Detection of Circulating Tumor Cells in Cerebrospinal Fluid of Patients with Suspected Breast Cancer Leptomeningeal Metastases: A Prospective Study

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BACKGROUND: The diagnosis of breast cancer (BC)-related leptomeningeal metastases (LM) relies on the detection of tumor cells in cerebrospinal fluid (CSF) using conventional cytology (gold standard). However, the sensitivity of this technique is low. Our goal was to evaluate whether circulating tumor cell (CTC) detection in CSF using the CellSearch® system could be used for LM diagnosis.

METHODS: This prospective, monocentric study included adult patients with suspected BC-related LM. The clinical sensitivity and specificity of CTC detection in CSF for LM diagnosis were calculated relative to conventional CSF cytology.

RESULTS: Forty-nine eligible patients were included and 40 were evaluable (CTC detection technical failure: n = 8, eligibility criteria failure: n = 1). Cytology was positive in 18/40 patients. CTCs were detected in these 18 patients (median: 5824 CTC, range: 93 to 45052) and in 5/22 patients with negative cytology (median: 2 CTC, range: 1 to 44). The detection of ≥1 CSF CTC was associated with a clinical sensitivity of 100% (95% CI, 82.4–100) and a specificity of 77.3% (95% CI, 64.3–90.3) for LM diagnosis. $HER2^+$ CTCs were detected in the CSF of 40.6% of patients with $HER2^-$ BC (median: 500 CTC, range: 13 to 28 320).

CONCLUSIONS: The clinical sensitivity of CTC detection in CSF with the CellSearch® system for LM diagnosis is higher than that of CSF cytology. CTC detection in patients with negative cytology, however, must be further investigated. The finding of $HER2^+$ CTCs in patients with $HER2^-$ BC suggests that the HER2 status of LM should be evaluated to increase the treatment opportunities for these patients.

Introduction

Leptomeningeal metastases (LM) from solid tumors occur in 5% to 19% of patients (1). Breast cancer (BC) is one of the most frequent causes. Similar to brain intraparenchymal metastases, LM incidence seems to be increasing due to the longer survival of patients with metastatic BC and the poor diffusion of therapeutic agents in the central nervous system (CNS).

The diagnosis of LM may be suspected in patients reporting headache with cranial and/or spinal nerve involvement. The two key exams to confirm the diagnosis of LM are conventional cytology of cerebrospinal fluid (CSF) samples (gold standard) and/or brain and medullar MRI. Imaging can show leptomeningeal gadolinium enhancement, sub-arachnoid nodes, cranial nerve enlargement, ventriculitis, and/or non-obstructive hydrocephalus. However, the clinical sensitivity of MRI for LM diagnosis remains poor (1). Therefore, diagnosis confirmation is based on the detection of tumor cells in the CSF using conventional cytology. The CSF sample volume has to be at least 3 mL and analysis must be performed rapidly because 90% of tumor cells are destroyed within 90 min after CSF sampling. Even with optimal CSF sample volume and analysis time, the efficiency of this technique is limited and

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Received April 21, 2022; accepted June 30, 2022.
https://doi.org/10.1093/clinchem/hvac127

repeated samples are required. The clinical sensitivity is approximately 45% for 1 CSF sample and 85% for 3 successive CSF samples (1–3). Moreover, this technique only allows a qualitative analysis (presence or absence of tumor cells in CSF) and not a quantitative evaluation of the tumor cell number.

Considering its paucicellularity and low content of cell-free DNA, CSF might be a good candidate for liquid biopsy to guide patient management: initial diagnosis, therapy choice (e.g., human epidermal growth factor receptor 2 [HER2]-targeted drugs), response monitoring, and prognosis. Previous studies analyzed several CSF biomarkers for LM diagnosis, including cell-free tumor DNA and circulating tumor cells (CTCs) (1, 3). However, the available data on the diagnostic or prognostic value of CTCs are not enough to consider them in clinical practice.

The CellSearch® system (Silicon Biosystems) is the only food and drug administration-cleared method to detect CTC in metastatic breast, prostate, and colorectal cancers (4). In this system, CTCs are EpCAM$^{(+)}$, cytokeratin$^{(+)}$, CD45$^{(-)}$, and nucleated cells (6-diamidino-2-phenylindole, DAPI+) (1, 4–6). The expression of an additional protein (e.g., HER2 (7)) can be analyzed in the fourth channel of the CellSearch instrument. This technique has been validated for CTC detection in several cancers, including metastatic BC (4, 5). In patients with metastatic BC, CTC detection in peripheral blood is a strong independent prognostic factor (5, 6, 8, 9) and a useful tool to guide treatment decisions and to monitor response, thus confirming the clinical validity and utility of this test (5, 9, 10). The feasibility of the detection of CSF CTCs with the CellSearch system has been demonstrated (1, 3, 11–15). These studies provided encouraging results and suggested higher clinical sensitivity and specificity (3) compared with cytology. This technique could facilitate LM diagnosis by reducing the number of samples needed and the organizational constraints of CSF sample processing. Indeed, CTC analysis with the CellSearch system can be reproducibly and robustly performed up to 96 h after sampling. The CellSearch technique also allows the reproducible quantitative measurement of rare tumor cells (13), unlike cytology. Since the CTC number in the blood of patients with metastatic BC is a well-established prognostic factor (5, 6, 8, 9), the CTC number in CSF at LM diagnosis could also have a prognostic value (16, 17). Moreover, CTC enumeration in CSF may give information on the patient response during LM treatment (11, 13, 17). Indeed, the assessment of the response to specific treatments is still problematic in LM, due to lack of standardization (18).

Lastly, the CellSearch system allows the characterization of additional proteins expressed by CTC (e.g., HER2, programmed cell death ligand 1). Importantly, discrepancies in HER2 expression between primary tumor and distant metastases and/or CTCs have been reported (19–24). In LM, data on HER2 expression concordance between CSF CTC and primary tumor are scarce, and only one small study (n = 16 patients) suggested high concordance (25).

In this prospective study, we evaluated the clinical sensitivity and specificity of CTC detection in CSF samples for BC-related LM diagnosis, and the concordance of HER2 expression between the last available primary BC tissue sample and CSF CTC.

Materials and Methods

The complete methodology of this prospective, monocentric study (promoted by the Institut du Cancer de Montpellier, France) is described in the online Supplemental Materials file that accompanies this article (NCT03252912). Adult patients with BC and clinical and/or radiological suspicion of LM were included before the first lumbar puncture. CTC detection with the CellSearch system was performed using an aliquot of the first CSF sample. Conventional cytology was performed using 1 to 3 CSF samples, according to the current guidelines. LM diagnosis was established if tumor cells were found in CSF by cytology. To evaluate the accuracy of CTC detection with the CellSearch system for LM diagnosis, the clinical sensitivity, specificity, negative predictive value, and positive predictive value were calculated using the cytology results as reference. The receiver-operating characteristics curve and area under the curve (AUC) were calculated using 2 different cutoff values: 0 vs ≥1 CTC, and the cutoff determined with the Youden index.

Results

PATIENT CHARACTERISTICS

In total, 53 patients were included between January 2017 and January 2020. Among them, 49 were eligible: 1 patient not affiliated to the French Social Security System, and 3 patients without CSF samples for conventional cytology (failed lumbar puncture) were excluded (Fig. 1).

Table 1 contains the clinical and biological characteristics of these 49 patients (95.9% women). The median age at CSF sampling was 63 y (range: 32 to 77). The most represented histological subtype was ductal carcinoma (71.4% of BC). BCs were classified in the following molecular groups: HER2^{-}/hormone receptors (HR)$^{+}$ (71.4%), HER2^{+}/HR^{+} (12.2%), HER2^{+}/HR^{-} (6.1%), and triple negative (10.2%). BC was already metastatic in 93.9% of patients at the time of CSF sampling.

LM suspicion occurred after a median of 78.1 months (range 2.2 to 316.2) following BC diagnosis and 32.8 months (range 0.6 to 217.7) following the diagnosis of metastatic disease. At the time of LM suspicion, the median

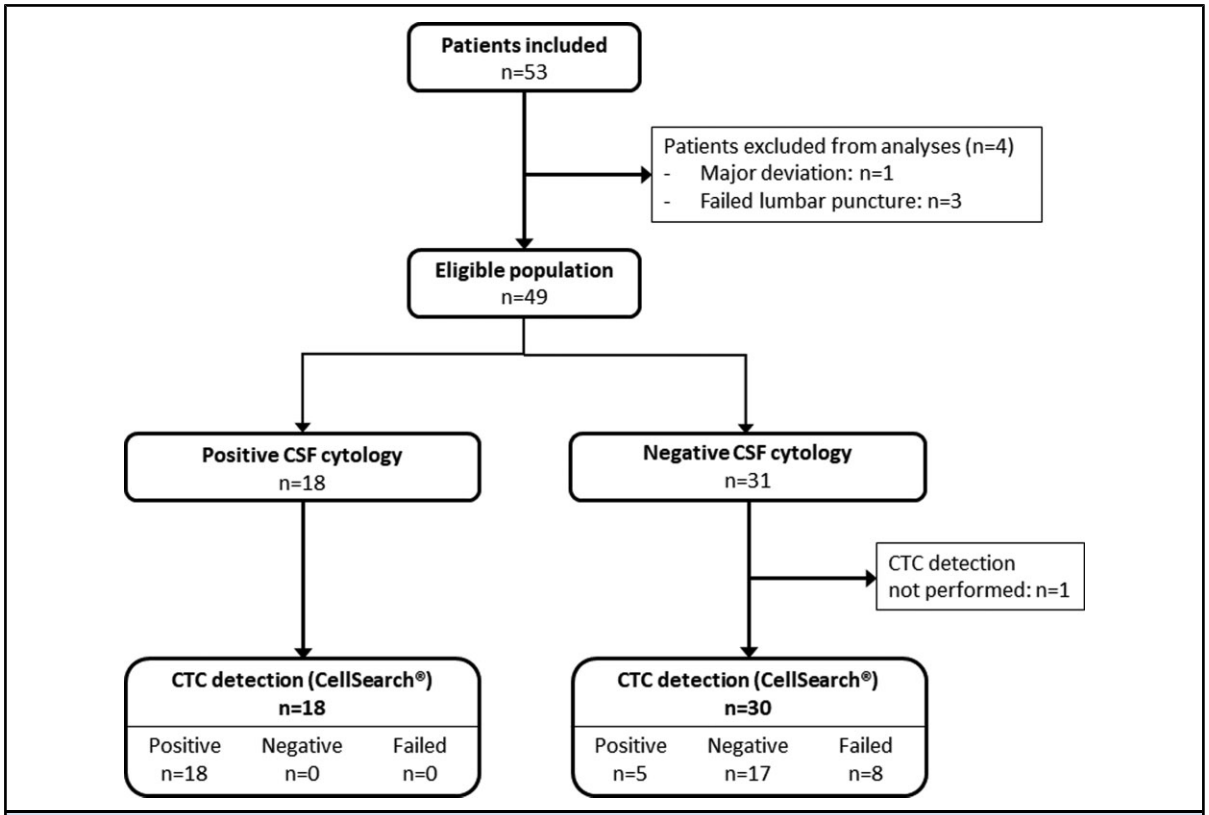

Fig. 1. Flowchart: included, eligible, and evaluable populations. Abbreviations: DAPI, 4′,6-diamidino-2-phénylindole; CD, cluster of differentiation.

number of metastatic sites was 3 (range 1 to 6), and 34.8% of patients had brain intraparenchymal metastases. The median number of previous chemotherapy lines (neoadjuvant and adjuvant chemotherapy excluded) was 1 (range 0 to 10; no previous chemotherapy line: 24.5%, 1 to 2 chemotherapy lines: 36.7%, and >2 chemotherapy lines: 38.8%); 44.4% of patients with $HER2^+$ BC had received anti-HER2 agents (neoadjuvant and adjuvant excluded).

At the time of LM suspicion, 79.6% of patients had an Eastern Cooperative Oncology Group performance status ≤2 and 89.8% of patients had clinical symptoms suggestive of LM. Gait disturbances (65.9% of patients), nausea (43.2%), headache (38.6%), and cognitive impairment (18.2%) were among the most frequently reported symptoms. Serum cancer antigen 15–3 was >30 U/mL in 77.6% of patients and carcinoembryonic antigen was >10 ng/mL in 46.9%. Brain and/or medulla imaging was performed in 45 patients (CT: n = 4; MRI: n = 41) (online Supplemental Table 1). CTCs (≥1) were detected in 78.3% and 21.7% of patients with and without leptomeningeal abnormalities on imaging, respectively ($P = 0.048$). Data on the diagnostic value of imaging are presented in online Supplemental Table 2.

LM DIAGNOSIS BASED ON CSF CYTOLOGY

Among the 49 eligible patients, 17 patients (34.7%) had 1 lumbar puncture, 6 (12.2%) 2 lumbar punctures, and 26 (53.1%) 3 lumbar punctures. Only one patient (2.2%) reported complications (grade 1 pain) following lumbar puncture. The median CSF sample volumes (first lumbar puncture) used for conventional cytology (median time to analysis: 22 min, range 1 to 214) and the CellSearch system (analysis performed within 96 h after collection) were 3.0 mL (range: 0.8 to 4.0 mL) and 3.3 mL (range 2.9 to 4.1 mL), respectively.

Tumor cells were detected by conventional cytology in 18 patients (36.7%; n = 16 in the first CSF sample and n = 2 in the second CSF sample) (Fig. 1).

CTC DETECTION IN CSF WITH THE CELLSEARCH SYSTEM

CTC detection with the CellSearch system could be performed in the CSF samples of 40 patients (i.e., the evaluable population). Specifically, CTCs could not be

Table 1 Patient characteristics (eligible population, n = 49) as a function of LM status (based on conventional cytology).[a]

	CSF cytology(gold standard)			
	Positive (n = 18)	Negative (n = 31)	Total (n = 49)	*P*-value
Initial characteristics				
Sex, n (%)				0.526
Female	18 (100.0)	29 (93.5)	47 (95.9)	
Male	0 (0.0)	2 (6.5)	2 (4.1)	
Median age at BC diagnosis in years (range)	54 (30–69)	47 (30–76)	51 (30–76)	0.199
Tumor molecular group, n (%)				0.780
$HER2^+$ HR^+	2 (11.1)	4 (12.9)	6 (12.2)	
$HER2^+$ HR^-	1 (5.6)	2 (6.5)	3 (6.1)	
$HER2^-$ HR^+	12 (66.7)	23 (74.2)	35 (71.4)	
Triple negative	3 (16.7)	2 (6.5)	5 (10.2)	
Histological subtype, n (%)				0.011
Ductal carcinoma	9 (50.0)	26 (83.9)	35 (71.4)	
Other subtypes	9 (50.0)	5 (16.1)	14 (28.6)	
Histological grade, n (%)				0.179
1 or 2	13 (72.2)	15 (48.4)	28 (57.1)	
3	4 (22.2)	15 (48.4)	19 (38.8)	
X	1 (5.6)	1 (3.2)	2 (4.1)	
Metastatic status at BC diagnosis, n (%)				0.456
M_0	11 (68.7)	19 (79.2)	30 (75.0)	
M_1	5 (31.2)	5 (20.8)	10 (25.0)	
M_x	2	7	9	
Adjuvant or neoadjuvant chemotherapy, n (%)				0.880
No	5(27.8)	8 (25.8)	13 (26.5)	
Yes	13 (72.2)	23 (74.2)	36 (73.5)	
Patient characteristics at the time of LM suspicion				
Median age in years (range)	62 (43-75)	63 (32-77)	63 (32-77)	0.868
Number of chemotherapy lines, n (%)				0.626
0	5 (27.8)	7 (22.6)	12 (24.5)	
1 or 2	7 (38.9)	11 (35.5)	18 (36.7)	
> 2	6 (33.3)	13 (41.9)	19 (38.8)	
Previous anti-HER2 treatment in patients with $HER2^+$ cancer (adjuvant and neoadjuvant excluded), n (%)	*n = 3*	*n = 6*	*n = 9*	1.000
No	2 (66.7)	3 (50.0)	5 (55.6)	
Yes	1 (33.3)	3 (50.0)	4 (44.4)	
Presence of metastases, n (%)				0.288
No	0 (0.0)	3 (9.7)	3 (6.1)	
Yes	18 (100.0)	28 (90.3)	46 (93.9)	
Median number of metastatic sites (range)	3 (1–5)	2 (0–6)	3 (1–6)	0.581

Continued

Table 1 (continued)

	CSF cytology(gold standard)			
	Positive (n = 18)	Negative (n = 31)	Total (n = 49)	*P*-value
Brain intraparenchymal metastases, n (%)	7 (38.9)	9 (32.4)	16 (34.8)	0.639
Liver metastases, n (%)	6 (33.3)	14 (50.0)	20 (43.5)	0.364
Bone metastases, n (%)	14 (77.8)	21 (75.0)	35 (76.1)	1.000
Lung metastases, n (%)	2 (11.1)	11 (39.3)	13 (28.3)	0.049
Lymph node metastases, n (%)	9 (50.0)	14 (50.0)	23 (50.0)	1.000
Subcutaneous metastases, n (%)	4 (22.2)	1 (3.6)	5 (10.9)	0.069
Pleural metastases, n (%)	2 (11.1)	5 (17.9)	7 (15.2)	0.688
Serous metastases, n (%)	8 (44.4)	7 (22.6)	15 (30.6)	0.109
Metastases at other sites, n (%)	3 (16.7)	2 (7.1)	5 (10.9)	0.366
ECOG performance status, n (%)				0.095
0–1	7 (38.9)	19 (61.3)	26 (53.1)	
2	4 (22.2)	9 (29.0)	13 (26.5)	
3	7 (38.89)	3 (9.7)	10 (20.4)	
Symptoms and signs at LM suspicion, n (%)				0.143
Absent	0 (0.0)	5 (16.1)	5 (10.2)	
Present	18 (100.0)	26 (83.9)	44 (89.8)	
Headache, n (%)	11 (61.1)	6 (23.1)	17 (38.6)	0.011
Nausea, n (%)	13 (72.2)	6 (23.1)	19 (43.2)	0.001
Altered vigilance, n (%)	4 (22.2)	3 (11.5)	7 (15.9)	0.419
Cognitive impairment, n (%)	6 (33.3)	2 (7.7)	8 (18.2)	0.048
Coordination disorder, n (%)	4 (22.2)	1 (3.8)	5 (11.4)	0.142
Gait disturbances, n (%)	16 (88.9)	13 (50.0)	29 (65.9)	0.010
Cranial nerve involvement, n (%)	6 (33.3)	5 (19.2)	11 (25.0)	0.288
Epilepsy, n (%)	0 (0.0)	1 (3.8)	1 (2.3)	1.000
Medullar involvement, n (%)	4 (25.0)	3 (14.3)	7 (18.9)	0.437

[a]Abbreviations: HR: hormone receptor. ECOG, eastern coopertive oncology group.

detected due to technical problems in 8 samples, and the CellSearch analysis could not be performed in 1 sample due to organizational constraints. Among the 40 evaluable patients, LM diagnosis was confirmed by cytology in 18 patients (i.e., confirmed LM population) (Fig. 1).

CTCs were detected in these 18 patients with confirmed LM (Fig. 2). The median CTC number was 5824 (range: 93 to 45 052). CTCs were also detected in 5/22 patients with negative conventional cytology (median: 2 CTCs, range 1 to 44). In these 5 patients, CSF protein concentration was <0.45 g/L in all patients but 1, and no leptomeningeal abnormality was detected on MRI. Among these 5 patients, the diagnosis of LM was confirmed by cytology in 1 patient (with 44 CTC) 9 months later, but not in the other four patients (1 to 3 CTC) who died due to extra-cerebral BC metastases after a median time of 5.2 months (range 0.9 to 25.9) (online Supplemental Table 3). The detection of CTCs according to the tumor subtype is detailed in online Supplemental Table 4.

Using the cytology results as reference, the detection of at least one CTC in CSF was associated with a sensitivity of 100.0% and a specificity of 77.3% (95% CI, 64.3–90.3) for LM diagnosis (Table 2).

To further evaluate the value of CTC detection with the CellSearch system for LM diagnosis, CTC number was considered as a quantitative value. Because there was no validated CTC cutoff in this setting, the CTC cutoff that maximized the Youden index (n = 93 CTC) was determined using the receiver-operating characteristics analysis. The detection of at least 93 CTCs in CSF was associated with a sensitivity and a specificity of 100.0%

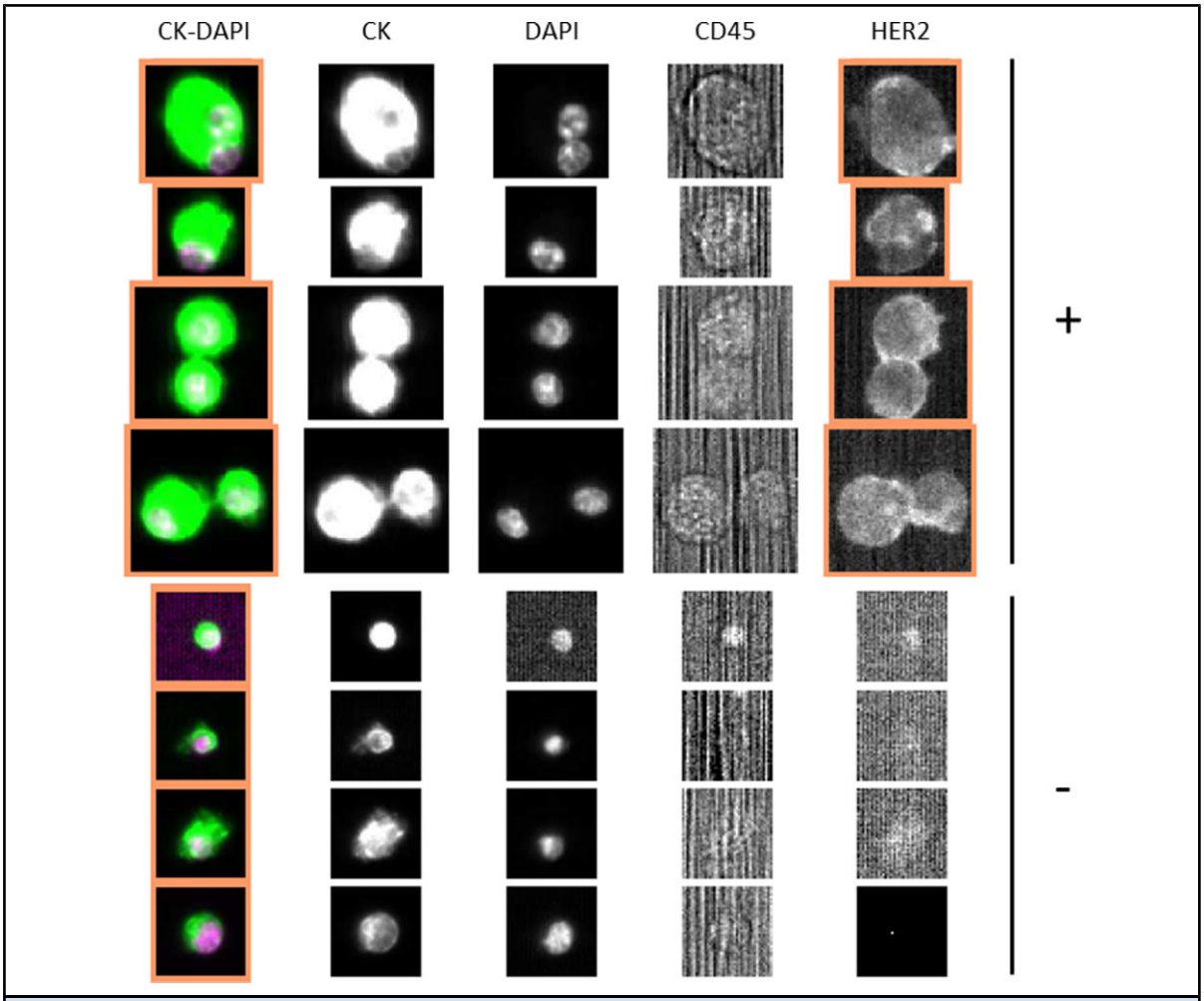

Fig. 2. Representative images of HER2^{+} (+) and HER2^{-} (–) of CTCs in CSF samples of patients with BC-related LM using the IVD CellSearch CTC Kit and the CellSearch system. CTCs are identified as EpCAM$^{(+)}$, CK$^{(+)}$, DAPI$^{(+)}$, and CD45$^{(-)}$. Abbreviations: EpCAM, epithelial cell adhesion molecule; CK, cytokeratins 8, 18, and 19; CD45, cluster of differentiation 45.

for LM diagnosis. With this cutoff, the AUC was 1.000 (95% CI, 1-1) (Table 2, Fig. 3).

HER2 STATUS IN CSF CTC USING THE CELLSEARCH SYSTEM

HER2^{+} CTCs were detected in 16/37 patients from the evaluable population (HER analysis not performed in 3 patients) (Table 3). Specifically, HER2^{+} CTCs in CSF were detected in 3 of the 6 patients with HER2^{+} tumor tissue (median: 33 HER2^{+} CTCs, range: 5 to 60). HER2^{+} CTCs (median: 500 HER2^{+} CTCs, range: 13 to 28 320) were also detected in the CSF samples from 40.6% of patients with HER2^{-} BC (13/32) (online Supplemental Table 5). When considering the tumor tissue HER2 status based on immunohistochemistry, IHC), HER2^{+} CTCs were detected in the CSF of 40.0% of patients with an IHC score 0 or 1+ (median: 395 HER2^{+} CTCs, range: 13 to 28 320) and in 45.4% of patients with an IHC score 2+ (median: 162 HER2^{+} CTCs, range: 33 to 8800) in BC tissue samples.

CSF PROTEIN CONCENTRATION

CSF protein concentration could be determined in 48/49 eligible patients. Protein was increased (≥0.45 g/L according to the laboratory cutoff) in 88.2% and 45.2% of patients with confirmed LM and with negative cytology, respectively ($P = 0.005$).

Using the Youden index, the CSF protein concentration cutoff for LM diagnosis was 0.7 g/L. With this cutoff, CSF protein concentration was associated with a sensitivity of 70.6% (95% CI, 57.7–83.5) and a specificity of

Table 2 Clinical sensitivity, specificity, and predictive values of CTC detection in CSF samples using the CellSearch system for LM diagnosis, with a cutoff of 1 (A) and 93 (B) CTC.[a]

	CSF cytology (gold standard)		
(A)	Positive	Negative	Total
CTC detection (CellSearch)			
Positive (CTC >0)	TP = 18 (78.3)	FP = 5 (21.4)	23
Negative (CTC = 0)	FN = 0 (0.0)	TN = 17 (100.0)	17
	18	22	40
Sensitivity		100.0%	
Specificity		77.3% (95% CI, 64.3–90.3)	
Positive predictive value		78.3% (95% CI, 65.5–91.0)	
Negative predictive value		100.0%	
Global concordance		87.5% (95% CI, 77.3–97.7)	
Kappa correlation coefficient		0.76	
	CSF cytology (gold standard)		
(B)	Positive	Negative	Total
CTC detection (CellSearch)			
Positive (CTC ≥93)	TP = 18 (72.0)	FP = 0 (0.0)	18
Negative (CTC < 93)	FN = 0 (0.0)	TN = 22 (100.0)	22
	18	22	40
Sensitivity		100.0%	
Specificity		100.0%	
Positive predictive value		100.0%	
Negative predictive value		100.0%	
Global concordance		100.0%	
Kappa correlation coefficient		1.0	

[a]Abbreviations: FN, false negative; FP, false positive; TN, true negative; TP, true positive.

87.1% (95% CI, 77.6–96.6) for LM diagnosis. The AUC was 0.841 (95% CI, 0.719–0.962) (online Supplementary Table 6 and Fig. 3).

The median CSF protein concentration was 0.48 g/L (range: 0.20 to 1.00) in patients without CTCs and 1.76 g/L (range: 0.20 to 8.40) in patients with CTCs ($P = 0.025$).

PATIENTS WITH PROBABLE LM

Seven patients with negative cytology met the criteria for probable LM according to the European Association of Neuro-Oncology/European Society for Medical Oncology (ESMO–EANO) guidelines (1). When considering patients with confirmed and probable LM (n = 25), at least one CTC was detected with the CellSearch system in 19 patients (79.2%) and no CTCs in 5 (20.8%) (technical failure in 1 patient). Using one CTC as cutoff, the sensitivity, specificity, positive predictive value, and negative predictive value for LM diagnosis were 79.2%, 75.0%, 82.6%, and 70.6%, respectively (online Supplemental Table 7). Using 93 CTCs as cutoff, the sensitivity, specificity, positive predictive value, and negative predictive value were all 100.0% and the AUC was 1. The median CSF protein concentration was 1.71 g/L (range: 0.2 to 8.4) in the confirmed + probable LM group, and 0.45 (range 0.2 to 1.0) in patients with possible LM ($P = 0.007$). The associated sensitivity, specificity, predictive values, and AUC are in online Supplemental Table 7.

PATIENT OUTCOMES

Patients with confirmed LM (n = 18) were followed prospectively. After LM diagnosis, 15 patients (83.3%) received a systemic treatment and 2 (11.1%) radiation therapy. Additionally, 17 patients received intrathecal chemotherapy (methotrexate) after a median time of 5 days (range 1 to 20)

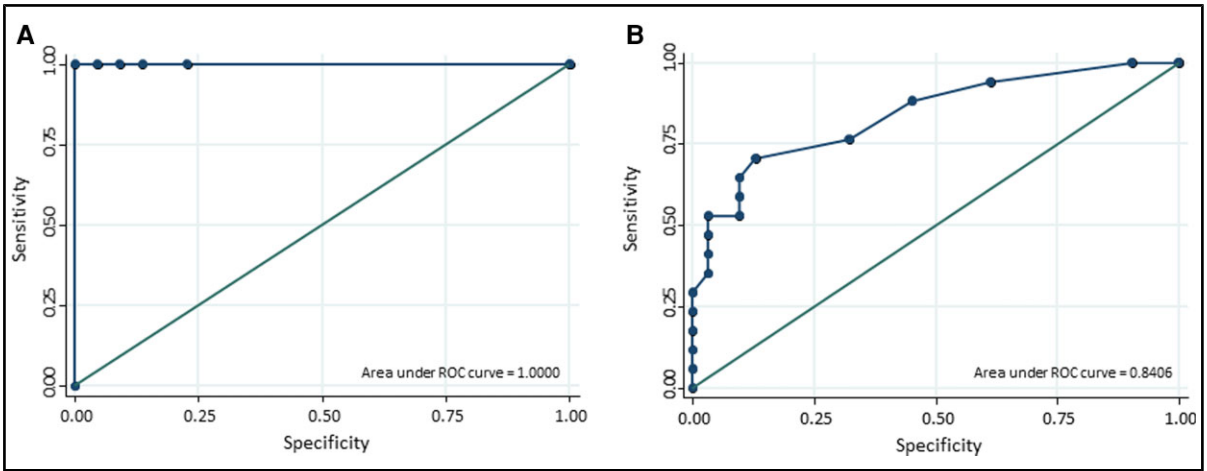

Fig. 3. Clinical sensitivity and specificity of (A) CTC detection using the CellSearch system for LM diagnosis (cutoff: 93 CTC) and (B) CSF protein concentration (cutoff: 0.7 g/L) for LM diagnosis.

following LM confirmation (median number of injections: 7, range: 1 to 45). In these 17 patients, 13 were evaluable for response according to the EANO–ESMO criteria: response (n = 4, 30.8%), stable disease (n = 4, 30.8%), and tumor progression (n = 5, 38.5%).

After a median follow-up of 22.4 months (95% CI, 20.3-can not be calculated, range 0.1 to 24.1), 17 patients (94.4%) had experienced deterioration of their neurological status. The median survival without neurological degradation was 1.2 months (95% CI, 0.7–4.0). The 6-month survival rate without neurological degradation was 5.9% (95% CI, 0.3–23.5).

In total, 15/18 patients with confirmed LM (83.3%) were dead at the study end. The median overall survival was 2.7 months (95% CI, 1.35–7.2), and the 6-month overall survival rate was 38.9% (95% CI, 17.5–60.0).

Discussion

In this prospective study, CTCs were detected in the CSF of all patients with a confirmed diagnosis of LM by cytology (n = 18). The detection of at least one CTC in CSF was associated with a sensitivity of 100.0% and a specificity of 77.3%, in line with previous studies (sensitivity 78% to100% and specificity 84% to 100% (6)). Due to its low sensitivity (3), conventional cytology in CSF frequently requires several CSF samples to confirm the diagnosis of LM. CTC detection in CSF could bring a clinical advantage by reducing the number of necessary CSF samples to reach the diagnosis. In our series, LM diagnosis by cytology was confirmed using the first CSF sample in most patients (16/18), possibly because samples were analyzed rapidly after lumbar puncture and due to the expertise of our pathologist. Nevertheless, the CellSearch system is associated with fewer organizational constraints because samples can be analyzed within 96 h after lumbar puncture (vs 1 h for cytology). Another advantage of the CellSearch system, compared with conventional cytology is the possibility of precise and reproducible quantification of CTCs (12, 13). This could allow use of CTC number variations to monitor LM response to specific treatments, a major issue in the management of patients with LM (1, 18). Indeed, in this context, response monitoring is challenging, particularly due to the frequent co-occurrence of extra-cerebral and/or brain metastases, and due to the difficulties concerning the definition of the clinical and radiological responses. In this context, a few studies suggested that survival in patients with BC-related LM is correlated with the cytologic response (i.e., the disappearance of tumor cells in CSF upon treatment) (26, 27). However, these studies might be biased by the use of cytology to identify tumor cells in CSF where false-negative results could have been misinterpreted as tumor cell disappearance. More recently, studies with small cohorts of patients suggested an association between a decrease of the CSF CTC count, determined with the CellSearch system, and the response to LM treatment in patients with various cancers (11, 13, 14).

Besides the question of the prognostic value of CTC clearance in CSF, it can be asked whether CTC count at LM diagnosis could also be a prognostic marker, as demonstrated for blood CTC in patients with metastatic BC (6, 28, 29). Data on this question are scarce (14, 15, 30). In a recent study in patients with lung cancer-associated LM, CSF CTCs were quantified

Table 3 Number of HER2$^+$ CTCs in CSF samples in function of the tumor tissue HER2 status (evaluable population).

	HER2 status (primary tumor)		
	HER2$^-$ (n = 34)	HER2$^+$ (n = 6)	Total (n = 40)
Number of HER2+ CTC in CSF			
0	19 (59.4%)	2 (40.0%)	21 (56.8%)
≥ 1	13 (40.6%)	3 (60.0%)	16 (43.2%)
Missing	2	1	3
Median number of HER2$^+$ CTC in CSF (range) if >0	500 (13-28320)	33 (5-60)	226 (5-28320)

in 16 patients at diagnosis (30). Clinical outcome (overall survival) was worse in patients with ≥50 CTCs/3 mL than in those with <50 CTCs/3 mL. In another study in 58 patients with newly diagnosed LM, the CSF CTC count was prognostic for CNS progression-free survival and overall survival (14).

CTCs were detected also in few patients in whom LM was not confirmed by cytology, in agreement with published data (14, 17, 31, 32). In one study, CTCs were detected with a flow cytometry immunophenotyping technique in 13 of 34 CSF samples with negative cytology (31). The patients' clinical course and laboratory changes (CSF cytology) were not reported. Our findings suggest that LM confirmation using the CellSearch system would had been associated with a clinical benefit (earlier LM treatment) only for only patient, and might have led to unnecessary treatment in the 4 patients who did not develop any clinical sign of LM during the follow-up. The prognosis of patients with cytology$^-$/CTC$^+$ needs to be thoroughly investigated in a larger cohort. These patients also raise the question of the choice of gold standard to investigate the diagnostic value of a new technique for LM. In our study, we chose conventional cytology because it is the only method used in clinical practice for the diagnosis of confirmed LM according to the EANO–ESMO criteria. However, due its low sensitivity, other authors used conventional cytology and imaging data in recent studies (14,17,32). Moreover, the concept of probable LM has been introduced recently to consider these patients (1). In our study, 7 patients received a diagnosis of probable LM. When considering patients with confirmed and probable LM, the CellSearch system sensitivity was 79.2% (cutoff: 1 CTC) and 75.1% (cutoff: 93 CTCs).

Compared with CSF cytology, the CellSearch system also allows the study of tumor heterogeneity at the single-cell level through CTC phenotypic and molecular characterization, a very important variable in the era of targeted therapies. In our study, we could analyze the HER2 status of CTC detected in CSF in 37 patients. We found that among the patients with a HER2$^-$ primary tumor and detectable CSF CTCs, 40.6% had HER2$^+$ CTCs. Previous studies have reported possible HER2 status changes between the primary BC tissue and metastatic BC tissue obtained with biopsies or CTCs detected in the peripheral blood (19–24). Conversely, the few studies on BC-related LM suggested a high concordance rate between primary BC tissue and CSF CTCs (17, 25). One study reported that in the 4 patients with HER2$^+$ primary BC (fluorescence in situ hybridization [FISH] and/or IHC 3+), CTCs in CSF were HER2$^+$ CTCs (using FISH). In the 12 patients with HER2$^-$ CTCs in CSF, the HER2 IHC score for the primary tumor was 0 or 1+ in 10, and 2+ without gene amplification (FISH) in 2 (25). Another study found that CTCs in CSF were HER2$^+$ in 75% of 8 patients with HER2$^+$ BC (17). The changes in HER2 phenotype might be explained by different reasons. First, HER2 amplification can be gained during tumor evolution. Second, the primary BC tissue (or non-LM metastatic tissue) may harbor minor clones of tumor cells with HER2 amplification, with a subclonal evolution that favors leptomeningeal invasion by HER2$^+$ tumor cells. Our findings could have important prognostic and therapeutic implications. Indeed, the prognostic impact of the HER2 status of CTCs in peripherical blood has been recently shown in patients with metastatic BC (9); however, this question has not been addressed yet for CSF CTCs. The detection of HER2$^+$ CTCs in CSF might also offer new treatment opportunities. Recent studies suggested that anti-HER2 agents might be effective in patients with HER2$^+$ CTCs in blood and HER$^-$ BC (10, 33). Following the development of new HER2-targeted drugs with efficacy in CNS metastases (34–37) and their possible administration directly in the CSF (38), the detection of HER2$^+$ CTCs in CSF could bring new therapeutic options for patients with a HER2$^-$ metastatic BC.

Besides HER2, the CellSearch system can also be used to characterize CTCs at the genetic level (4, 39, 40). Ultimately, it might allow better understanding of the

biology and physiopathology of the metastatic cascade in the CNS.

The limitations of this study include the relatively small number of patients, particularly in the group with confirmed LM. This did not allow an accurate evaluation of the prognostic impact of CTC detection and quantification in CSF, reported in recently published studies (14, 15), to be made. Moreover, our study was not designed to include a longitudinal evaluation of CSF CTC count in patients receiving a LM-specific treatment, despite recent data suggesting a possible predictive impact on outcome of decreased CTC count in CSF (14). We also did not evaluate the detection of CTCs in peripheral blood for the diagnosis of LM, which might be interesting in the light of our results regarding the HER2 status of CSF CTCs, and for prognostication (even tough recent reports have failed to identify an impact of blood CTC count on outcome in patients with LM (14)).

Finally, our analyses were weakened by the fact that CTC detection technically failed in 8/40 CSF samples. The CellSearch system is not designed for CSF samples in which only few leukocytes are present. However, CTC detection was impossible only in paucicellular samples, with negative cytology. Finally, the presence of *HER2* gene amplification was not confirmed by FISH/chromogenic in situ hybridization (CISH) because CTCs were not isolated for further characterization. However, this characterization was performed in different clinical trials a decade ago (8), allowing us to base our conclusions only on the phenotype of $HER2^+$ CTCs (immunocytochemistry).

In summary, we detected CTCs with the CellSearch system in all patients with cytologically proven LM, and also in a few patients without cytological confirmation of LM. The prognosis of these patients with $cytology^-/CTC^+$ in CSF needs to be thoroughly investigated in a larger cohort. Moreover, we detected $HER2^+$ CTCs in 40.6% of patients with $HER2^-$ BC. These results need to be confirmed in an independent and larger cohort. In the era of anti-HER2 therapies, these data suggest that the HER2 status of CSF CTC should be routinely evaluated in LM to propose new treatment opportunities.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: BC, breast cancer; LM, leptomeningeal metastases; CSF, cerebrospinal fluid; CTC, circulating tumor cell; HER2, human epithelial growth factor receptor 2; AUC, area under the curve; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Conception and design of the study: A. Darlix, S. Pouderoux, W. Jacot, L. Cayrefourcq, C. Leaha, S. Thezenas, C. Alix-Panabières; Inclusion of patients and CSF samples: A. Darlix, S. Pouderoux; Analysis of CSF samples: L. Cayrefourcq, C. Leaha, C. Alix-Panabières; Imaging data review: N. Menjot de Champfleur; Analysis of results: A. Darlix, S. Pouderoux, N. Menjot de Champfleur, S. Thezenas, A. Bievelez, W. Jacot, L. Cayrefourcq, C. Leaha, C. Alix-Panabières; Manuscript drafting: A. Darlix, L. Cayrefourcq, C. Alix-Panabières, W. Jacot; Revision of the manuscript for intellectual content: S. Pouderoux, N. Menjot de Champfleur, S. Thezenas, C. Leaha.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: C. Alix-Panabières, Menarini.
Stock Ownership: None declared.
Honoraria: C. Alix-Panabières, Menarini.
Research Funding: C. Alix-Panabières is supported by the National Institute of Cancer (INCa, http://www.e-cancer.fr) and the SIRIC Montpellier Cancer Grant INCa Inserm DGOS 12533. W. Jacot, National Institute of Cancer (INCa).
Expert Testimony: None declared.
Patents: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

References

1. Le Rhun E, Weller M, Brandsma D, Van den Bent M, de Azambuja E, Henriksson R, et al. EANO-ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up of patients with leptomeningeal metastasis from solid tumours. Ann Oncol 2017;28-(Suppl 4):iv84–99.
2. Glantz MJ, Cole BF, Glantz LK, Cobb J, Mills P, Lekos A, et al. Cerebrospinal fluid cytology in patients with cancer: minimizing false-negative results. Cancer 1998;82:733–9.
3. Boire A, Brandsma D, Brastianos PK, Le Rhun E, Ahluwalia M, Junck L, et al. Liquid biopsy in central nervous system metastases: a RANO review and proposals for clinical applications. Neuro Oncol 2019;21:571–84.
4. Alix-Panabières C, Pantel K. Liquid biopsy: from discovery to clinical application. Cancer Discov 2021;11:858–73.
5. Banys-Paluchowski M, Fehm TN, Grimm-Glang D, Rody A, Krawczyk N. Liquid biopsy in metastatic breast cancer: current role of circulating tumor cells and circulating tumor DNA. Oncol Res Treat 2022;45:4–11.
6. Cristofanilli M, Pierga JY, Reuben J, Rademaker A, Davis AA, Peeters DJ, et al. The clinical use of circulating tumor cells (CTCs) enumeration for staging of metastatic breast cancer (MBC): international

expert consensus paper. Crit Rev Oncol Hematol 2019;134:39–45.
7. Riethdorf S, Müller V, Zhang L, Rau T, Loibl S, Komor M, et al. Detection and HER2 expression of circulating tumor cells: prospective monitoring in breast cancer patients treated in the neoadjuvant GeparQuattro trial. Clin Cancer Res 2010; 16:2634–45.
8. Bidard FC, Peeters DJ, Fehm T, Nolé F, Gisbert-Criado R, Mavroudis D, et al. Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. Lancet Oncol 2014;15: 406–14.
9. Müller V, Banys-Paluchowski M, Friedl TWP, Fasching PA, Schneeweiss A, Hartkopf A, et al. Prognostic relevance of the HER2 status of circulating tumor cells in metastatic breast cancer patients screened for participation in the DETECT study program. ESMO Open 2021;6: 100299.
10. Fehm T, Müller V, Banys-Paluchowski M, Fasching PA, Friedl TWP, Hartkopf A, et al. Efficacy of the tyrosine kinase inhibitor lapatinib in the treatment of patients with HER2-negative metastatic breast cancer and HER2-positive circulating tumor cells – results from the randomized phase III DETECT III trial [Abstract]. Cancer Res 2021;81(Suppl 4): PD3-12.
11. Patel AS, Allen JE, Dicker DT, Peters KL, Sheehan JM, Glantz MJ, et al. Identification and enumeration of circulating tumor cells in the cerebrospinal fluid of breast cancer patients with central nervous system metastases. Oncotarget 2011;2:752–60.
12. Le Rhun E, Massin F, Tu Q, Bonneterre J, Bittencourt Mde C, Faure GC. Development of a new method for identification and quantification in cerebrospinal fluid of malignant cells from breast carcinoma leptomeningeal metastasis. BMC Clin Pathol 2012;12:21.
13. Le Rhun E, Tu Q, De Carvalho Bittencourt M, Farre I, Mortier L, Cai H, et al. Detection and quantification of CSF malignant cells by the CellSearch technology in patients with melanoma leptomeningeal metastasis. Med Oncol 2013;30:538.
14. Wijetunga NA, Boire A, Young RJ, Yamada Y, Wolden S, Yu H, et al. Quantitative cerebrospinal fluid circulating tumor cells are a potential biomarker of response for proton craniospinal irradiation for leptomeningeal metastasis. Neurooncol Adv 2021;3: vdab181.
15. Diaz M, Singh P, Kotchetkov IS, Skakodub A, Meng A, Tamer C, et al. Quantitative assessment of circulating tumor cells in cerebrospinal fluid as a clinical tool to predict survival in leptomeningeal metastases. J Neurooncol 2022;157:81–90.
16. Subirá D, Simó M, Illán J, Serrano C, Castañón S, Gonzalo R, et al. Diagnostic and prognostic significance of flow cytometry immunophenotyping in patients with leptomeningeal carcinomatosis. Clin Exp Metastasis 2015;32:383–91.
17. Malani R, Fleisher M, Kumthekar P, Lin X, Omuro A, Groves MD, et al. Cerebrospinal fluid circulating tumor cells as a quantifiable measurement of leptomeningeal metastases in patients with HER2 positive cancer. J Neurooncol 2020; 148:599–606.
18. Le Rhun E, Devos P, Boulanger T, Smits M, Brandsma D, Rudà R, et al. The RANO Leptomeningeal Metastasis Group proposal to assess response to treatment: lack of feasibility and clinical utility and a revised proposal. Neuro Oncol 2019;21: 648–58.
19. Fehm T, Müller V, Aktas B, Janni W, Schneeweiss A, Stickeler E, et al. HER2 status of circulating tumor cells in patients with metastatic breast cancer: a prospective, multicenter trial. Breast Cancer Res Treat 2010;124:403–12.
20. Niikura N, Liu J, Hayashi N, Mittendorf EA, Gong Y, Palla SL, et al. Loss of human epidermal growth factor receptor 2 (HER2) expression in metastatic sites of HER2-overexpressing primary breast tumors. J Clin Oncol 2012;30:593–9.
21. Beije N, Onstenk W, Kraan J, Sieuwerts AM, Hamberg P, Dirix LY, et al. Prognostic impact of HER2 and ER status of circulating tumor cells in metastatic breast cancer patients with a HER2-negative primary tumor. Neoplasia 2016;18:647–53.
22. Aktas B, Kasimir-Bauer S, Müller V, Janni W, Fehm T, Wallwiener D, et al. Comparison of the HER2, estrogen and progesterone receptor expression profile of primary tumor, metastases and circulating tumor cells in metastatic breast cancer patients. BMC Cancer 2016;16:522.
23. Jakabova A, Bielcikova Z, Pospisilova E, Matkowski R, Szynglarewicz B, Staszek-Szewczyk U, et al. Molecular characterization and heterogeneity of circulating tumor cells in breast cancer. Breast Cancer Res Treat 2017;166:695–700.
24. Grüntkemeier L, Khurana A, Bischoff FZ, Hoffmann O, Kimmig R, Moore M, et al. Single HER2-positive tumor cells are detected in initially HER2-negative breast carcinomas using the DEPArray™-HER2-FISH workflow. Breast Cancer 2022;29:487–97.
25. Park IH, Kwon Y, Ro JY, Lee KS, Ro J. Concordant HER2 status between metastatic breast cancer cells in CSF and primary breast cancer tissue. Breast Cancer Res Treat 2010;123:125–8.
26. Clatot F, Philippin-Lauridant G, Ouvrier MJ, Nakry T, Laberge-Le-Couteulx S, Guillemet C, et al. Clinical improvement and survival in breast cancer leptomeningeal metastasis correlate with the cytologic response to intrathecal chemotherapy. J Neurooncol 2009;95:421–6.
27. Oechsle K, Lange-Brock V, Kruell A, Bokemeyer C, de Wit M. Prognostic factors and treatment options in patients with leptomeningeal metastases of different primary tumors: a retrospective analysis. J Cancer Res Clin Oncol 2010;136:1729–35.
28. Ramirez JM, Fehm T, Orsini M, Cayrefourcq L, Maudelonde T, Pantel K, et al. Prognostic relevance of viable circulating tumor cells detected by EPISPOT in metastatic breast cancer patients. Clin Chem 2014;60:214–21.
29. Müller V, Riethdorf S, Rack B, Janni W, Fasching PA, Solomayer E, et al. Prognostic impact of circulating tumor cells assessed with the CellSearch System™ and AdnaTest Breast™ in metastatic breast cancer patients: the DETECT study. Breast Cancer Res 2012;14:R118.
30. Nevel KS, DiStefano N, Lin X, Skakodub A, Ogilvie SQ, Reiner AS, et al. A retrospective, quantitative assessment of disease burden in patients with leptomeningeal metastases from non-small-cell lung cancer. Neuro Oncol 2020;22:675–83.
31. Lee JS, Melisko ME, Magbanua MJ, Kablanian AT, Scott JH, Rugo HS, et al. Detection of cerebrospinal fluid tumor cells and its clinical relevance in leptomeningeal metastasis of breast cancer. Breast Cancer Res Treat 2015;154:339–49.
32. van Bussel MTJ, Pluim D, Milojkovic Kerklaan B, Bol M, Sikorska K, Linders DTC, et al. Circulating epithelial tumor cell analysis in CSF in patients with leptomeningeal metastases. Neurology 2020; 94:e521–e528.
33. Jacot W, Cottu P, Berger F, Dubot C, Venat-Bouvet L, Lortholary A, et al. Actionability of HER2-amplified circulating tumor cells in HER2-negative metastatic breast cancer: the CirCe T-DM1 trial. Breast Cancer Res 2019;21:121.
34. Ricciardi GRR, Russo A, Franchina T, Schifano S, Mastroeni G, Santacaterina A, et al. Efficacy of T-DM1 for leptomeningeal and brain metastases in a HER2 positive metastatic breast cancer patient: new directions for systemic therapy - a case report and literature review. BMC Cancer 2018;18:97.
35. Freedman RA, Gelman RS, Anders CK, Melisko ME, Parsons HA, Cropp AM, et al. TBCRC 022: A phase II trial of neratinib and capecitabine for patients with human epidermal growth factor receptor 2-positive breast cancer and brain metastases. J Clin Oncol 2019;37:1081–9.
36. Jerusalem GHM, Park YH, Yamashita T, Hurvitz SA, Modi S, Andre F, et al. Trastuzumab deruxtecan (T-DXd) in patients with HER2 + metastatic breast cancer with brain metastases: a subgroup analysis of the DESTINY-Breast01 trial. J Clin Oncol 2021;39:526.
37. Lin NU, Borges V, Anders C, Murthy RK, Paplomata E, Hamilton E, et al. Intracranial efficacy and survival with tucatinib plus trastuzumab and capecitabine for previously treated HER2-positive breast cancer with brain metastases in the HER2CLIMB trial. J Clin Oncol 2020; 38(23):2610–9.
38. Zagouri F, Zoumpourlis P, Le Rhun E, Bartsch R, Zografos E, Apostolidou K, et al. Intrathecal administration of

anti-HER2 treatment for the treatment of meningeal carcinomatosis in breast cancer: a metanalysis with meta-regression. Cancer Treat Rev 2020;88:102046.
39. Zhang L, Ridgway LD, Wetzel MD, Ngo J, Yin W, Kumar D, et al. The identification and characterization of breast cancer CTCs competent for brain metastasis. Sci Transl Med 2013;5:180ra48.
40. Cayrefourcq L, Mazard T, Joosse S, Solassol J, Ramos J, Assenat E. Establishment and characterization of a cell line from human circulating colon cancer cells. Cancer Res 2015;75:892–901.
41. Obuchowski NA, McClish DK. Sample size determination for diagnostic accuracy studies involving binormal ROC curve indices. Stat Med 1997;16:1529–42.