

## Supplementary Online Content

White MD, Klein RH, Shaw B, et al. Detection of leptomeningeal disease using cell-free DNA from cerebrospinal fluid. *JAMA Netw Open*. 2021;4(8):e2120040.  
doi:10.1001/jamanetworkopen.2021.20040

**eFigure 1.** Inferred Segmental Copy Number Estimates, Cancer Fraction, and Tumor Ploidy Obtained by ichorCNA for Two Patients Previously Diagnosed With LMD by Cytology (A, B) and a Patient With a Parenchymal Brain Tumor, and No Known LMD (C)

**eFigure 2.** The Breakdown of Patients and Samples Obtained and Analyzed During This Study

**eFigure 3.** Confusion Matrix Indicating the Predicted vs True Diagnostic Labels for the CSF cfDNA Samples (A) and the CSF Cytology Samples (B)

**eFigure 4.** Results for Patient DFCI-022

**eFigure 5.** The Fragmentation Patterns of cfDNA Are Different for Plasma and CSF

**eFigure 6.** Using the Differences in Fragmentation Between Plasma cfDNA and CSF cfDNA, There Does Not Appear to be Evidence for Plasma Contamination of Patient CSF-050s CSF cfDNA Samples

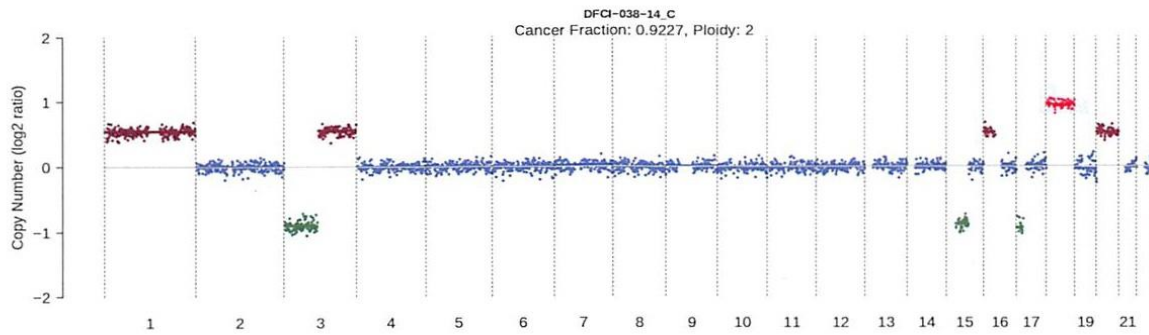
**eTable 1.** The Cancer Fraction, DNA Concentration, Peak Ratio, and Cell Counts Observed in Patient CSF-050s CSF Draws

**eTable 2.** Outcomes for Matched cfDNA and Cytologic Assessments for Patients With LMD Without PTACSF

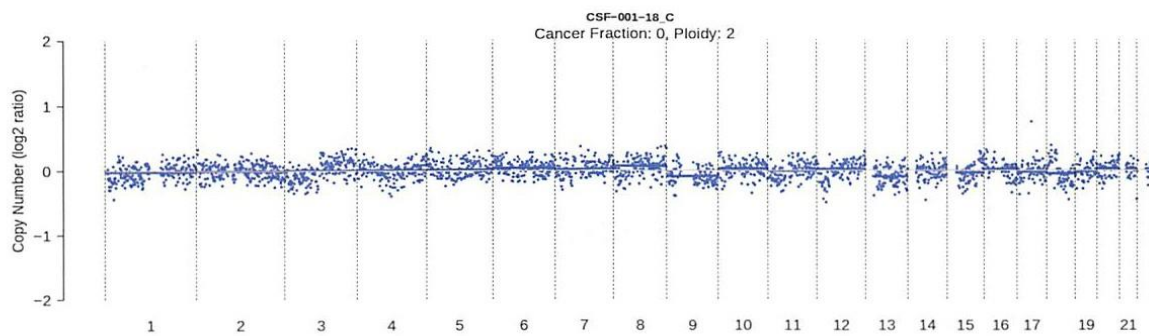
**eTable 3.** Outcomes for cfDNA and Cytologic Assessments Based on Sampling Location for Patients With LMD Without PTACSF

This supplementary material has been provided by the authors to give readers additional information about their work.

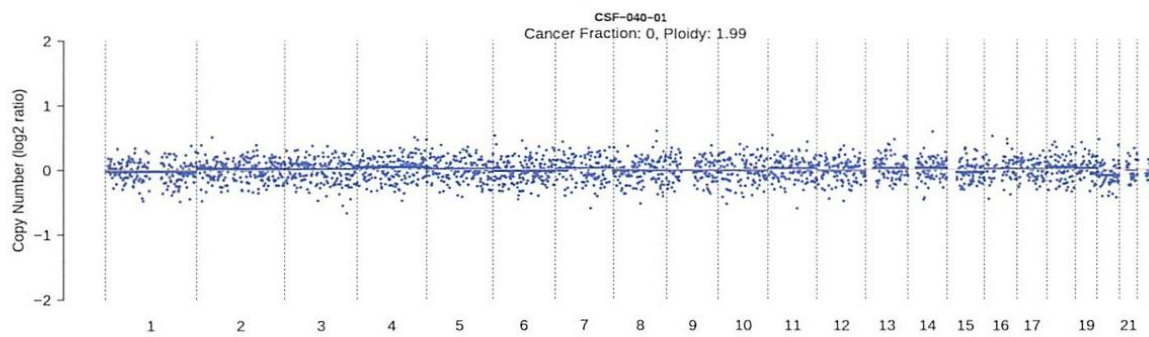
**eFigure 1.** Inferred Segmental Copy Number Estimates, Cancer Fraction, and Tumor Ploidy Obtained by ichorCNA for Two Patients Previously Diagnosed With LMD by Cytology (A, B) and a Patient With a Parenchymal Brain Tumor, and No Known LMD (C)



**A**



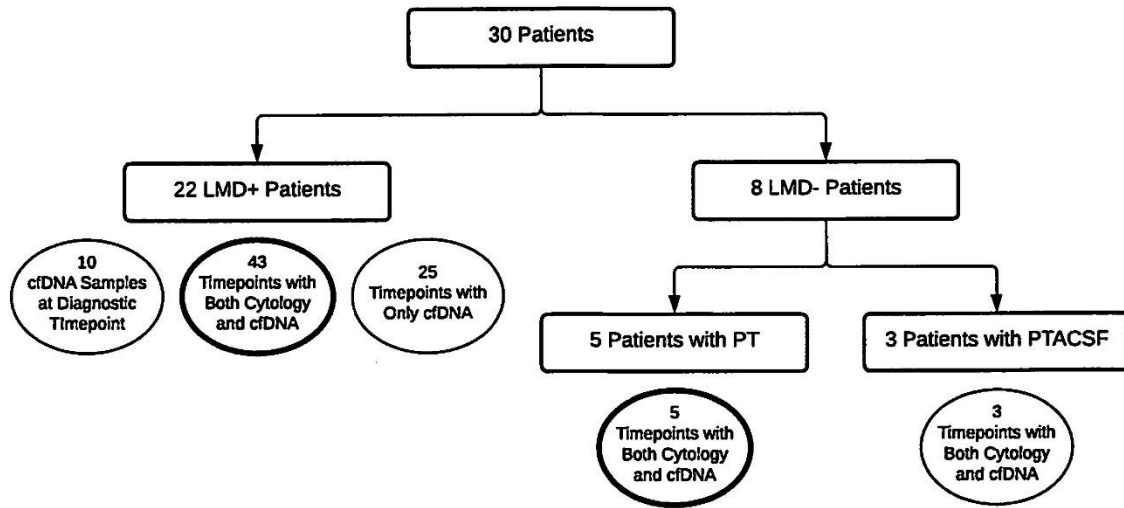
**B**



**C**

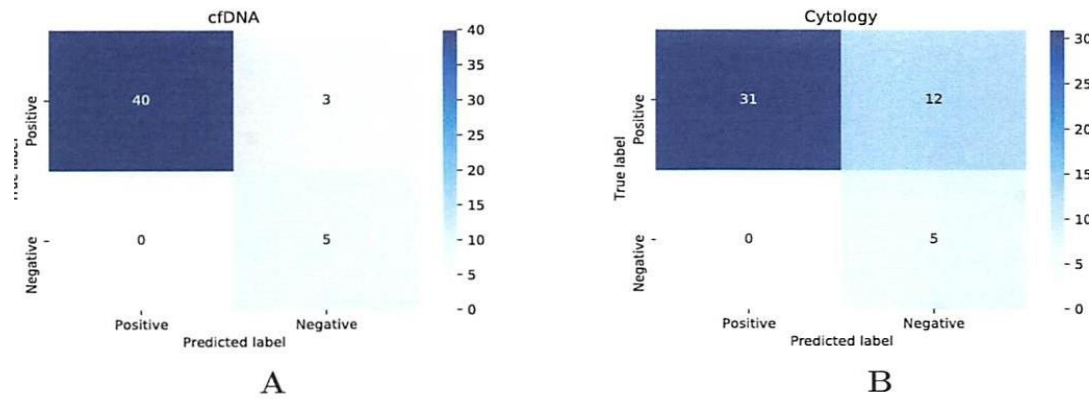
## Supplementary Figure 1

**eFigure 2.** The Breakdown of Patients and Samples Obtained and Analyzed During This Study. Shown in bold are the samples used to estimate sensitivity, specificity, diagnostic accuracy, and other secondary measure of diagnostic performance.



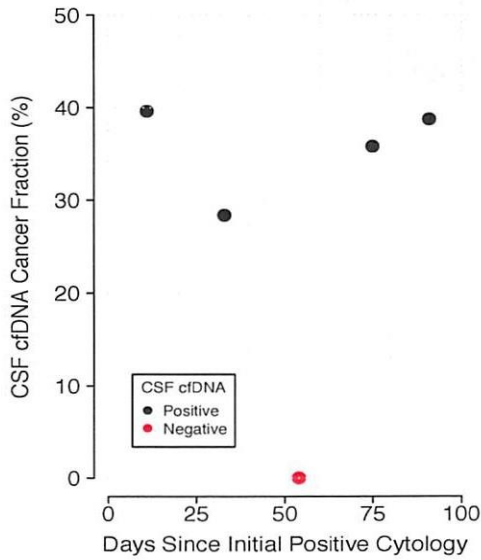
## Supplementary Figure 2

**eFigure 3.** Confusion Matrix Indicating the Predicted vs True Diagnostic Labels for the CSF cfDNA Samples (A) and the CSF Cytology Samples (B)

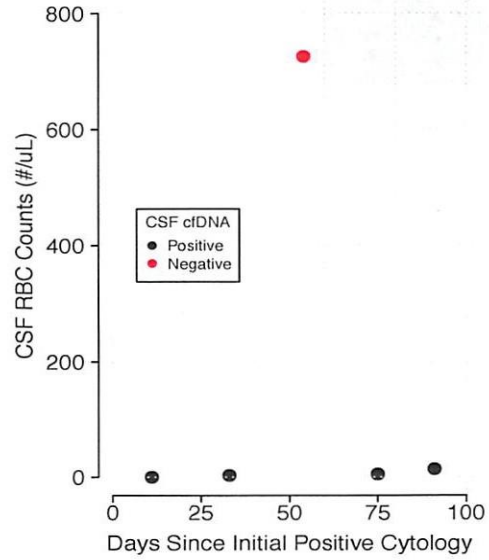


### Supplementary Figure 3

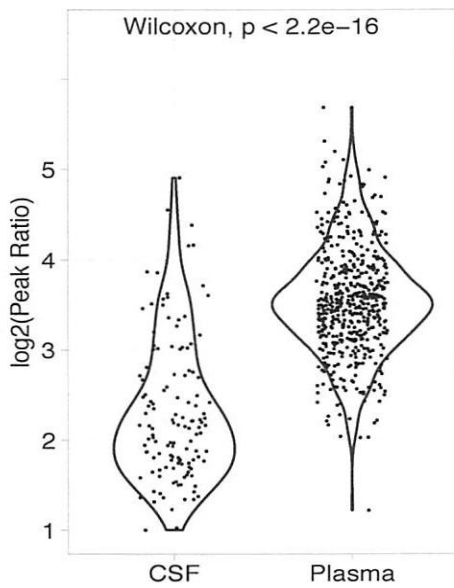
**eFigure 4.** Results for Patient DFCI-022. The inferred cancer fraction in patient DFCI-022's samples is consistent over time with the exception of her third sample (A); the outlier sample also had unusually high erythrocyte counts measured in the CSF (B); the peak ratio, produced from DNA fragmentation patterns, is significantly different in plasma-derived cfDNA vs CSF cfDNA (C); and the outlier sample also shows a larger peak ratio than the other samples, corroborating the possibility of contamination of CSF cfDNA by plasma cfDNA (D).



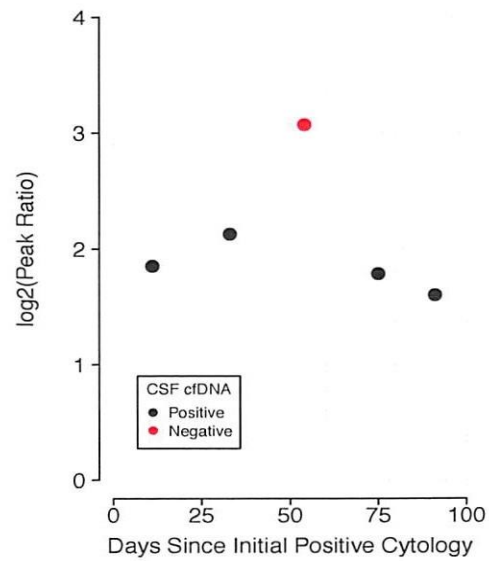
A



B

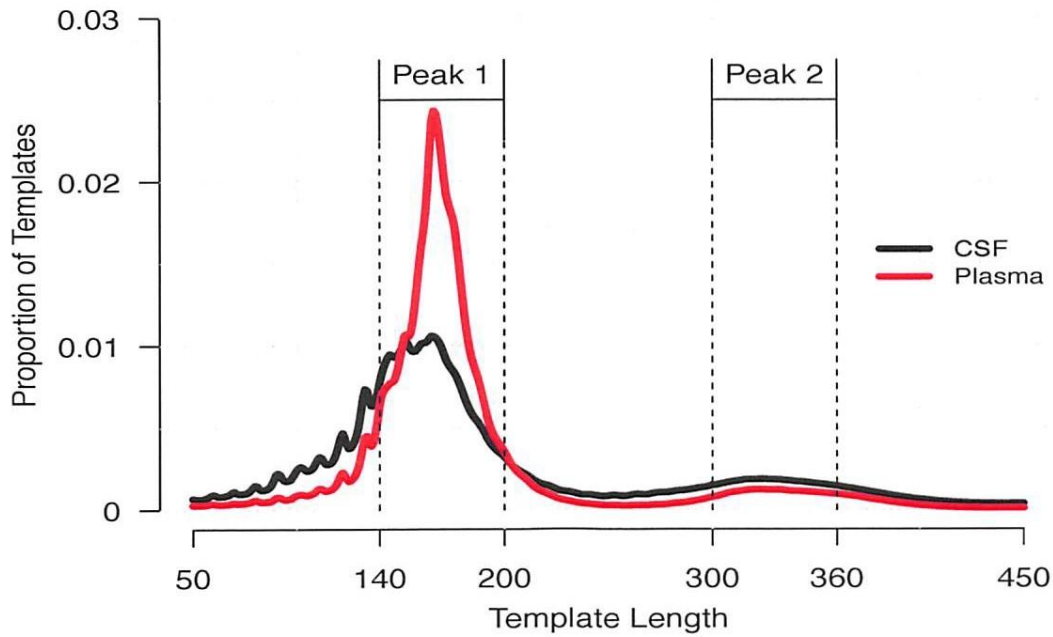


C



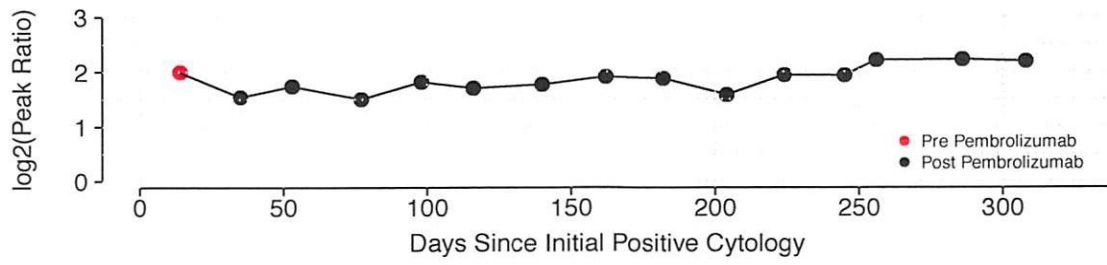
D

**eFigure 5.** The Fragmentation Patterns of cfDNA Are Different for Plasma and CSF. To describe this difference, we define peak 1 to be fragments with lengths in [140-200] base pairs and peak 2 to be fragments with length in [300-360] base pairs. The ratio of fragments in peak 1 to peak 2 is higher in plasma cfDNA than in CSF cfDNA.



Supplementary Figure 5

**eFigure 6.** Using the Differences in Fragmentation Between Plasma cfDNA and CSF cfDNA, There Does Not Appear to be Evidence for Plasma Contamination of Patient CSF-050s CSF cfDNA Samples



## Supplementary Figure 6

**eTable 1.** The Cancer Fraction, DNA Concentration, Peak Ratio, and Cell Counts Observed in Patient CSF-050s CSF Draws

Days Since Initial Positive Cytology	Cancer Fraction (%)	DNA Concentration (ng/uL)	Red Blood Cells (#/uL)	Nucleated Cells (#/uL)	Lymphocytes (#/uL)	Unclassified Cells (#/uL)	log2(Peak Ratio)
14	68.3	0.501	40	2	0.52	0.28	1.99
35	90.3	0.848	0	1	0.70	0.21	1.54
53	91.4	0.674	2	5	4.35	0.30	1.74
77	90.4	1.416	0	10	5.50	1.30	1.50
98	91.4	1.154	0	35	30.80	2.45	1.82
116	89.9	0.754	0	19	14.44	2.09	1.70
140	85.4	0.804	0	5	4.25	0.15	1.77
162	91.2	1.395	2	53	49.82	1.59	1.91
182	84.1	1.224	0	57	50.16	3.42	1.88
204	87.3	0.923	0	48	43.68	3.36	1.58
224	90.3	1.289	0	18	16.20	0.72	1.94
245	88.7	NA	2	27	24.57	0.54	1.94
266	86.1	1.545	0	34	30.60	0.68	2.21
286	84.6	1.749	3	28	26.04	0.84	2.23
308	85.7	2.000	0	32	29.44	0.32	2.19



**eTable 2.** Outcomes for Matched cfDNA and Cytologic Assessments for Patients With LMD Without PTACSF

		cfDNA	
		Negative	Positive
Cytology	Negative	2	10
	Positive	1	30

**eTable 3.** Outcomes for cfDNA and Cytologic Assessments Based on Sampling Location for Patients With LMD Without PTACSF

<b>Cytology</b>	<b>Shunt</b>	<b>LP</b>
<b>Positive</b>	24	9
<b>Negative</b>	5	6
<b>cfDNA</b>	<b>Shunt</b>	<b>LP</b>
<b>Positive</b>	44	14
<b>Negative</b>	1	2