Prolonged response to treatment based on cell-free DNA analysis and molecular profiling in three patients with metastatic cancer: a case series

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Abstract

Background: Patients with advanced and/or metastatic solid tumors have limited treatment options. Mutations that serve as biomarkers of carcinogenesis can be found in cell-free DNA of patients' plasma. Analysis of circulating tumor DNA (ctDNA) was developed as a non-invasive, cost-effective alternative to tumor biopsy when such biopsy is not technically feasible or it is associated with high risk for complications. The role of ctDNA in precision oncology is promising but its clinical significance across tumor types remains to be validated. We report a case series of three heavily pretreated patients with advanced solid tumors who received matched targeted therapy based on ctDNA analysis and/or tumor molecular profiling.

Case presentation: Three patients with advanced, metastatic cancer and the following characteristics are presented: a 71-year-old woman with ovarian cancer and *BRCA2* mutation identified in ctDNA and tumor tissue was treated with a PARP inhibitor and achieved partial response by RECIST (Response Evaluation Criteria in Solid Tumors) for 22.6+ months; a 40-year-old woman with adenoid cystic carcinoma of the parotid gland was treated with a MEK/RAF pathway inhibitor on the basis of *RAF1* amplification on ctDNA analysis and had stable disease for 20.2 months; and a 56-year-old woman with breast cancer and a *BRCA1* mutation identified by ctDNA analysis was treated with a PARP inhibitor and achieved stable disease for 9.1 months. All three patients are alive at the time of this report.

Conclusions: These results suggest that ctDNA analysis can contribute to selection of targeted therapy in patients with advanced, metastatic cancer. Prospective clinical trials to evaluate and optimize ctDNA biomarkers, as well as the integration of novel and/or alternative targeted therapies, are warranted to fully assess the role of ctDNA analysis in cancer therapy.

Trial registration: www.clinicaltrials.gov (NCT02152254). Registered May 28, 2014. https://www.clinicaltrials.gov/ct2/show/NCT02152254. MD Anderson protocol # PA12-1161 (approval ID IRB1 FWA00000121) and # PA11-0377 (approval ID IRB4 FWA00005015).

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Introduction

Genomic alterations play an important role in carcinogenesis, disease progression, resistance and response to targeted therapy. The genetic heterogeneity across tumor types complicates the management of these diseases. Using the genomic profiles of individual patient tumors, precision medicine helps optimize treatment selection to improve clinical outcomes for patients with cancer.[1] Mutations that serve as biomarkers of tumor progression and response can be found in cell-free DNA collected from the plasma of individual

patients. Analysis of this circulating tumor DNA (ctDNA), therefore, offers a promising, non-invasive, and personalized diagnostic approach to selecting treatment and patients for enrollment in clinical trials.

Tumor biopsy samples are typically analyzed to determine the tumor characteristics, but biopsy is not feasible for some patients because their tumors are not easily accessible or because the biopsy procedure is associated with significant risk. Also, samples obtained from biopsies can be insufficient for tumor analysis. The proportion of patients who are ineligible for molecular screening due to inaccessibility or high risk of tumor biopsy, and therefore are excluded from certain clinical trials offering targeted treatment based on molecular alterations, has not been systematically analyzed. In the BATTLE (Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination) study, it was estimated that 10% to 15% of patients with lung cancer did not have sufficient material for next-generation molecular testing.[2] In an interim analysis of IMPACT2, an ongoing randomized study evaluating genomic profiling and targeted agents in metastatic cancer, of 391 patients who were enrolled in the first part of the study, 19 (4.86%) patients had inadequate tumor cells for analysis and biopsy was not feasible or tumor was not accessible in 15 (3.84%) patients. Overall, 213 (54.48%) of 391 received anticancer therapy (Tsimberidou *et al.*, *Npj Precision Oncology*, in Press).

CtDNA analysis was developed as a non-invasive, cost-effective alternative to tumor biopsy when such biopsy is associated with significant risk, when tumor tissue is insufficient or inaccessible, and/or when repeated assessment of tumor molecular abnormalities is needed to optimize treatment.[3]

We previously reviewed the role of ctDNA in guiding targeted therapy in clinical trials that involved ctDNA analysis of specific tumors and across tumor types.[3] CtDNA analysis based on epidermal growth factor receptor (EGFR) and v-Ki-ras2 kirsten rat sarcoma viral oncogene homolog (KRAS) has been well established for selecting treatment for patients with advanced non-small cell lung cancer (NSCLC) and colorectal cancer (CRC), respectively.[4,5] However, the assessment of ctDNA and clinical trials that involve ctDNA analysis in other tumor types are still needed. Herein, we describe encouraging clinical outcomes of three patients with advanced metastatic cancer who received matched targeted therapy based on the results of ctDNA analysis.

Case presentation

Case presentation 1: Patient (ID 001)

A 71-year-old woman was diagnosed with metastatic ovarian cancer in October 2010. She had been experiencing symptoms of vaginal dryness, dyspareunia, and abdominal and lower back pain. Magnetic resonance imaging (MRI) of the pelvis demonstrated a complex adnexal mass and computed tomography (CT) imaging studies demonstrated a lesion in the upper lobe of the left lung, and an adenoma of the left adrenal gland. Transvaginal ultrasonography confirmed a multilocular mass ($6.0 \times 5.0 \times 5.6$ cm) centered in the right adnexal region. The mass had multiple irregular thick septa and a soft tissue component with definite vascular flow in the regions of echogenic soft tissue and was highly suggestive of cystadenocarcinoma. The patient underwent a laparoscopic bilateral salpingo-oophorectomy, and the surgical pathology report demonstrated ovarian high-grade serous carcinoma with extension to the fallopian tube surface and fimbrial end in the right ovary and tube, and metastatic to the omentum. In November 2010, the patient was treated with six cycles of carboplatin and paclitaxel followed by 11 cycles of paclitaxel consolidation therapy. CT imaging studies demonstrated no evidence of disease at 5 months after treatment and annually for 3 years. Three years after treatment, she presented to the clinic with increasing pain and pressure in the lower abdomen, including pain with urination. CT scans demonstrated diffuse peritoneal carcinomatosis, evidenced by multiple nodular masses noted within the peritoneum and the supracolic omentum, and small volume ascites. She was retreated with six cycles of carboplatin and paclitaxel, and restaging scans demonstrated improvement in the peritoneal carcinomatosis and no gross CT evidence of residual disease. She had no evidence of disease for 17 months, and then CT scans showed interval development of a heterogeneous soft tissue lesion in the pelvis compatible with recurrent disease. She underwent tumor reductive surgery followed by six cycles of carboplatin and paclitaxel. Molecular analysis using formalin-fixed paraffin-embedded (FFPE) tissue of the most recently resected pelvic mass demonstrated tumor protein p53 (*TP53*) mutation and breast cancer type 2 (*BRCA2*) somatic mutation (Table 1). The disease responded to treatment

Table 1. Genomic analysis using tissue and ctDNA samples.

Pt. ID	Collection date	Sample source	Genomic alterations	Panel, no. of clinically relevant genes
001	16 June 2015	Pelvic mass	*TP53 V157F*	*Solid tumor genomic assay V2, 146 genes*
			BRCA2 R3052W	
	20 July 2017	Blood	***BRCA2 R3052W***	*Guardant360, 73 genes*
			NF1 splice site	
			TP53 V157F	
			GNAS R201H	
002	21 July 2017	Blood	***RAF1* amplification**	*Guardant360, 73 genes*
	25 September 2017	Liver	None	*Solid tumor genomic assay V1, 134 genes*
	22 November 2019	Liver	*BRAF* V600dup	*Tempus × T assay, 596 genes*
			MYB-NFIB chromosomal rearrangement	
			KMT2D Q2696 Stop gain – LOF	
003	29 January 2015	Lung	*ATM* loss exons 57–63	*FoundationOne, 315 genes*
			NOTCH2 A3F	
			ESR1 Y537S	
			CCND1 amplification	
			EMSY amplification	
			FGF19 amplification	
			FGF3 amplification	
			FGF4 amplification	
			MCL1 amplification - equivocal	
	15 October 2018	Blood	*ATM M3011T*	*Liquid biopsy panel, 70 genes*
			BRCA1 Q356*	
			ESR1 Y537S	
			TP53 A159V	
			TP53 P152L	

ATM, ATM serine/threonine kinase; BRAF, rapidly accelerated fibrosarcoma homolog B; BRCA1, breast cancer type 1; BRCA2, breast cancer type 2; CCND1, Cyclin D1; EMSY, BRCA2 interacting transcriptional repressor; ESR1, estrogen receptor 1; FGF, fibroblast growth factor; GNAS, guanine nucleotide binding protein, alpha stimulating activity polypeptide; KMT2D, histone-lysine N-methyltransferase 2D; LOF, loss of function; MCL1, myeloid cell leukemia 1; MYB-NFIB, myeloblastosis virus oncogene – nuclear factor 1 B-type; NF1, neurofibromatosis type 1; NOTCH2, notch receptor 2; RAF1, rapidly accelerated fibrosarcoma 1; TP53, tumor protein p53.
Molecular alterations identified by ctDNA analysis and used to select matched therapy are presented in bold text.

with carboplatin and paclitaxel but recurred after 20 months and the patient was referred to the Department of Investigational Cancer Therapeutics in July 2017. She was enrolled on a clinical trial that included carboplatin, paclitaxel and immunotherapy. Her best response was immune-related

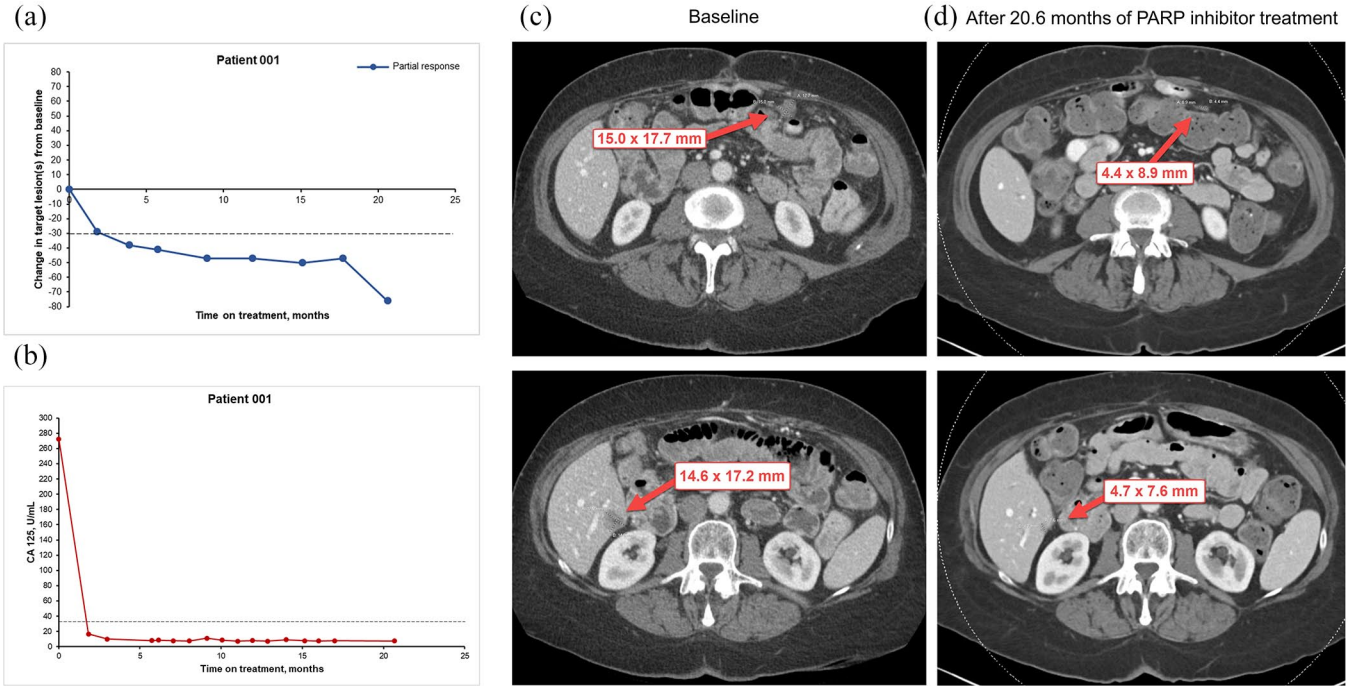

Figure 1. Changes in tumor measurement from baseline in patient 001, who underwent treatment with PARP inhibitor on the basis of ctDNA analysis showing *BRCA2 R3052W* mutation. (a) Scatter plot illustrates changes in lesion tumor burden. Computed tomography (CT) scan data at the most recent time prior to cycle 1, day 1 is chosen as baseline (time 0). The horizontal (*x*) axis shows time at restaging CT scans as months from baseline. The vertical (*y*) axis shows percentage of change in tumor measurement from baseline. Each dot represents a data point collected at each restaging CT scan. The blue line represents PR (⩾30% decrease in tumor measurements from baseline, based on RECIST 1.1). (b) Scatter plot illustrates changes in tumor marker levels over time. (c) Representative CT images from the patient at baseline. (d) Representative CT images from the patient after 20.6 months of targeted therapy with PARP inhibitor.
Red arrows indicate target lesions.

partial response (PR). On cycle 19, day 22, CT images demonstrated increasing size and number of nodules within the peritoneum consistent with increasing metastatic disease; a growing metastasis was also noted in segment five of the liver. ctDNA analysis performed 13 months prior to disease progression demonstrated the following alterations: neurofibromin 1 (*NF1*), splice site SNV 1.9, *TP53 V157F* 0.2, guanine nucleotide binding protein, alpha stimulating activity polypeptide (*GNAS*) *R201H* 0.2, *BRCA2 R3052W* 0.1 (Table 1). On the basis of this information and evidence of a *BRCA2* somatic mutation in the pelvic mass, the patient was enrolled on a clinical trial of a poly (ADP-ribose) polymerase (PARP) inhibitor administered daily. She underwent genetic counseling, and testing for germline *BRCA1* and *BRCA2* mutations was negative. Thus far, she has received 21 cycles of the PARP inhibitor, and her best RECIST response was PR (76% decrease from baseline per RECIST 1.1) (Figure 1). Her treatment is ongoing [progression-free survival (PFS) = 22.6+ months] (Table 2). The patient experienced grade 3 anemia that started during cycle 3 and was treated with transfusion of red blood cells (average, 2 units monthly). On cycle 7, day 1, she required a dose adjustment consisting of a 25% reduction in the PARP inhibitor dose and a decrease of days of treatment to 3 weeks on, 1 week off, after which she tolerated the treatment without the need for transfusion.

Case presentation 2: Patient (ID 002)

A 40-year-old woman was first diagnosed in 2007 with adenoid cystic carcinoma of the left parotid gland and bone metastases. She was a non-smoker and had a pertinent family history of multiple cancers, notably of the brain, stomach, and skin. The patient underwent superficial parotidectomy with apparently negative margins followed by postoperative adjuvant radiotherapy. Subsequent imaging revealed a lytic lesion at the L3 vertebral body; biopsy did not demonstrate metastatic tumor. She had no evidence of disease until 2013, when a positron emission tomography (PET)/CT revealed

Table 2. Clinical outcomes of three patients with advanced solid tumors who received matched therapy based on ctDNA analysis.

Pt. ID	ctDNA biomarker	Matched therapy	Best RECIST response	PFS*, months	Progr. status	Subsequent Rx	Survival status	OS†, months
001	*BRCA2 R3052W*	PARP inhibitor	PR	22.6+	N/A	N/A	Alive	22.6+
002	*RAF1* amplification	MEK/RAF pathway inhibitor	SD	20.2	PD	Investigational	Alive	28.4+
003	*BRCA1 Q356**	PARP inhibitor	SD	9.1	PD	Doxorubicin; radiotherapy	Alive	14.8+

N/A, non-applicable; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; Progr., progression; Rx, therapy; SD, stable disease.
BRAC1, breast cancer type 1; BRAC2, breast cancer type 2; MEK, mitogen-activated protein kinase kinase; PARP, poly (ADP-ribose) polymerase; RAF1, rapidly accelerated fibrosarcoma 1.
*Progression-free survival is measured in months from cycle 1, day 1 to time of radiologic scan showing progressive disease.
†Overall survival is measured in months from cycle 1, day 1 to the most recent time of this report.

increased activity in the L3 vertebra. The patient experienced back pain while lifting heavy objects or when the lesion was percussed, post-operative paresthesia in the lobule and tragus of the left ear and stiffening of the left neck. In October 2013, she received stereotactic radiation therapy to the L3 vertebra (24 Gy to the gross tumor volume and 16 Gy to the clinical target volume). Three months later, PET/CT imaging studies demonstrated no evidence of FDG-avid metastasis.

She had no evidence of active disease for 33 months, until October 2016, when MRI and PET/CT imaging studies demonstrated left pulmonary metastasis, an enlarging lesion at L3–L4 consistent with progressive disease. She was referred to the Department of Investigational Cancer Therapeutics in February 2017. She was treated with an investigational combination therapy comprising a STAT3 inhibitor and nivolumab for eight cycles. Her best RECIST response was stable disease (SD), with a 13% decrease in tumor measurements from baseline to cycle 3, day 26 (RECIST 1.1). She was taken off protocol in July 2017 owing to disease progression that included liver metastases. Possibly related adverse events included grade 1 diarrhea and abdominal cramps.

Molecular diagnostic solid tumor genomic assay using FFPE slides from a liver biopsy performed in September 2017 did not identify any somatic mutations (Table 1). However, ctDNA analysis using the patient's blood sample demonstrated a strongly positive rapidly accelerated fibrosarcoma 1 (*RAF1*) amplification, with a magnitude in the 50th to 90th percentile (Table 1). On the basis of this finding, the patient was started on a clinical trial with a mitogen-activated protein kinase kinase/ rapidly accelerated fibrosarcoma (MEK/RAF) pathway inhibitor in January 2018. Her best RECIST response was SD with an 18% decrease in tumor measurements (RECIST 1.1) after 10 cycles (Figure 2). She received 29 cycles of the study drug and was taken off study owing to progressive disease confirmed by CT imaging studies. PFS duration was 20.2 months and overall survival duration was 28.4+ months. Adverse events possibly associated with MEK/RAF pathway inhibitor were all grade 1 and included intermittent diarrhea and vomiting; maculopapular and acneiform rash; neutropenia; blurry vision; and hair thinning.

In November 2019, the patient underwent a core needle biopsy of the liver for completion of molecular profiling, which demonstrated rapidly accelerated fibrosarcoma homolog B (*BRAF*) *V600dup* and myeloblastosis virus oncogene – nuclear factor 1 B-type (*MYB-NFIB*) chromosomal rearrangement, biologically relevant histone-lysine n-methyltransferase 2D (*KMT2D*) genomic variant, negative programmed death-ligand 1 (*PDL1*) expression, normal DNA mismatch repair protein expression, and RNA sequencing demonstrating fibroblast growth factor receptor 2 (*FGFR2*) and fibroblast growth factor receptor 1 (*FGFR1*) overexpression (Table 1). Two months later, she underwent transcatheter arterial hepatic embolization of the larger liver metastases. The patient was alive at the time of this report (Table 2). She did not experience any serious adverse events and did not require any blood transfusions.

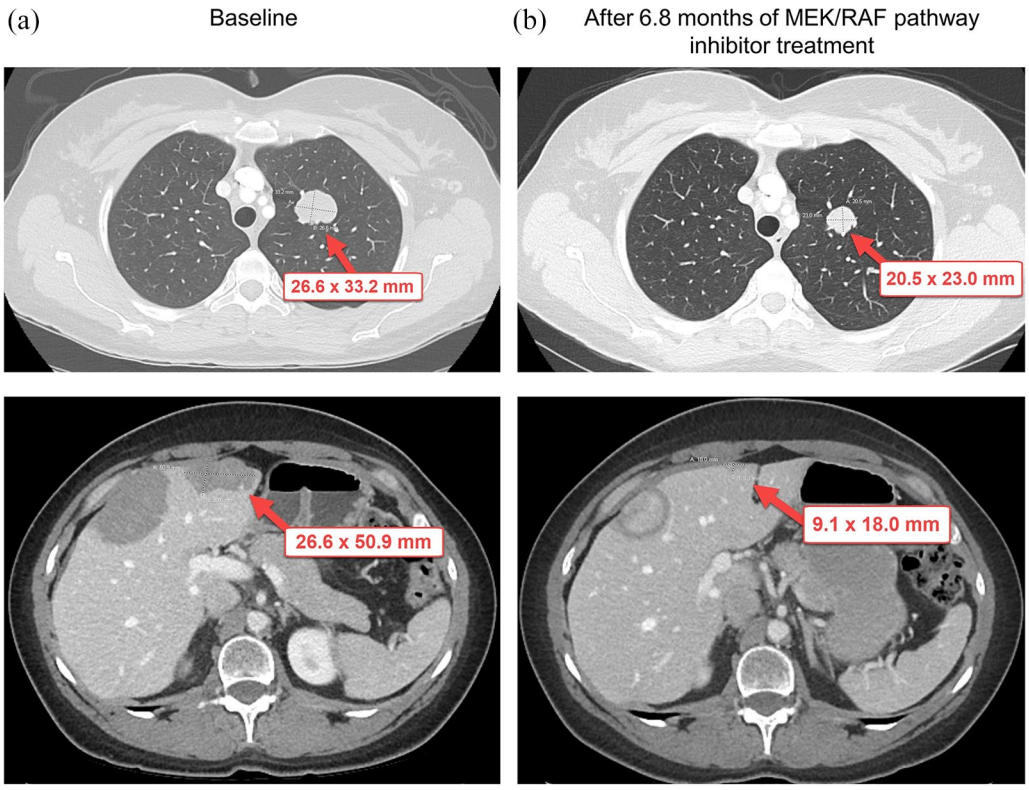

Figure 2. Changes in tumor measurement from baseline in patient 002, who underwent treatment with MEK/RAF pathway inhibitor on the basis of ctDNA analysis showing *RAF1* amplification. (a) Representative computed tomography (CT) images from the patient at baseline. (b) Representative CT images from the patient after 6.8 months of treatment with MEK/RAF pathway inhibitor.
Red arrows indicate target lesions.

Case presentation 3: Patient (ID 003)

A 56-year-old woman with a history of breast cancer was referred to the Department of Investigational Cancer Therapeutics in January 2015. She had no pertinent medical history and was in good health until early 2005, when she felt a mass in her left breast. She was a non-smoker and had a family history of breast cancer and prostate cancer. Physical examination demonstrated a 3 × 3 cm mass in the 10 to 11-o'clock position in the superior aspect of the left breast and a 2 cm lymph node in the left axilla. Ultrasound-guided core needle biopsy performed in February 2005 demonstrated an estrogen receptor- and progesterone receptor-positive, human epidermal growth factor receptor 2 (HER-2/neu)-negative invasive mammary carcinoma of the left breast [modified Black's nuclear grade 1 (well differentiated)], carcinoma *in situ*, low grade, solid type, without necrosis). CT scans indicated no evidence of metastasis; however, fine-needle aspiration of the left axilla lymph nodes demonstrated metastatic carcinoma consistent with the primary breast tumor.

In March 2005, the patient was enrolled on a clinical trial that included weekly paclitaxel chemotherapy for 12 cycles followed by six cycles of 5-fluorouracil, epirubicin, and cyclophosphamide. Four months later, imaging studies indicated a reduction in tumor size. In September 2005, she underwent a left skin-sparing total mastectomy with axillary lymph node dissection followed by delayed reconstruction using a submuscular tissue expander. Then, from October to December 2005, she underwent post-operative adjuvant radiation therapy (50 Gy in 25 fractions to the left central chest and left lateral chest wall with an additional 10 Gy boost to the tumor bed). She also intermittently received Tamoxifen between 2005 and 2008. In December 2006, imagining studies demonstrated bone metastasis and the patient was treated with radiation therapy and anastrozole plus fulvestrant from December 2006 to March 2014. The bone disease stabilized, but she had disease progression in her lungs and lymph nodes. Although her tumor did not harbor an alteration in the PI3K/Akt/mTOR pathway, she was treated

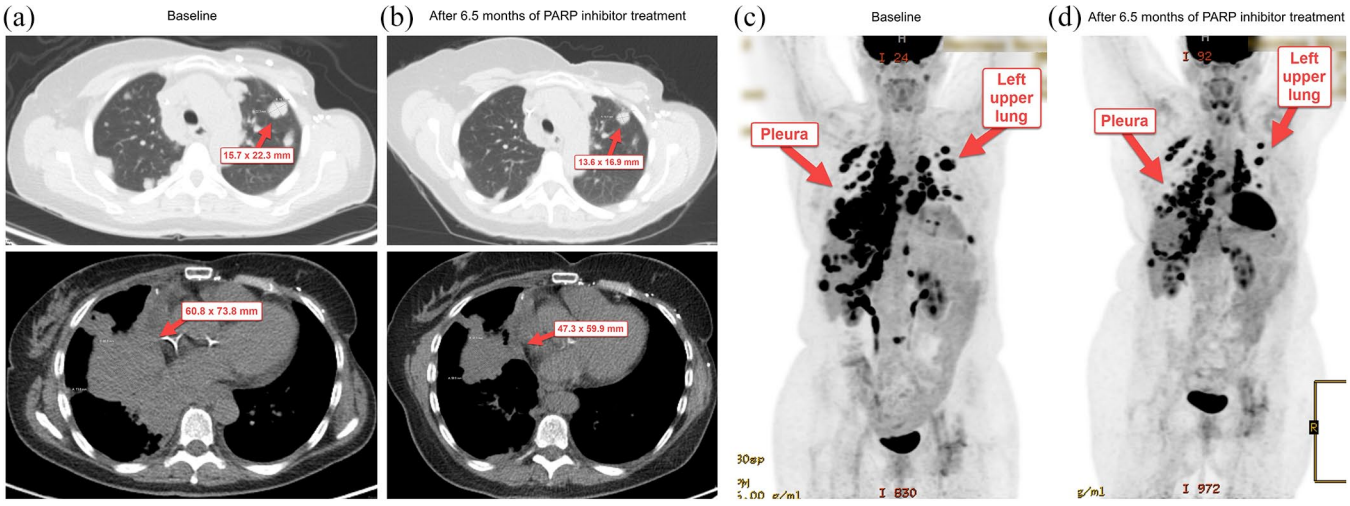

Figure 3. Changes in tumor measurement from baseline in patient 003, who underwent treatment with PARP inhibitor on the basis of ctDNA analysis showing *BRCA1 Q356** mutation. (a) Representative computed tomography (CT) images from the patient at baseline. (b) Representative CT images from the patient after 6.5 months of treatment with PARP inhibitor. (c) Representative positron emission tomography (PET) overview image from the patient at baseline. (d) Representative PET overview image from the patient after 6.5 months of treatment with PARP inhibitor.
Red arrows indicate target lesions.

with the standard-of-care exemestane and everolimus from March 2014 to January 2015.

In January 2015, CT scans demonstrated increasing thoracic lymphadenopathy and pulmonary metastatic disease, and a nuclear medicine bone scan demonstrated bone metastasis involving the left ilium, acetabulum, and ischium. The patient was referred to the Department of Investigational Cancer Therapeutics in January 2015 and underwent biopsy of a lung tumor. Molecular profiling of the tumor demonstrated the following genomic alterations: ATM serine/threonine kinase (*ATM*) loss in exons 57 to 63, notch receptor 2 (*NOTCH2*) *A3F*, estrogen receptor 1 (*ESR1) Y537S*, and amplification of the following genes: cyclin D1 (*CCND1*), myeloid cell leukemia 1 (*MCL1*), BRCA2 interacting transcriptional repressor *(EMSY)*, fibroblast growth factor 19 (*FGF19*), fibroblast growth factor 3 (*FGF3*), and fibroblast growth factor 4 (*FGF4*) (Table 1). Early assessments suggested that preclinical models with increased cyclin D1 or cyclin-CDK-Rb pathway activation may have increased sensitivity to CDK4/6 inhibitors.[6,7] However, the patient did not receive a CDK4/6 inhibitor because clinical trials were not available at that time. She was presented at the multidisciplinary conference for optimization of treatment selection. On the basis of tumor molecular profiling demonstrating multiple mutations in *FGF* (*FGF19*, *FGF3*, and *FGF4*), the patient was offered an investigational therapy comprising FGFR inhibitor, but she pursued treatment with eribulin; after four cycles she developed disease progression. Subsequently, she was treated with 11 cycles of fulvestrant and palbociclib and was taken off study owing to progressive disease in August 2017.

On the basis of her tumor's *NOTCH2 A3F* alteration, in October 2017, the patient was enrolled on a clinical trial consisting of a NOTCH inhibitor, cisplatin, and gemcitabine. Her best RECIST response was PR per RECIST 1.1 criteria on cycle 12, day 19. She received 22 cycles of the investigational drug combination for 19 months and was taken off study owing to disease progression. Adverse events included impaired hearing and thrombocytopenia.

The patient underwent genetic counseling, and testing for germline *BRCA1* and *BRCA2* mutations was negative. CtDNA analysis in October 2018 demonstrated multiple somatic mutations which included a breast cancer type 1 (*BRCA1*) nonsense mutation on exon 10 (Table 1). On the basis of these findings, the patient was enrolled on a clinical trial with a PARP inhibitor. Her best RECIST response was SD with a 14% decrease in tumor measurement from baseline per RECIST 1.1, and the PFS duration was 9.1 months (Figure 3 and Table 2). Imaging studies in January 2020

demonstrated evidence of progressive disease and she was taken off study. Subsequently, she received radiotherapy and doxorubicin. At the time of this report, the patient was still alive, and the overall survival duration was 14.8+ months (Table 2).

Discussion and conclusions

The clinical significance of ctDNA analysis is evolving. The FDA has approved the use of next-generation sequencing (NGS) analysis as a companion diagnostic device for multiple biomarkers detected by ctDNA analysis using plasma specimens of patients with NSCLC (*EGFR* mutations for the use of gefitinib, osimertinib and erlotinib; and *ALK* rearrangements for treatment with alectinib), prostate (*BRCA1* and *BRCA2* alterations for the use of rucaparib and olaparib; and *ATM* alterations for the use of olaparib), ovarian (*BRCA1* and *BRCA2* alterations for the use of rucaparib), and breast cancer (*PI3K* mutations for the use of alpelisib). CtDNA analysis is also used for *KRAS* in CRC[5] and in the management of resistance to ALK inhibitors in lung cancer.[8] However, ctDNA analysis has not been systematically validated for patients with other tumor types. If the specific genomic alterations associated with these FDA-approved drugs are not detected in the blood, then tumor biopsies should be performed to identify these alterations.

In this case series, the use of ctDNA analysis alone or in combination with tumor molecular profiling was associated with encouraging clinical outcomes in three patients with advanced, metastatic cancer treated with targeted therapy. One patient had a durable PR of 22.6+ months as evidenced by CT imaging and tumor marker monitoring (Figure 1); the other two patients had SD lasting for 20.2 months and 9.1 months, respectively (Table 2). Our data demonstrate that in heavily pretreated patients with advanced, metastatic cancer, targeted therapy selected on the basis of ctDNA and/or tumor genomic analysis was associated with encouraging results. Our observation emphasizes the need to systematically analyze ctDNA in correlation with tumor genomic analysis. Further, the role of ctDNA analysis should be assessed in prospective clinical trials for selection of personalized therapy in individual patients.

In our study, patient ID 002, who received MEK/RAF pathway inhibitor on the basis of ctDNA analysis demonstrating a strongly positive *RAF1* amplification, had disease stabilization for 20.2 months and overall survival (OS) >28.4+ months. Genomic analysis using tumor biopsy also demonstrated *BRAF_V600dup* alteration. Tumor harboring both *RAF* amplification and *BRAF V600dup* may have sensitivity to treatment with MEK/RAF inhibitors, and our results warrant further investigation. There is minimal evidence on *RAF1* amplification and enhanced tumor response to targeted therapies. Overexpression of *RAF1* promotes c-Raf signaling *in vitro*, and preclinical studies have suggested that tumors with activating c-Raf signaling may be more sensitive to MEK inhibition.[9–11] Tumors with *RAF1* amplification therefore might benefit from MEK inhibitor therapy. Studies conducted by other investigators in a phase III, randomized clinical trial demonstrated that patients with melanoma harboring either *RAF1*, *KRAS* or *CCND1* amplification who received carboplatin, paclitaxel, and sorafenib, had a longer OS and PFS compared with only carboplatin and paclitaxel.[12] The outcomes were attributed to targeting of RAF1 by sorafenib. Hence, it is possible that *RAF1* amplification in this tumor type might sensitize cells to sorafenib and other multi-tyrosine kinase inhibitors.

BRAF_V600dup has been reported to be an activating alteration. This alteration is a duplication of codon V600, a "hotspot" codon located within the BRAF kinase domain (amino acids 457–717), which is commonly mutated in cancer. Codon V600 is important for maintaining the Asp-Phe-Gly (DFG) motif of BRAF in an inactive state.[13] Alterations at codon V600 increase BRAF activity by stabilizing the active conformation of BRAF. Several studies conducted by other investigators demonstrated that multiple missense mutations at V600 promote constitutive activation of BRAF and alterations at K601 and T599I promote BRAF kinase activity.[13–17] These alterations and other overlapping deletion and/or insertion events have been reported to be activating *in vitro* as evidenced by the activation of BRAF kinase activity, increase in MEK and ERK signaling, and transformation in NIH-3T3 cells.[18]

The effect of BRAF inhibitors on tumors harboring activating *non-BRAF V600* mutations is unclear. Limited preclinical data suggest that the majority of *non-BRAF V600* mutations are associated with decreased response or resistance to BRAF inhibitors, vemurafenib and dabrafenib.[19,20] In contrast, other investigators reported that activating *non-V600* variants may retain some

sensitivity to dabrafenib.[21] Some preclinical data indicate that MEK and ERK inhibitors may be effective for tumors harboring *non-BRAF V600* mutations.[21–23]

Plasma ctDNA analysis may address tissue heterogeneity by capturing somatic alterations that represent multiple metastatic sites; therefore, it may be more informative than tissue molecular profiling. These alterations may indicate tumor sensitivity to targeted therapies or resistance to treatment. ctDNA analysis is associated with a shorter turnaround time compared with tumor tissue molecular analysis and eliminates the risks associated with invasive biopsies. Identification of tumor genomic alterations using ctDNA analysis also may help select patients for enrollment in clinical trials.

The use of different panels and genomic assays in our study may contribute to the differences in molecular alterations found in tumor biopsy *versus* ctDNA. Other factors may include the methodology used for genomic assays, tumor heterogeneity, and tumor burden at the time of diagnosis and at the time of disease progression. Notably, in patient ID 002, RAF1 amplification detected using ctDNA analysis was not found in tumor analysis (Table 1). The results are consistent with the concept that ctDNA analysis may be more representative of the overall tumor molecular profiling, regardless of the location of the tumor.

The practice of ordering ctDNA analysis when response to ongoing therapy starts to wane may allow for optimized treatment selection without delay at the time of disease progression. Patients with advanced, metastatic cancer typically have a higher total systemic tumor burden compared with that of patients with early stage disease and, therefore, higher ctDNA levels.[24] For instance, in CRC, the ctDNA levels decreased after tumor resection and generally increased with the presentation of new lesions evidenced by imaging scans.[24]

CtDNA analysis and selected targeted treatments have been well established[4,5,25,26] in CRC and NSCLC, and the concordant rates between ctDNA and matched tissue biopsies across other advanced/metastatic solid tumors range from 48% to 100%. The respective published data include patients with breast cancer,[27] castration-resistant prostate cancer,[28] pancreatic cancer,[29] neuroblastoma,[30] and melanoma.[31]

Analysis of ctDNA and tumor tissue samples in patients with metastatic breast cancer using droplet digital polymerase chain reaction analysis reported an overall concordance rate of 74.3% in *ESR1* mutation status,[27] indicating acquired resistance to endocrine therapy.[27] In patients with metastatic, castration-resistant prostate cancer, the concordance between ctDNA and matched metastatic tissue biopsies was 100% in somatic mutations and 88.9% for individual copy number calls of the driver genes androgen receptor (*AR*), *BRCA2*, *ATM*, phosphatase and tensin homolog (*PTEN*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta (*PIK3CB*), phosphoinositide-3-kinase regulatory subunit 1 (*PIK3R1*), *TP53*, and RB transcriptional corepressor 1 (*RB1*).[28] In patients with advanced pancreatic ductal adenocarcinoma, the concordance rates between ctDNA and tumor tissue DNA alterations were 61% for TP53 and 52% for *KRAS* mutations.[29] For *KRAS* alterations, concordance between ctDNA and tumor tissue DNA was significantly higher for metastatic than for primary tumors.[29]

One 83-year-old male patient with advanced pancreatic ductal adenocarcinoma (PDAC) treated with trametinib based on evidence of MEK pathway (*GNAS*, *KRAS*, and *NF1*) mutations, remained on treatment for 6+ months; although he was unable to undergo CT due to renal insufficiency, the levels of total ctDNA and CA19-9 significantly decreased.[29]

In patients with neuroblastoma, the concordance rate between ctDNA and tumor tissue for one of three anaplastic lymphoma kinase (*ALK*) mutational hotspots, the *ALK F1174L* mutation, was 100%.[30] In patients with metastatic melanoma, the concordance rate between ctDNA from plasma compared with tumor tissue ranged from 75% to 100% (average, 89%).[31]

Serial monitoring of ctDNA can be used to assess clonal evolution, predict progressive disease, and identify resistant clones. Other investigators have reported on ctDNA analysis in diverse solid tumors including advanced breast, ovarian, lung, and colorectal cancers.[32–35] CtDNA clonal mutations were assessed to monitor tumor burden and disease progression in patients with advanced gastric cancer.[36] For instance, serial monitoring of ctDNA samples from patients with gastric cancer and evaluation of the molecular tumor burden

index (mTBI) identified progressive disease a mean of 18 weeks before it was seen on radiographic results. In patients who received anti-HER2 therapy, ctDNA analysis identified 32 expanding mutations potentially related to trastuzumab resistance. In patients who received chemotherapy, prediction of progressive disease using mTBI was validated with 94% sensitivity. Higher mTBI (⩾1%) in pre-treatment ctDNA was associated with shorter PFS.[36]

Microsatellite instability (MSI) and high tumor mutation burden (TMB-High) are promising pan-tumor biomarkers for the selection of patients to receive checkpoint blockade immunotherapy. Studies conducted by other investigators demonstrated the feasibility of using ctDNA analysis to assess MSI and TMB-high status.[37,38] However, sensitive detection of MSI using ctDNA is still in early phases of development owing to technical and bioinformatics challenges including efficient molecular capture, sequencing, mapping, variant calling, error correction at microsatellite loci, the highly repetitive genomic context and low tumor fraction in circulation, high level of normal cell contamination, and technical noise due to polymerase slippage. Other factors affecting the accuracy of MSI detection using ctDNA and associated with discrepancies between studies include the number and type of microsatellite markers used, tumor cell fraction, and intratumor and intertumor heterogeneity.[39] In patients with gastric cancer, high MSI status, as assessed by ctDNA analysis, was associated with favorable clinical response to immunotherapy.[38] MSI detection using ctDNA sequencing demonstrated high specificity, precision, and sensitivity up to a limit of 0.1% tumor content. CtDNA analysis detected 87% of tissue with high MSI and 99.5% of microsatellite stable tissue, with an overall accuracy of 98.4% and a 95% positive predictive value. Objective responses were noted in 63% of these patients.[38] Ongoing studies are investigating the role of ctDNA in assessment of MSI and TMB-high status as predictive biomarkers for the selection of checkpoint inhibitors.

Encouraging results have been reported using ctDNA analysis to select targeted therapy across tumor types. The TARGET (Tumour chARacterisation to Guide Experimental Targeted therapy) study, which was initiated in 2015, matches patients with diverse advanced tumors to early-phase clinical trials based on the tumor molecular abnormalities identified by ctDNA analysis.[40] In 100 patients (Part A) with advanced colorectal cancer, breast cancer, NSCLC, small-cell lung carcinoma, sarcoma, prostate cancer, cholangiocarcinoma, small bowel cancer, melanoma, adrenal cancer, solitary fibrous tumor, cancer of unknown primary, or other cancers, the concordance rate between mutations identified in ctDNA and in the tumor tissue was 70%. Genomic profiling of ctDNA using NGS identified actionable mutations in 41 patients, of whom 11 patients received a matched therapy and 17 patients received a non-matched therapy.[40] Four of the 11 patients treated with targeted therapy had a PR lasting for 8, 12, 18, and 20 months, respectively; and four of the 17 patients treated with non-matched therapy had SD (no objective response was noted). The study is accruing patients in Part B with a planned enrollment of 450 patients over 3 years to compare clinical outcomes between matched and non-matched therapies.[40] The TARGET study indicates the feasibility of using ctDNA analysis to guide targeted therapy in early-phase clinical trials. Notably, in August 2020, the FDA approved Guardant360 CDx, the first liquid biopsy companion diagnostic approved to provide information on multiple solid tumor biomarkers.[41] In October and November 2020, the FDA also approved FoundationOne Liquid CDx test (Foundation Medicine, Inc.) as a companion diagnostic device for multiple additional biomarkers detected in cell-free DNA isolated from plasma specimens.[42]

Clinical trials that allow ctDNA analysis for selection of targeted therapy are in development, including the TAPUR (Targeted Agent and Profiling Utilization Registry) study, a non-randomized, open-label, multi-basket, pragmatic phase II precision oncology trial that integrates tumor genomic profiling using tumor biopsy and/or liquid biopsy to match patients with diverse advanced cancers to targeted anticancer agents outside of their FDA-approved indications (NCT02693535).[43] The NEXT-2 [Next Generation pErsonalized tX (Therapy) with plasma DNA] trial is another ongoing prospective study being conducted in Korea that evaluates the role of ctDNA in refractory solid tumors (NCT02140463).[44] The investigators reported that 30 (15.5%) of 195 patients who underwent comprehensive ctDNA genomic profiling were enrolled on clinical trials with matched therapy and noted objective responses in six of nine patients with gastric cancer, 13 of 15 patients with NSCLC, one of two patients with melanoma,

and zero of one patient in the other tumor types category.[44]

Limitations to ctDNA analysis are associated with discordance between ctDNA and tumor tissue genomic analysis due to biologic and technical differences (sensitivity and specificity), metastatic sites, histology, time of treatment administration prior to blood/plasma collection, low tumor burden, and absence of a primary tumor.[25,45–47] CtDNA is secreted by tumor cells, phagocyte-engulfed tumor cells, and necrotic or apoptotic tumor cells, and therefore reflects tumor from all sites of disease.[3] In addition, genomic analysis using only ctDNA may exclude other biomarkers. Analysis of circulating RNAs, circulating tumor cells, and exosomes may overcome this challenge.[45]

In conclusion, ctDNA and tumor profiling for treatment optimization for patients with advanced solid tumors requires greater precision. Although the role of ctDNA in guiding targeted treatments is well established in NSCLC and in CRC,[4,5] the assessment of ctDNA and its clinical significance in other tumor types remains to be validated, despite some encouraging results. Prospective clinical trials involving ctDNA analysis and targeted agents are ongoing and/or awaiting results. ctDNA analysis at the time of diagnosis, or early evidence of progressive disease during ongoing treatment, will yield significant insights into the evolution of the patient's tumor biology, accelerate drug development, and contribute to the implementation of precision medicine to improve clinical outcomes.

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Authors' contributions

MFN and HHC contributed to literature search, data acquisition, data analysis, data interpretation and writing the manuscript. DV contributed to data acquisition, data analysis, data interpretation, and review of the manuscript. AMT (corresponding author) conceptualized the manuscript, contributed to patient enrollment, patient treatment and assessment, data analysis, data interpretation and writing the manuscript. All authors read and approved the final manuscript.

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Conflict of interest statement

Apostolia M. Tsimberidou: Clinical Trial Research Funding/Grant Support: IMMATICS, Parker Institute for Cancer Immunotherapy, Tempus, OBI Pharma, EMD Serono, Baxalta, ONYX, Bayer, Boston Biomedical, Placon Therapeutics, Karus Therapeutics, Tvardi, CPRIT; Travel: ASCO, Japanese Society of Medical Oncology.

Consulting or Advisory Role: Covance, Genentech, Tempus

David J. Vining: Other ownership interests: VisionSR, Majority owner and CEO, multimedia structured reporting for use in advancing medical research; Bracco Diagnostics, Inventor, virtual colonoscopy-related products for colorectal cancer imaging.

The remaining authors have no competing interests.

Ethics approval and consent to participate

The protocol was approved by the FDA and the Institutional Review Board at The University of Texas MD Anderson Cancer Center. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. All the study participants provided written informed consent before enrollment on the individual clinical trials in which they participated.

Consent for publication

Patient consent for publication is not required. Patients consented to participate in the study.

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Availability of data and material

Data are available upon reasonable request. The datasets used and/or analyzed during the current study are available from the corresponding author

on reasonable request and approval from study sponsor and institution according to available guidelines at time of request.

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