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Liquid biopsies in the clinic

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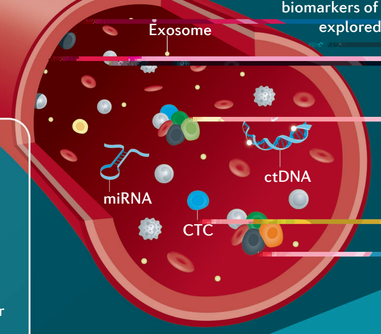
The concept of 'liquid biopsy' refers to the detection of molecules that originate from tumours, by analysis of intact circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), microRNAs (miRNAs) and exosomes. A number of cellular processes, such as epithelial-to-mesenchymal transition, collective migration, cell co-operation or vasculogenic mimicry, are associated with intravasation and extravasation of tumour components[1]. The mechanisms whereby ctDNA enters the bloodstream remain understudied; however, the prevailing view is that ctDNA is released from dying cells[2]. Exosomes are small microvesicles that are released into the blood by normal and malignant cells, and contain proteins, nucleic acids and lipids[3].

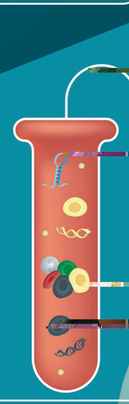
CTCs can disseminate as single cells or as clusters, and are thought to be harbingers of metastases[2]. DNA released from tumour cells into the circulation can also be also detected in the blood of patients with cancer and reveal information on the molecular evolution of cancer[2]. Exosomes that are present in liquid biopsies (such as blood plasma) are promising novel candidate blood-based biomarkers of tumour progression and resistance to therapy[3]. miRNAs have good stability and are being explored as biomarkers that enable screening and/or early detection[4].



Red blood cell
White blood cells
Mutated DNA
CTC cluster
Tumour heterogeneity

Studying liquid biopsies at different times of tumour progression offers a wealth of information on:

- disease stage and tumour heterogeneity
- selection of targeted therapies
- response to treatment and
- the emergence of therapy resistance and its underlying mechanisms



Identification of therapeutic targets for

Liquid biopsies are beginning to fulfil their potential as prognostic, predictive, and pharmacodynamic biomarkers, and are being used for longitudinal monitoring of tumour evolution and emergent treatment resistance. Examples of biomarkers detected in liquid biopsies to guide precision medicine approaches include:

- Breast cancer: oestrogen receptor (*ESR1*), *HER2* oncogene and immune-checkpoint regulators, such as *PDL1* in CTCs[7]
- Prostate cancer: expression, activation and signalling mediated by the androgen receptor (*AR*) as a target of antiandrogen therapy (for example, *AR* splice variant 7) in CTCs[8]

Mutations in genes encoding therapeutic targets and downstream effectors can affect drug efficacy: for example, *EGFR* mutations in ctDNA from lung cancer, *KRAS* mutations in CTCs from colorectal cancer, *ESR* and *PIK3CA* mutations in ctDNA and CTCs from breast cancer, *AR* amplifications and mutations in CTCs and ctDNA from prostate cancer, and *BRAF* mutations in CTCs and ctDNA from melanoma.

Sequential CTC and ctDNA analyses have the potential to inform on the optimal time to switch therapies:

- Breast cancer: CTC counts to predict chemotherapy effectiveness; ctDNA analysis to map subclonal evolution[9]
- Prostate cancer: CTC counts as an individual-level surrogate for patient survival in metastatic castration-resistant prostate cancer in the context of antiandrogen therapy; detection of *AR* gene aberrations in ctDNA to enabled prediction of the outcomes of androgen-deprivation therapy
- Melanoma: dynamic changes of *BRAF* mutations in ctDNA to monitor therapy response[10]
- Colorectal cancer: ctDNA analysis to track clonal evolution and acquired resistance to *EGFR*-targeted therapies (often associated with emergence of *KRAS* mutations[11])

Liquid biopsy assays now need to be standardized and cross-validated within international consortia (for example, the European IMI consortium (www.cancer-id.eu) and the utility of liquid-biopsy-based clinical decision-making now needs to be demonstrated in randomized clinical interventional studies incorporating established end points (such as time-to-progression or overall survival).

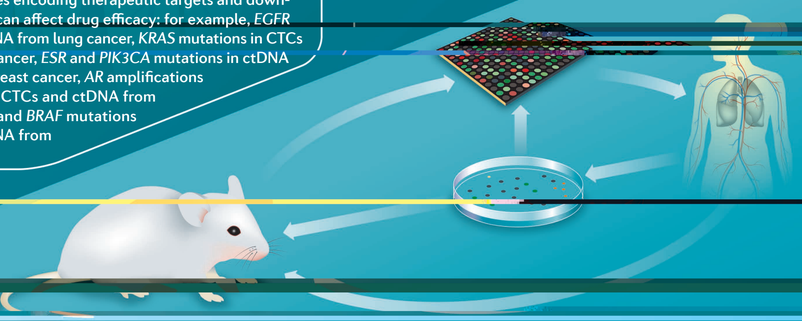
Circulating biomarkers for cancer screening and early detection have often emerged from comparative longitudinal studies of healthy individuals, patients with benign disease and patients with cancer. The process of biomarker validation is facilitated by focussing on individuals at a high risk of developing cancer.

Owing to low CTC numbers and ctDNA concentrations in the bloodstream of individuals at risk, liquid biopsy assay sensitivity is a technical challenge for detection of early malignant lesions. The clinical challenge is to minimize false-positive test results that can be associated with anxiety[5], and provoke additional unnecessary diagnostic procedures. Developing the 'right' panel of cancer-specific genomic aberrations to distinguish individuals with cancer from healthy individuals or from those with benign disease is of utmost importance, but requires substantial resources.

Tumour staging (estimation of an individual's risk of progression at the time of diagnosis) guides the decision on the most appropriate therapy aimed to eradicate all tumour cells and prevent local and/or disseminated disease relapse. Numerous studies have shown the prognostic value of CTCs expressing epithelial marker proteins (such as EpCAM and/or cytokeratin) in patients with solid tumours without clinical or radiological signs of overt distant metastases at primary diagnosis, in particular, in patients with breast cancer[6].

Liquid biopsies enable post-surgical surveillance of minimal residual disease (MRD) aiming to detect relapse before imaging or clinical symptoms report potential overt metastatic disease. However, CTC numbers and ctDNA concentrations are often low, requiring ultrasensitive technologies.

Analysis of liquid biopsy samples from the proximal draining veins of cancerous organs at the time of surgical resection with curative intent might enable identification of those patients at risk of recurrence. If candidate biomarkers have been first defined in the resected tumour, longitudinal profiling of 'early' CTCs and/or ctDNA might inform on treatment of recurrent disease. Studies on the kinetics of MRD can also provide valuable insights into the biology of cancer dormancy.



Patient-derived CTCs can be cultured *in vitro* and show substantial promise for real-time assessment of treatment efficacy as part of a personalized medicine approach[12,13].

CTC-derived explant models (CDX) are generated directly from CTC-enriched blood samples from patients and have been developed for breast cancer, small-cell lung cancer and melanoma. These models do not constitute 'avatars' because the time to model development is lengthy, but they do provide a faithful platform for the identification of new therapeutic targets and therapy testing[14].

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