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Liquid biopsy for evaluating mutations and chromosomal aberrations in cerebrospinal fluid from patients with primary or metastatic CNS tumors

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ABSTRACT

Background: Cytopathology analysis of cerebrospinal fluid (CSF) is limited in detecting tumors in patients with suspected primary or metastatic central nervous system (CNS) malignancy. We investigated the use of CSF liquid biopsy (LBx) to detect neoplastic processes in the CNS.

Methods: Cell-free DNA (cfDNA) from the CSF of patients with suspected metastatic (N = 106) or primary CNS (N = 23) tumors was deep sequenced using a 302-gene panel.

Results: Four samples (3 %) (3 metastatic and 1 primary) failed sequencing quality control criteria. Metastatic tumor was confirmed in 84 (82 %) of the 103 patients suspected of metastatic tumor. Primary CNS tumor was confirmed in 11 of 22 (50 %) patients suspected of CNS tumor. Chromosomal abnormalities were detected in 55 samples (54 %). Germline mutations were detected in 23 (22 %) patients with metastatic tumors and in 1 (5 %) with a primary CNS tumor. Of the 29 patients with metastatic breast cancers, 2 (7 %) had mutations in ESR1 and 9 (31 %) had mutations in PIK3CA. Of the 21 patients with metastatic lung cancer, 9 (43 %) had EGFR mutations and 5 (24 %) had KRAS mutations. Upon comparing CSF LBx with peripheral blood LBx in 14 patients, 13 (93 %) showed only CHIP and one patient showed CNS primary tumor mutation. Serial samples from 14 patients demonstrate that CSF LBx can be used for monitoring therapy efficacy.

Conclusions: LBx using CSF is clinically reliable and provides informative results in a substantial proportion of patients with metastatic CNS tumors and to a lesser degree in patients with primary CNS tumors.

1. Introduction

Cerebral spinal fluid (CSF) from patients with suspected primary or metastatic central nervous system (CNS) malignancy is frequently analyzed for the presence of absence of tumor [1]. Cytologic examination by conventional means is often inadequate due to the paucity of tumor cells in CSF samples [2]. The limited number of cells obtained from CSF samples also limits the ability to perform additional testing to assess immunophenotype, chromosomal abnormalities, and genomic deviations [3]. For evaluating CNS involvement by hematologic malignancies, chromosomal flow cytometry and cytogenetic data are crucial for diagnosis and determining therapy [4]. For primary and metastatic solid tumors, molecular evaluation of genomic abnormalities is also important for targeted therapy and evaluating resistance mechanisms [2,5,6]. However, in most types of cancers involving the CNS, especially solid tumors, it is rare to find adequate circulating tumor cells in the CSF [7]. Furthermore, even if tumor cells are identified by microscopic examination, frequently there are too few for ancillary studies to identify specific biological characteristics for determining a precise therapeutic approach [8].

Tumor cells by their nature have higher turn-over and shed their DNA and RNA at higher rates than normal cells [9,10] Because of these characteristics, peripheral blood cell-free DNA (cfDNA) and RNA (cfRNA) have been used extensively for evaluating metastatic and localized tumors in specific organs [11,12]. However, because of the blood-brain barrier, cfDNA from CNS tumors is rarely detectable in peripheral blood [13]. Testing CSF for cfDNA or cfRNA might provide a more reliable approach for detecting neoplastic processes within the CNS [14–16].

Toward this goal, we analyzed cfDNA and cfRNA in CSF collected from patients suspected of having CNS involvement by primary or metastatic tumors.

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2. Materials and methods

2.1. Patients and CSF samples

This retrospective study used consecutive CSF samples that were submitted as part of clinical testing for mutations in cfDNA using a targeted DNA panel of 302 genes (Supplement 1) and a targeted RNA panel of 1600 genes. All results were reported to both clinicians and patients/families. This retrospective study of data was performed under an approved IRB protocol (WCG IRB # 1-1476184-1) and was conducted in accordance with the principles of the Declaration of Helsinki and its later amendments.

2.2. cfDNA and cfRNA extraction

We used the Apostle MiniMax High-Efficiency total nucleic acid isolation Kit (Beckman Coulter, Brea, CA, USA) and followed the protocol recommended by the manufacturer as previously described in detail [17]. After extraction, half of the cell-free total nucleic acid was treated with DNase to obtain cfRNA, and the other half was used for cfDNA analysis.

2.3. Next-generation cfDNA and cfRNA sequencing

DNA sequencing was based on the KAPA HyperCap protocol (Roche, Pleasanton, CA, USA). The DNA panel included 302 cancer-related genes. DNA was quantified using the Varioskan LUX. The KAPA Universal UMI Adapters were implemented with the KAPA Unique Dual-Indexed (UDI) Primer Mixes to remove duplicates and sequencing error correction.s. After library amplification and cleanup, KAPA HyperChoice MAX custom probes and the HyperCapture reagent kit were used for target enrichment. The final library was quantified and loaded on a NovaSeq 6000 system and run with 150x2 cycles. The depth of sequencing was between 25,000X and 30,000X. More than 50–100 ng of DNA were used in sequencing when possible, but lower quantities were used for samples with low levels of cfDNA. Sequencing data were analyzed using the Dragen v3.10.8 – Somatic DNA-Seq pipeline with UMI analysis. The VCF file generated was annotated and analyzed following a rigorous protocol that included BAM file inspection for every reported mutation. The CNVkit software was used to evaluate chromosomal abnormalities. The software compares binned read depths in on- and off-target regions to pooled normal reference and estimates the copy number at various resolutions. RNA was sequenced using similar hybrid capture approach. The targeted cfRNA sequencing panel covered 1600 genes including all immunoglobulin heavy and light chain genes and all T-cell receptors genes. Sequencing was performed using illumina NovaSeq 6000 instrument. More than 80 million reads were required for accepting results. The required percentage of spliced reads was above 20 %. cfRNA data was accepted and evaluated only when these two conditions were met. Therefore, cfRNA is not further discussed in this study.

3. Results

During the study period, 129 CSF samples from patients with suspected metastatic (#105) or primary (#23) CNS tumors were tested. The median patient age was 58 years (range: 19 to 86). Of the 129 CSF sample, 4 were excluded because the cfDNA sequencing data did not meet the quality criteria: 3 from patients with suspected metastatic CNS tumors and 1 from a patient with a suspected primary CNS tumor. Except for one sample from a patient with a diagnosis of breast cancer, none of the tested samples showed diagnostic results on routine cytology testing. In 14 patients, peripheral blood (PB) was also collected and cfDNA and cfRNA were tested using the same technology and the same approach. In addition two or more consecutive samples were collected and tested from 14 patients In 92 % of samples, cfRNA data did not meet our quality control in achieving 80 million reads and spliced read of greater than 20 %.

3.1. Higher positive rate in metastatic tumors than in primary CNS neoplasms

Of the 103 CSF samples from patients with suspected metastatic tumors, 84 (82 %) showed mutations or chromosomal abnormalities that are typically seen in solid tumors or lymphoma, confirming the diagnosis of a CNS metastasis (Table 1). Such abnormalities were detected in a high proportion of CSF samples from patients with a diagnosis of breast cancer (91 %), lung cancer (95 %), or lymphoma (71 %). In contrast, primary CNS tumors were detected in only 11 (50 %) (P-value = 0.004, Kruskal-Wallis test) of samples from patients with suspected primary CNS tumors (Table 1). Glioblastoma showed a higher rate of positivity as compared with less aggressive tumors (Table 1). The detection of the presence of solid tumor in CSF does not distinguish between leptomeningeal disease and tumor in brain parenchyma.

3.2. Detected chromosomal abnormalities in CSF

Chromosomal structural abnormalities were detected in 55 (54 %) of the 103 samples from patients with metastatic tumors but only in 4(18 %) of the 23 with primary CNS tumors (Table 2; Figs. 1 and 2). Only one of the metastatic tumors showed chromosomal abnormalities without somatic mutations. This case showed gain in 5q involving PDGRB and loss on 16q, likely due to amplification in PDGFRB and possible homozygous loss on 16q. Some of the detected abnormalities are very relevant for therapy. Twenty samples showed amplification in one or more genes with clinical relevance for therapy, including PDGFRB, ERBB2, CCND1, MDM2, FGFR1, MYC, KRAS, and others (Table 2; Fig. 1). As shown in Table 3, when CNS was compared with peripheral blood samples that were collected at the same time, chromosomal abnormalities were not detectable in any of the tested peripheral blood samples.

Table 1
Studies with informative results from CSF liquid biopsy.

Metastatic tumors (primary tumor site)	Number	Positive cases	% positive
Breast[a]	32	29	91
DLBCL	2	1	50
Pancreas	1	1	100
Lymphoma	17	12	71
neuroma	1	0	0
Carcinoma	6	6	100
Cervical	1	1	100
Urothelial	1	0	0
Colorectal	2	2	100
Gastric	3	3	100
Lung (NSCLC)	22	21	95
Melanoma	4	4	100
Esophageal	1	1	100
Ovarian	1	1	100
Neuroendocrine	2	1	50
Sarcoma	1	0	0
Testicular	1	0	0
Brain mass (not otherwise specified)	5	1	20
Total metastatic	103	84	82
Primary CNS tumors			
Glioma grade 1 and 2	4	2	50
Glioblastoma	6	4	67
Spinal tumor[b]	4	2	50
Ependymoma	2	0	0
Brain tumor	5	2	40
Midline astrocytoma	1	1	100
Total primary CNS tumors	22	11	50

[a] Of the 29 positive cases, 2 were HER2 positive and 10 were triple negative.
[b] Two ofhe spinal tumors were schwannomas, one was meningioma and the fourth was unknown.

Table 2
Detected Chromosomal gains or losses.

Metastatic tumors	Diagnosis
6p+, 6q-, +7, +12, +16, and 19q+	Lymphoma
1q+, 6q-, 10q+ (RET amplification), 12q+ (MDM2 amplification), 13q-, 14q-, 15q-18q-, 20q+ and 21q-	Lung
+8 and −18.	Lung
6p-(Telomeric) and 6q-.	Breast
1p-, 2p-, 3p-, 4q-, +5, −6, −7, −9, 13q+, 14q-, +15, +16, +17, −18, 19p-, 19q+ and −22.	Carcinoma
trisomy 12	Lymphoma
1p+ (amplification of MYCL and others), 1q+, 2p+, 3q+, 6p+, 7p+(Amplification of EGFR), 7q+, 9q+, +10, +11, +12, +15 and others.	Breast
1q+, 2q+, −3, 4p+, 5p+, +7, −8, 9p-, −10, +11, +12, 13q-, 14q-, 15q-, +17, 18q+, 19q+, +20 and others	Lung
1q+ and 12q+ (MDM2 amplification)	Carcinoma
1p-, 1q+, 6q-, 8p-, 9p-, −10, 11p+, 11q1, +12, −13, −14, −15, 16p+, 16q-, and −22.	Breast
1q+, 3q- (distal), +5, 6p+, +7, 8p+ (proximal), −9, +11 13q-, 14q+, 16p+, 20q+21q+ (distal) and others	Lung
Distal 1p-, proximal 1p+, 1q+, distal 2q+, 3p-, −4, −5, proximal 7p+, +8, −9 (CDKN2A deletion), 10p+, 10q- (PTEN deletion), 11p-, 12p+, −14, 17p- (TP53 deletion) & others	Breast
1p-, distal 2q+, distal 5p+ (TERT amp), proximal 5p-, 6p+ (CCND3 amp), 8p-, 8q+, distal 10q-, distal 11p-, 16q-, 17p- (TP53 deletion), small 17q+ (ERBB2 amp), 18p+, 19q+, +20, −21 & others	Gastric
Monosomy 2, -3, -4, +7 (SAMD9 gain), −9, −10,11q-, 12q+ (proximal, MDM2 and CDK4 amplification), 13q-, 19q+ (proximal), and 22q-.	Neuroendocrine
1q+, 3p-, distal 4p+, 11p-, 13q-, and 17q+	Lymphoma
1q+, 3q+, 4q-, −7, 8q+ (MYC gain), 9p- (distal, CD274 deletion), 11p+ (proximal), 13q+ (distal), 17q+ (ERBB2 gene gain), 20q+ and others	Gastric
1q+, 3q+, 5p+, 5q-, +7, +8 (MYC amplification), +10, −15, +16, 17p+, 18p+, 19q+, +20, +21 and others.	Breast
1q+, 13q-, 15p+, 16p+, and 19q+	Breast
+1, +5, 7p+, 11p+, 14q+, 17p-(distal), 17p+ (proximal (FLCN amplification), and 20q+.	Lung
Monosomy 6, partial deletion of 9p- (deletion of CDKN2A/2 B), 13q+, +18 and 19q+	Lymphoplasmacytic lymphoma
1q+ and 17p-	Lung
17p deletion	Lung
+1 (DDR2 and AKT3 gain), 2p-, 2q+, +7, 8p- (distal), 8p+ (proximal), 8q+ (MYC gain), 10p+ (GATA3 gain), 10q- (distal), 11p-, 13q+ (proximal, BRCA2 gain), 16q-, 17p-, 17q- (ERBB2 and CDK12 amplification), +20, 21q+ and multiple others	Breast
10p+ and 13q-.	DLBCL
5q+ (distal, PDGFRB and CSF1R gain) and 16q- (distal)	Breast
1p-, 2q-, 3q+, −4, 5q-, 7p+, 8p+ (proximal, FGFR1 gain), 8q+ (MYC gain), 9q+ (proximal), 11q+, 13q-, 15q+ (proximal), 17p- (TP53 deletion), 17q+, 20q+ and others	Lung
1q+, 2p+, 3q+, +6 (ROS1, ESR1 and CCND3 gain), 10p+, +11, 12p+ (proximal, KRAS amplification), 15q+ (distal, IDH2 gain), 17q+ and +20	Lung
proximal1p-, distal 2q+, distal 5p+ (TERT amp), proximal 5p-, 6p+ (CCND3 amp), 8p-, 8q+, distal 10q-, 12p+, 12q-, 16q-, 17p- (TP53 deletion), proximal 17q+ (ERBB2 amp), 18p+, 19q+, +20, 21q- & others	Gastric
1q+ (DDR1 gain), 5p+ (TERT gain), 6q-, +7, −9, 12q- (distal), 14q+,	Breast
1q+, 3q+ (distal), −4, +5, 6p+, 7p+, 8p-, 9p- (distal, CDKN2A/B deletion), +11, 12p+ (KRAS gain), 15q-, 16p+, 17p-, 18q-, 19p-, 20q+, 22q- and others	Lung
1q+, 6p+, 6q-, 8p-, 10p-, 13q-, 16q-, 21q-, 22q+ (distal) and others	Melanoma
4p-, 5p+, 8p-, 8q+ (MYC gain), +10, −11, −12, 13q-, 15q+ (distal), 17q+ (ERBB2 and CDK12 amplification), 18q-, +20 and others	Breast
loss of CDKN2A/B on 9p and significant loss on 19p involving SMARCA4 and NOTCH3 genes.	Carcinoma
1p-, 1q+, 4p-, 5p+, 7p+, −18 and others	Lung
+1, 5p+, 5q- (proximal), 7p+, −8, −9, 14q+ (proximal), 17p+ (proximal)	Lung
1p-, 1q+, 2q-, 3p-, 3q+, 4p-, 4q+, 7q-(terminal), 7q+ (proximal, CD6 amplification), +8, 10p-, +13, 14q-, +15, 17p-, 17q+, 19p-, 19q+, +20 and others.	Pancreas
+1, 4p-, 5p+, 6p+, +7 (EGFR amplification), 8p-, 9p- (CDKN2A/B deletion), 13q-, 14q+, 16q-, 17p-, 17q+, 19q+, +20 and others	Lung
1q+, 3q+, 8p+(FGFR1 amplification), 10p-, +12 (MDM2 amplification), 17p-, −18, 20q+ and others.	Breast
1q+, +3, 5p+, 5q-, +6, +7, 8q+, +9, 10p+, 10q-, +11, +13, −14, 18q-, +20 and others.	colorectal
2p-, 3q+, −4, 5p+, 5q-, 6p+, 7q-, 7p+, 8p-, 8q+, 10q-, 11p-, 11q+12q-, 13p-, 13q-(proximal), 13q+(distal), 15q-, 16p-, 17p-, 18q-, 19p-, −22 and others.	Ovarian
1q+, +4, and 16p+.	Melanoma
Trisomy 1, 3p+ (distal), 5p+, 5q-, 6q (ESR1 and ARID1B gain), 8p+ (proximal), 9p+ (JAK2 and CD274 gain), 10p+, +18, +19 (CCNDE1 gain) and others	Breast
1q+ (distal, AKT3 gain), 8p+ (proximal, FGFR1 amplification), 8q+ (MYC amplification), +10, 11q+ (proximal, CCND1 amplification), 11q- (distal), 13q-, +20	Breast
1p-, −4, 5q+, 6q-, +7,8p-, 8q+, 13q-, 17p-, 18p+, 18q-, 20p-, 20q+ and others	Colorectal
4p-, and 16p+	Breast
1p-, 1q+, +2, 3q+, 4q-, +5, 6p+, 7p+, 8p-, 8q+, 9q+, +11, 12p+(amplification of KRAS gene), 17p-, 19p-, 20q+, −22 and others.	Lung
1q+, 2q- (proximal), 3p-, 3q+, 6q+ (ESR1 gain), 8q+ (MYC gain), 9p- (CDKN2A/B deletion), 13q-, 17p-, 21q- and multiple others	Breast
4p-, 7p-, 8p-, 9p-, 11p-, −13q, −14q, 18q-, 21q- and others	Breast
1q+, 2q+, 3p-, 3q+, 4q-, 5q-, 5q-, 7p+, 8p-, 8q+, 9p-, 10q+, 11p-, 11q+(CCND1 and FGF4 amplification), 12q-, 13q+, +16, 17q+, 18p-, +20, +21 and others.	Breast
1q+, 3q+(MECOM and TERC amplification), 5p+, 5q+ (distal, PDGFRB amplification)), 7p+, 13q+, +16, 17p-, 19q+, and others.	Lung
1p+(MTOR amplification), 1q+, 8p-, +14, 16p+, and 22q-.	Lung
1q+, 3q- (proximal), 3q+ (distal), 5p+, 7p+, 8p+ (proximal, FGFR1 gain), 10p-, −12, 13q-, 15q-, 17p-, 19q+ (CCNE1 and AKT2 gain) and others	Lung
1q+, 8p+ (proximal, FGFR1 amplification), 11q+ (proximal, CCND1 gain), 16p+	Breast
1p+, 2q-, −3, −6, +7, 8q+(MYC amplification), −9, 10p+, 10q-, 11q+(CCND1 amplification), −13, +14, −15, 16p+, 17p-, 19q+ and others.	Lung
1p-, 1q+, 4q-, 6p+, 6q-, 7p+, 8p-, 8q+, 12p-, −13, 16p+, 16q-, −17, −18, +20–22 and others.	Breast
Primary CNS tumors	
4p+, −5, 6p-, −8, −11, −13, −14, 17p-, −18 and others.	Glioma
+1, +2 with MYCN amplification), +7, −10, +12 (Amplification of CD4 and MDM2 genes), +15, +18, +19, +20, +21 and + 22.	Glioblastoma
1p-, −2, −4, 6q-, 9p-(Homozygous deletion of CDKN2A/B), −10, −11, −13, 15q-, and 16q-.	Glioblastoma
2p-, 5q-, 9p- (distal), 10p-, 12p-, 16q-, 17p-, −18, 19q-, 20p-, 21q-	Glioblastoma

The demonstration of the presence or absence of chromosomal abnormalities is relevant for diagnosis as well as for prognosis. For example, Table 4 shows lymphoma patient (patients # 2) with single abnormality (trisomy 12) who achieved remission and remained in remission after 406 days while patient 8 who had multiple chromosomal abnormalities (complex abnormalities) relapsed within 285 days.

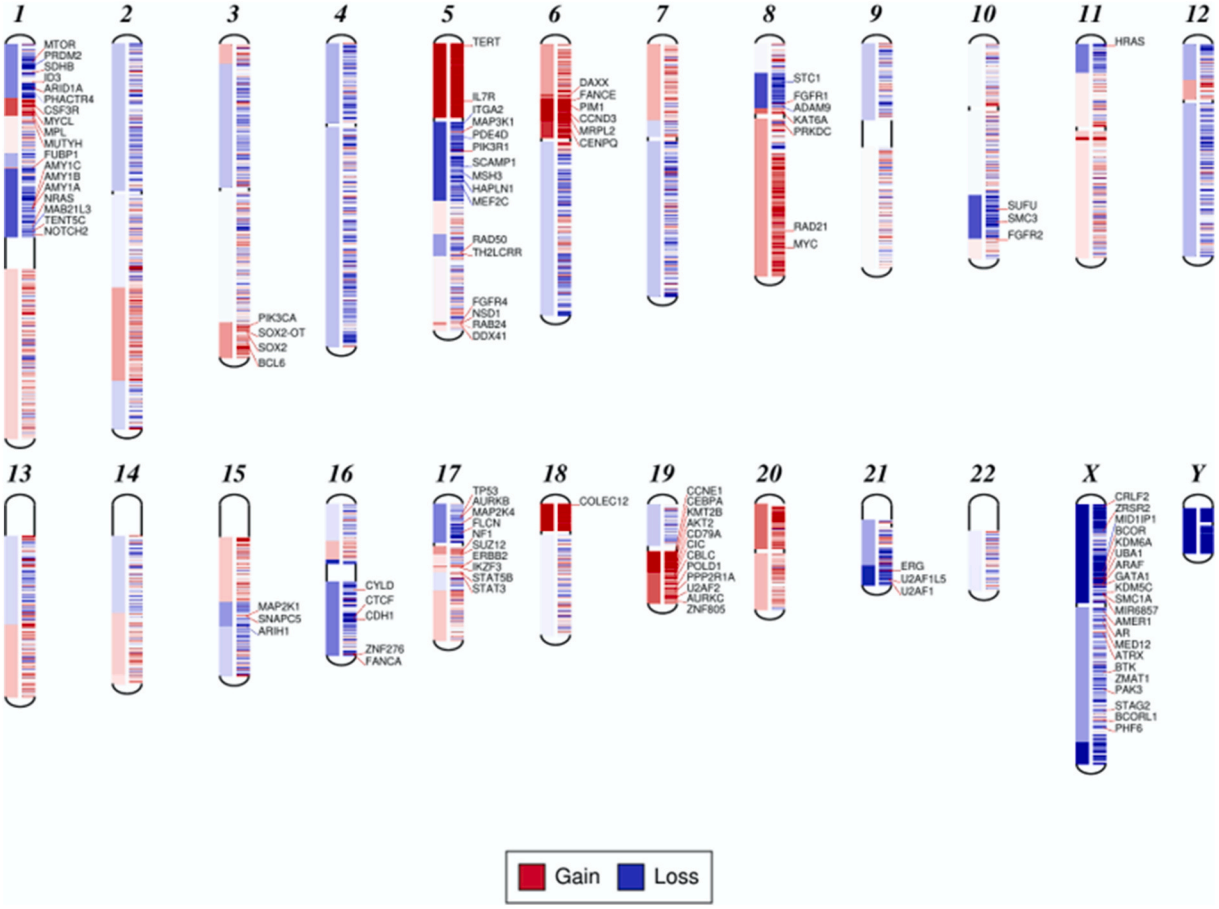

Fig. 1. Example of chromosomal aberrations detected in CSF of a patient with metastatic breast cancer with amplification of *ERBB2* (*HER2*) along with other abnormalities.

3.3. Mutations profiles in CSF

The detected mutations varied with the type of tumor. However, 21 of the 125 (17 %) tested samples showed mutations in ASXL1, TET2, and DNMT3A that are likely represent CHIP (clonal hematopoiesis of indeterminate potential). Two of these cases showed strictly CHIP without other abnormalities and were considered negative for molecular evidence of metastatic or primary CNS tumor. Upon comparing PB with CSF, 13 of the 14 compared samples showed low level mutations in PB that were mainly CHIP or germline. One patient with diffuse midline glioma showed mutation in *H3-3A(H3K27M)* mutation in both PB and CSF. However, the level of the mutation was significantly higher in CSF than in PB (variant allele frequency 36 % vs 0.001 %). More importantly none of the testing PB samples showed chromosomal abnormalities while 8 of the 14 cases showed significant chromosomal abnormalities in CSF.

Twenty-four of the 125 informative cases (19 %) had mutations detected at high variant allele frequencu (VAF) (40 %–50 %), suggesting germline mutations. Of these, 2 had mutations in BRCA1, 3 in BRCA2, 3 in PRKDC, 5 in CHEK2, 2 in FANCC, 2 in PALB2, 1 in ALK, 1 in BARD1, 1 in TP53, 1 in PTEN, 1 in MSH2, 1 in MPL, and 1 in SRC gene. Most of these germline mutations are involved in DNA repair and lead to homologous recombination deficiency (HRD). Distinguishing germline mutations from somatic can be difficult when tumor fraction in the analyzed sample is significantly high. However, in most of the tested liquid biopsy samples, tumor fraction was very low and VAF of the germline mutation is called only when significant difference is present between the germline mutation and the various somatic mutations.

Of the 29 breast cancer metastatic tumors with positive results, 2 (7 %) had ESR1 mutations and 9 (31 %) had PIK3CA mutations. Of the 21 positive lung cancer cases, 9 (43 %) had EGFR mutations and 5 (24 %) had KRAS mutations.

3.3.1. Clinical relevance of CSF liquid biopsy testing

Although the demonstration of the presence of molecular abnormalities associated with cancer in CSF serves as confirmatory test for CNS involvement by cancer, the findings are relevant for therapy and monitoring. Mutations associated with resistance can be detected in the CSF and selection for new actionable mutation can also be detected in testing CSF. Table 4 shows examples of monitoring patients using CSF liquid biopsy testing. As shown, the first 4 patients converted from positive to negative with treatment while patient 8 showed low level of mutation suggesting possible lymphoma but converted to overt diffuse large B-cell lymphoma at subsequent testing. The level of abnormalities is reported quantitively as molecular per 1 mL of CSF (data not shown) and this level can be monitored quantitively. While long follow up is not available but from the limited data converting to negative CSF is likely associated with better outcome. For example, patients #2 in Table 4 remained in CR after 406 days suggesting better progression free survival and possibly overall survival.

4. Discussion

CSF provides a special environment that is different from peripheral blood plasma. CSF contains significantly lower levels of cells, protein and sugar [18]. Furthermore, the protein profile and response elements to various tumors or inflammatory processes differ between CSF and peripheral blood plasma [19,20]. CSF is specifically enriched with

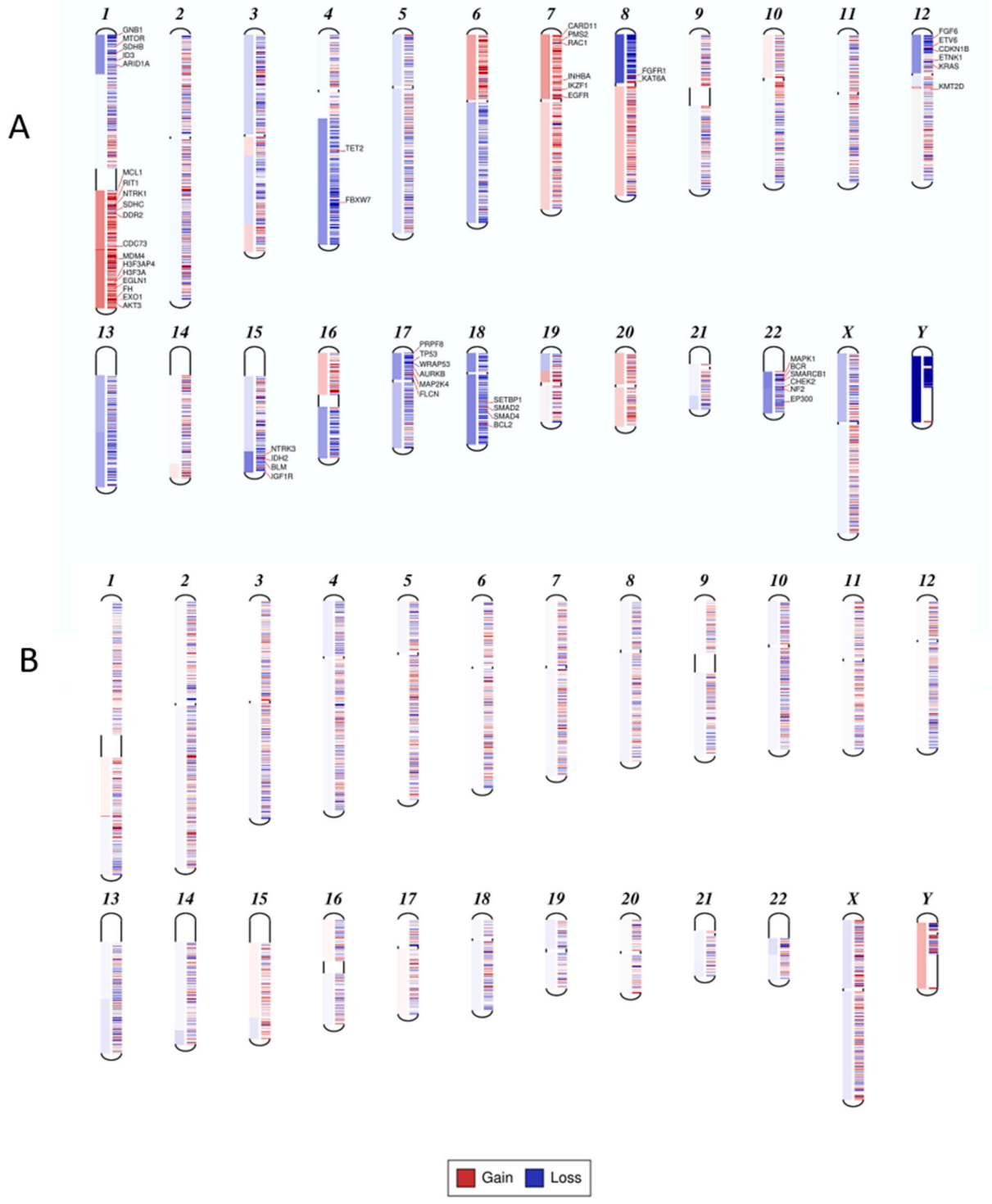

Fig. 2. Chromosomal aberrations detected in cfDNA from a cerebrospinal fluid (CSF) but not in peripheral blood (PB) sample collected at the same time.

T-cells [21]. Therefore, CSF in general does not provide a good environment for cells to survive and be examined through routine cytology, and evaluation of CFS cytology is frequently inconclusive or negative in the presence of CNS metastasis [22]. Next-generation sequencing of cfDNA and cfRNA in CSF may represent a good alternative. More importantly, genomic analysis of the molecular abnormalities may provide information that can be used for targeted therapy or predicting resistance to specific therapy [6,23–25].

The findings of this study demonstrate that sequencing cfDNA in CSF is a reliable approach for evaluating CNS involvement by a metastatic tumor. However, our attempts to evaluate cfRNA showed that in most cases, cfRNA is degraded and unreliable for molecular evaluation or quantification. Unlike peripheral blood [17], cfRNA is detected at very low levels despite the cfDNA is easily detected and at relatively high levels. Sequencing of the low level cfRNA results in biased RNA levels for the highly expressed genes, which are frequently house keeping genes, while the important genes that are involved in the oncogenesis process are not detected (zero level). In our study, we demonstrate that of patients with metastatic tumors, 82 % of tested CSF samples showed clinically useful information when cfDNA is used. The studied metastatic

Table 3
Comparing cerebrospinal fluid (CSF) with peripheral blood (PB).

Patient	CSF	PB
2746	KRAS (G12V), MRE11A, BRCA2, KIT (exon 17), SETD2, KMT2C, NOTCH2, PRPF8, KMT2D, RET, CUX1, FGFR2, SAMD9L, POT1, AXIN2, IDH1, CREBBP, DDR2, EXO1, CARD11, MRE11A (Germline), B-cell clonality (IgHV3-7/IgKV3D-20), 1q+, 3q+ (distal), −4, +5, 6p+, 7p+, 8p-, 9p- (distal, CDKN2A/B deletion), +11, 12p+ (KRAS gain), 15q-, 16p+, 17p-, 18q-, 19p-, 20q+, 22q- and others	DNMT3, TET2, ERBB3, MRE11A (germline)
2752	KMT2C	KMT2C, EZH2, NBN, ROS1, DNMT2A.
2821	BRAF (V600E),KMT2C, 1q+, 6p+, 6q-, 8p-, 10p-, 13q-, 16q-, 21q-, 22q+ (distal)	KMT2C, DNMT3A, CREBBP, NOTCH2
3091	None	None
3193	H3-3A, NF1, and TP53, ERBB2 (germline), 4p+, −5, 6p-, −8, −11, −13, −14, 17p-, −18 and others.	H3-3A, ERBB2(germline)
3290	SMARCA4, STK11, TGFBR2, AKT3, ALK, PRDM1, LRP1B, INHBA, BCR, KEAP1, GPRC5D, CACA. loss of CDKN2A/B on 9p and significant loss on 19p involving SMARCA4 and NOTCH3 genes 9p-(loss of CDKN2A/B), −19p(loss of SMARCA4 and NOTCH3)	DNMT3A, CREBBP
7751	KRAS, MRE11A, KIT (exon 17), BRCA2, SETD2, KMT2C, CUX1, AXIN2, SAMD9L, KMT2D, CARD11, PRPF8, POT1, NOTCH2, RET, CREBBP, EXO1, IDH1, FGFR2, and DDR2, MRE11A (germline) 1p-, 1q+, 3p-, 3q+, −4, +5,6p+, 7p+, 8q+, 9p- (distal, CDKN2A/B deletion), 11q+, 15q-, 16p+, 17p-, 20q+ and multiple others	DNMT3A, DNMT3A, TET2, DNMT3A, TP53, ERBB3, MRE11A (germline)
8167	TP53 (2 mutations), WHSC1	TP53 (2 mutations), WHSC1
8172	TERT (2 mutations), STK11	TERT (1 mutation), STK11
9735	NF1, DDX41(Germline), WT1 (Germline), NTRK2, ARID1A, FANCA (Germline), BRCA2, ASXL1, SPEN, EPC1, 1q+, 2p-, 3p-, 5q-, 6q-, 8p+ (proximal, FGFR1 amplification), 8p- (distal), 9p- (CDKN2A/B deletion), 10q- (distal), 11q+ (proximal, CCND1 amplification), 13q- (RB1 deletion), 16p+, 17p-, +20, 21q	PPM1D, DNMT3A, SETD3, WT1 (germline), FANCA(germline), DDX41(germline)
10163	PIK3CA, CDH1, TET2, CBLB, SMARCA4, PRKDC, AKT3, SMC1A, 1q+, 3p-, 4q-, 6p+, 7p+, 8p-, 8q+, 13q-, 15q-, 16p+, 16q-, 17p-, −18, +20, 22q-	PIK3CA, PHF6, KMT2B
10599	STK11, APC, MET, TG(germline)	FLT3-ITD(VAF = 0.35), TG (germline)
11039	TP53, EGFR, BRCA1, and RAD21 (2 mutations), 3p+ (proximal), 5q+ (proximal), 9p-, 13q+ (distal) and others	SF3B1, NOTCH3, PPM1D, NKX2-1, RAD21 (3 mutations), EP300, TERT
11204	KMT2C	KMT2C

Table 4
Changes in the CSF findings while on treatment.

Patients	Days since baseline	Mutated genes and chromosomal abnormalities
1	0	KMT2C, MYD88
	85	KMT2C
	143	KMT2C
2	0	SOCS1, EGFR, PRKDC, XPO1, ROS1, ERBB4, PIM1 (2 mutations), EP300, CD79B, FUBP1, B-cell clonality (IgKV 9-1), trisomy 12
	36	No evidence of mutations, No B-clonality
	406	No evidence of mutations, No B-clonality
3	0	BRCA1, TP53, CBL, DPYD (Germline), B cell clonality (IGKV1D-39)
	118	No evidence of mutations, No B-clonality
4	0	CHEK2 (Germline), BRCA2, GNAS CUX1, LRP1B, (1q+, 13q-, 15p+, 16p+, and 19q+)
	24	CHEK2 (Germline) No evidence of somatic mutations
	107	CHEK2 (Germline) No evidence of somatic mutations
5	0	ERBB2 amplification, PIK3CA, PRKDC (Germline), NTRK2 CIC, CDH1 (1p-, distal 2q+, distal 5p + TERT amp, proximal 5p-, 6p + CCND3 amplification, 8p-, 8q+, distal 10q-, distal 11p-, 16q-, 17p-, small 17q+ (ERBB2 amp), 18p+, 19q+, +20, −21).
	59	ERBB2 amplification, PIK3CA, PRKDC (Germline), NTRK2 CIC, CDH1 (1p-, distal 2q+, distal 5p + TERT amp, proximal 5p-, 6p + CCND3 amplification, 8p-, 8q+, distal 10q-, distal 11p-, 16q-, 17p-, small 17q+ (ERBB2 amp), 18p+, 19q+, +20, −21).
6	0	BRCA1(Germline) TP53, NF1, CDK12, TSHR, ACVR1B, ABCB1 (Germline), HPV viral RNA (Type 82), (2p-, 3q+, −4, 5p+, 5q-, 6p+, 7q-, 7p+, 8p-, 8q+, 10q-, 11p-, 11q+12q-, 13p-, 13q-(proximal), 13q+(distal), 15q-, 16p-, 17p-, 18q-, 19p-, −22).
	99	BRCA1(Germline) TP53, NF1, CDK12, TSHR, ACVR1B, ABCB1 (Germline), (2p-, 3q+, −4, 5p+, 5q-, 6p+, 7q-, 7p+, 8p-, 8q+, 10q-, 11p-, 11q+12q-, 13p-, 13q-(proximal), 13q+(distal), 15q-, 16p-, 17p-, 18q-, 19p-, −22).
7	0	EGFR,TP53, TET2, EBV viral RNA, HPV viral RNA(type 82), (+1, +5, 7p+, 11p+, 14q+, 17p-(distal), 17p+ (proximal (FLCN amplification), and 20q+).
	182	EGFR,TP53, TET2, (+1, +5, 7p+, 11p+, 14q+, 17p-(distal), 17p+(proximal (FLCN amplification), and 20q+).
8	0	KMT2C
	285	CBL (2 mutations), NF1, KMT2C (2 mutations), BCORL1, EP300, (1q+, 3q-, −4, 7p-, 7q+, 8q+, −14, 15q-, and 19q- B-cell clonality (IgHV 3–30/IgKV 1–5)
9	0	DNMT3A, TP53
	103	No evidence of mutations
10	0	KRAS (G12V), MRE11A (2 mutations), BRCA2, KIT (exon 17), SETD2, NOTCH2, RET, FGFR2, AXIN2, IDH1, CREBBP, DDR2 KMT2C, PRPF8, KMT2D, CUX1, SAMD9L, POT1, EXO1, CARD11 -(1q+, 3q+ (distal), −4, +5, 6p+, 7p+, 8p-, 9p- (distal, CDKN2A/B deletion), +11, 12p+ (KRAS gain), 15q-, 16p+, 17p-, 18q-, 19p-, 20q+, 22q- and others)
	14	MRE11A (2 mutations), KRAS (G12V), KIT (exon 17), NOTCH2, CREBBP, BRCA2, DDR2 KMT2C, SAMD9L, KMT2D, EBV viral RNA, (1p-, 1q+, 4p-, 5p+, 7p+, −18 and others)
	56	KRAS (G12V), MRE11A (2 mutations), BRCA2, KIT, AXIN2, RET, SETD2, NOTCH2, DDR2, FGFR2, CREBBP, IDH1, TET2 CUX1, KMT2C, PRPF8, SAMD9L, EXO1, CARD11, KMT2D, POT1 (1p-, 1q+, +2, 3q+, 4q-, +5, 6p+, 7p+, 8p-, 8q+, 9q+, +11, 12p+(amplification of KRAS gene), 17p-, 19p-, 20q+, −22 and others).
	105	KRAS (G12V), MRE11A (2 mutations), KIT (exon 17), BRCA2, SETD2, AXIN2, NOTCH2, RET, CREBBP, IDH1, FGFR2, DDR2 KMT2C, CUX1, SAMD9L, KMT2D, CARD11, PRPF8, POT1, EXO1, EBV viral RNA, (1p-, 1q+, 3p-, 3q+, −4, +5,6p+, 7p+, 8q+, 9p- (distal, CDKN2A/B deletion), 11q+, 15q-, 16p+, 17p-, 20q+ and multiple others)
	147	KRAS (G12V), BRCA2, MRE11A (2 mutations), SETD2, FGFR2, KIT (exon 17), CREBBP, AXIN2, IDH1,

(*continued on next page*)

tumors included solid tumors and lymphoma (Table 1). Breast cancer was the most common, followed by lung cancer; the rate of detectable tumors by liquid biopsy in these cancers was 91 % and 95 %, respectively. A high proportion (68 %) of lymphomas (DLBCL and other types) were also positive by CSF liquid biopsy.

More importantly, the findings from liquid biopsy were often clinically relevant for selecting therapy [26]. For example, 31 % of breast cancer showed mutations in PIK3CA, suggesting that they can be targeted by PI3K inhibitors [27]; 9 % showed the emergence of mutations in ESR1, suggesting possible benefit from therapy with fulvestrant [28,

Table 4 (*continued*)

Patients	Days since baseline	Mutated genes and chromosomal abnormalities
		NOTCH2, RET, CARD11, DDR2 KMT2C, KMT2D, SAMD9L, CUX1, PRPF8, EXO1, EBV viral RNA, (1q+, 3q+ (distal), −4, +5, 6p+, 7p+, 8p-, 9p-, +11, 12p+, 15q-, 16p+, 17p-, 18q-, 19p-, 20q+, 2q- and others)
	161	MRE11A (2 mutations), KRAS (G12V), BRCA2, KIT (exon 17), SETD2, NOTCH2, RET, DDR2, AXIN2, CREBBP, IDH1, FGFR2 KMT2C, CARD11, CUX1, PRPF8, SAMD9L, EXO1, KMT2D, POT1, (1q+, 3p-, 4p-, 5p+, 7p+, 8p-, 10p-, 11q+, 16p+, 19p-, 22q-)
11	0	KMT2C
	91	KMT2C
12	0	EGFR (exon 19), MECOM::PLD1 fusion mRNA, (1q+, 3q+(MECOM and TERC amplification), 5p+, 5q+ (distal, PDGFRB amplification), 7p+, 13q+, +16, 17p-, 19q+, and others).
	140	MECOM::PLD1 fusion mRNA, (1q+, 2q+, 5p+, 5q+ (distal), 7p+, 11p+, 13q+, +16, 17p-, 17q+, 19q+ and others).
13	0	PIK3CA, PRKDC, TET2, SMARCA4 CDH1, (1p-, 1q+, 4q-, 6p+, 6q-, 7p+, 8p-, 8q+, 12p-, −13, 16p+, 16q-, −17, −18, +20–22 and others).
	77	PIK3CA, PRKDC, TET2, SMARCA4, CDH1 (1p-, 1q+, 4q-, 6p+, 6q-, 7p+, 8p-, 8q+, 12p-, −13, 16p+, 16q-, −17, −18, +20–22 and others).
	133	PIK3CA, SMARCA4, TET2, SMC1A, PRKDC, AKT3, CDH1, CBLB (1q+, −3, 4q-, 6p+(CCND3 gain), +7 (EGFR gain), 8p-, 8q+, 12p-, 13q-, 15q-, 16p+, 16q-, 17p-, −18, +20, 22q- and others)
14	0	PIK3R2, ARID2, UBA1, (1q+, 6q-, 10q+ (RET amplification), 12q+ (MDM2 amplification), 13q-, 14q-, 15q-18q-, 20q+ and 21q-)
	83	MDM2 amplification only

29] and CDK4/6 targeted therapies [30]. In lung cancer cases, 43 % showed mutations in EGFR that can be targeted, and 24 % showed mutations in KRAS. These findings support the concept that lung cancer with EGFR and KRAS mutations have greater tendency to involve the CNS [31,32]. Furthermore, liquid biopsy using CSF can be used for monitoring patients and evaluating efficacy of therapy. The data shown in Table 4 is clinically useful and help physician tailor their therapy properly.

Our testing panel includes most of the genes known to be involved in inherited cancer (Supplement 1). While the presence or absence of germline mutation should be confirmed by testing skin biopsy or peripheral mononuclear cells if they are not involved in cancer, alerting the treating physician to the potential of the presence of a germline mutation is very important. Germline mutations are expected when the variant allele frequency (VAF) is between 40 % and 50 %, while other mutations are suspected when the VAF is significantly lower [33]. Interestingly, in this series of patients, germline mutations were detected in 22 % of the tested samples with metastatic tumors, markedly higher than the expected prevalence in all types of cancers (between 6 % and 10 %) [34]. While it has been suggested that patients with germline mutations in genes involved in predisposition to cancer may have a higher tendency for involving CNS [35,36], this may reflect the type of cancers that have higher tendency to metastasize to CNS. High percentage of the metastatic tumors in our study had breast cancer and germline mutations play a significant role in the oncogenesis of breast cancer, which possibly explains the high rate of germline mutations in our cases.

Our assay also detected significant chromosomal gains and losses in CSF that can be clinically relevant not only for diagnosis, but also for therapeutic approaches. More than half (54 %) of metastatic tumors showed chromosomal gains or losses detectable in CSF. One sample, from a patient with metastatic breast cancer, showed chromosomal changes without a single gene point mutation. Breast cancer is one of the tumors in which oncogenesis is more likely to be driven by chromosomal structural abnormalities than single-gene mutations [33,37,38]. Detecting such abnormalities is important for diagnosis as well as therapy selection [39]. For example, detecting evidence of gene amplification in ERBB2, CCND1, MDM2, and FGFR1 is relevant for selecting therapy that targets these genes [40].

Despite the small sample size in our study, our data show that primary CNS tumors can be detected using CSF, albeit at a much lower detection rate as compared to metastatic tumors (55 % in primary vs 82 % in metastatic) (Table 1). Of the 22 informative cases involving suspected primary CNS tumors, 11 showed evidence of tumor and 4 showed chromosomal structural abnormalities that are relevant for classification and prognosis. Only one patient with an informative sample showed a germline mutation, in CHEK2.

Making treatment decisions based on molecular findings in CSF is new clinical practice that should be implemented carefully. Decision for treatment should consider the overall clinical presentation and history. In evaluating molecular findings in CSF, CHIP should be excluded. As shown in Table 3, frequently, some CHIP mutations can also be detected in CSF but solid tumor abnormalities are frequently dominant when the CNS is involved with tumor. Comparing findings in CSF with those in PB might be a good standard practice. In addition, making sure that the CSF findings are consistent with expected findings of the primary tumor is very important.

In summary, liquid biopsy performed on CSF is reliable for detecting CNS involvement in patients with metastatic tumors and, to a lesser degree, in those with primary brain tumors. Our conclusions are limited by the relatively small number of cases, and further studies correlating CSF molecular findings with outcome are needed.

Ethics

This retrospective study of data was performed under an approved IRB protocol (WCG IRB # 1-1476184-1) and was conducted in accordance with the principles of the Declaration of Helsinki and its later amendments.

Author contributions

Conceptualization, M.A.-A.G. and A.P.; Methodology, M.A.-A.C.-S.A. and A.M., Analysis, M.A. A.C.-S.A. and A.M.; Investigation, M.A., A.I., and A.G.- Resources, M.A. and A.G.; data curation, A.C. and M.A.; writing—review and editing, A.C.-S.A.-A.M.-A.I.-A.G. and M.A. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data presented in this study is available on request from the corresponding author.

Ethical approval

The study protocol was approved by the Western Copernicus Group Institutional Review Board (New England IRB, Aspire IRB, and Midlands IRB) (Number 1-1476184-1). Patient informed consent was waived due to incidental collection and lack of risk. This study was conducted in accordance with the principles of the Declaration of Helsinki and its later amendments.

Submission declaration and verification

The manuscript has not been published previously and is not under consideration for publication elsewhere. The submission of this manuscript has been approved by all authors. If accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the

Declaration of generative AI in scientific writing

No generative AI is used in writing the paper.

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Declaration of competing interest

AC, SA, AM and MA work for (employed) a diagnostic company offering clinical liquid biopsy testing.

MA, AP and AG own stocks in a diagnostic company offering clinical liquid biopsy testing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jlb.2024.100281.

References

[1] Ballester LY, Lu G, Zorofchian S, Vantaku V, Putluri V, Yan Y, Arevalo O, Zhu P, Riascos RF, Sreekumar A, et al. Analysis of cerebrospinal fluid metabolites in patients with primary or metastatic central nervous system tumors. Acta Neuropathol. Commun 2018;6. https://doi.org/10.1186/s40478-018-0588-z.

[2] Weston CL, Glantz MJ, Connor JR. Detection of cancer cells in the cerebrospinal fluid: current methods and future directions. Fluids Barriers CNS 2011;8:14. https://doi.org/10.1186/2045-8118-8-14.

[3] Hickman RA, Miller AM, Arcila ME. Cerebrospinal fluid: a Unique source of circulating tumor DNA with broad clinical applications. Transl. Oncol. 2023;33: 101688. https://doi.org/10.1016/j.tranon.2023.101688. 101688.

[4] Deak D, Gorcea-Andronic N, Sas V, Teodorescu P, Constantinescu C, Iluta S, Pasca S, Hotea I, Turcas C, Moisoiu V, et al. A narrative review of central nervous system involvement in acute leukemias. Ann Transl Med 2021;9:68. https://doi.org/10.21037/atm-20-3140. 68.

[5] Min H-Y, Lee H-Y. Molecular targeted therapy for anticancer treatment. Exp Mol Med 2022;54:1670–94. https://doi.org/10.1038/s12276-022-00864-3.

[6] Regulska K, Stanisz B, Regulski M. Individualization of anticancer therapy; molecular targets of novel drugs in oncology. Postepy hig. Med Doswiadczalna 2012;66:855–67. https://doi.org/10.5604/17322693.1019649.

[7] Millner LM, Linder MW, Valdes R. Circulating tumor cells: a review of present methods and the need to identify heterogeneous phenotypes. Ann Clin Lab Sci 2013;43:295–304.

[8] Lin X, Fleisher M, Rosenblum M, Lin O, Boire A, Briggs S, Bensman Y, Hurtado B, Shagabayeva L, DeAngelis LM, et al. Cerebrospinal fluid circulating tumor cells: a novel tool to diagnose leptomeningeal metastases from epithelial tumors. Neuro Oncol 2017;19:1248–54. https://doi.org/10.1093/neuonc/nox066.

[9] Baba Al, Câtoi C. Tumor cell morphology. Comparative oncology. Bucharest, Romania: The Publishing House Of The Romanian Academy; 2007. ISBN 9789732714577.

[10] Borek E, Baliga BS, Gehrke CW, Kuo CW, Belman S, Troll W, Waalkes TP. High turnover rate of transfer RNA in tumor tissue. Cancer Res 1977;37:3362–6.

[11] Kan C-M, Pei XM, Yeung MHY, Jin N, Ng SSM, Tsang HF, Cho WCS, Yim AK-Y, Yu AC-S, Wong SCC. Exploring the role of circulating cell-free RNA in the development of colorectal cancer. Int J Mol Sci 2023;24:11026. https://doi.org/10.3390/ijms241311026.

[12] Hassan S, Shehzad A, Khan SA, Miran W, Khan S, Lee Y-S. Diagnostic and therapeutic potential of circulating-free DNA and cell-free RNA in cancer management. Biomedicines 2022;10:2047. https://doi.org/10.3390/biomedicines10082047.

[13] Otsuji R, Fujioka Y, Hata N, Kuga D, Hatae R, Sangatsuda Y, Nakamizo A, Mizoguchi M, Yoshimoto K. Liquid biopsy for glioma using cell-free DNA in cerebrospinal fluid. Cancers 2024;16:1009. https://doi.org/10.3390/cancers16051009.

[14] McEwen AE, Leary SES, Lockwood CM. Beyond the blood: CSF-derived CfDNA for diagnosis and characterization of CNS tumors. Front Cell Dev Biol 2020;8. https://doi.org/10.3389/fcell.2020.00045.

[15] Gaitsch H, Franklin RJM, Reich DS. Cell-free DNA-based liquid biopsies in neurology. Brain 2023;146:1758–74. https://doi.org/10.1093/brain/awac438.

[16] Wang P, Zhang Q, Han L, Cheng Y, Sun Z, Yin Q, Zhang Z, Yu J. Genomic instability in cerebrospinal fluid cell-free DNA predicts poor prognosis in solid tumor patients with meningeal metastasis. Cancers 2022;14:5028. https://doi.org/10.3390/cancers14205028. 5028.

[17] Albitar M, Zhang H, Charifa A, Ip A, Ma W, McCloskey J, Donato M, Siegel D, Waintraub S, Gutierrez M, Pecora A, Goy A. Combining cell-free RNA with cell-free DNA in liquid biopsy for hematologic and solid tumors. Heliyon 2023 May 16;9(5): e16261. https://doi.org/10.1016/j.heliyon.2023.e16261. eCollection 2023 May. PMID: 37251903.

[18] Hladky SB, Barrand MA. Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. Fluids Barriers CNS 2014;11. https://doi.org/10.1186/2045-8118-11-26.

[19] Huang J, Khademi M, Fugger L, Lindhe Ö, Novakova L, Axelsson M, Malmeström C, Constantinescu C, Lycke J, Piehl F, et al. Inflammation-related plasma and CSF biomarkers for multiple sclerosis. Proc Natl Acad Sci USA 2020;117:12952–60. https://doi.org/10.1073/pnas.1912839117.

[20] Vasunilashorn SM, Ngo LH, Dillon ST, Fong TG, Carlyle BC, Kivisäkk P, Trombetta BA, Vlassakov KV, Kunze LJ, Arnold SE, et al. Plasma and cerebrospinal fluid inflammation and the blood-brain barrier in older surgical patients: the role of inflammation after surgery for elders (RISE) study. J Neuroinflammation 2021;18: 1–10. https://doi.org/10.1186/s12974-021-02145-8.

[21] Cordone I, Masi S, Giannarelli D, Pasquale A, Conti L, Telera S, Pace A, Papa E, Marino M, de Fabritiis P, et al. Major differences in lymphocyte subpopulations between cerebrospinal fluid and peripheral blood in non-hodgkin lymphoma without leptomeningeal involvement: flow cytometry evidence of a cerebral lymphatic system. Front Oncol 2021;11:685786. https://doi.org/10.3389/fonc.2021.685786.

[22] Sung Bae Yoon, Cheong J-W, Chang Won Seok, Kim S, Oh Eun Ji. Se hoon kim diagnostic accuracy of cerebrospinal fluid (CSF) cytology in metastatic tumors: an analysis of consecutive CSF samples. Korean J. Pathol. 2013;47:563. https://doi.org/10.4132/koreanjpathol.2013.47.6.563. 563.

[23] Friedman JS, Hertz AJ, Karajannis MA, Miller AM. Tapping into the genome: the role of CSF CtDNA liquid biopsy in glioma. Neurooncol. Adv 2022;4:ii33–40. https://doi.org/10.1093/noajnl/vdac034.

[24] Miller AM, Karajannis MA. Current role and future potential of CSF CtDNA for the diagnosis and clinical management of pediatric central nervous system tumors. J Natl Compr Cancer Netw 2022;20:1363–9. https://doi.org/10.6004/jnccn.2022.7093.

[25] Orzan F, De Bacco F, Lazzarini E, Crisafulli G, Gasparini A, Dipasquale A, Barault L, Macagno M, Persico P, Pessina F, et al. Liquid biopsy of cerebrospinal fluid enables selective profiling of glioma molecular subtypes at first clinical presentation. Clin Cancer Res 2023;29:1252–66. https://doi.org/10.1158/1078-0432.CCR-22-2903.

[26] Sivapalan L, Murray JC, Canzoniero JV, Landon B, Jackson J, Scott S, Lam V, Levy BP, Sausen M, Anagnostou V. Liquid biopsy approaches to capture tumor evolution and clinical outcomes during cancer immunotherapy. J. Immunother. Cancer 2023;11:e005924. https://doi.org/10.1136/jitc-2022-005924.

[27] Ellis H, Ma CX. PI3K inhibitors in breast cancer therapy. Curr Oncol Rep 2019;21: 53. https://doi.org/10.1007/s11912-019-0846-7.

[28] Dustin D, Gu G, Fuqua SAW. ESR1 mutations in breast cancer. Cancer 2019;125: 3714–28. https://doi.org/10.1002/cncr.32345.

[29] Turner NC, Swift C, Kilburn L, Fribbens C, Beaney M, Garcia-Murillas I, Budzar AU, Robertson JFR, Gradishar W, Piccart M, et al. ESR1 mutations and overall survival on fulvestrant versus exemestane in advanced hormone receptor–positive breast cancer: a combined analysis of the phase III SoFEA and efect trials. Clin Cancer Res 2020;26:5172–7. https://doi.org/10.1158/1078-0432.ccr-20-0224.

[30] Sammons SL, Topping DL, Blackwell KLHR+. HER2– advanced breast cancer and CDK4/6 inhibitors: mode of action, clinical activity, and safety profiles. Curr Cancer Drug Targets 2017;17. https://doi.org/10.2174/1568009617666170330120452.

[31] Tomasini P, Serdjebi C, Khobta N, Metellus P, L'Houcine Ouafik, Nanni I, Laurent Greillier, Loundou A, Fina F, Céline Mascaux, et al. EGFR and KRAS mutations predict the incidence and outcome of brain metastases in non-small cell lung cancer. Int J Mol Sci 2016;17:2132. https://doi.org/10.3390/ijms17122132. 2132.

[32] Zhao W, Zhou W, Rong L, Sun M, Lin X, Wang L, Wang S, Wang Y, Hui Z. Epidermal growth factor receptor mutations and brain metastases in non-small cell lung cancer. Front Oncol 2022;12:912505. https://doi.org/10.3389/fonc.2022.912505.

[33] Yap TA, Ashok A, Stoll J, Mauer E, Nepomuceno VM, Blackwell KL, Garber JE, Meric-Bernstam F. Prevalence of germline findings among tumors from cancer types lacking hereditary testing guidelines. JAMA Netw Open 2022;5:e2213070. https://doi.org/10.1001/jamanetworkopen.2022.13070.

[34] Stout LA, Kassem N, Hunter C, Philips S, Radovich M, Schneider BP. Identification of germline cancer predisposition variants during clinical CtDNA testing. Sci Rep 2021;11. https://doi.org/10.1038/s41598-021-93084-0.

[35] Wang Q. Cancer predisposition genes: molecular mechanisms and clinical impact on personalized cancer care: examples of lynch and HBOC syndromes. Acta Pharmacol Sin 2015;37:143–9. https://doi.org/10.1038/aps.2015.89.

[36] Jovanović A, Tošić N, Marjanović I, Komazec J, Zukić B, Nikitović M, Ilić R, Grujičić D, Janić D, Pavlović S. Germline variants in cancer predisposition genes in pediatric patients with central nervous system tumors. Int J Mol Sci 2023;24: 17387. https://doi.org/10.3390/ijms242417387.

[37] Kou F, Wu L, Ren X. Chromosome abnormalities: new insights into their clinical significance in cancer. Mol. Ther. Oncolytics 2020;17:562–70. https://doi.org/10.1016/j.omto.2020.05.010.

[38] Privitera AP, Barresi V, Condorelli DF. Aberrations of chromosomes 1 and 16 in breast cancer: a framework for cooperation of transcriptionally dysregulated genes. Cancers 2021;13:1585. https://doi.org/10.3390/cancers13071585.

[39] Testa U, Castelli G, Pelosi E. Breast cancer: a molecularly heterogenous disease needing subtype-specific treatments. Med Sci 2020;8. https://doi.org/10.3390/medsci8010018.

[40] Kadota M, Sato M, Duncan B, Ooshima A, Yang HH, Diaz-Meyer N, Gere S, Kageyama S-I, Fukuoka J, Nagata T, et al. Identification of novel gene amplifications in breast cancer and coexistence of gene amplification with an

activating mutation of PIK3CA. Cancer Res 2009;69:7357–65. https://doi.org/10.1158/0008-5472.can-09-0064.