OXFORD

J Natl Cancer Inst Monogr (2015) 2015(51): lgv006

doi:10.1093/jncimonographs/lgv006

Circulating Biomarkers for Prediction of Treatment Response

Vera Cappelletti, Valentina Appierto, Paola Tiberio, Emanuela Fina, Maurizio Callari, Maria Grazia Daidone

Affiliation of authors: Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy (VC, VA, PT, EF, MC, MGD).

Correspondence to: Maria Grazia Daidone, MSc, PhD, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Amadeo 42, Milan 20133, Italy (e-mail: mariagrazia.daidone@istitutotumori.mi.it).

Abstract

For cancer management, predicting and monitoring response to treatment and disease progression longitudinally is crucial due to changes in tumor biology and therapy responsiveness over time. However, solid tumors are usually sampled only at time of initial diagnosis, as obtaining tissue biopsies is an invasive procedures with associated risks. Thus, there is a pressing need for approaches able to serially detect function-related reliable biomarkers reflecting treatment response and/or disease progression through easy noninvasive procedures, amenable for longitudinal analysis of tumor molecular features. Recent evidences indicate that blood and other body fluids could replace invasive surgical biopsies and represent a "liquid biopsy" containing cells and nucleic acids released by primary and metastatic lesions, reflecting their biological features and allowing identification of clinically useful biomarkers and treatment-induced cancer adaption processes. The development of new and highly sensitive technologies that allow to detect and characterize circulating tumor cells, to identify cell-free nucleic acids (circulating tumor-associated microRNAs and cancer-specific mutations in circulating DNA) and to measure their eventual dynamic changes represents therefore a major achievement for disease monitoring. However, notwithstanding preliminary findings support the prognostic and/or predictive role of this new generation of biomarkers, there are a number of technical and biological caveats that still require additional studies to demonstrate and validate their clinical utility. A unique opportunity to rapidly assess the contribution of circulating tumor cells and cell-free nucleic acids to patient management and to personalized medicine could derive by their combined consideration in the neoadjuvant setting.

The identification of biomarkers that can be repeatedly assessed through noninvasive approaches acting as reliable read-outs of functional and molecular features of the primary tumor and of its metastatic lesions still represents an issue of utmost importance in clinical practice as it would allow to collect real-time information on disease progression and treatment response. In such a context, blood-based biomarkers as those derived from circulating tumor-released cells and free nucleic acids represent ideal noninvasive tools which already provided promising results (1).

Circulating Tumor Cells

Detection of circulating tumor cells (CTCs) proved to play an important role in predicting disease-free and overall survival and to provide an early assessment of treatment response, mainly in advanced breast cancer (BC) (2). So far CTCs have generally been considered as a tumor burden marker, as shown by the focus on CTC enumeration rather than on their biological characterization. However, by combining detection approaches with molecular characterizations (by in situ hybridization [ISH], array comparative genomic hybridization [aCGH], gene and microRNA [miRNA] expression profiling, DNA methylation, etc.) heterogeneity and phenotypic changes in CTCs can be identified (Table 1) and exploited to investigate the biology of tumor progression and to better monitor treatment efficacy (3,4).

Evidence of advantages offered by such characterization derives from HER2 determination, where HER2 status discrepancies between primary tumors and CTCs were reported

Table 1. Circulating biomarkers to monitor disease progression and response to therapy*

Circulating biomarker	Strength	Weakness
CTC enumeration	Possibility to recover cells for molecular analyse Predictive value already demonstrated Clinical validation ongoing	Low frequency and not easy detection Lack of concordance among the different approaches Not satisfactory specificity, sensitivity, and inter-lab reproducibility Possible underestimation of cell subpopulations Not suitable for retrospective studies
CTC molecular profile	Tissue heterogeneity-independence Possibility to analyze distinct cell subpopulations Information on tumor biology and heterogeneity Suitable for investigating target/actionable genes	Feasibility dependent on capture efficiency Contamination by blood cells Possible missing of clinically relevant subpopulations Need of prior amplification approaches for omic analyses Few correlative studies with clinical outcome
microRNAs	Feasible on small sample amount Tissue heterogeneity-independence Accounting for microenvironment interactions Rapid and low cost (by RT-PCR) Suitable for retrospective studies	Individual variability (eg, effect of diet, physical activity, etc.) Blood/plasma/serum issue Normalization problems Blood cell confounding effects Few correlative studies with clinical outcome Discordant results among different studies
Mutations in cell-free DNA	Feasible on small sample amount Tissue heterogeneity-independence High specificity High dynamic range Identification of clonal resistance Identification of actionable targets Suitable for retrospective studies	Possibly not suitable for early stage tumors Standard "mutation panel" unlikely applicable to all breast cancer patients Timing of blood collection (eg, apoptosis-confounding effect, etc.) Need of high bioinformatics expertise Few correlative studies with clinical outcome

*CTC = circulating tumor cell; RT-PCR = reverse transcription-polymerase chain reaction.

along with a benefit from trastuzumab treatment in women with HER2-negative primary (5) and metastatic tumors but with HER2-positive CTCs (6). To comparatively evaluate the clinical relevance of HER-2 detected on CTCs the prospective multicenter DETECT III study has been recently activated to challenge the additional benefit of lapatinib to standard treatment in women with metastatic HER2-negative tumors but with HER2-positive CTCs.

Although directly involved in the metastatic cascade not all CTC subpopulations are likely to have the same metastatic potential. Recently, in patients with metastatic BC serially monitored through diseases progression, the occurrence of changes in CTC epithelial and mesenchymal composition—a crucial feature for tumor cell dissemination (7)—has been demonstrated and associated to treatment resistance (3). Such a study represents a proof-of-concept that dynamic changes of specific CTC subpopulations can trace the pace in cancer adaption during treatment and might act to identify new treatment targets and resistance markers, possibly anticipating the clinical evidence of progression.

Case reports on molecular characterization of CTCs, although still in their infancy and challenging due to low CTC numbers and potentially high contamination by leukocytes (Table 1), are raising the important question of CTC definition. The operative CTC definition as a CD45-negative, epithelial cell adhesion molecule (EpCAM)-positive, and cytokeratin (CK)-positive cell (8) becomes inadequate when CTCs are considered not only as markers of tumor burden, but as direct triggers of cancer adaption. Addition of epithelial-mesenchymal transition (EMT) or stemness markers to CTC positivity criteria seems to improve their biological relevance (9) but the clinical role of such subpopulations still needs a confirmation. In this regard capture strategies should also be revised to enrich the CTC fraction not only based on epithelial-specific or tumor-specific markers: only in this way CTC molecular characterization could become a powerful tool also in early BC.

Circulating miRNAs

The recent discovery that miRNAs—highly conserved small RNAs acting in gene regulatory networks—have altered expression in different tumor types, generated a great deal of interest as potentially robust biomarkers for cancer diagnosis, and for predicting prognosis and treatment efficacy. In BC, miRNAs proved to be deregulated and many data are currently available on their expression, biological role, and association with patient outcome, in terms of prognosis and treatment response. In addition, recent *in vitro* and *in vivo* studies have demonstrated the involvement of specific miRNAs in resistance to taxol [miR-21, Mei et al. (10)], to epirubicin with or without docetaxel [miR-200c, Chen et al. (11)] and, in HER-2 positive BC, to trastuzumab [miR-21, Gong et al. (12)] and to tyrosine kinase inhibitors lapatinib and gefitinib [miR-205, Iorio et al. (13)].

More recently, miRNAs have been shown to be detectable, quantifiable, and highly stable also in cell-free components of blood and body fluids, thus potentially fulfilling the characteristics of ideal candidates as cancer biomarkers. In this context, recent correlative studies within neoadjuvant chemotherapy trials identified many circulating miRNAs as associated with response to treatment and drug resistance. Specifically, miR-221 plasma level was identified as an independent predictive factor for taxane- and anthracycline-based therapies (14), whereas high expression levels of serum miR-125b were correlated to

resistance to fluorouracil, epirubicin, and cyclophosphamide treatment (15). In HER2-positive BC, at baseline plasma miR-210 levels were directly associated to resistance to trastuzumab while they decreased after surgery (16). Moreover, through de novo sequencing low levels of miR-375 and high baseline levels of miR-122 were found in serum of patients successively not responding to trastuzumab-based treatment (17).

The above evidences indicate the potential of circulating miRNAs in predicting and possibly monitoring treatment response. However, before translation in clinical practice, these findings require further steps of validation due to poor overlap of results among different studies and to potential technical bias such as individual variability, hemolysis confounding effect, and absence of consensus for data normalization (Table 1) (18). In fact specific miRNAs suitable as reference for data normalization in circulating miRNA studies have not been found and this could, at least in part, explain the widespread inconsistency among different studies. In addition, other critical issues are represented by nonuniform sample choice (blood, serum, or plasma), handling and processing as well as by blood cell contamination in sample preparation. All these variables could generate artifacts and studies are needed to assess whether the introduction of standard operating procedures in circulating miRNA studies will generate concordant results.

Circulating DNA

The presence of extracellular DNA in human blood was first described in 1948 (19) and it is now known that cancer patients have higher circulating DNA levels than healthy individuals. However, due to the lack of reference DNA concentrations in healthy individuals, the simple quantification of circulating DNA cannot be considered a tumor-specific marker (20). Independent studies have recently demonstrated that circulating mutant tumor DNA (ct-DNA), containing representation of the entire tumor genome, can be specifically detected in the blood of cancer patients, although it represents a small fraction of total circulating DNA. The ability to precisely measure the level of ct-DNA may have tremendous innovative potential in cancer management allowing developing minimally invasive and sensitive tumor biomarkers for treatment response and disease progression monitoring (1). This concept, together with the development of massively parallel sequencing technologies, has generated a great deal of enthusiasm in the scientific communities and a number of studies have probed the potential of approaches based on ct-DNA analysis to personalize treatment of BC patients (21). Importantly, contrary to other cancer types characterized by frequent tumor-specific aberrations (eg, TP53 in ovarian cancer), BC does not display commonly mutated single *loci*. Hence, a considerable sequencing effort of primary tumors is required for the identification of somatic alterations in individual patients to be monitored in plasma (Table 1).

By tracking the dynamics of 10 tumor-specific concomitant mutations (identified by whole-genome sequencing of tumor tissue) in serial plasma samples of a metastatic BC patient undergoing chemotherapy, ct-DNA proved to mirror the clinical course of the disease (22). An important proof-of-concept showing that ct-DNA could represent a specific and sensitive biomarker of tumor burden in metastatic BC comes from a recent study showing that somatic genomic alterations identified in the primary tumor could also be detected and monitored in the majority of plasma samples collected during the course of endocrine or cytotoxic therapy (23). Eventual changes of ct-DNA frequency were closely associated with treatment response, showing a higher dynamic range and correlation with tumor burden (evaluated by radiographic imaging) than CA15-3 or CTC enumeration. Moreover, whole-exome sequencing of ct-DNA in serial plasma samples of metastatic patients allowed tracking genomic evolution associated with acquired drug resistance (24). Intriguingly, one BC patient, following paclitaxel treatment, displayed an activating mutation in PIK3CA, previously showed to confer increased resistance to paclitaxel in mammary epithelial cells (25).

The possibility to monitor ct-DNA during anticancer therapy in BC has been so far probed in a limited number of patients with advanced cancers and high systemic tumor burden (22–24). Hopefully, improvements in sequencing and related methodologies may enable to translate similar concepts to early BC in which the study of ct-DNA dynamics may be a specific noninvasive tool to predict and monitor treatment efficacy.

Conclusions

The opportunity to identify reliable circulating biomarkers mirroring tumor behavior represents a great paradigm shift in personalized clinical care. However, notwithstanding initial encouraging results and the rapid development of novel and sensitive technologies, weaknesses of this new generation of biomarkers still outperform strengths (Table 1), and only for CTC enumeration studies are available with a high level of evidence and prospective clinical validation is ongoing. Development and planning of neoadjuvant studies in which CTCs, ct-DNA, and miRNAs are evaluated, singly and in association, to monitor disease progression and treatment response could represent a unique opportunity to rapidly assess whether such new biomarkers will be able to affect the chances of cure in selected patients or—conversely—represent unfulfilled promises.

Funding

This work was supported by grants from the Associazione Italiana per la Ricerca sul Cancro (AIRC, the Italian Association for Cancer Research, grant 5/1000 No. 12162 and grant No. 10611), by a grant from Regione Lombardia-Call ricerca indipendente 2010 (grant No. 11902), and by EurocanPlatform (European Community, 7th Framework Programme, grant No. 260791). This work was in part supported also by funds obtained through an Italian law that allows taxpayers to allocate the “5x1000” share of their payments to support a research institution of their choice.

References

1. De Mattos-Arruda L, Cortes J, Santarpia L, et al. Circulating tumour cells and cell-free DNA as tools for managing breast cancer. *Nat Rev Clin Oncol*. 2013;10(7):377–389.
2. Pantel K, Riethdorf S. Pathology: are circulating tumor cells predictive of overall survival? *Nat Rev Clin Oncol*. 2009;6(4):190–191.
3. Yu M, Bardia A, Wittner BS, et al. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science*. 2013;339(6119):580–584.
4. Magbanua MJ, Sosa EV, Roy R, et al. Genomic profiling of isolated circulating tumor cells from metastatic breast cancer patients. *Cancer Res*. 2013;73(1):30–40.
5. Paik S, Kim C, Wolmark N. HER2 status and benefit from adjuvant trastuzumab in breast cancer. *N Engl J Med*. 2008;358(13):1409–1411.
6. Fehm T, Becker S, Duerr-Stoerzer S, et al. Determination of HER2 status using both serum HER2 levels and circulating tumor cells in patients with recurrent breast cancer whose primary tumor was HER2 negative or of unknown HER2 status. *Breast Cancer Res*. 2007;9(5):R74.
7. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009;119(6):1420–1428.
8. Allard WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clin Cancer Res*. 2004;10(20):6897–6904.
9. Aktas B, Tewes M, Fehm T, Hauch S, Kimmig R, Kasimir-Bauer S. Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients. *Breast Cancer Res*. 2009;11(4):R46.

10. Mei M, Ren Y, Zhou X, et al. Downregulation of miR-21 enhances chemotherapeutic effect of taxol in breast carcinoma cells. *Technol Cancer Res Treat*. 2010;9(1):77–86.
11. Chen J, Tian W, Cai H, He H, Deng Y. Down-regulation of microRNA-200c is associated with drug resistance in human breast cancer. *Med Oncol*. 2012;29(4):2527–2534.
12. Gong C, Yao Y, Wang Y, et al. Up-regulation of miR-21 mediates resistance to trastuzumab therapy for breast cancer. *J Biol Chem*. 2011;286(21):19127–19137.
13. Iorio MV, Casalini P, Piovan C, et al. microRNA-205 regulates HER3 in human breast cancer. *Cancer Res*. 2009;69(6):2195–2200.
14. Zhao R, Wu J, Jia W, et al. Plasma miR-221 as a predictive biomarker for chemoresistance in breast cancer patients who previously received neoadjuvant chemotherapy. *Onkologie*. 2011;34(12):675–680.
15. Wang H, Tan G, Dong L, et al. Circulating MiR-125b as a marker predicting chemoresistance in breast cancer. *PLoS One*. 2012;7(4):e34210.
16. Jung EJ, Santarpia L, Kim J, et al. Plasma microRNA 210 levels correlate with sensitivity to trastuzumab and tumor presence in breast cancer patients. *Cancer*. 2012;118(10):2603–2614.
17. Wu X, Somlo G, Yu Y, et al. De novo sequencing of circulating miRNAs identifies novel markers predicting clinical outcome of locally advanced breast cancer. *J Transl Med*. 2012;10:42.
18. Leidner RS, Li L, Thompson CL. Dampening enthusiasm for circulating microRNA in breast cancer. *PLoS One*. 2013;8(3):e57841.
19. Mandel P, Métais, P. Les acides nucléiques du plasma sanguin chez l'homme. *C. R. Acad. Sci. Paris*. 1948;142(3–4):241–243.
20. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer*. 2011;11(6):426–437.
21. Leary RJ, Sausen M, Kinde I, et al. Detection of chromosomal alterations in the circulation of cancer patients with whole-genome sequencing. *Sci Transl Med*. 2012;4(162):162ra154.
22. Forshew T, Murtaza M, Parkinson C, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. *Sci Transl Med*. 2012;4(136):136ra68.
23. Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N Engl J Med*. 2013;368(13):1199–1209.
24. Murtaza M, Dawson SJ, Tsui DW, et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature*. 2013;497(7447):108–112.
25. Isakoff SJ, Engelman JA, Irie HY, et al. Breast cancer-associated PIK3CA mutations are oncogenic in mammary epithelial cells. *Cancer Res*. 2005;65(23):10992–11000.