Application of Liquid Biopsies in Cancer Targeted Therapy

S Sumanasuriya[1,2], MB Lambros[1] and JS de Bono[1,2]

As a growing body of evidence demonstrates intertumoral and intratumoral heterogeneity and clonal evolution, both during carcinogenesis and also throughout treatment resulting in acquired drug resistance, the utility of blood-based assays or "liquid biopsies" is becoming increasingly recognized in clinical practice and trial design. "Liquid biopsies" provide a less invasive approach to the current gold standard of interrogating tumors by tissue biopsies, which are frequently unfeasible, associated with morbidity, and cannot be performed as often.

Over the past several years, two main forms of "liquid biopsy" have emerged:

1. Circulating (plasma) cell free (cf) nucleic acids, including DNA (cfDNA) shed by tumor cells into blood, and usually it is probably encapsulated in some way, such as with exosomes, cell fragments, or microparticles;
2. Circulating tumor cells (CTCs), intact rare cells found in blood, which can be separated and counted as well as genomically characterized.

These two main types of analyses provide complementary information; cfDNA is unable to characterize intrapatient heterogeneity at a single time point as well as single CTC analyses, but acquiring cfDNA for analysis is more easily feasible in the majority of patients with advanced cancer. Methods of increasing CTC capture and improving cfDNA analyses are now transforming this field. Other types of circulating biomarker analyses key to cancer medicine include whole blood transcriptome analyses and circulating immune and endothelial cell characterization that may shed light on tumor-stromal cell interactions.

CIRCULATING NUCLEIC ACIDS

DNA, mRNA, and microRNA (miRNA) are shed into the blood of patients with cancer from both primary and metastatic disease sites through necrosis, apoptosis, and probably active release.

Cell-free DNA

The cfDNA concentrations vary considerably between patients and tumor types; for example, glioblastoma and ovarian cancer frequently have lower cfDNA levels, likely due to their disease localization and pattern of spread. Quantitative and qualitative analyses carried out on cfDNA have demonstrated utility as a prognostic, predictive, and response biomarker. Studying cfDNA has the ability to interrogate disease clonal evolution and resistance mechanisms in serial and disease progression samples. CfDNA detection can also be used to identify minimal residual disease and early relapse, with array comparative genomic hybridization (aCGH) carried out on plasma cfDNA being comparable to aCGH on the same patient tissue samples (see **Figure 1a**).

We have recently shown, for example, that decreases in cfDNA concentrations and mutation allele frequency from baseline significantly associate with response to treatment with poly ADP-ribose polymerase inhibition in advanced prostate cancer. Plasma cfDNA analyses have been used to identify second hit mutations in BRCA2 and PALB2 restoring DNA repair gene function as mechanisms of resistance to treatment at progression.[1] Despite showing promise as a biomarker from a liquid biopsy, cfDNA analyses are not currently used routinely in clinical practice. However, in nonsmall cell lung cancer, the US Food and Drug Administration has approved plasma epidermal growth factor receptor testing from cfDNA for the presence of a T790M mutation, which can be used to detect acquired resistance to first and second generation tyrosine kinase inhibitors (e.g., erlotinib and naratanib) but also confer sensitivity to third generation tyrosine-kinase inhibitors, such as osimertinib.

Dilution by normal DNA can complicate and limit cfDNA analyses but DNA stability in the circulation can allow tumor genomic characterization by targeted, exome, and whole genome sequencing. Orthogonal assays, such as digital droplet polymerase chain reaction and aCGH, can also be used and provide analytic validation.

RNA and microRNAs

RNA is generally unstable in blood, but miRNA comprises stable, short, noncoding molecules made of 9–25 nucleotides. Tumor-derived miRNA is detectable in plasma, serum, urine, and even

[1]The Institute of Cancer Research, Sutton, London, UK; [2]The Royal Marsden, Sutton, London, UK. Correspondence: JS de Bono (johann.de-bono@icr.ac.uk).

Received 10 May 2017; accepted 1 June 2017; advance online publication 29 July 2017. doi:10.1002/cpt.764

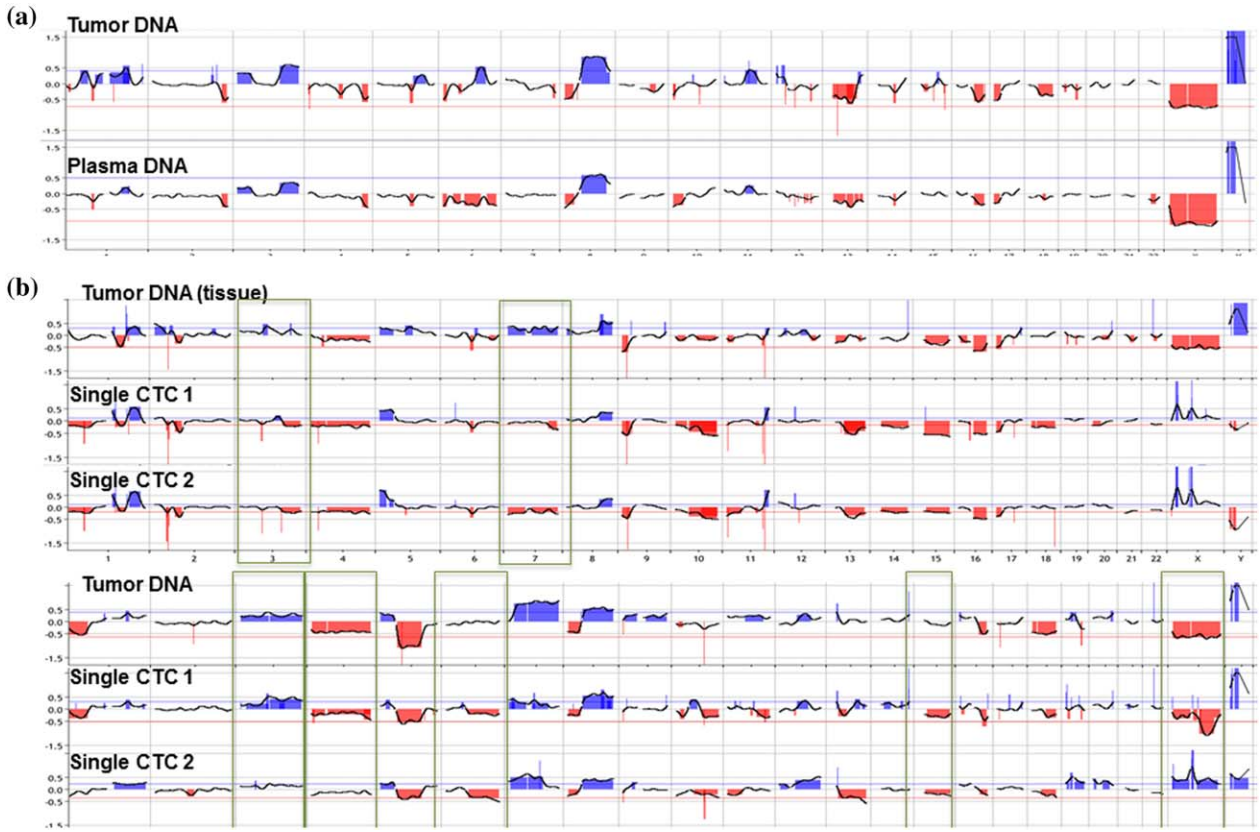

Figure 1 Array comparative genomic hybridization (aCGH) performed on plasma cell-free DNA (**a**) and on multiple circulating tumor cells (CTCs) (**b**), reflecting intrapatient heterogeneity compared to aCGH on diagnostic tumor samples.

saliva and semen. These miRNAs can be analyzed by targeted or RNA sequencing methods, with miRNA signatures identified as significantly deregulated in patients with cancer compared with healthy volunteers, perhaps leading to utility in cancer diagnosis. MiRNA signatures have been reported to be response biomarkers for chemotherapy and radiotherapy, although reproducibility remains a challenge. Additional studies are warranted to further study the role of miRNA signatures for disease detection, prognostication, identification of minimal residual disease, tumor recurrence, and as a response biomarker.

Tumor educated platelet mRNA

Reports indicate, but need validation, that we may also be able to acquire data from noncancer-derived cells, including tumor educated platelets. Tumor cells are hypothesized to interact with circulating platelets, "educating" them by activating surface receptors and altering cytokine expression and altering platelet mRNA. Investigating the mRNA profiles of tumor educated platelets may provide information on cancer type, and work is ongoing to validate these data.

CIRCULATING TUMOR CELLS

These are rare cells found in the blood of patients with cancer, detectable at 1 CTC[2,3] per milliliter or less of blood in patients with advanced cancer. Precise identification of these rare cells is difficult; the only US Food and Drug Administration cleared platform for identifying CTCs remains the CellSearch platform that identifies CTCs based on epithelial cell adhesion molecule capture and cytokeratin-positivity and CD-45 negativity. Multiple other CTC assays have, however, been reported with different degrees of analytic validation and clinical qualification pursued to date. CTC counts are highly prognostic, serve as post-treatment response measures associating with survival, and may be true surrogate biomarkers of treatment benefit. Molecular characterization of CTCs allows better insight into tumor heterogeneity than cfDNA, with single cells being amenable to most assays, including immunofluorescence, array CGH, next generation sequencing of both DNA and RNA, and fluorescence in situ hybridization. These may have clinical utility for use in trials for patient selection, pharmacodynamics, and response biomarkers. One major limitation, however, of CTC analyses has been the very small numbers of cells generally captured from these studies.

We have recently demonstrated that identified and captured CTC numbers can be enriched easily and safely using diagnostic leukapheresis over 1–3 h.[4] This procedure is well tolerated, with the increased CTC yield allowing the genomic analyses of many pure single CTCs and the study of intrapatient CTC genomic heterogeneity. We have shown that, although these CTC analyses are comparable to tumor biopsy genomics, biopsy analyses, which usually analyze millions of cells, frequently miss the heterogeneity identified in the study of many single CTCs[4] (see **Figure 1b**).

Further prospective trials evaluating CTC enumeration as a surrogate biomarker of response in prostate cancer are currently being undertaken (TOPARP-B: ISRCTN15124653, CTC-STOP:

ISRCTN82499869) as are trials incorporating techniques to maximize CTC yield in order to fully realize the full potential of CTCs as multipurpose biomarkers.

IMMUNE CELL STUDIES

Neutrophil-to-lymphocyte ratio

An inexpensive, easily available, but nonspecific assay, an immune cell study[5] has previously been documented to be a highly prognostic factor in studies of >50,000 patients with cancer with many solid tumors. This inexpensive biomarker of cancer inflammation can predict likelihood of response to treatment, associating with response to abiraterone and taxanes in advanced prostate cancer. Significant changes in neutrophil-to-lymphocyte ratio (NLR) during treatment also correlate with response. High NLR levels may correlate with increased myeloid-derived suppressor cell (MDSC) counts.

Myeloid derived suppressor cells

These originate from primitive hematopoietic precursors as a result of tumor-generated endocrine and paracrine factors, and can be immunosuppressive and support tumor growth, survival, and treatment resistance. High MDSC levels correlate not only with high NLR levels but also with decreased response to treatment and shorter survival. Peripheral MDSC subsets have been identified and these can be sorted by flow cytometry, and the proteins they release studied *ex vivo*. Studies into MDSC subsets and their cytokine release may help direct anticancer treatment.

CONCLUSION

Circulating biomarkers may aid diagnosis, prognostication, identify minimal residual disease, and allow disease molecular characterization, with serial sample analyses allowing response assessment, clonal evolution, and studies of therapeutic resistance. Circulating tumor cells and cfDNA are complementary noninvasive approaches for such biomarker studies. We envision that rigorous validation, including healthy volunteer blood analyses studies and prospective clinical trials for clinical qualification, will allow these biomarkers to transform cancer care.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

1. Goodall, J. *et al*. Circulating free DNA to guide prostate cancer treatment with PARP inhibition. *Cancer Discov.* (2017); (e-pub ahead of print).
2. Mehra, N., Zafeiriou, Z., Lorente, D., Terstappen, L.W. & de Bono, J.S. CCR 20th anniversary commentary: circulating tumor cells in prostate cancer. *Clin. Cancer Res.* **21**, 4992–4995 (2015).
3. de Bono, J.S. *et al*. Potential applications for circulating tumor cells expressing the insulin-like growth factor-I receptor. *Clin. Cancer Res.* **13**, 3611–3616 (2007).
4. Lambros, M. Diagnostic leukapheresis (DLA): molecular characterization and organoid culture of circulating tumor cells (CTC) from metastatic castration resistant prostate cancer (mCRPC). Oral Presentation #993 at the American Association for Cancer Research (AACR) annual meeting, Washington, DC, April 1–5, 2017.
5. Yap, T.A., Smith, A.D., Ferraldeschi, R., Al-Lazikani, B., Workman, P. & de Bono, J.S. Drug discovery in advanced prostate cancer: translating biology into therapy. *Nat. Rev. Drug Discov.* **15**, 699–718 (2016).