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Genotyping tumour DNA in cerebrospinal fluid and plasma of a HER2-positive breast cancer patient with brain metastases

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ABSTRACT

Background Central nervous system (CNS) involvement contributes to significant morbidity and mortality in patients with human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer (mBC) and represents a major challenge for clinicians. Liquid biopsy of cerebrospinal fluid (CSF)-derived circulating tumour DNA (ctDNA) harbours clinically relevant genomic alterations in patients with CNS metastases and could be effective in tracking tumour evolution.

Methods In a HER2-positive mBC patient with brain metastases, we applied droplet digital PCR (ddPCR) and next-generation whole exome sequencing (WES) analysis to measure ctDNA dynamic changes in CSF and plasma collected during treatment.

Results Baseline CSF-derived ctDNA analysis revealed *TP53* and *PIK3CA* mutations as well as *ERBB2* and c*MYC* amplification. Post-treatment ctDNA analysis showed decreased markers level in plasma, consistent with extra-CNS disease control, while increased in the CSF, confirming poor treatment benefit in the CNS.

Discussion Analysis of ctDNA in the CSF of HER2-positive mBC is feasible and could represent a useful companion for clinical management of brain metastases.

Key questions

What is already known about this subject?
Cerebrospinal fluid (CSF)-derived circulating tumour DNA (ctDNA) harbours clinically relevant genomic alterations in breast cancer patients with central nervous system (CNS) metastases and could be effective in tracking tumour evolution in the CNS (liquid biopsy).

What does this study add?
In this clinical case, ctDNA dynamic changes in plasma and CSF were more informative than traditional imaging in monitoring the disease. Furthermore, ctDNA from the CSF provided hints on potential biomarkers of CNS metastatisation.

How might this impact on clinical practice?
Paired plasma and CSF-based liquid biopsy could be useful to optimise the management of women with metastatic, human epidermal growth factor receptor 2-positive breast cancer who often show differential treatment benefits in CNS and extra-CNS sites.

INTRODUCTION

Survival of patients with metastatic, human epidermal growth factor receptor 2 (HER2)-positive breast cancer has been considerably extended due to the introduction of HER2-directed therapies such as the monoclonal antibodies trastuzumab and pertuzumab, the antibody-drug conjugate trastuzumab emtansine (T-DM1) and the tyrosine-kinase inhibitor lapatinib.[1] Unfortunately, HER2-positive breast cancer cells have tropism for the central nervous system (CNS), which is considered a sanctuary site where the activity of current anti-HER2 treatment is suboptimal.[2 3] Consequently, although the most common initial clinical presentation of metastatic HER2-positive breast cancer is with extra-CNS metastases (ie, lung and liver), CNS progression occurs in a substantial proportion of patients during the course of the disease.[4] As a matter of fact, enhanced and prolonged extra-CNS disease control, achieved by HER2 targeting, results in high incidence of CNS progression. This occurrence, that here we call 'neurosystemic dissociation' of HER2-positive metastatic breast cancer (mBC), remains a major challenge for medical oncologists, and research is striving to find strategies to prevent overt CNS metastases or to treat them more effectively.[5] Markers that could allow early diagnosis and treatment of this disease are mandatory. Liquid biopsy is a minimally invasive procedure that allows the identification and analysis of circulating

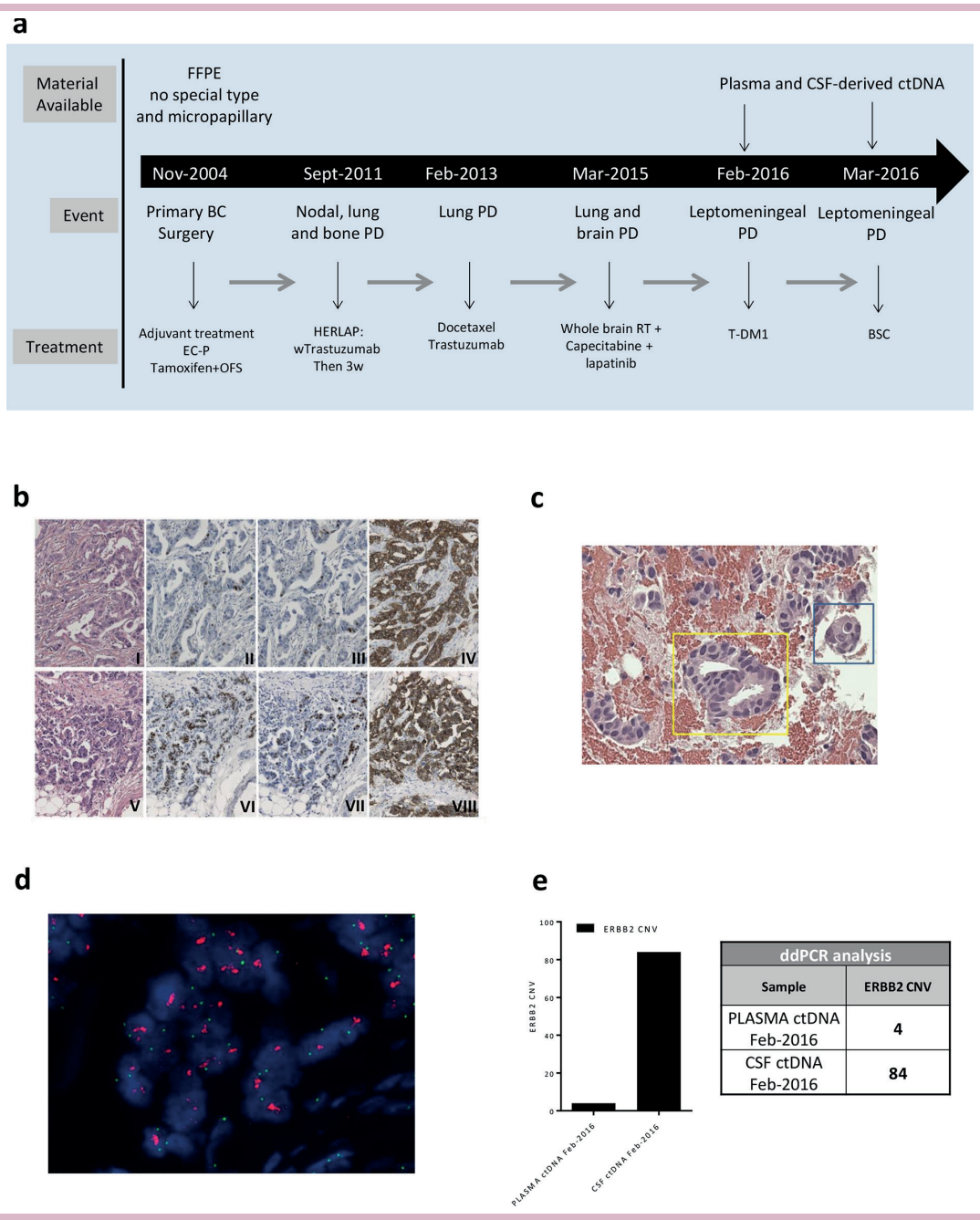
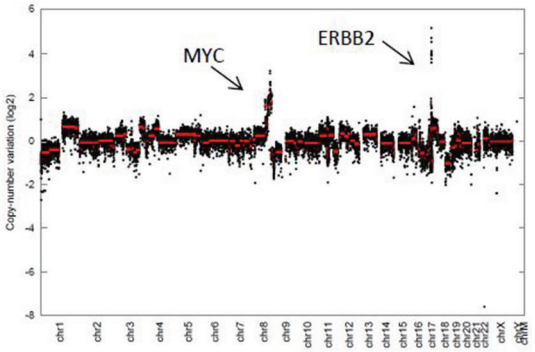


Figure 1 (A) Timeline of the patient's clinical events and interventions. (B) H&E of ductal non-special type (NST) (I) and micropapillary (V) components of the primary mixed invasive breast cancer. Immunostaining: Oestrogen and progesterone receptors are positive in about 10% of the nuclei of ductal NST (II and III) and in 70% (VI) and 40% (VII) of micropapillary subtype respectively. HER2 immunostaining is intense and complete (score 3+) in both histotypes (IV, VIII). (C) H&E staining of cell block section of fine needle aspiration (FNA) of supraclavicular lymph node showing micropapillary (blue) and glandular structures (yellow). (D) *ERBB2* gene amplification by FISH in breast cancer cells. Red dots indicate the *ERBB2* gene probe, and green signals represent the CEP17 reference probe. Magnification: 100x. (E) ddPCR analysis of matched plasma and CSF-derived ctDNA prior to T-DM1 treatment. BC, breast cancer; BSC, best supportive care; CSF, cerebrospinal fluid; ctDNA, circulating tumour DNA; EC-P, Epi-doxorubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2 q21 days for four cycles followed by paclitaxel 175 mg/m^2 q21days for four cycles; FFPE, formalin fixed, paraffin embedded; FISH, fluorescence in situ hybridisation; HERLAP, HERceptin or LAPatinib trial; OFS, ovarian function suppression; PD, progressive disease; RT, radiation therapy; T-DM1, trastuzumab emtansine; CNV, copy number variation.

tumour-derived DNA (ctDNA) that is shed from tumour cells in body fluids.[6 7] Here we describe a clinical case of HER2-positive mBC patient with a 'neurosystemic dissociation' documented by paired ctDNA analysis in the cerebrospinal fluid (CSF) and in the plasma, and we discuss the potentialities of

Cosmic	Gene	coord	AAchange	var_effect	% mutant reads
842	TP53	chr17:7577538	p.R248Q	nonsynonymous	56.16
3	SYNE1	chr6:152644680	p.A5284T	nonsynonymous	3.51
2	PIK3CA	chr3:178916890	p.R93W	nonsynonymous	44.18
2	WHSC1	chr4:1918624	p.H263Y	nonsynonymous	19.21
2	AP2A1	chr19:50295254	p.S179L	nonsynonymous	12.58
1	GSX2	chr4:54967859	p.R229*	stopgain	49.30
1	NFYB	chr12:104519899	p.T75M	nonsynonymous	15.79
1	WDR25	chr14:100996223	p.D494N	nonsynonymous	15.32
1	C17orf102	chr17:32906212	p.Q30*	stopgain	13.93
1	WDR60	chr7:158664076	p.K105E	nonsynonymous	13.38
1	MAML1	chr5:179193437	p.R476W	nonsynonymous	13.33
1	FAM186A	chr12:50745783	p.Q1611L	nonsynonymous	13.19
1	WWC1	chr5:167850747	p.S495*	stopgain	12.96



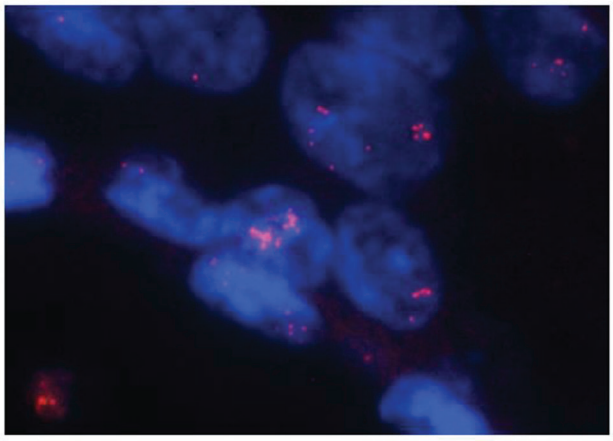


Figure 2 (A) WES of CSF-derived ctDNA collected prior to T-DM1 treatment. Only point mutations reported in COSMIC database are shown (left panel) together with complete copy number profile (right panel). (B) *MYC* gene amplification by fluorescence in situ hybridisation (FISH). Multiple red signals relative to *MYC* gene are present in breast cancer nuclei. Magnification: 100x. CSF, cerebrospinal fluid; ctDNA, circulating tumour DNA; FISH, fluorescence in situ hybridisation; PBMC, peripheral blood mononuclear cells; T-DM1, trastuzumab emtansine; WES, whole exome sequencing.

this approach in the management of patients with HER2-positive breast cancer.

METHODS

Study oversight

The patient provided informed consent for an institutional review board-approved protocol for longitudinal collection of plasma and CSF and genomic profiling on tumour DNA.

Plasma and cerebrospinal fluid collection

At least 10 mL of whole blood and 4 mL of CSF were collected by blood draw (using EDTA as anticoagulant) and lumbar puncture, respectively. Plasma was separated and CSF was cleaned within 4 hours through two different centrifugation steps (the first at room temperature for 10 min at 1600×g and the second at 3000×g for the same time and temperature). Plasma and CSF were stored at −80°C until ctDNA extraction.

Circulating DNA (ctDNA) isolation

ctDNA was extracted from plasma and CSF using the QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's instructions.

Additional and detailed materials and methods are provided in online supplementary file 1.

Results

Report of the case

A 32-year-old woman presented in October 2004 with a left-sided breast invasive ductal carcinoma (figure 1A). Modified radical mastectomy was performed and the pathology report documented a stage IIB (pT1c, pN1a), poorly differentiated (G3), invasive mixed carcinoma (not-special type-40% and micropapillary-60%, figure 1B, C) estrogen receptor (ER), progesterone receptor (PR) and HER2 positive (figure 1D). Adjuvant therapy consisted of anthracycline-taxane-based chemotherapy, followed by tamoxifen plus ovarian function suppression for 5 years. Seven years later, a fine-needle aspiration of an enlarged supraclavicular lymph-node confirmed breast cancer recurrence, with a cytological (figure 1C) and immunophenotypical pattern consistent with that of the primary tumour (ER 11%, PR 12%, HER2 3+). Restaging by CT scan detected both lung and bone metastases. The patient was treated with a chemo-free approach within a phase 2 trial conducted at our institution with

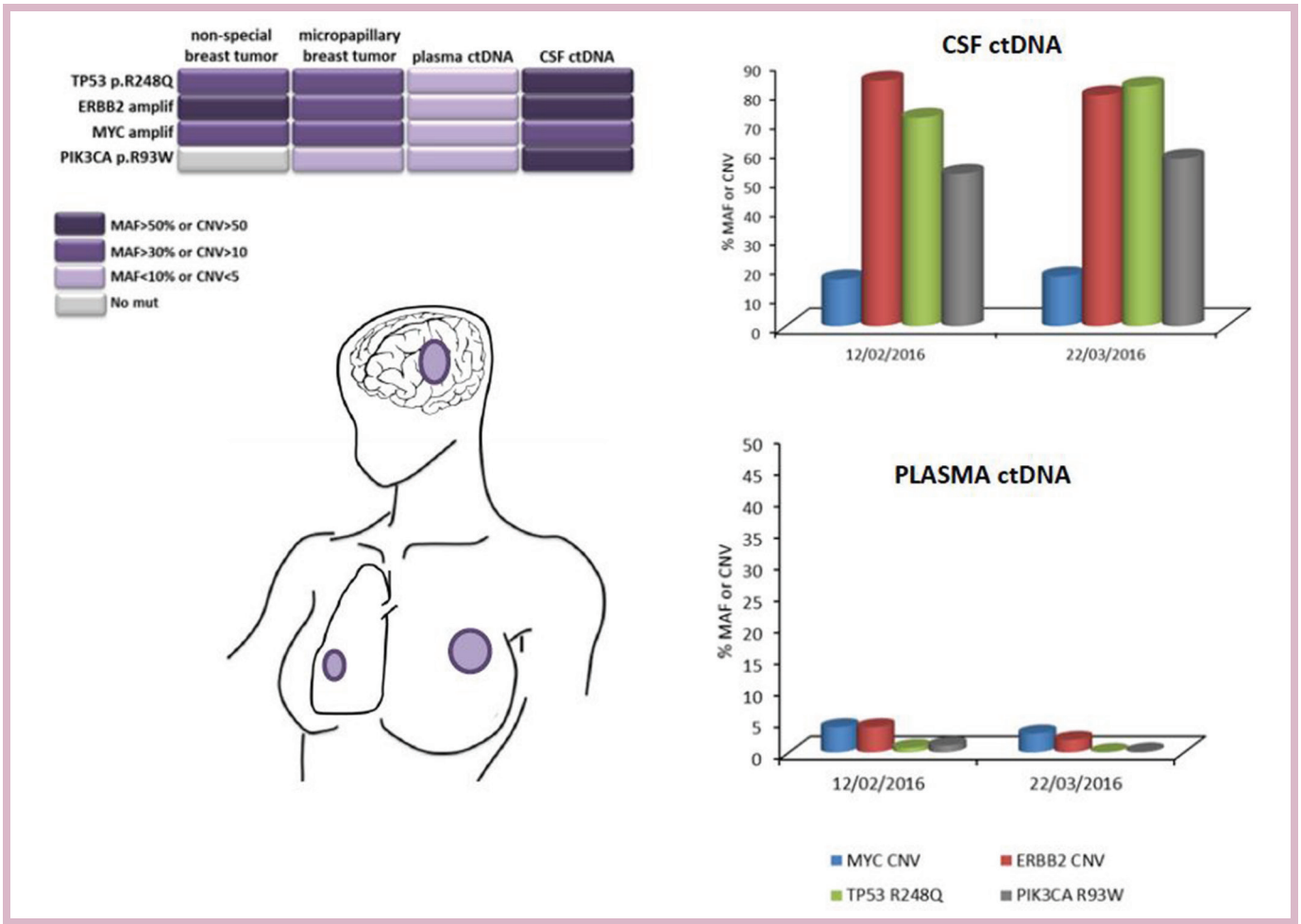


Figure 3 Analysis of CSF and plasma-derived ctDNA and primary breast tumours. Heatmap of the non-silent genetic alterations (top panel) from each of the specimens collected is shown. Violet-scale colour key for MAFs is shown. CSF, cerebrospinal fluid; ctDNA, circulating tumour DNA; MAFs, mutant allelic frequencies; PBMC, peripheral blood mononuclear cells.

Trastuzumab (T) single agent, for a total of 51 3-weekly doses of T, achieving a partial response as best response.[8] Then, for lung disease progression, she started a first-line chemotherapy with docetaxel (75 mg/m^2)+T for eight cycles and then T maintenance therapy. After 2 years she presented with headache, reduced flexion of lower limbs and gait disturbance. CT scan showed multiple cerebral and cerebellar cortico-subcortical contrast-enhancing lesions, compatible with CNS metastases and regrowth of bone and lung metastases. She then received whole brain radiotherapy (30 Gy/10 fractions) and oral corticosteroids. On completion of radiation therapy, she started a third-line systemic treatment with capecitabine (2000 mg/m^2, from day 1 to day 14)-lapatinib (1250 mg, every day) achieving partial response within 4 months. Nine months later, epileptic seizures occurred and a CT scan and MRI documented a CNS progression with leptomeningeal carcinomatosis. She therefore started a fourth line of treatment with the antibody-drug conjugate T-DM1, which was stopped after three cycles for worsening of neurological symptoms. At the time of neurological deterioration, MRI showed stable disease in the CNS and CT scan showed a sustained partial remission in extra-CNS disease sites (neurosystemic dissociation). Unfortunately, the patient died 20 months after the initial diagnosis of CNS metastases. Since the evidence of CNS progression we collected multiple plasma samples and two paired CSF samples by lumbar puncture, one before and the other after the three cycles with T-DM1.

Molecular-based liquid biopsy analyses

We first analysed CSF ctDNA collected before T-DM1 treatment. Whole exome sequencing (WES) revealed a *TP53* p.R248Q mutation (FA 56.16%), a *PIK3CA* p.R93W mutation (FA 44.18%), c*MYC* amplification (3.6X). Positivity for HER2 overexpression was confirmed by the detection of high copy number of the *ERBB2* exons (15.2X) (figure 2A). *MYC* amplification was later confirmed by FISH analysis (figure 2B).

We next analysed the corresponding primary tumour tissue. In both the not-special type and micropapillary components, WES analysis confirmed the presence of

the *TP53* p.R248Q mutation and amplification of *ERBB2* and c*MYC* (figure 3 and see online supplementary figure 1). Notably, the *PIK3CA* p.R93W variant found in the CSF was detected only in the micropapillary component of the tumour and not in the non-special tumour tissue (figure 3 and see online supplementary figure 2).

To describe clonal dynamics in plasma ctDNA collected during treatment, we designed ddPCR assays for each of the molecular alterations found in CSF and tumour tissue. c*MYC* amplification was not detected in plasma ctDNA samples. Conversely, *ERBB2* amplification, *TP53* and *PIK3CA* mutations were detectable at the time of concomitant CNS and lung progression (figure 3). Plasma ctDNA collected before and after treatment with T-DM1 showed continuously decreasing *ERBB2* levels and *TP53* and *PIK3CA* frequency of mutations, which was consistent with imaging showing extra-CNS disease control. In parallel, CSF genomic profiling recapitulated the clinical course of the disease in the CNS, with no benefit from T-DM1 treatment. Indeed, CSF-derived ctDNA analysis revealed high copy number for *MYC* and *ERRB2* and high frequency of mutations for *TP53* and *PIK3CA* at both timepoints, confirming a molecular non-response to T-DM1 treatment (figure 3).

DISCUSSION

This case report underscores the potentialities of CSF-derived ctDNA analysis in the management of patients with HER2-positive mBC. The emergence of CNS metastases in the context of extracerebral disease control represents one of the major challenges for medical oncologists dealing with this disease (neurosystemic dissociation). Newer tools to personalise and monitor the efficacy of treatments are therefore needed in this particular population.

In this clinical case, at the time of the second CSF progression, we opted for T-DM1 having the patient exhausted other treatment options and because of preclinical and clinical data suggesting T-DM1 potential activity in CNS metastases.[9 10]

Although we collected paired blood and CSF ctDNA samples over a narrow window in this patient's medical history, we show that CSF ctDNA levels follow tumour burden variation, being, in this specific case, even more informative and sensitive than traditional imaging. Indeed, persistence of high levels of ctDNA in the CSF predicted neurological deterioration during T-DM1 treatment. Conversely, extra-CNS sustained response was paralleled by a significant drop of molecular alterations detectable in plasma samples, as an effect of the antitumour activity of T-DM1.

An interesting finding of our analyses was that WES of CSF detected the repertoire of genetic alterations of this patient's mixed histology tumour, including one mutation (*PI3KCA* p.R93W) which was exclusively present in the micropapillary component. We speculate that the micropapillary component of her tumour, known for being more aggressive than the ductal type,[11] may have metastasised systemically and in CNS sites. This outlines the potentialities of ctDNA analysis of CSF in designing strategies to identify subjects at increased risk of CNS involvement. While exome sequencing in paired plasma ctDNA failed to identify this *PIK3CA* mutation, the latter could be detected in the baseline plasma sample by targeted ddPCR.

CONCLUSION

We report that paired analysis of plasma and CSF ctDNA might be useful in the management of women with HER2-positive mBC. CSF ctDNA levels follow tumour burden variations, being potentially more informative and sensitive than traditional imaging. Analysis of CSF ctDNA could represent, therefore, a minimally invasive approach for monitoring progression and response to treatment of CNS lesions, in particular within the 'neurosystemic dissociation' phenomenon, which frequently occurs during HER2 targeted treatments. Furthermore, next generation sequencing of CSF ctDNA may be highly sensitive in identifying the mutational repertoire of a metastatic tumour. This may be valuable in designing tailored treatments for CNS disease, in generating hypotheses on the clonal evolution of metastatic disease and in identifying molecular markers of CNS-tropism.

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Contributors GS, A Bardelli, EG and FM designed the study and wrote the manuscript. GS, BM, A Bartolini, GC, LC and IS performed the experiments and acquired the data. A Bardelli, AS and FM supervised the study. All authors revised and approved the manuscript.

Competing interests FM has received speakers Honoraria from Novartis, Astra Zeneca and Roche and travel grants from Astra Zeneca and Roche. All other authors declare no conflicts of interests.

Patient consent Obtained.

Ethics approval Ethical Committee of Candiolo Cancer Institute.

Provenance and peer review Not commissioned; internally peer reviewed.

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