Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence


Abstract

Purpose: Up to 30% of patients with breast cancer relapse after primary treatment. There are no sensitive and reliable tests to monitor these patients and detect distant metastases before overt recurrence. Here, we demonstrate the use of personalized circulating tumor DNA (ctDNA) profiling for detection of recurrence in breast cancer.

Experimental Design: Forty-nine primary patients with breast cancer were recruited following surgery and adjuvant therapy. Plasma samples (n = 208) were collected every 6 months for up to 4 years. Personalized assays targeting 16 variants selected from primary tumor whole-exome data were tested in serial plasma for the presence of ctDNA by ultradeep sequencing (average >100,000X).

Results: Plasma ctDNA was detected ahead of clinical or radiologic relapse in 16 of the 18 relapsed patients (sensitivity of 89%); metastatic relapse was predicted with a lead time of up to 2 years (median, 8.9 months; range, 0.5–24.0 months). None of the 31 nonrelapsing patients were ctDNA-positive at any time point across 156 plasma samples (specificity of 100%). Of the two relapsed patients who were not detected in the study, the first had only a local recurrence, whereas the second patient had bone recurrence and had completed chemotherapy just 13 days prior to blood sampling.

Conclusions: This study demonstrates that patient-specific ctDNA analysis can be a sensitive and specific approach for disease surveillance for patients with breast cancer. More importantly, earlier detection of up to 2 years provides a possible window for therapeutic intervention.

Introduction

Breast cancer is one of the most commonly diagnosed cancers worldwide and the second leading cause of cancer-related deaths in women (1). The current standard of care for women with primary (nonmetastatic) breast cancer is surgery, often followed with adjuvant therapy to eliminate microscopic minimal residual disease (2, 3). Unfortunately, up to 30% of women who present with no evidence of disease following treatment with curative intent eventually relapse and die of metastatic breast cancer (4). Current guidelines for disease surveillance recommend regular imaging and physical examinations, and additional testing upon presentation of symptoms (5–7). However, imaging tests such as mammography, MRI, and PET/CT often suffer from significant false-negative and false-positive results (8–10). Biochemical methodologies such as serum levels of cancer antigen 15-3 (CA 15-3) have limited sensitivity and accuracy in detecting micrometastases (11–13) and have not been widely incorporated into clinical guidelines (14). Therefore, there is a compelling need to develop more sensitive technologies capable of detecting preclinical metastases and identifying patients with disease recurrence earlier.

Circulating tumor DNA (ctDNA) detectable in blood plasma has been shown to reflect the mutational signatures of the primary tumor and is emerging as a potential noninvasive biomarker for monitoring tumor progression across different cancer types. In breast cancer, previous studies have shown the potential utility of ctDNA to detect preclinical metastases and predict relapse following surgery and/or adjuvant therapy in patients with specific hotspot mutations (15, 16), chromosomal rearrangement...
Translational Relevance

Currently, there are no sensitive and specific clinical tests available to follow patients with breast cancer after primary treatment. We developed a patient-specific method to analyze circulating tumor DNA (ctDNA) that allows for monitoring of these patients regardless of molecular genotype. In this study, we analyzed 208 blood samples from 49 patients monitored longitudinally for up to 4 years after completion of adjuvant chemotherapy to determine whether personalized ctDNA assays can allow for more effective monitoring than current clinical tests such as CA 15-3. Remarkably, for the patients that recurred, our test detected molecular relapse up to 2 years ahead of clinical relapse (median, 8.9 months) with 89% sensitivity and 100% specificity. This may provide a critical window of opportunity for additional therapeutic intervention.

Materials and Methods

Patients and samples
EBLIS is a multicenter, prospective cohort study, funded by Cancer Research UK and the National Institute for Health Research. Blood sample collection was conducted in accordance with the Declaration of Helsinki. All the patients provided written-informed consent prior to entry into the trial. The trial protocol was approved by the Riverside Research Ethics Committee-informed consent prior to entry into the trial. The trial with the Declaration of Helsinki. All the patients provided written-informed consent prior to entry into the trial. The trial protocol was approved by the Riverside Research Ethics Committee.

We previously reported a personalized ctDNA profiling approach for early detection of lung cancer recurrence (21, 22). In this study, we applied an improved version of the assay for tracking breast cancer recurrence following surgery and adjuvant therapy. The primary objective was to determine the "lead interval" between detection of ctDNA in plasma and clinical detection of overt metastatic disease. A secondary objective was to determine whether ctDNA in plasma can detect recurrent disease earlier than current clinical methods, such as imaging or biochemical markers.

At the midpoint of the study (2 years), 50% of the predicted events (18 relapses, comprising one local recurrence and 17 patients with distant relapse) were observed. Following an interim analysis, the EBLIS Trial Management group recommended an interim laboratory assessment of serial plasma in the first 49 patients. The 49 patients included all 18 relapses and comprised an approximately 1:2 ratio of relapsed to nonrelapsed patients. Relapsed and nonrelapsed patients were sequential patients recruited over the same time frame. We performed whole-exome analysis of the archived primary tumor for each patient for custom assay design. Serial plasma samples were analyzed in a blinded manner. Detailed description of blood sample collection, plasma cell–free DNA isolation and quantitation, and whole-exome sequencing (WES) is provided in the Supplementary Methods.

Custom assay design
Patient-specific somatic variants were identified by comparison of paired primary tumor and matched white blood cell DNA WES profiles for all 49 patients. Clonality of variants was inferred based on the estimated proportion of cancer cells harboring the variant as described in McGranahan and colleagues (23). Note that clonality inference from samples with low tumor cell fraction was limited due to a fairly flat distribution of variant allele frequency (VAF). The observed VAF in tumor DNA and sequence context of variants were used to prioritize somatic SNVs (single nucleotide variants) and short INDELs identified for each tumor. The bespoke amplicon design pipeline was used to generate PCR primer pairs for the given set of variants. For each patient, 16 highly ranked compatible amplicons were selected for the custom patient-specific panel. The PCR primers were ordered from Integrated DNA Technologies. Details on steps of the workflow are provided in the Supplementary Methods.

Statistical analyses

This study was designed to measure the average time between detection of ctDNA and overt metastases. The target sample size for the EBLIS prospective cohort study was 194, using a 20% patient dropout rate and assuming that 20% of patients will relapse within 2 years. With such a sample size, the mean lead time interval was measured with a precision that extends to ±3 months, with a 95% probability.

All data are presented descriptively as means, medians, or proportions. Relapse-free survival from the day of surgery was determined using the Kaplan–Meier method. Cox proportional hazard regression was used to model the time to disease relapse. Sensitivity is defined as number of patients with preclinical metastasis detected by ctDNA over the total number of patients with clinical relapse. Specificity is defined as the number of patients that were ctDNA negative during the clinical follow-up period over the total number of patients who did not relapse. All statistical analyses were performed using Stata, release 12.0 (Stata Corp.), and survival plots were generated using R version 3.5.1 (‘survminer’ package version 0.4.2.99; refs. 24, 25).

Results

Here, we report the analysis of the first 49 patients that entered into the EBLIS study (Fig. 1). The cohort comprised three main subtypes: 34 patients with estrogen receptor–positive, termed herein hormone receptor–positive (HR⁺) and human epidermal growth factor receptor 2–negative (HER2⁻) tumors, eight patients
with HR+ and HER2+ tumors, and seven patients with triple-negative breast cancer (TNBC; Table 1; Supplementary Table S1). Ten patients received neoadjuvant chemotherapy (NACT) prior to breast cancer resection, whereas 39 received no systemic therapy prior to surgery. All except seven patients received adjuvant or NACT with an anthracycline/taxane regimen (Table 1; Supplementary Table S2). Thirty-eight patients were receiving adjuvant endocrine therapy throughout the time of blood sampling (Supplementary Table S2). Although repeat scans were not required prior to study entry, all except three patients had radiologic imaging performed at diagnosis or at the time of entry into the study, and all were within normal limits (Supplementary Table S2). At the reporting census date (June 30, 2018), 18 of the 49 patients had relapsed and 31 remained disease-free (Fig. 2A).

Clinical validity of ctDNA detection and lead interval

To assess the presence of ctDNA for each patient, assays specific to tumor signatures were designed targeting 16 SNVs and indels from the somatic mutation profile of the primary tumor (Supplementary Fig. S2; Supplementary Table S3A and S3B). For each patient, we then tested their personalized 16-plex assays across each of their respective plasma samples (range, 1–8 serial samples per patient). Plasma ctDNA was detected in 89% (16 of 18) of the patients who relapsed (Fig. 2). By subtype, the sensitivity of the assay was 82%, 100%, and 100% in HR+/HER2−, HR+/HER2+, and TNBC, respectively (Fig. 2B). Two relapsed patients were not detected by ctDNA analysis. The first patient

Table 1. Characteristics of the patients at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N = 49</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at diagnosis (range), year</td>
<td>57 (38–81)</td>
</tr>
<tr>
<td>Estrogen receptor status, N (%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>37 (76)</td>
</tr>
<tr>
<td>Negative</td>
<td>12 (24)</td>
</tr>
<tr>
<td>Progesterone receptor status, N (%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>32 (65)</td>
</tr>
<tr>
<td>Negative</td>
<td>17 (35)</td>
</tr>
<tr>
<td>HER2 status, N (%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Negative</td>
<td>41 (84)</td>
</tr>
<tr>
<td>Tumor stage, N (%)</td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>1 (2)</td>
</tr>
<tr>
<td>IIA</td>
<td>1 (2)</td>
</tr>
<tr>
<td>IIB</td>
<td>14 (29)</td>
</tr>
<tr>
<td>IIIA</td>
<td>17 (35)</td>
</tr>
<tr>
<td>IIIB</td>
<td>2 (4)</td>
</tr>
<tr>
<td>IIBC</td>
<td>14 (28)</td>
</tr>
<tr>
<td>Size of tumor, mean (range), cm</td>
<td>3.8 (0.9–10)</td>
</tr>
<tr>
<td>Treatment, N (%)</td>
<td></td>
</tr>
<tr>
<td>NACT</td>
<td>10 (20)</td>
</tr>
<tr>
<td>ACT</td>
<td>32 (65)</td>
</tr>
<tr>
<td>None</td>
<td>7 (14)</td>
</tr>
</tbody>
</table>

NOTE: After screening and recruitment, patients were followed up with six monthly blood samples.

Abbreviation: ACT, adjuvant chemotherapy. *HER2 status was determined by IHC and FISH assays. A patient was considered to have HER2-positive cancer if either assay was positive.

Figure 1.

Patient recruitment and collection of clinical samples. For the 49 women with breast cancer monitored in this study, exonic alterations were determined through paired-end sequencing of formalin-fixed, paraffin-embedded tumor tissue specimens and matched normal DNA. Patient-specific panels were designed to include 16 somatic mutations identified from WES data. Serial plasma samples were analyzed with the corresponding custom 16-plex assay panels using the Signatera RUO workflow in a blinded manner. A total of 208 samples were analyzed for ctDNA detection.

www.aacrjournals.org Clin Cancer Res; 25(14) July 15, 2019 4257

Published OnlineFirst April 16, 2019; DOI: 10.1158/1078-0432.CCR-18-3663
Coombes et al.

(E009) had three separate foci of primary breast cancer. Exome analysis confirmed a clonal origin with 69 overlapping SNVs between the three tumors. The patient was also recruited at an earlier time point than other patients, with the baseline blood sample drawn just 13 days after the completion of chemotherapy. The 16 SNVs selected from clonal tumor SNVs were undetected in this plasma sample despite sequencing 64 ng total cfDNA at >80,000X. Just 4 months after the patient was recruited, a bone scan showed bony metastases in the sternum, pelvis, and vertebralae. The other patient (E010) had a small resectable local recurrence in the sternum (Supplementary Table S1). The assay achieved 100% specificity, as ctDNA was not detected in any of the 156 plasma samples collected from the 31 patients who did not relapse (Fig. 2).

All ctDNA-positive patients relapsed within 50 months after surgery, and molecular relapse through ctDNA analysis was detected up to 2 years prior to clinical relapse with a median of 266 days (range, 14–721 days) or 8.9 months (Fig. 2B). When stratified by subtype, the median lead times for HR+/HER2−, HR+/HER2+, and TNBC were 301, 164, and 258 days, respectively.
Personalized ctDNA Detection of Breast Cancer Recurrence

ctDNA and other monitoring tests
Concurrent with the plasma ctDNA analyses, additional monitoring tests were also performed, including CT imaging, liver function tests (LFT), and CA 15-3. All CT imaging that was performed prior to clinical relapse was negative (Supplementary Table S2). Interestingly, for seven patients who had scans within 4 months of their first ctDNA-positive time point, all scans were negative at this point. Similarly, other tests, such as LFTs, were also negative until clinical relapse.

CA 15-3 levels were monitored in 43 of the 49 patients. Of the 18 patients who relapsed, 13 had CA 15-3 measurements; seven had normal CA 15-3 levels, six (30%) had positive CA 15-3 values, but only two patients had progressively rising CA 15-3 levels. Six patients (three relapsed and three nonrelapsed) had an occasional blood sample with slightly elevated CA 15-3, but the levels fluctuated and did not reflect disease progression, whereas all 31 patients who did not relapse were ctDNA negative in 100% of 156 plasma samples. Interestingly, even for the patients that were positive for CA 15-3, ctDNA was detected on average over 200 days ahead of significant CA 15-3 levels (Supplementary Table S2; Fig. 4A, and Supplementary Fig. S4C).

In addition to the presence or absence of ctDNA, the levels of ctDNA can also be used to approximate and track disease burden over time as demonstrated by the ctDNA plots for each patient (Fig. 4; Supplementary Fig. S4). In Fig. 4, we illustrate five patients, representing three cancer subtypes: HR+/HER2− (E017 and E026), HER2+ (E040), and TNBC (E029 and E033). Three patients were initially ctDNA-negative and became positive over time and subsequently had a rise in the ctDNA ahead of clinical relapse (Fig. 4A–C). The other two patients were ctDNA positive at the earliest monitored time point, and levels increased corresponding to an expected increased tumor burden (Fig. 4D and E).

In all cases, ctDNA was detected ahead of clinical relapse (Fig. 4; Supplementary Fig. S4). Overall, disease progression can be monitored by both the VAF and the number of detected variants (Fig. 4F). The median VAF increased from 0.09% in the first positive time point (range, 0.01%–9.22%) to 3.88% (range, 0.05%–64.44%) at the last time point, and the median number of variants detected at the first time point was 5 (range, 2–12) compared with 12 variants (range, 5–15) at the last time point. The low number of variants detected at early time points, each present at very low copy numbers (Supplementary Table S3A), points to the importance of testing multiple mutations present in a patient’s tumor to maximize the probability of early detection.

The individual patient graphs also show the low limit of detection of the assay. For example, patients E017, E029, E033, and E040 had VAFs detected in the range of 0.01% to 0.02% (Fig. 4B–E; Supplementary Fig. S4). The lowest VAF of 0.01% corresponds to the detection of a single mutant molecule in the plasma sample (Supplementary Figs. S5 and S6). The average number of mutant molecules per mL of plasma was measured to be less than two in 13 ctDNA-positive samples (Supplementary Table S3A). A low limit of detection with high specificity was
Figure 4.
Personalized profiling detects rising ctDNA ahead of clinical relapse. A–E, Plasma levels of ctDNA across serial plasma time points for five patients with breast cancer (one per panel). Mean VAFs are denoted by a dark blue circle, and solid lines represent the average VAF profile over time. The lead time is calculated as the time interval between clinical relapse (red triangle) and molecular relapse (blue triangle). CA 15-3 levels are graphed over time (teal circle), and the baseline levels (32 U/mL) are marked in light blue. F, Summary of percent VAF and number of targets detected at molecular and clinical relapse for all ctDNA-positive samples. Data are from 13 relapsed patients, excluding three patients with only one plasma time point.
achieved by requiring two or more of the 16 variants to be measured above the selected confidence threshold (Supplementary Methods), underscored by the fact that all 156 plasma samples from the 31 nonrecurring patients were negative.

**Discussion**

Here, we show noninvasive detection of preclinical metastases and earlier identification of breast cancer recurrence across breast cancer subtypes through personalized ctDNA analysis. The approach uses tumor exome data to design patient-specific 16-plex assays and ultradense sequencing of plasma ctDNA at extremely high depth (average of >100,000 reads per target) to achieve a detection sensitivity down to a single mutant molecule.

In this study, we detected ctDNA in 16 of 17 patients with a lead time of up to 2 years (range, 0.5–24 months) prior to distant metastatic relapse, demonstrating the ability of the assay to predict breast cancer recurrence earlier than imaging, CA 15-3, clinical examination, and liver function tests. Previously, Olssoon and colleagues profiled patient-specific chromosomal rearrangement breakpoint junctions in 20 patients and demonstrated accurate discrimination between patients with and without clinical detection of recurrence (17). In 12 of 14 patients, molecular relapse through ctDNA preceded clinical detection of occult metastasis with an average lead time of 11 months (range, 0–37 months). Similarly, Garcia-Murillas and colleagues followed patient-specific point mutations using digital droplet PCR, and in 12 of 15 patients, ctDNA was detected ahead of metastatic relapse with a median lead time of 7.9 months (range 0.03–13.6 months; ref. 16). Our data therefore provide further demonstration that ctDNA can be detected in most patients with breast cancer several months before clinical relapse and with excellent specificity. This presents a window of opportunity for the introduction of non-cross-resistant therapies to prevent overt metastatic relapse. Importantly, once detected, ctDNA remained positive throughout all subsequent monitoring timepoints. In addition to ctDNA detection, circulating tumor cells (CTC) have also shown prognostic significance for detection of preclinical metastases in patients with breast cancer (26–29). Both ctDNA and CTCs could therefore complement conventional recurrence monitoring tests that have limited specificity and frequently result in considerable anxiety and expensive follow-up testing.

Using an upfront exome profiling of tumor tissue followed by a personalized targeted multiplex plasma sequencing has distinct advantages. First, this strategy enables detection of ctDNA in all patients regardless of molecular subtype, irrespective of known molecular driver genes. In our study, 100% of all eligible patients were monitored. Previous studies have profiled a small number of genes (i.e., 2–14) to determine the personalized variants for tracking; however, only 57% to 78% of women have any trackable mutations using such an approach (16, 19, 20). In our cohort, only 33 of 49 (67%) patients had one or more driver mutations identified in their exome profile, indicating that 16 of 49 patients in this study would not have been able to be monitored using a predetermined gene panel approach based on driver genes.

Second, the outlined approach enables simultaneous interrogation of 16 somatic variants, whereas technologies such as droplet digital PCR or BEAMing are usually designed to monitor either one target or a handful of targets (30–33). Increasing evidence suggests that the evolution of cancer is complex and often results in high levels of inter- and intratumor heterogeneity (34). Therefore, measuring 16 tumor-specific variants provides a more robust representation of the tumor, enabling more accurate tracking of disease burden.

Third, sequencing a highly targeted panel at a depth of read (>100,000X) allows for a very low limit of detection at <0.01%. Larger gene panels used for this purpose rarely reach this depth or limit of detection due to the high cost. However, this low level of detection is critical for assessing preclinical metastases as we have shown that variants can be within the range of 0.01% to 0.02% VAF. By using a large volume of plasma (up to 5 mL), we were able to achieve a high assay sensitivity and detect down to 1 ctDNA molecule in 5-mL plasma with a lead time of up to 2 years prior to distant metastatic relapse.

As with all technologies, there are limitations to the outlined approach. The test is not suitable for detecting a second primary breast cancer unless it recurred from the original tumor; this is exemplified by patient E010, where a second contralateral primary cancer was detected (Supplementary Table S1). Second, relying on ctDNA requires that sufficient molecules are present in the plasma at the time of collection, which may not be the case in patients with smaller and less aggressive breast cancers. This is exemplified by patient E010 who relapsed with local resectable disease but was ctDNA negative.

There are some important implications for the future of breast cancer treatment and drug evaluation from our study. In the past, systemic treatment with targeted or cytotoxic therapies has been shown to be curative only when administered in the adjuvant setting; treatment of overt metastatic disease is rarely, if ever, curative (35). The approach described here offers an alternative—that of attempting to salvage patients who are ctDNA-positive with second-line therapies. In conclusion, we present a sensitive and specific clinical test that can be used to identify preclinical metastases and follow all patients with breast cancer after therapy irrespective of molecular subtype. It outperforms conventional means of monitoring and shows promise as a tool for guiding future precision medicine. Future studies will address the issue of the effects of therapy on ctDNA levels in patients with breast cancer.

**Disclosure of Potential Conflicts of Interest**

A. Armstrong holds ownership interest (including patents) in Astra Zeneca. R.C. Coombers reports speaker fees from Pfizer and has shares in Carrick Ltd. L. Kenny reports receiving speakers bureau honoraria from Pfizer, and is a consultant/advisory board member for Celgene, Novartis, and Lilly. J. Stebbing is the editor-in-Chief of Oncogene, sat on SABs for Cellonin, Vor Biopharma and Benevolent AI, and Chairs the Board of Directors for BB Biotech Healthcare Trust and Xerion Healthcare. H. Sethi, Z.J. Ansa, H.T. Wu, S. Dashner, A.S. Tin, M. Balcigu, S.V. Shcgegora, A. Olson, D. Hafez, A. Aleshin, R. Salar, C.H. Jimmy Lin, P. Natarajan, and B.G. Zimmermann hold ownership interest (including patents) in Natera, Inc. R. Swerenten holds ownership interest (including patents) in Natera, Inc. and Coladex. P. Billings holds ownership interest (including patents) in Natera, Inc. and is a consultant/advisory board member for Clear Gene. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

**Conception and design:** R.C. Coombers, R. Salar, A. Armstrong, S. Cletor, J. Stebbing, H. Sethi, R. Swervent, M.C. Louie, C.-H.J. Lin, B.G. Zimmermann, J.A. Shaw


www.aacrjournals.org Clin Cancer Res; 25(14) July 15, 2019 4261

Downloaded from clinccancerres.aacrjournals.org on June 28, 2021. © 2019 American Association for Cancer Research.


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.C. Coombes, K. Page, J. Stebbing, H. Sethi, A. Boydell, R. Swenerton, D. Fernandez-Garcia, K. Goddard, L. Kenny, J.A. Shaw


Other [led the translational aspects of the study]: J.A. Shaw

Acknowledgments

We thank the Cancer Research UK Imperial Centre, the Leicester and Imperial Experimental Cancer Medicine Centers (ECMC), the Imperial College Tissue Bank, NIHR BRC and the clinical teams at Charing Cross Hospital London, the Christie Hospital Manchester, and the Leicester Royal Infirmary for supporting patient recruitment and sample collection. We thank Dr. David Moore and Dr. Gerald Saldanha for reviewing tissue histology and hematoxylin and eosin slides. This research used the ALICE and SPECTRE High Performance Computing Facilities at the University of Leicester. We thank Samantha Navarro, John So, and Dr. Agust Egilsson for their technical support and Shrutti Goel for her assistance with the figures. We are grateful to Marc Dantone, Solomon Moshkovich, and Dr. Matthew Rabinowitz for their insightful discussions and critical reading of the article.

This study was supported by program grant funding from Cancer Research UK to J.A. Shaw and R.C. Coombes (C14315/A13462 and C14315/A23464). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 8, 2018; revised February 6, 2019; accepted April 11, 2019, published first April 16, 2019.

References

Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence

Raoul Charles Coombes, Karen Page, Raheleh Salari, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-18-3663

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2019/04/16/1078-0432.CCR-18-3663.DC1

Cited articles
This article cites 33 articles, 13 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/25/14/4255.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/25/14/4255.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/25/14/4255.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.