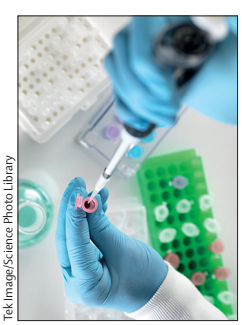
technology assessment mechanisms, identifying costs across whole pathways, developing new and context-appropriate models of care, and strengthening governance of clinical practice in both public and private sectors. India needs to revisit and revise its national cancer control programme with a focus on better overall care, not just access to new technologies.[4] Ayushman Bharat offers an important opportunity—and responsibility—to improve care meaningfully for patients across the health system.

Carlo Caduff, Christopher M Booth, C S Pramesh, Richard Sullivan
Department of Global Health & Social Medicine, King's College London, London WC2B 4BG, UK (CC); Canada Research Chair in Population Cancer Care, Queen's University, Kingston, ON, Canada (CMB); Tata Memorial Centre, Parel, Mumbai, India (CSP); and Institute of Cancer Policy, Conflict & Health Research Group, King's College London, King's Health Partners Comprehensive Cancer Centre, London, UK (RS)
carlo.caduff@kcl.ac.uk

We declare no competing interests.

1 Mallath MK, Taylor DG, Badwe RA, et al. The growing burden of cancer in India. Epidemiology and social context. *Lancet Oncol* 2014; **15:** e205–12.
2 Pramesh CS, Badwe RA, Borthakur BB, et al. Delivery of affordable and equitable cancer care in India. *Lancet Oncol* 2014; **15:** e223–33.
3 Pramesh CS, Chaturvedi H, Reddy VA, et al. Choosing wisely India. *Lancet Oncol* 2019; **20:** e218–23.
4 Sullivan R, Pramesh CS, Booth CM. Cancer patients need better care, not just more technology. *Nature* 2017; **549:** 325–28.

The liquid biopsy: towards standardisation in preparation for prime time


Tek Image/Science Photo Library

The liquid biopsy holds potential as a more cost-effective, easier, and less invasive method for diagnosing and monitoring cancer, as well as predicting response, than currently available approaches (such as tissue biopsies or imaging scans). Circulating tumour cells (CTCs) and circulating cell-free DNA (cfDNA) are currently the most intensely investigated analytes, with some tests already approved in clinical practice (eg, the Cobas EGFR mutation test v2, Roche, Burgess Hill, UK). Despite the potential of the liquid biopsy for managing patient therapy, no widely accepted consensus has been reached regarding pre-analytical blood sample handling and technologies used for extracting cfDNA and isolating CTCs, or optimal workflows for their molecular analyses, all of which are required before liquid biopsies can become routinely used in the clinic. In this Comment, we discuss the current status of liquid biopsy testing in patients with solid tumours, and the variation in blood sample handling and isolation or extraction methods, focusing on cfDNA and CTCs.

Several pre-analytical variables can affect downstream data obtained from cfDNA analysis. The first is specimen type (plasma or serum). Plasma and serum constitute the non-cellular fraction of whole blood; however, serum is obtained by allowing whole blood to clot at room temperature before processing. This process results in substantial leukocyte and haematopoietic cell lysis, diluting the concentration of circulating tumour DNA (ctDNA) present in cfDNA. To obtain plasma, whole blood is processed as soon as possible post-venepuncture, reducing contamination by genomic DNA and is, therefore, considered optimal for ctDNA analysis. The second, and arguably most crucial, pre-analytical variable is blood sample processing, further stratified by the type of blood collection tube and time to centrifugation, and the speed and number of centrifugations.

The type of blood collection tube is the most diverse pre-analytical variable. It is well documented in published literature that an increase in total cfDNA yield with increasing time before centrifugation is observed when blood is drawn into edetic acid (EDTA)-stabilising blood collection tubes, mainly because of leukocyte lysis. Therefore, it is recommended that time to processing is within 2 h of blood collection, minimising the risk of genomic DNA contamination and subsequent dilution of ctDNA.[1,2] To circumvent the need for immediate blood processing, specialised preservative blood collection tubes are available, permitting longer-term storage (from 1 day to several days) at ambient temperatures (room temperature to 37°C), but at a higher cost than EDTA tubes. These blood collection tubes allow unprocessed samples to be transported before processing, with studies suggesting that preservative blood collection tubes prevent leukocyte lysis for up to 7 days at room

temperature,[3] with no reduction in plasma volume for up to 5 days.[2] However, a significant effect on leukocyte lysis has been observed when storing blood at extreme temperatures (4°C or 40°C) for 5 days or longer. Therefore, to ensure the success of cfDNA-based multicentric clinical trials involving hospitals with no equipment to double spin and freeze the plasma, "special care has to be taken to maintain a defined room temperature range to obtain reliable mutation testing results".[2]

A systematic review[4] concluded that centrifugation speed was not crucial in determining the cfDNA extraction yield; however, double centrifugation is recommended to ensure removal of cells carried over from the first spin. Once isolated, plasma can be stored for up to 3 h at 4°C if processed immediately without affecting downstream extraction efficiency, or should be aliquoted and stored at –80°C for long-term storage, avoiding more than three freeze–thaw cycles.[5] An annual 30% reduction in cfDNA yield from plasma stored long term at –80°C has been suggested;[6] therefore, it is preferable to extract cfDNA as soon as possible post-venepuncture.

Many commercial kits are available for cfDNA extraction; however, numerous protocol modifications exist, all varying in plasma volume required, DNA elution volumes, yields, and cost. Furthermore, the method of choice depends on the throughput requirements and availability of associated equipment. Regarding sample storage, it is recommended that cfDNA is quantified immediately and no later than 3 months post-storage at –20°C because of sample fragmentation.[6] However, storage is less crucial for analysis of specific sequences or high-frequency mutations and, therefore, samples can be stored at –20°C for several years,[7] although it might affect low-frequency variants.

The final pre-analytical variable is total cfDNA quantitation; methods used include fluorescence, spectrophotometry, and quantitative and digital PCR. Fluorescence and spectrometry have inherent disadvantages; they are less sensitive than PCR, are affected by impurities (eg, phenols, RNA, and organic compounds), and cannot distinguish between different DNA sources (such as from bacterial, viral, or fungal infections). Quantitative PCR and digital PCR, although more expensive, offer a highly sensitive and specific method for quantitation because the analysis depends on sequence-specific amplification across a small sequence (typically 60–120 base pairs), thereby accurately quantifying the aggregate of tumour-derived DNA and DNA derived from apoptosis of normal cells.

Although numerous clinical trials are using ctDNA for guiding patient therapy, a joint review[8] by the American Society for Clinical Oncology and the College of American Pathologists, published in 2018, concluded that ctDNA assays currently do not offer any evidence of clinical validity or utility despite the potential for them to be used throughout the patient's diagnosis, treatment, and follow-up. At present, only the Cobas v2 ctDNA assay for non-small-cell lung cancer has been clinically validated and granted regulatory approval in the USA and Europe; however, the joint review raised concerns around its clinical utility because the evidence for its approval was based on retrospective analyses and might not be representative of the population targeted for clinical use of the ctDNA assay (ie, validation of clinical utility should be done with randomised controlled trials).

CTCs offer the potential for multi-omics analysis, while also having preclinical potential for guiding patient therapy. However, CTCs are rare (typically 1–10 CTCs in 10^6–10^8 leukocytes), heterogeneous, and are expensive to enumerate and isolate. To obtain live CTCs, blood samples must be processed on-site within hours of collection to retain viability (which many hospital sites cannot do). Consequently, transportation of live CTCs in blood samples between sites is often not possible because of high CTC senescence. As a result, blood is usually taken into preservative blood collection tubes, allowing storage for up to 3 days. However, this process also kills the cells, reducing the number of potential downstream applications.

The US Food and Drug Administration-approved CellSearch System (Menarini, Bologna, Italy) provides prognostic information based on enumeration of CTCs that are EpCAM-positive, CK-positive, and CD45-negative. However, this technology cannot be used to enumerate other EpCAM-negative CTC populations and, therefore, might not represent the entire CTC population. Other technologies use physical properties, such as cell size, density, deformability, and electrical charge to enumerate CTCs, whereas microfluidic methods produce intact cell suspensions suitable for downstream immunofluorescence imaging or single cell isolation. Technologies isolating CTCs based on their size, although representative of a wider population (including CTC clusters), are limited because they miss

smaller CTCs implicated in progressive disease, which requires a different course of therapy. Similarly, isolation based on density is also difficult because very small CTCs might have a similar density to red blood cells and, therefore, be lost with low-density separation media. Technologies based on deformability are hampered because CTCs can be mechanically similar to other blood cells in some cancer types (such as prostate cancer).[9]

At present, the technology used for isolation and enumeration of CTCs depends entirely on the hypothesis investigated. The ideal CTC marker will be expressed on every CTC (including clusters) but is absent from other blood-derived cells and is constitutively expressed throughout disease progression. To establish CTCs as a clinical biomarker, optimised workflows are essential to generate robust, cost-efficient, and reproducible data that can inform clinical decisions. A consensus cutoff regarding the number of CTCs per mL as a clinical biomarker for stratification is undetermined in most advanced cancers, although a recent meta-analysis[10] in metastatic breast cancer has made great progress towards establishing this aim. Overall, analysis of CTCs for prognosis and therapeutic stratification is still not routine in the clinic; however, they are increasingly used in prospective clinical trials for guiding therapy (eg, CTC-STOP [ISRCTN82499869]).

For more on **CANCER-ID** see www.cancer-id.eu

Various multicentre efforts are ongoing to establish the best practice in the field, including CANCER-ID, which aims to provide consensus workflows for sample processing, specimen storage, biobanking, and molecular analysis of ctDNA, CTCs, and microRNAs. Future studies should focus on validating ctDNA assays in compliance with international standards and local legislation, guided by a standardised framework that describes the necessary procedures for validating potential biomarkers. Overall, the liquid biopsy holds promise for patient diagnostics and therapy monitoring. We anticipate that studies reporting over the next few years will help to progress the liquid biopsy from the laboratory to the clinic, to be ready for prime time.

*Karen Page, Jacqueline A Shaw, *David S Guttery*
The Leicester Cancer Research Centre, University of Leicester, Leicester Royal Infirmary, Leicester LE2 7LX,UK (KP, JAS, DSG)
dsg6@le.ac.uk

We declare no competing interests.

1 Franczak C, Filhine-Tresarrieu P, Gilson P, Merlin JL, Au L, Harle A. Technical considerations for circulating tumor DNA detection in oncology. *Expert Rev Mol Diagn* 2019; **19:** 121–35.
2 Medina Diaz I, Nocon A, Mehnert DH, Fredebohm J, Diehl F, Holtrup F. Performance of Streck cfDNA blood collection tubes for liquid biopsy testing. *PloS one* 2016; **11**: e0166354.
3 Parpart-Li S, Bartlett B, Popoli M, et al. The effect of preservative and temperature on the analysis of circulating tumor DNA. *Clin Cancer Res* 2017; **23:** 2471–77.
4 Trigg RM, Martinson LJ, Parpart-Li S, Shaw JA. Factors that influence quality and yield of circulating-free DNA: a systematic review of the methodology literature. *Heliyon* 2018; **4:** e00699.
5 El Messaoudi S, Rolet F, Mouliere F, Thierry AR. Circulating cell free DNA: preanalytical considerations. *Clin Chim Acta* 2013; **424:** 222–30.
6 Sozzi G, Roz L, Conte D, et al. Effects of prolonged storage of whole plasma or isolated plasma DNA on the results of circulating DNA quantification assays. *J Natl Cancer Inst* 2005; **97:** 1848–50.
7 Kopreski MS, Benko FA, Kwee C, et al. Detection of mutant K-ras DNA in plasma or serum of patients with colorectal cancer. *Br J Cancer* 1997; **76:** 1293–99.
8 Merker JD, Oxnard GR, Compton C, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists joint review. *J Clin Oncol* 2018; **36:** 1631–41.
9 Shaw Bagnall J, Byun S, Begum S, et al. Deformability of tumor cells versus blood cells. *Sci Rep* 2015; **5:** 18542.
10 Cristofanilli M, Pierga JY, Reuben J, et al. The clinical use of circulating tumor cells (CTCs) enumeration for staging of metastatic breast cancer (MBC): international expert consensus paper. *Crit Rev Oncol Hematol* 2019; **134:** 39–45.

Cancer hospital advertising and outcomes: trust the messenger?


Lewis Houghton/SPL

Hospitals have made substantial investments in advertising for cancer services in the past two decades, totalling over US$200 million in 2016 alone.[1,2] Advertisements promoting cancer centres are unavoidable in the USA. They hang on highway billboards and on air during prime-time programming. Some advertisements claim superior outcomes, others highlight access to clinical trials, and many present heart-warming patient stories that might be non-representative of actual outcomes.[3] Data suggest that patients are highly aware of advertisements and are likewise influenced by them.[4]

Decades of research have shown wide and consistent variations in cancer care outcomes between US hospitals.[5,6] Although patients might wish to select their cancer care provider based on objective measures of cancer care quality and outcomes,[7,8] few measures are publicly available. Advertising is designed to improve cancer centre recognition and attract patients in an