
|------------------|---------------------|--------------|
| Oxygen | 41 | 100 |
| Protein | 0.5 | 100 |
| Sugar | 66 | 100 |
| Fats | 0.2 | 100 |
| Genomic DNA | 0.1 | 100 |



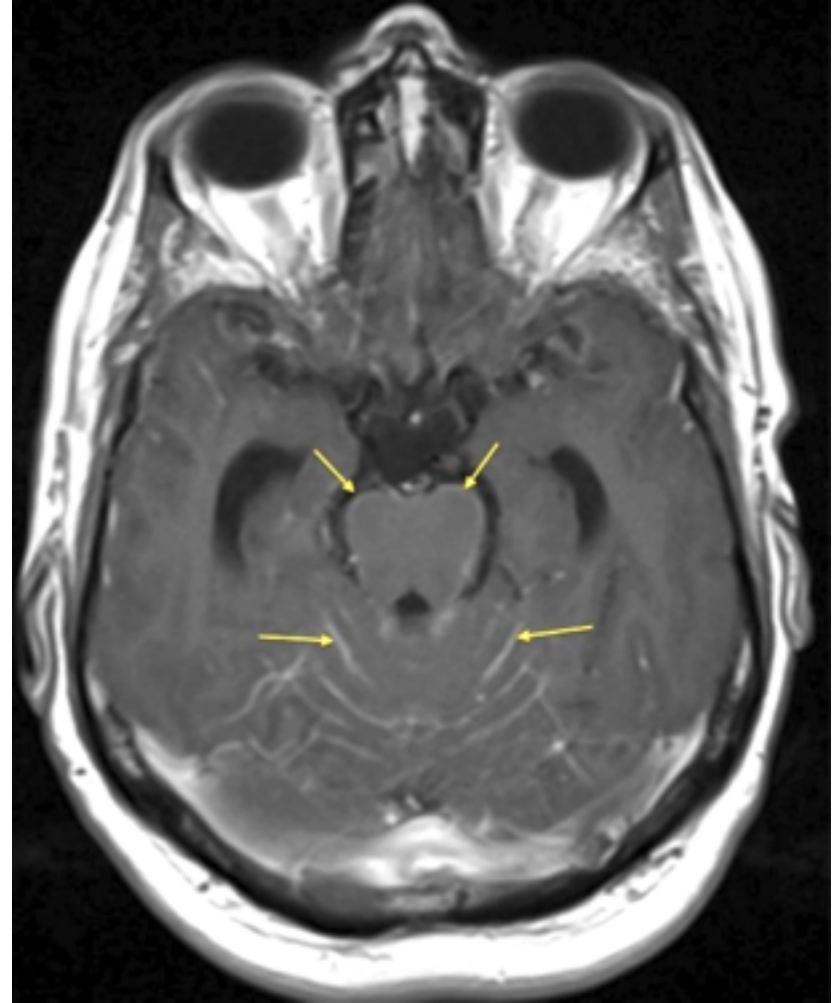
Cell-Free Tumor DNA sequencing from plasma vs. CSF: CNS metastases

| Study | Site of CNS Malignancy | n | Primary | Biological Fluid Sampled | Sequencing Method | CNS Malignancy Mutation Detection Rate |
|-----------------------|------------------------|----|--|--------------------------|---------------------------------|---|
| De Mattos et al. 2015 | Parenchyma | 12 | 6 breast cancer, 2 lung cancer, 4 glioblastoma | CSF Plasma | Targeted Sequencing | CNS disease only: (58% CSF, 0% plasma); CNS and non-CNS disease: (60% CSF, 55.5% plasma) |
| Marchio C et al. 2017 | Leptomeninges | 2 | Lung Adenocarcinoma | CSF Plasma | Targeted Sequencing | KRAS mutations detectable in CSF 2/2 (100%) |
| Siravegna et al, 2017 | Parenchyma | 1 | HER 2 + breast CSF adenocarcinoma | CSF plasma | ddPCR Whole exome sequencing | ERBB2 CNYC TP53 PIK3CA |

CNS Metastasis: Compartment



Parenchymal

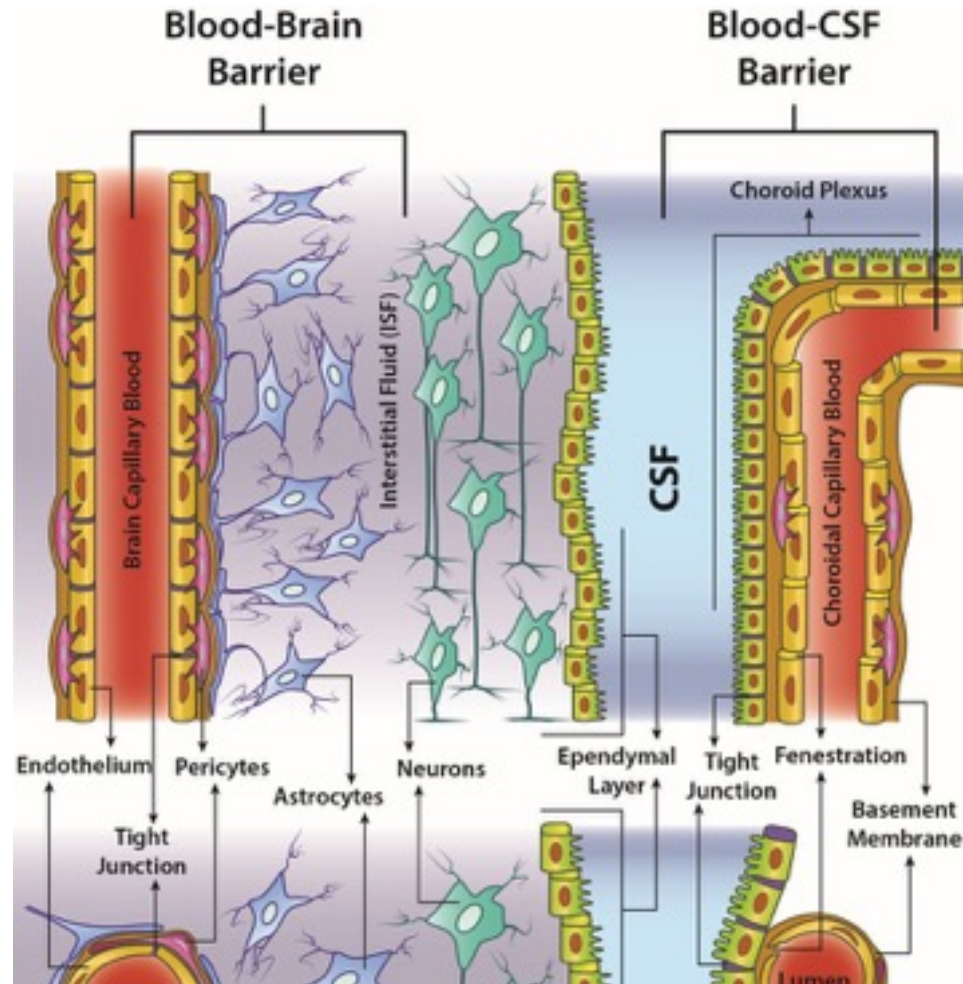
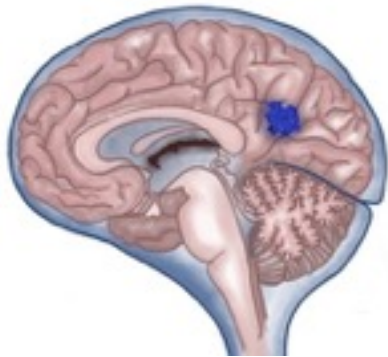


Leptomeningeal

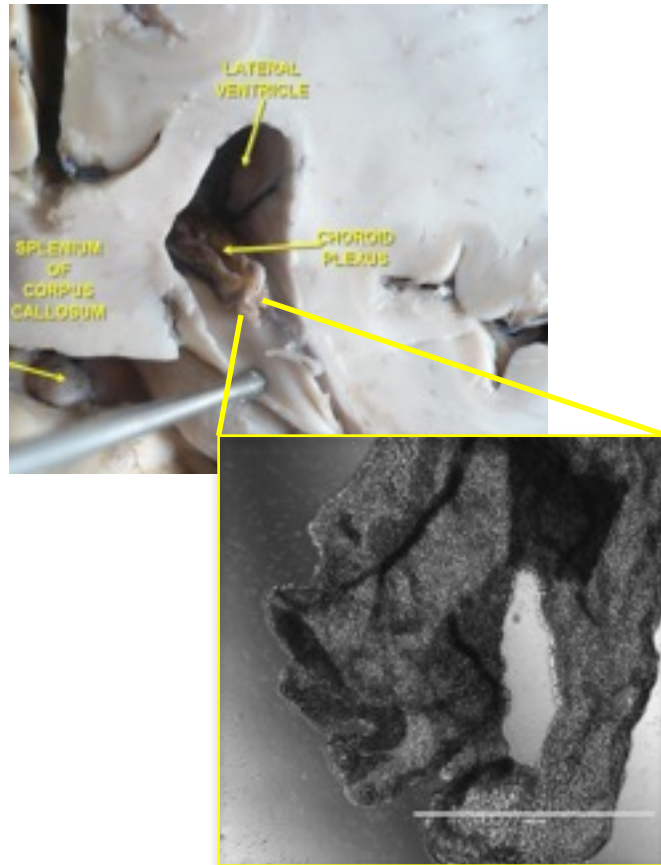
Classical View: Two Barriers, Two Compartments

Parenchymal

Leptomeningeal



CSF Circulation: Classical View

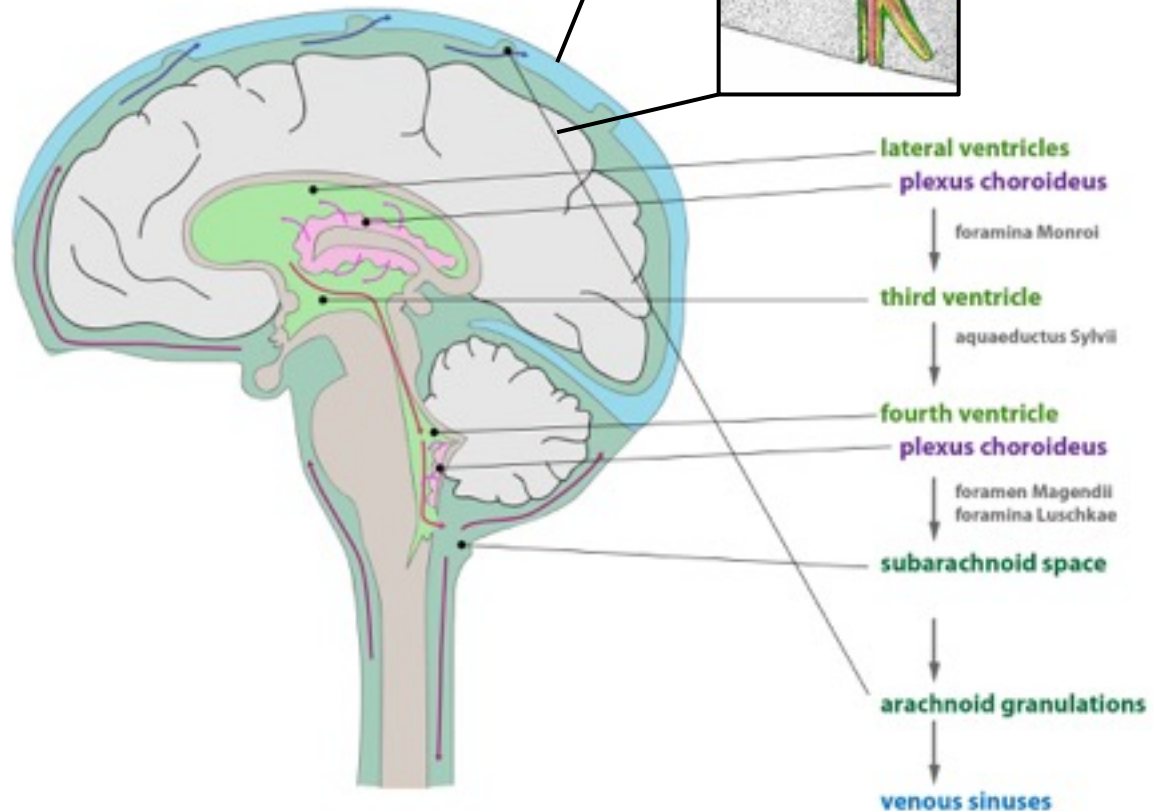
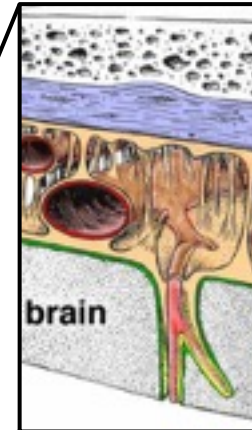


Virchow-Robin Spaces:

Potential spaces

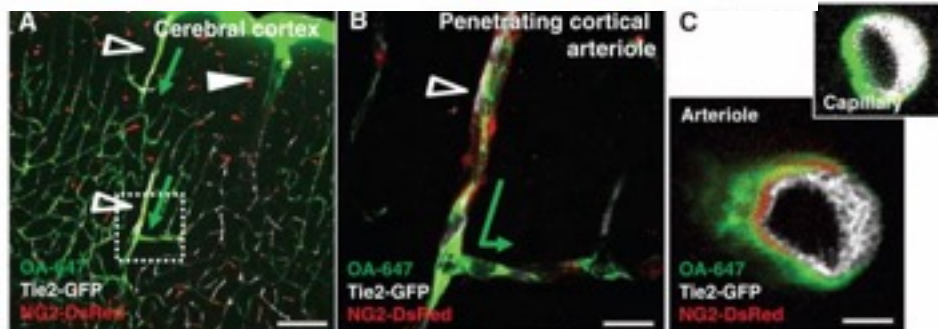
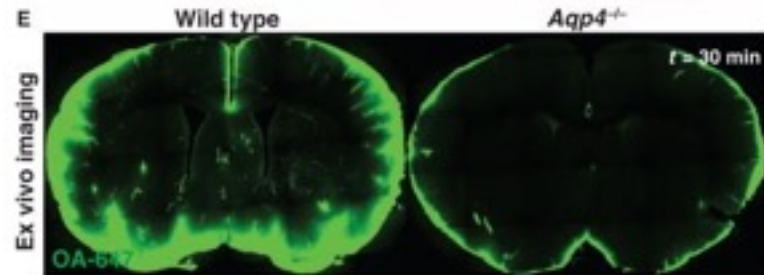
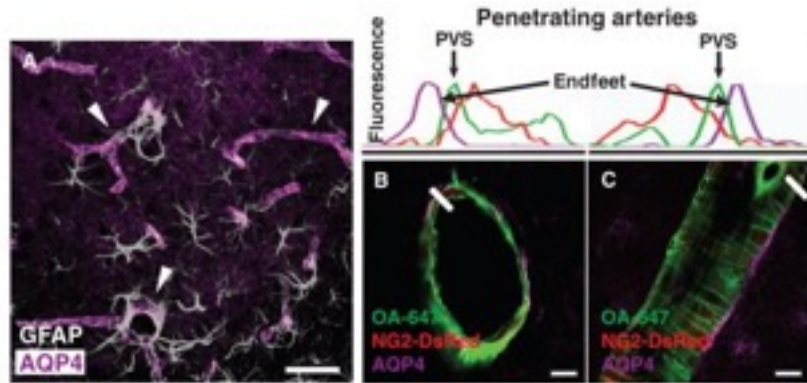
Extending into cortex (but not beyond)

CSF-containing in pathologic contexts

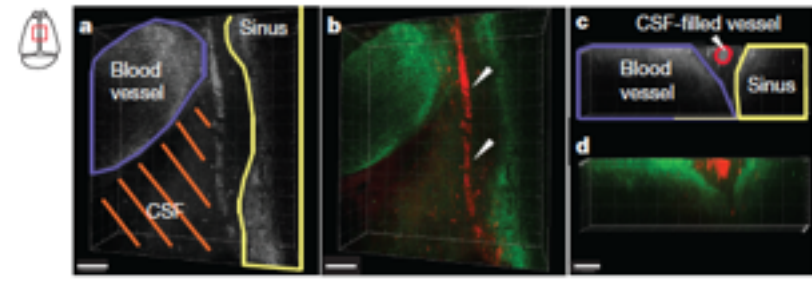


New Communications Uncovered Between CSF and Systemic Circulation

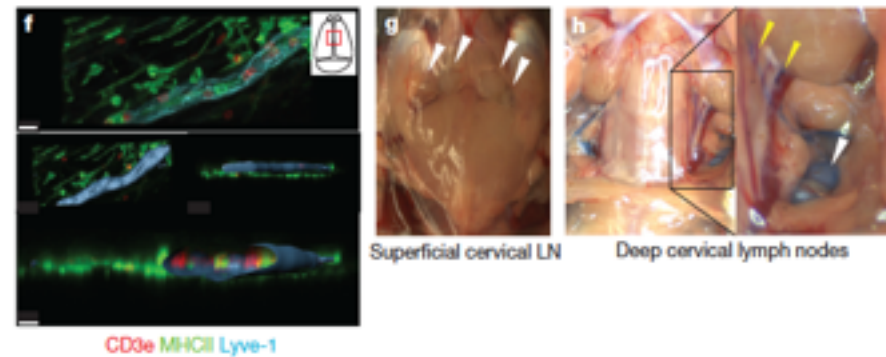
A. VRS Extend deep into the parenchyma



B. Communication between CSF and LN



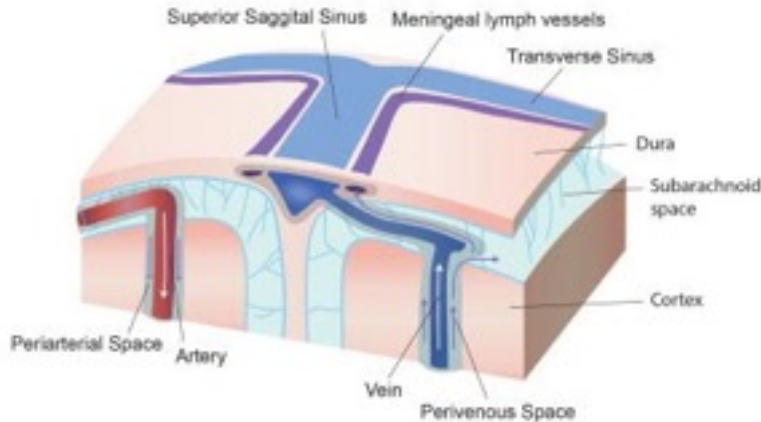
QDot 655 (ICV) Fluorescein (IV)



Louveau A et al Nature 2015 523:337

New view of CSF circulation: Nedergaard & Kipnis

A.



CSF is not a closed circulation

Communicates with systemic immune system through meningeal lymph vessels draining to LN

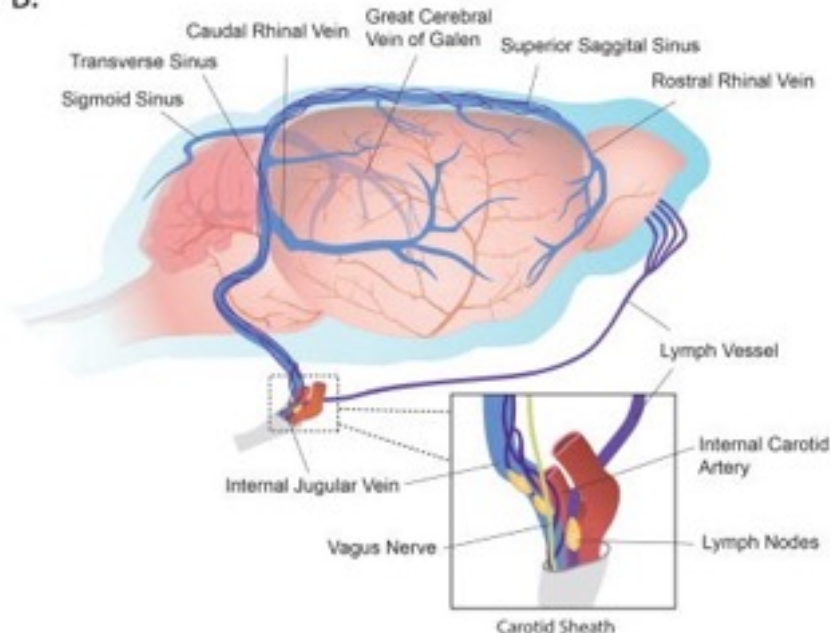
Primarily demonstrated through the use of small molecules (45 kDa or less).

Circulating immune cells? Macrophage?

Where are the meningeal vessels (really)- intradural or extradural?

VERY small, sparse vessels, is this vestigial?

B.



Cell-Free DNA sequencing in CSF: Site of Metastasis

| Study | Site of CNS Malignancy | n | Primary | Biological Fluid Sampled | Sequencing Method | CNS Malignancy Mutation Detection Rate |
|-------------------------|---------------------------|----|--|--------------------------|--|---|
| Momtaz P et al. 2016 | Parenchyma, Leptomeninges | 11 | Patients with BRAF-mutated malignancies | CSF | Targeted Sequencing | BRAF mutations detected in CSF of 6/11 (54%) |
| Pentsova EI et al. 2016 | Parenchyma, Leptomeninges | 41 | Various | CSF | Targeted Sequencing | Mutations detectable in CSF of 20/32 (63%) patients with Parenchymal Mets 3/4 (75%) patients with LM |
| Swinkels DW et al. 2000 | Leptomeninges | 2 | Lung Adenocarcinoma | CSF | Mutant-allele-specific Amplification (PCR) | Kras mutation detectable in CSF 2/2 (100%) |
| Marchio C et al. 2017 | Leptomeninges | 2 | Lung Adenocarcinoma | CSF Plasma | Targeted Sequencing | KRAS mutations detectable in CSF 2/2 (100%) |
| Fan Y et al 2018 | Leptomeninges | 11 | EGFR-mutated NSCLC | CSF | Targeted Sequencing | EGFR mutations detectable in CSF 11/11 (100%); mutations were not concordant in 1/11 (9%) |
| Li YS et al 2018 | Leptomeninges | 42 | EGFR-mutated NSCLC | CSF | Targeted Sequencing | EGFR mutations detectable in CSF of 92% (n = 28) |
| Huang WT et al, 2018 | Leptomeninges | 1 | CUP Adenocarcinoma | CSF | Targeted sequencing | HER 2 and MPL amplification PIK3CA, CDKN2A and P53 mutations |
| Siravegna et al, 2017 | Parenchyma | 1 | HER 2 + breast CSF adenocarcinoma | CSF plasma | ddPCR Whole exome sequencing | ERBB2 CNYC TP53 PIK3CA |
| De Mattos et al. 2015 | Parenchyma | 12 | 6 breast cancer, 2 lung cancer, 4 glioblastoma | CSF Plasma | Targeted Sequencing | CNS disease only: (58% CSF, 0% plasma); CNS and non-CNS disease: (60% CSF, 55.5% plasma) |

Potential Applications of this Technology



- Diagnosis of LM metastases when CSF cytology is negative or inconclusive.
- Diagnosis of brain metastasis, especially from unknown primary tumor or multiple lesions.
- Quantification of residual tumor following surgical resection.
- Differential diagnosis between pseudoprogression/radionecrosis and tumor progression following stereotactic radiosurgery
- Evaluation of tumor response following cytotoxic or targeted agents
- Early diagnosis of tumor relapse.
- Prediction of resistance to targeted agents.
- Monitoring of treatment of resistance mutations with specific targeted agents.
- Better evaluation of prognosis (based on number of cells and molecular features).

Ongoing clinical studies on CNS metastases including cfDNA

| Study number | Patient population | Type of study | Fluid sample | Technique | Primary outcome |
|--------------|--|---|-------------------------------|--|--|
| NCT02607605 | 10 patients with advanced lung cancer with LM | Observational Prospective | CSF | cfDNA using QIAamp Circulating Nucleic Acid kit (Qiagen) | Correlation between cfDNA and CSF cytology |
| NCT02803619 | 60 patients with EGFRm+ NSCLC and LM | Observational Prospective | CSF | Not reported | OS after LM diagnosis in NSCLC patients |
| NCT03029065 | 50 patients with BM or LM from NSCLC | Observational Prospective | CSF Plasma | cfDNA using Next-generation Sequencing technique | Compare cfDNA in CSF with Plasma |
| NCT03257735 | 50 patients with BM from NSCLC | Observational Prospective | CSF Plasma Tumor tissue | cfDNA using Next-generation Sequencing technique | Compare cfDNA in CSF and blood with tumor tissue at baseline and after 2 months of treatment |
| NCT03257124 | 80 patients with EGFR T790M Mutated NSCLC and BM and/or LM who failed Tyrosine Kinase Inhibitors | Phase II trial Experimental arm: AZD9291 (160 mg PO QD) in BM or LM cohort in T790M positive | CSF Plasma Tumor tissue | Not reported | OS in BM and LM cohorts |
| NCT02071056 | 22 patients with LM from metastatic solid tumors | Observational Prospective | CSF Plasma Tumor tissue | Not reported | Tumor DNA detectability and cytological confirmation of leptomeningeal metastasis. |

Acknowledgements



Boire Lab



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RANO



MEMORIAL SLOAN KETTERING | EQUINOX

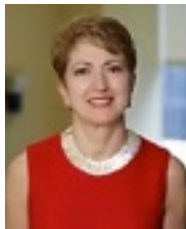
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THE
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DeAngelis



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Parada



Elena
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