Nano-omics: nanotechnology-based multidimensional harvesting of the blood-circulating cancerome

Lois Gardner, Kostas Kostarelos, Parag Mallick, Caroline Dive and Marilena Hadjidemetriou

Abstract | Over the past decade, the development of 'simple' blood tests that enable cancer screening, diagnosis or monitoring and facilitate the design of personalized therapies without the need for invasive tumour biopsy sampling has been a core ambition in cancer research. Data emerging from ongoing biomarker development efforts indicate that multiple markers, used individually or as part of a multimodal panel, are required to enhance the sensitivity and specificity of assays for early stage cancer detection. The discovery of cancer-associated molecular alterations that are reflected in blood at multiple dimensions (genome, epigenome, transcriptome, proteome and metabolome) and integration of the resultant multi-omics data have the potential to uncover novel biomarkers as well as to further elucidate the underlying molecular pathways. Herein, we review key advances in multi-omics liquid biopsy approaches and introduce the 'nano-omics' paradigm: the development and utilization of nanotechnology tools for the enrichment and subsequent omics analysis of the blood-circulating cancerome.

Despite exciting advances in treatment strategies, cancer remains the cause of one in six deaths globally[1]. The lack of early cancer detection tools is one of the main reasons for this high mortality. Tests that enable the detection of tumour signatures in the blood at an early disease stage offer enormous, untapped potential for patients with cancer to receive effective treatment before the tumour burden becomes incurable. Hence, liquid biopsy techniques are being developed apace, not only to enable non-invasive tumour profiling but also to detect the onset of cancer in asymptomatic individuals[2].

On the basis of a rationale similar to that underlying the use of combinations of therapeutic modalities to treat cancer (for example, surgery, radiotherapy and chemotherapy), the synergy of multiple blood-circulating analytes as 'cancer fingerprints' has led to a paradigm shift in early cancer detection. Liquid biopsy samples contain a repertoire of proteins, nucleic acids, circulating tumour cells (CTCs) and extracellular vesicles (EVs) that are shed into the blood circulation from multiple tumour sites and collectively reflect the spatial and temporal heterogeneity of the tumour biology. Although much remains to be learned about the dynamics of secreted and circulating tumour materials, serial liquid biopsies offer the possibility to longitudinally capture systemic biomolecular alterations as they develop dynamically during the evolutionary trajectory of tumour progression.

Examining multidimensional molecular alterations (genomic, epigenomic, proteomic and others) in a variety of blood constituents and integrating the resultant multi-omics datasets holds the potential not only to elucidate cancer-specific molecular mechanisms and thus potential therapeutic targets, but also to uncover novel combinations of biomarkers for early cancer detection (FIG. 1). To date, integrated analyses of the cancerome have been limited in scope by the extremely low concentrations of liquid biopsy analytes, especially in patients with non-metastatic disease. Indeed, one of the major bottlenecks in blood-based multi-omics biomarker discovery is the large sample volumes required (typically 10–15 ml) to separately enrich and extract the different types of liquid biopsy analytes. Moreover, the development of multiple analyte extraction protocols compromises the analytical reproducibility and comparability of the resultant omics datasets[3].

Herein, we appraise advances made over the past decade in multi-omics approaches for early cancer detection. We also introduce the concept of 'nano-omics', an emerging paradigm using nanotechnology to address current technological constraints associated with the enrichment and analysis of the blood-circulating cancerome. Specifically, nano-omics utilizes biofluid-incubated nanomaterials as scavenging platforms to enrich and isolate cancer-derived analytes prior to their omics analysis, with the ultimate goal of identifying novel multi-omics panels of biomarkers for early cancer detection.

The multi-omics biomarker landscape

Currently, most liquid biopsy tests are based on either protein or cell-free DNA (cfDNA) analytes and are clinically used to detect prognostic and predictive biomarkers, mainly to aid in the selection of an optimal therapeutic strategy. For example, serum cancer antigen 15-3 is often used to monitor treatment response in patients with advanced-stage breast cancer[4], and *EGFR* mutation testing of plasma cfDNA can be used to predict responsiveness to EGFR tyrosine-kinase inhibitors in patients with non-small-cell lung cancer[5]. As such assays gain traction in the clinic, ongoing biomarker discovery efforts are gradually moving towards the development of multi-analyte tests intended for cancer screening and early detection. Although assays evaluating a single protein (for example, prostate-specific antigen for prostate cancer screening)[6] or multiple proteins (such as the OVA1 panel for ovarian cancer detection during the preoperative work-up of women with a known pelvic mass)[7] have been successfully translated into the clinic, (epi)genomic approaches are currently dominating the early cancer detection arena[8,9].

Circulating tumour DNA (ctDNA), enclosed within CTCs or released into

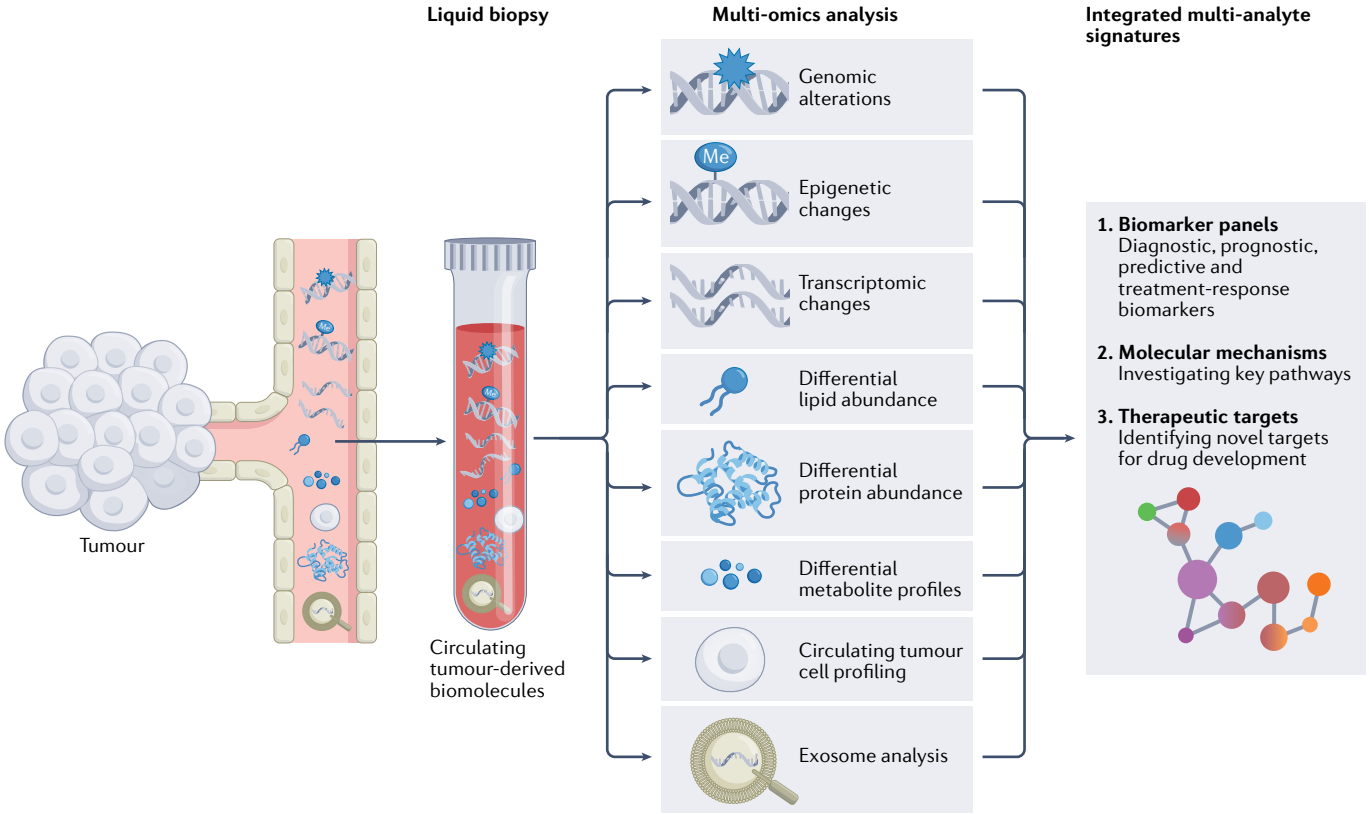


Fig. 1 | **Translational potential of multi-omics liquid biopsy.** Schematic representation of the multiple biomolecular layers of tumour-specific information that can be captured through blood-based liquid biopsy. The complex biomolecule signatures present in blood highlight the opportunity to develop methodologies enabling the detection of tumour-specific multi-omics profiles from a single blood sample. The multi-omics signatures identified have potential applications in cancer biomarker and drug development.

the bloodstream as a result of tumour cell apoptosis and/or necrosis, is emerging as one of the most promising sources of biomarkers for early cancer detection[8,10]. Although ctDNA constitutes only a subfraction of the total cfDNA, next-generation sequencing (NGS) methods enable amplification of the ctDNA signal and therefore outperform mass spectrometry (MS)-based, protein biomarker discovery approaches[11]. Currently, more than 30 ongoing large-cohort clinical trials are evaluating ctDNA-based biomarkers in blood[10]. Single-gene analysis has gradually evolved into multi-gene NGS assays and, more recently, into multimodal liquid biopsy approaches. The integration of different classes of biomarker molecules has the potential not only to increase the sensitivity and specificity of cancer detection but also to enable the localization of tumours at specific anatomical sites[12–17].

In the vanguard of liquid biopsy development for multi-cancer early detection, two different multiplex biomarker-signature platforms are currently being tested in prospective clinical studies: CancerSEEK and the GRAIL test. The CancerSEEK test uses a proteogenomic biomarker panel and was first evaluated prospectively in patients with no history of cancer in the Detecting cancers Earlier Through Elective mutation-based blood Collection and Testing (DETECT-A) study, after initial clinical evaluation in a retrospective study involving 1,005 patients with clinically detected non-metastatic cancers of 8 different types[16,17]. The initial proof-of-concept retrospective study evaluated a multi-analyte panel comprising 16 genes and 8 proteins and demonstrated a median test sensitivity of 70% (with considerable variation between the 8 different cancer types as well as between disease stages) and a specificity greater than 99%. Moreover, the application of a supervised machine learning algorithm correctly identified the organ of origin in a median of 63% of patients with a positive CancerSEEK test[16]. The subsequent DETECT-A study, which was the first-ever prospective and interventional trial to evaluate a multi-analyte (16 genes and 9 proteins) and multi-cancer blood test, involved 10,006 women (aged 65–75 years) without known cancer at enrolment[17]. A total of 96 cancer diagnoses were made during the study, of which 26 were exclusively detected using the CancerSEEK blood test, 24 were detected as a result of standard-of-care screening, and the remaining 46 were detected based on symptoms or other means[17]. The sensitivity for the CancerSEEK test was reported to be 27.1% across all cancer types when the test was used alone and 52.1% when combined with standard-of-care testing[17]. It should be noted, however, that the CancerSEEK test was reliant on diagnostic PET–CT scans to confirm all positive cases and to localize cancers to a specific anatomical site. Nevertheless, this trial illustrated that a multi-analyte blood test coupled with PET–CT and standard cancer screening protocols can not only effectively be incorporated into routine clinical care, but can also prompt surgery with an intent to cure. Validation of the latest version of CancerSEEK is currently under way within a prospective, observational study in 1,000 individuals with known or suspected cancer and 2,000 with no known cancer, named Detecting Cancers Earlier Through Elective Plasma-based CancerSEEK Testing–Ascertaining Serial Cancer Patients to Enable New Diagnostic (ASCEND; NCT04213326).

The GRAIL test uses an alternative assay approach based on DNA-methylation patterns in plasma cfDNA determined through bisulfite sequencing of >100,000 informative methylation regions[18]. This platform is currently being tested for multi-cancer screening across an ambitious clinical programme encompassing five prospective trials: the Circulating Cell-free Genome Atlas (CCGA) study (NCT02889978), STRIVE (NCT03085888), SUMMIT (NCT03934866), PATHFINDER (NCT04241796) and PATHFINDER 2 (NCT05155605). The foundational CCGA study demonstrated that this targeted DNA-methylation assay can detect more than 50 cancer types while also predicting the tissue of origin of the cancer signal with 93% accuracy[18]. Cancers were detected across all disease stages (stage I–III sensitivity: 43.9%; stage I–IV sensitivity: 54.9%) with a specificity of >99%[18]. Through a collaboration with the UK National Health Service, the clinical and economic performance of the latest version of the GRAIL test, known as Galleri, will be evaluated prospectively in a pilot screening study including 140,000 participants 50–77 years of age[19]. Of note, both CancerSEEK and the GRAIL test have been awarded FDA Breakthrough Device status, highlighting the substantial potential of multi-analyte tests for the early detection of multiple cancer types.

Looking beyond cell-free genomic and proteomic cancer biomarkers, attempts have also been made to purify and characterize CTCs and tumour-derived EVs from blood, mainly for real-time monitoring of therapy response. The CELLSEARCH system is the first FDA-approved platform designed to capture, purify and enumerate CTCs of epithelial origin for prognostication in patients with metastatic breast, colorectal or prostate cancer[20–22]. Currently, enumeration of the vanishingly small numbers of CTCs (typically 1–10 per millilitre of blood in patients with metastatic disease) is based on the expression of epithelial markers, such as epithelial cell adhesion molecule (EpCAM) and cytokeratins 8, 18 and/or 19, and relies on antibody-based cell capture and staining methodologies that fail to maintain the viability of CTCs. At present, the clinical utility of CTCs is solely based on enumeration and is limited to predicting clinical outcome rather than enabling cancer detection[23]. However, a plethora of CTC enrichment technologies are under development to enable the sequential sampling and molecular profiling of heterogeneous CTC populations[23]. The transition from bulk-cell strategies to single-cell analyses of viable and intact patient-derived CTCs has fuelled the development of microfluidic technologies with integrated downstream molecular analysis functionalities, including the ClearCell FX1 System[24].

Tumour-secreted EVs have not only been implicated in tumour growth and metastasis but might also stably encapsulate a treasure trove of cancer-associated proteins, nucleic acids and lipids[25]. In comparison with CTCs, EVs are present in larger quantities in biological fluids, although reproducible isolation and enrichment of EVs from the background molecular constituents of biofluids remains notoriously difficult[26]. DNA barcoding labelling[27], 3D-nanopatterned microfluidic chips[28] and label-free purification platforms (for example, exosome detection via the ultrafast-isolation system (EXODUS))[29] are only a few examples of the approaches currently under development to overcome the limitations associated with conventional ultracentrifugation-based and antibody-based EV-purification protocols in terms of purification efficiency, yield, speed and robustness. The integration of biomolecular and/or biophysical enrichment with multiplexed detection of EV-encapsulated biomarkers (such as proteins and microRNAs) within a single microfluidic platform (for example, the template plasmonics for exosomes technology (TPEX)) has shown great promise in separating EVs from non-vesicular biofluid components[30].

Attempts have also been made for the dual isolation and profiling of both CTCs and EVs from a single sample using immunoaffinity-based microfluidic interfaces[31,32]. For example, the dual-utilization OncoBean (DUO) microfluidic device has been shown to enable the simultaneous isolation of CTCs and EVs from blood samples obtained from patients with melanoma as well as subsequent molecular profiling of these analytes using a multiplex real-time quantitative reverse transcription PCR (RT-qPCR) test for the expression pattern of a panel of 96 melanoma-associated genes[31]. The enrichment of multiple cancer analytes using a single device or platform is considered the next frontier in the field of multi-omics liquid biopsies.

Data analysis and integration

Despite the increasing availability of omics datasets, translating biomarker discoveries into clinical assays remains challenging and requires computational manipulation and interpretation of multi-omics datasets. Large-scale international research networks are beginning to realize the vast potential of capturing data on integrated layers of the cancerome. The Cancer Genome Atlas (TCGA)[33], a pan-cancer genomics consortium initiated in 2005, has now expanded into a multi-omics framework, comprising over 2.5 petabytes of genomic, epigenomic, transcriptomic and proteomic data[34–37]. The US National Cancer Institute's Clinical Proteomic Tumour Analysis Consortium (CPTAC) is another example of a multi-institution initiative aiming to leverage the complementarity of proteogenomic datasets to provide new molecular insights across different cancer types[38].

The integration of multi-omics datasets generated from a single patient sample offers enormous potential to discover disease-specific molecular signatures in the blood. However, multi-omics data analysis is exponentially more challenging than 'single-omic' analysis, and the following six key issues remain to be addressed: (1) nomenclature differences (for example, gene centric versus protein centric) and identifier deprecation can unintentionally conflate distinct molecular species[39]; (2) each data modality is subject to its own specific noise and distribution characteristics, which requires the use of numerous, inter-dependent software tools in the analysis workflows[40,41]; (3) extensive domain knowledge is required to develop and execute multi-omics workflows; (4) the complexity of the workflows makes them difficult to optimize and prone to errors[42,43]; (5) findings can be highly dependent on the design of the analysis workflow[44]; and (6) reproducing and comparing results can be complicated by subtle variations in workflows[45].

A number of workflow solutions, such as GalaxyP and WINGS[46,47], have been developed to enable the correlation of multi-omics data, yet currently no consensus exists regarding the selection of key biomarkers from such datasets. The available tools and methods for multi-omics data analysis and integration have been thoroughly reviewed elsewhere[44,48,49].

Nano-enrichment of the cancerome

Technological advances in MS and NGS have enormously advanced the analysis of proteogenomic signatures in the blood, yet only a few blood-based cancer biomarker assays have been approved by the FDA[50]. The extraction and purification of cancer-associated analytes from blood remain a major bottleneck limiting the integration of liquid biopsies into routine clinical practice[51].

The quest for novel early-detection biomarkers has led to the development of nanotechnology-based platforms engineered to enrich different components of the blood cancerome (including proteins, ctDNA, CTCs and EVs)[52]. Most of these 'nano-enrichment' strategies rely on the high surface-to-volume ratio of nanoparticles as well as on their surface engineering and functionalization capacity. All of these strategies exploiting the properties of nanoscale technologies or materials are encompassed in the nano-omics paradigm. Here, we discuss current technological challenges impeding the clinical translation of liquid biopsies and highlight examples of nano-omics platforms that have been utilized to overcome such challenges (TABLE 1).

'Targeted nano-omics' is predicated on functionalization of the nanoparticle surface with targeting moieties as recognition elements for specific cancer-related analytes. By contrast, the 'untargeted nano-omics' approach relies on the non-specific adsorption of cancer-associated analytes onto the nanoparticle surface upon incubation with a biological fluid (FIG. 2). A number of targeted nano-omics approaches have been developed, mainly for the enrichment of EVs and CTCs (FIGS 2 and 3), whereas the spontaneous adsorption of cancer analytes onto the surface of biofluid-incubated nanoparticles has only been exploited in the past 5 years, mainly for the enrichment and analysis of proteins and cfDNA (TABLE 1). We emphasize that the incorporation of nanoparticle-based probes in immunoassays and biosensors, although extensively investigated[53–58], falls outside of the scope of the nano-omics approach given that the outcome signal of such biosensors is based on the unique optical and electrochemical properties of the nanoparticle–analyte complex rather than on the downstream omics analyses of the nanoparticle-enriched analytes.

Protein and ctDNA harvesting. Among blood-circulating biomolecules, proteins are the biological end points of cellular processes and, therefore, have historically attracted the most interest as molecular biomarkers[59]. However, the discovery of novel protein biomarkers directly from blood has been complicated by the overwhelming masking effect of highly abundant proteins (for example, albumin accounts for approximately 50% of the total protein content)[60]. Despite considerable improvements in label-free MS-based proteomics, this signal-to-noise issue substantially hinders the identification of disease-specific protein signatures in the blood. Plasma immunoaffinity-based depletion columns are widely used to overcome the issue of albumin masking but can result in substantial loss of the low-molecular-weight (LMW) proteome (for example, proteins <60 kDa) along with the highly abundant carrier proteins[61].

The use of harvesting nanoparticles to enhance the proteomic analysis of the LMW cancer proteome in the blood was first suggested in 2003[61], yet this concept only attracted the interest of the nanoscience community over the past decade (TABLE 1). The Nanotrap technology developed by Liotta, Petricoin and co-workers[62] uses core–shell affinity-bait hydrogel nanoparticles as protein harvesters. Similarly to the aforementioned immunoaffinity columns, the Nanotrap technology enables separation of highly abundant high-molecular-weight (HMW) proteins from LMW proteins. Specifically, the porous outer shell of the nanoparticles blocks the entry of HMW but not of LMW proteins, while the internal core contains covalently attached chemical affinity baits that capture the LMW proteins for harvesting and subsequent analysis. Notably, although preliminary feasibility studies demonstrated the potential use of Nanotrap particles as a platform for protein biomarker discovery, this technology has mainly been utilized to capture and enrich known biomarker proteins[63,64].

The spontaneous and untargeted adsorption of proteins onto the surface of nanoparticles upon incubation with biological fluids, known as the 'protein corona'[65,66] (BOX 1), has also been exploited for protein biomarker discovery. During the past decade, we have learnt that a complex protein corona forms rapidly on the surfaces of all nanoscale materials to varying degrees, depending on their physicochemical properties and surface characteristics. Indeed, the binding affinity of nanoparticles for blood proteins has been shown to be determined by a number of different factors, including their size, surface charge and functionalization as well as the nanoparticle–biofluid incubation conditions[66–69] (BOX 1).

The nanoparticle corona-enabled enrichment and analysis of low-abundance proteins were first investigated in vivo through intravenous administration of lipid-based nanoparticles into tumour-bearing mice and to patients with ovarian carcinoma[70,71]. Subsequent recovery of corona-coated nanoparticles from blood and purification of the nanoparticle-bound proteins from highly abundant background molecules (which are of no diagnostic value) by size exclusion chromatography enabled high-resolution analysis of the LMW fraction of the plasma proteome[70–72]. This initial paradigm-shifting work sparked interest in the clinical exploitation of ex vivo-formed protein corona fingerprinting as a novel tool for proteomic analyses of plasma samples obtained from cohorts of patients with cancer[73–78]. Comprehensive comparisons between 'healthy' and 'diseased' nanoparticle corona samples through label-free proteomic techniques can enable the identification of multiple previously unrecognized candidate biomarker proteins (TABLE 1).

On the basis of these principles, the Proteograph platform, which uses a combination of magnetic nanoparticles with different surface properties and thus distinct corona profiles, has been developed for deep analysis of the plasma proteome[76]. Considering the plethora of 2D and 3D nanomaterials at our disposal, more work is needed to investigate whether the combination of various types of nanoparticles offers substantial 'broadening' of the blood proteome coverage in MS analyses. The purification and retrieval of corona-coated nanoparticles from plasma samples, the synthesis and stability of nanoparticle formulations, and the sample volume required are some of the remaining technical challenges that are likely to impede the development of such biofluid pre-processing protocols and will, therefore, need to be addressed.

More recently, nanoparticle protein corona formation has conceptually morphed into the multi-molecular self-assembly of layers composed of proteins, lipids, polysaccharides and nucleic acids, termed the 'biomolecule corona'[66,79] (BOX 1). For example, we demonstrated the interaction of cfDNA with lipid-based nanoparticles upon their incubation with human plasma samples[79]. The discovery of this additional omics dimension as well as the markedly higher abundance of nanoparticle corona cfDNA identified in samples from women with advanced-stage ovarian cancer (compared with age-matched women without cancer)[79] pave the way for further investigations of the potential of the nanoparticle biomolecule corona to enrich ctDNA. Interestingly, proteomic analysis of the same nanoparticle corona samples revealed cancer-specific elevations in histone proteins, suggesting a nucleosome-mediated nanoparticle–cfDNA interaction[79]. While the nanoparticle surface adsorption of microRNAs (either in protein complexes or encapsulated within EVs) remains to

Table 1 | **Example studies using nano-omics approaches for the analysis of liquid biopsy analytes**

Nanomaterial platform	Enrichment mechanism	Biofluid sampled (volume, if reported)	Downstream analysis	Refs
Proteins				
Nanotrap hydrogel core–shell nanoparticles	Affinity capture	Serum from patients with ovarian or prostate cancer (200 µl)	LC-MS/MS	62
PEGylated liposomal doxorubicin	Protein corona	Plasma from patients with ovarian cancer (1 ml)	LC-MS/MS	73
Anionic liposomes	Protein corona	Plasma from patients with NSCLC	LC-MS/MS	74
Anionic, neutral and cationic liposomes	Protein corona	Plasma from patients with pancreatic ductal adenocarcinoma	LC-MS/MS	75
Proteograph superparamagnetic iron oxide nanoparticles	Protein corona	Plasma from patients with NSCLC (100 µl)	LC-MS/MS	76
Gold nanoparticles	Protein corona	Serum from patients with breast cancer	SWATH-MS	77
Silver nanoparticles	Protein corona	Serum from patients with bladder cancer	SWATH-MS	78
cfDNA				
GMACS chip (silica magnetic nanoparticles)	Label free	Serum from patients with breast cancer (500 µl)	Total DNA quantification (qPCR)	81
Polypyrrole-coated gold nanowires	Label free	Plasma from patients with breast or lung cancer (200 µl to 1 ml)	Total DNA quantification (qPCR)	82
CTCs				
CELLSEARCH magnetic nanoparticles	Immunocapture (anti-EpCAM)	Blood from patients with breast, colorectal or prostate cancer (7.5 ml)	Enumeration (ICC)	20–22
First-generation NanoVelcro chip (silicon nanowires)	Immunocapture (anti-EpCAM)	Blood from patients with prostate cancer (1 ml)	Enumeration (ICC)	92
Second-generation NanoVelcro chip (PLGA nanofibres)	Immunocapture (anti-CD146)	Blood from patients with melanoma (1 ml)	Enumeration (ICC) and Sanger sequencing	113
Third-generation NanoVelcro chip (thermosensitive PIPAAm silicon nanowires)	Immunocapture (anti-EpCAM)	Blood from patients with NSCLC (1 ml)	Enumeration (ICC) and Sanger sequencing	91
Fourth-generation NanoVelcro chip (PEDOT nanosubstrate)	Immunocapture (anti-EpCAM)	Blood from patients with prostate cancer	RT-qPCR	114
Biomimetic immuno-magnetic Fe_3O_4 nanoparticles coated with graphene sheets and leukocyte membrane fragments	Immunocapture (anti-EpCAM)	Blood from patients with epithelial cancers (1.5 ml)	Enumeration (ICC)	84
Magnetic nanowires	Immunocapture (anti-EpCAM, anti-EGFR, anti-N-cadherin, anti-TROP2 and anti-vimentin)	Blood from patients with non-metastatic, early stage breast cancer (200 µl to 1 ml)	Enumeration (ICC)	85
MagRC chip (magnetic nanoparticles)	Immunocapture (anti-EpCAM)	Blood from patients with prostate cancer (1 ml)	Enumeration (ICC)	86
Magnetic nanoparticles	Immunocapture (anti-EpCAM)	Blood from patients with prostate cancer (1 ml)	Enumeration (ICC)	87
PEGylated immuno-magnetic Fe_3O_4 nanospheres coated with quantum dots and PEG	Immunocapture (anti-EpCAM)	Blood from patients with epithelial-phenotype cancers (1.5 ml)	Enumeration (ICC)	88
NP-^{HB}CTC-chip (gold nanoparticles)	Immunocapture (anti-EpCAM, anti-HER2 and anti-EGFR)	Blood from patients with metastatic breast cancer (3.5 ml)	Enumeration (ICC) and RNA sequencing	90
TiO_2 nanofibres	Immunocapture (anti-EpCAM)	Blood from patients with colorectal or gastric cancer (1 ml)	Enumeration (ICC)	95

Table 1 (cont.) | **Example studies using nano-omics approaches for the analysis of liquid biopsy analytes**

Nanomaterial platform	Enrichment mechanism	Biofluid sampled (volume, if reported)	Downstream analysis	Refs
CTCs (cont.)				
PL–PEG–NH_2-functionalized graphene oxide nanosheets	Immunocapture (anti-EpCAM)	Blood from patients with lung, breast or pancreatic cancer (1 ml)	Enumeration (ICC) and RT-qPCR	96
Carbon nanotube-chip	Label free	Blood from patients with breast cancer (4.0–8.5 ml)	Enumeration (ICC)	97
EVs				
Nano-interfaced microfluidic exosome (nanoIMEX) chip (graphene oxide–polydopamine coated)	Immunocapture (anti-CD81)	Plasma from patients with ovarian cancer (2 µl)	ELISA	98
Nano-HB chip (silicon nanoparticles)	Immunocapture (anti-CD81)	Plasma from patients with ovarian cancer (100 µl)	ELISA and RT-ddPCR	28
Magnetic nanowires	Immunocapture (anti-CD9, anti-CD63 and anti-CD81)	Plasma from patients with lung cancer (250 µl)	RT-qPCR	89
NanoVilli chip (silicon nanowires)	Immunocapture (anti-EpCAM)	Blood from patients with NSCLC (200 µl)	RT-ddPCR	93
EV Click chip (silicon nanowires)	Immunocapture (anti-EpCAM, anti-ASGPR1 and anti-CD147)	Plasma from patients with hepatocellular carcinoma (0.5 ml)	RT-ddPCR	94
Multi-analyte				
PEGylated liposomes	Biomolecule corona (proteins and cfDNA)	Plasma from patients with ovarian cancer (1 ml)	Protein: LC-MS/MS cfDNA: total DNA quantification (qPCR)	79
Magnetic polypyrrole nanowires	cfDNA: label-free (using PEI-conjugated nanowires) CTCs: immunocapture (antibody cocktail)	Blood from patients with NSCLC (cfDNA: 300 µl; CTCs: 1 ml)	CTCs enumeration (ICC and IHC) cfDNA and CTC-derived DNA: ddPCR	80

ASGPR1, asialoglycoprotein receptor 1; cfDNA, circulating cell-free DNA; CTC, circulating tumour cell; ddPCR, droplet digital PCR; ELISA, enzyme-linked immunosorbent assay; EpCAM, epithelial cell adhesion molecule; EV, extracellular vesicle; ICC, immunocytochemistry; IHC, immunohistochemistry; LC-MS/MS, liquid chromatography with tandem mass spectrometry; nano-HB, nanostructured herringbone; NP-^{HB}CTC-chip, nanoparticle herringbone circulating tumour cell chip; NSCLC, non-small-cell lung cancer; PEDOT, poly(3,4-ethylenedioxythiophene); PEG, polyethylene glycol; PEI, polyethylenimine; PIPAAm, poly(*N*-isopropylacrylamide); PLGA, poly(lactic-co-glycolic acid); PL, phospholipid; qPCR, quantitative PCR; RT-ddPCR, reverse transcription droplet digital PCR; RT-qPCR real-time quantitative reverse transcription PCR; SWATH-MS, sequential window acquisition of all theoretical mass spectra; TROP2, tumour-associated calcium signal transducer 2.

be investigated, these findings highlight an opportunity for the development of a nano-proteogenomic harvesting platform technology able to simultaneously enrich and purify both plasma proteins and cell-free nucleic acids.

Only a few alternative approaches using nanoparticles to purify cfDNA from blood are being explored, including the development of cationic magnetic nanowire systems[80–82]. In a proof-of-principle study, such a nano-purification method outperformed the gold-standard QIAamp Circulating Nucleic Acid Kit in the harvesting of cfDNA for detection of *EGFR* mutations by droplet digital PCR[80]. Moreover, the co-isolation of CTCs and cfDNA from the blood of patients with non-small-cell lung cancer exemplified the potential to enrich for multiple analytes using a single nanoparticle platform[80]. Other studies demonstrating the interaction of gold nanoparticles with methylated DNA have also laid the groundwork for the exploitation of the bio–nano interface to detect cancer-specific methylation patterns in cfDNA[83].

CTC and EV isolation. Key to the clinical translation of CTCs and EVs as liquid biopsy analytes is their efficient retrieval and purification from the blood of patients with cancer, which presents nanotechnologists with an engineering innovation challenge. The gold-standard CTC immunocapture-based approaches fail to harvest heterogeneous populations of functionally viable CTCs. As a result, the current clinical utility of CTCs is simply based on their detection and enumeration among vast quantities of haematopoietic cells, and only in patients with high-burden, metastatic disease. Despite the greater abundance of EVs in blood, their small size and low density pose a unique set of technical challenges. Conventional benchtop EV-purification techniques (such as ultracentrifugation, polymer-induced precipitation and others) mostly rely on their physical properties, take several hours and fail to distinguish cancer-derived EVs from those released by non-malignant cells[25].

To successfully capitalize on the cancer specificity of CTCs and certain EV subsets, numerous attempts have been made to enhance the capture and isolation of blood CTCs and EVs, along with their genomic, transcriptomic and proteomic cargoes, using nano-omics approaches. The majority of these harvesting strategies entail coating the nanoparticle surface with antibodies targeting well-known CTC and EV surface antigens (such as EpCAM, HER2, CD9, CD81 and CD63). An extensive range of nanotechnologies have been developed to capture blood CTCs and EVs (TABLE 1 and FIG. 3), including magnetic[84–89], gold[90], silicon[28,89,91–94], titanium dioxide (TiO_2)[95] and carbon[96–98] nanomaterial platforms, with varying degrees of design sophistication and success. To address the issue relating to the inherent heterogeneity of CTCs and

to increase capture efficiency, functionalization of the same nanoparticle platforms with mixtures of different antibodies has been also suggested[85,90]. For example, magnetic nanowires labelled with antibody cocktails have been shown to effectively isolate early stage, non-metastatic breast cancer-derived CTCs from a 250-μl blood sample with 100% efficiency (29 out of 29 patients)[85].

Antibody-targeted nanoparticles have also been integrated into microfluidic devices, which require lower sample volumes and have a higher sensitivity of detection compared with standard methods of CTC or EV isolation, and can be engineered to have multistep functionality (for example, analyte separation, identification and detection)[28,86]. Examples of such nanoparticle-based platforms include the magnetic ranking cytometry-based microfluidic chip designed by Poudineh et al.[86] to profile CTCs on the basis of their surface protein expression phenotype, and the Nano-HB microfluidic chip with self-assembled 3D herringbone nanopatterns that Zhang et al.[28] developed to detect low levels of tumour-associated exosomes in plasma from patients with ovarian cancer. Microfluidic chips that combine nanoparticle-enabled isolation of CTCs and/or EVs and downstream intracellular and/or vesicular omics profiling are also in development and are gradually evolving into integrative multispecies analysis platforms[52].

The multimodal engineering capacity that nanomaterials offer has enabled the simultaneous capture and visualization of cancer analytes from complex biological fluids as well as stimulus-responsive detachment and sampling of the captured analytes for further analysis. An example of a multifunctional nanoparticle platform is the luminescent polyethylene glycol-functionalized immuno-magnetic nanospheres developed by Zhou et al.[88] to enable high-resolution visualization of CTCs isolated from peripheral blood samples of patients with $EpCAM^+$ epithelial cancers. The deposition of quantum dots onto these magnet-responsive Fe_3O_4 nanoparticles enabled monitoring of the CTC recovery process in real time in addition to their magnetic separation from blood[88]. Finally, a disulfide bond-containing linker was used for the attachment of the anti-EpCAM antibody onto the surface of these nanoparticle constructs to enable glutathione-mediated release of viable CTCs[88].

Beyond these epithelial marker-dependent technologies, marker-independent approaches that exploit the high affinity of CTCs for bare carbon-based nanoparticle surfaces have also been reported and are expected to capture a wider range of CTC subtypes, permitting characterization of their unique metastatic potential. For example, in a proof-of-concept study, Loeian et al.[97] developed a carbon nanotube-CTC-chip able to successfully capture heterogeneous CTCs with various phenotypes (according to differential expression of cytokeratin 8 and/or 18, EGFR, and HER2) from 4-ml or 8.5-ml blood samples (0.5–28 CTCs per millilitre of blood obtained from 7 patients with stage I–IV breast cancer). More optimization work will be needed to enable the adhered CTCs to be released from the nanotube-CTC-chip and purified from contaminant white blood cells for subsequent omics analyses.

Thus, ample evidence indicates that nanotechnology solutions can enhance sampling of the blood-circulating cancerome. Nevertheless, more rigorous work is needed on the downstream proteogenomic analysis of the harvested CTCs and EVs for the promise of nano-omics approaches to be fully realized in the context of early cancer detection.

The nano-omics vision and challenges

The rise of multi-omics liquid biopsy analyses is gradually revolutionizing the way in which we capture the vast complexity of the cancerome. Blood-based multi-omics profiling of cancer has the potential to ultimately encompass genomics, epigenomics, proteomics, lipidomics and

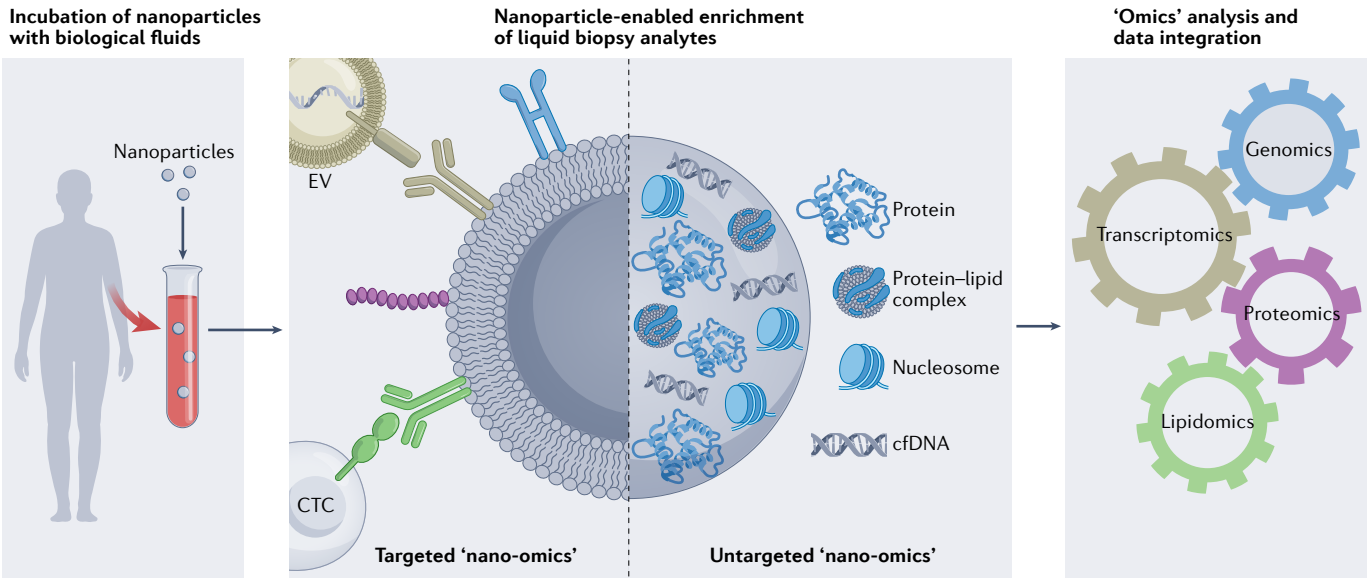


Fig. 2 | **The nano-omics paradigm.** Schematic summarizing the 'nano-omics' approach, whereby nanomaterials are utilized as scavenging platforms to capture, enrich and isolate cancer-associated analytes from biological fluids for downstream omics analyses. 'Targeted nano-omics' requires functionalization of the nanomaterial surface with targeting moieties to capture specific cancer analytes, whereas 'untargeted nano-omics' relies on the non-specific, spontaneous adsorption of cancer analytes onto the nanoparticle surface (known as biomolecule corona formation). Nanomaterial-based harvesting platforms can simultaneously enrich cancer-specific genomic, transcriptomic, proteomic and lipidomic signatures from a single peripheral blood sample (and potentially other biological fluids). The nano-omics approach seeks to apply the knowledge garnered at the bio–nano interface in order to enable the multi-omics analysis of complex biological fluids, with the ultimate goal of unveiling novel multi-analyte biomarker panels for early cancer detection. cfDNA, circulating cell-free DNA; CTC, circulating tumour cells; EV, extracellular vesicle.

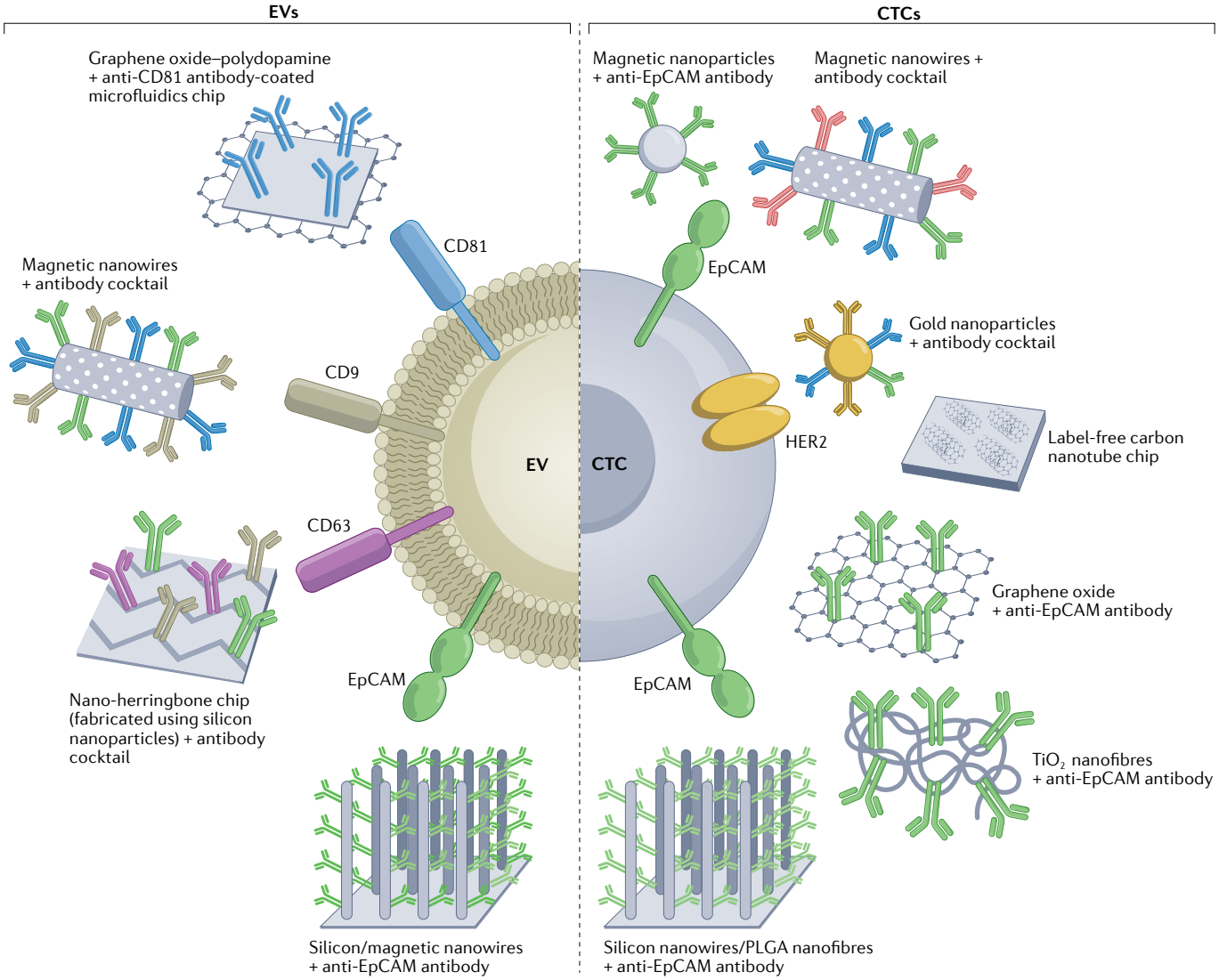


Fig. 3 | **Nanomaterial-based isolation of blood EVs and CTCs.** Schematic summary of several nanotechnologies developed to facilitate the enrichment of extracellular vesicles (EVs) and circulating tumour cells (CTCs) from blood samples. The majority of EV and CTC enrichment strategies are based on surface functionalization of nanoparticles or nanowires with specific targeting moieties (typically antibodies); however, label-free enrichment methods have also been proposed. Specific surface ligands targeted on CTCs and EVs include epithelial cell adhesion molecule (EpCAM), HER2, CD9, CD63 and CD81. PLGA, poly(lactic-co-glycolic acid).

metabolomics signatures, providing greater insight into tumorigenesis and increased sensitivity for early detection[99] (FIG. 1). The vanishingly low amounts of liquid biopsy analytes in blood mandate the development of novel technologies to enable the enrichment of the cancerome, while also minimizing the sample volume required.

The nano-omics approach is herein introduced and defined as the nanotechnology-enabled isolation of analytes from biological fluids for subsequent (multi)-omics analysis (FIG. 2). Nano-omics seeks to apply the knowledge garnered at the bio–nano interface to offer a comprehensive analysis of the disease-specific analytes or analyte signatures present in blood and other biological fluids. The ultimate goal of nano-omics is to generate integrated multi-omics knowledge with high informative power and to unveil novel panels of molecular biomarkers.

Nanotechnology-based platforms have shown great promise in the enrichment of CTCs and EVs from blood and, more recently, in unveiling the previously hidden blood proteome. While targeted nano-omics approaches — through functionalization of nanoparticles with targeting moieties — have been developed mainly to capture blood CTCs and EVs, more recent efforts utilizing nanoparticles for blood proteomic analysis have been based on the non-targeted, spontaneous phenomenon of protein corona formation (BOX 1). According to this strategy, nanoparticles act as 'fishing nano-nets' that capture the LMW blood proteome, thereby addressing the signal-to-noise challenge that has plagued label-free proteomics analyses to date[70,71,73,100].

The nanotechnology community has begun to look beyond the well-characterized protein corona and is now interrogating the spontaneous interactions of nanoparticles with other biomolecule species (including lipids, metabolites and cfDNA) that collectively constitute the so-called biomolecule corona[101–110]. The complex molecular fingerprint offered by the biomolecule corona presents nanotechnologists with an exciting

opportunity to develop nanoscale platforms for the multi-omics analysis of blood. Although much work remains to be done, we envision that future nanoparticle-based scavenging platforms will simultaneously capture cancer-specific genomic, transcriptomic, proteomic and lipidomic information from a single biofluid specimen (FIG. 2).

The utility of the nanoparticle biomolecule corona as a tool for biomarker discovery at multiple omics layers could be deployed across a range of biomarker applications and pressing clinical challenges[71,73,100,111]. For early disease detection, in particular, nano-omics offers an integrated solution to analyse the entire circulating cancerome via a single blood draw, while also exploring the role of alternative circulating biomolecules that are poorly understood in cancer such as lipids and metabolites. Unlike other nanoparticle-based biosensing technologies designed to capture and quantify already-known cancer-associated analytes, the nano-omics 'blood-mining' approach has the potential to accelerate the discovery phase of the biomarker development pipeline. To drive the development of such nanoscale blood-based scavenging platforms, the nanoscience community needs to focus on the translational potential of the extensive catalogue of nanomaterials at their disposal.

While nano-omics could address some of the technological obstacles associated with liquid biopsy analyses, other challenges are emerging as limiting factors that impede the clinical translation of cancer biomarkers. These hurdles include the need for high-dimensional machine learning-based bioinformatics approaches to integrate the large and distinct datasets obtained from multi-omics analyses of individual samples as well as the development of multi-analyte devices applicable for clinical use. Indeed, the Cancer Research UK roadmap for early cancer detection highlights the need for a holistic approach in the intersectional research space of basic and molecular biology, assay technology, and machine learning[112]. Transitioning from bench to bedside requires the amalgamation of multisector networks encompassing academic research, industry, research funders, regulators and health-care professionals[112]. The discovery phase of biomarker development is often initiated in academic labs and frequently results in the identification of multiple biomarker candidates. Translating these discoveries into clinical assays with multiplexing capabilities requires tremendous resources for analytical and clinical validation studies in large cohorts of patients, necessitating substantial input from all stakeholders mentioned above[112].

Last but not least, the validation phase of the biomarker pipeline is largely dependent on specimen availability, which can present a particular challenge for studies of early stage cancers (given that blood samples are not routinely collected from patients with such cancers). Specimen collection, processing and storage processes pose additional challenges with regards to analytical reproducibility in the validation phase. Finally, an important consideration with cancer screening approaches relates to the value of integrating liquid biopsy analyses with standard imaging-based screening practices. Such multimodal early detection approaches hold the greatest potential to provide precise information on the localization and size of the tumour and to address the issue of overdiagnosis.

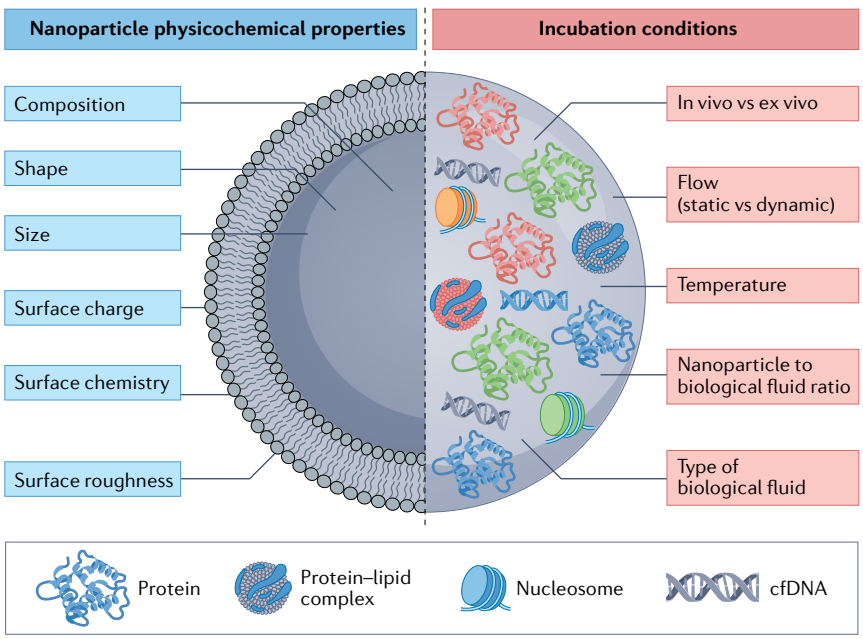
Conclusions

It is becoming clear that integrated multi-omics signatures derived from liquid biopsy samples are the future of precision medicine and early cancer detection. Thanks to major advances in both omics analytical tools and machine learning-based bioinformatics approaches, liquid biopsy has the potential to overcome many of the limitations associated with tissue biopsy sampling, including by better capturing and reflecting tumour heterogeneity. The use of nanotechnology for cancer biomarker discovery remains in its infancy, yet the use of nanoparticles as harvesting agents of the blood-circulating cancerome (proteins, ctDNA, CTCs, EVs and others) offers enormous potential and could redefine the future of early cancer detection. The nano-omics approach that we have defined herein exploits targeted and untargeted interactions at the bio–nano interface to unveil potentially novel multi-omics biomarker panels and decipher multidimensional information embedded in the omics data. The development of integrative bioinformatics data analysis tools as well as the availability of human biospecimens and multi-analyte tests needed for the validation phase of the biomarker pipeline will be key to the clinical translation of this nano-omics paradigm.

Box 1 | **The nanoparticle biomolecule corona**

The 'biomolecule corona' refers to the spontaneous adsorption and self-assembled layering of various biomolecules onto the surface of nanoparticles upon their incubation with a biological fluid. The adsorption of proteins onto nanoparticles is referred to as the 'protein corona'.

The composition of the biomolecule corona is affected by numerous factors. Specifically, the composition is defined by the various physicochemical properties of the nanoparticles as well as the incubation conditions of the nanoparticles in the biological fluid (Figure). cfDNA, cell-free DNA.



Lois Gardner[1,2], Kostas Kostarelos[1,3], Parag Mallick[4], Caroline Dive[2] and Marilena Hadjidemetriou[1]✉

[1]Nanomedicine Lab, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK.

[2]Cancer Research UK Manchester Institute Cancer Biomarker Centre, The University of Manchester, Manchester, UK.

[3]Catalan Institute of Nanoscience & Nanotechnology (ICN2), UAB Campus, Barcelona, Spain.

[4]Canary Center at Stanford for Cancer Early Detection, Stanford University, California, USA.

✉*e-mail: marilena.hadjidemetriou@manchester.ac.uk*

https://doi.org/10.1038/s41571-022-00645-x

Published online: 23 June 2022

1. Ferlay, J. et al. Cancer statistics for the year 2020: an overview. *Int. J. Cancer* **149**, 778–789 (2021).
2. Lo, Y. M. D. & Lam, W. K. J. Towards multi-cancer screening using liquid biopsies. *Nat. Rev. Clin. Oncol.* **17**, 525–526 (2020).
3. Hristova, V. A. & Chan, D. W. Cancer biomarker discovery and translation: proteomics and beyond. *Expert. Rev. Proteom.* **16**, 93–103 (2019).
4. Duffy, M. J. Serum tumor markers in breast cancer: are they of clinical value? *Clin. Chem.* **52**, 345–351 (2006).
5. Keedy, V. L. et al. American Society of Clinical Oncology provisional clinical opinion: epidermal growth factor receptor (EGFR) mutation testing for patients with advanced non-small-cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy. *J. Clin. Oncol.* **29**, 2121–2127 (2011).
6. Tarone, R. E., Chu, K. C. & Brawley, O. W. Implications of stage-specific survival rates in assessing recent declines in prostate cancer mortality rates. *Epidemiology* **11**, 167–170 (2000).
7. Zhang, Z. & Chan, D. W. The road from discovery to clinical diagnostics: lessons learned from the first FDA-cleared in vitro diagnostic multivariate index assay of proteomic biomarkers. *Cancer Epidemiol. Biomark. Prev.* **19**, 2995 (2010).
8. Chen, X. et al. Non-invasive early detection of cancer four years before conventional diagnosis using a blood test. *Nat. Commun.* **11**, 3475 (2020).
9. Olivier, M., Asmis, R., Hawkins, G. A., Howard, T. D. & Cox, L. A. The need for multi-omics biomarker signatures in precision medicine. *Int. J. Mol. Sci.* **20**, 4781 (2019).
10. Campos-Carrillo, A. et al. Circulating tumor DNA as an early cancer detection tool. *Pharmacol. Ther.* **207**, 107458 (2020).
11. Cayer, D. M., Nazor, K. L. & Schork, N. J. Mission critical: the need for proteomics in the era of next-generation sequencing and precision medicine. *Hum. Mol. Genet.* **25**, R182–R189 (2016).
12. Calsina, B. et al. Integrative multi-omics analysis identifies a prognostic miRNA signature and a targetable miR-21-3p/TSC2/ mTOR axis in metastatic pheochromocytoma/ paraganglioma. *Theranostics* **9**, 4946–4958 (2019).
13. Wang, H. et al. Deep multiomics profiling of brain tumors identifies signaling networks downstream of cancer driver genes. *Nat. Commun.* **10**, 3718 (2019).
14. Ankney, J. A., Xie, L., Wrobel, J. A., Wang, L. & Chen, X. Novel secretome-to-transcriptome integrated or secreto-transcriptomic approach to reveal liquid biopsy biomarkers for predicting individualized prognosis of breast cancer patients. *BMC Med. Genomics* **12**, 78 (2019).
15. Zhang, B. et al. Proteogenomic characterization of human colon and rectal cancer. *Nature* **513**, 382–387 (2014).
16. Cohen, J. D. et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science* **359**, 926–930 (2018).
17. Lennon, A. M. et al. Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention. *Science* **369**, eabb9601 (2020).
18. Liu, M. C. et al. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. *Ann. Oncol.* **31**, 745–759 (2020).
19. NHS-Galleri Trial. https://www.nhs-galleri.org/about-the-trial (2021).
20. Hayes, D. F. et al. Circulating tumor cells at each follow-up time point during therapy of metastatic breast cancer patients predict progression-free and overall survival. *Clin. Cancer Res.* **12**, 4218–4224 (2006).
21. Cohen, S. J. et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J. Clin. Oncol.* **26**, 3213–3221 (2008).
22. De Bono, J. S. et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin. Cancer Res.* **14**, 6302–6309 (2008).
23. Cristofanilli, M. 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.* **134**, 39–45 (2019).
24. Lim, S. et al. Liquid biopsy: one cell at a time. *NPJ Precis. Oncol.* **3**, 23 (2019).
25. Dai, J. et al. Exosomes: key players in cancer and potential therapeutic strategy. *Signal. Transduct. Target. Ther.* **5**, 145 (2020).
26. Wang, J., Ma, P., Kim, D. H., Liu, B. F. & Demirci, U. Towards microfluidic-based exosome isolation and detection for tumor therapy. *Nano Today* **37**, 101066 (2021).
27. Ko, J. et al. Single extracellular vesicle protein analysis using immuno-droplet digital polymerase chain reaction amplification. *Adv. Biosyst.* **4**, 1900307 (2020).
28. Zhang, P. et al. Ultrasensitive detection of circulating exosomes with a 3D-nanopatterned microfluidic chip. *Nat. Biomed. Eng.* **3**, 438–451 (2019).
29. Chen, Y. et al. Exosome detection via the ultrafast-isolation system: EXODUS. *Nat. Methods* **18**, 212–218 (2021).
30. Wu, X. et al. Exosome-templated nanoplasmonics for multiparametric molecular profiling. *Sci. Adv.* **6**, eaba2556 (2020).
31. Kang, Y.-T. et al. Dual-isolation and profiling of circulating tumor cells and cancer exosomes from blood samples with melanoma using immunoaffinity-based microfluidic interfaces. *Adv. Sci.* **7**, 2001581 (2020).
32. Lim, J., Choi, M., Lee, H. J., Han, J. Y. & Cho, Y. A novel multifunctional nanowire platform for highly efficient isolation and analysis of circulating tumor-specific markers. *Front. Chem.* **7**, 664 (2019).
33. McLendon, R. et al. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* **455**, 1061–1068 (2008).
34. Corces, M. R. et al. The chromatin accessibility landscape of primary human cancers. *Science* **362**, eaav1898 (2018).
35. Kahles, A. et al. Comprehensive analysis of alternative splicing across tumors from 8,705 Patients. *Cancer Cell* **34**, 211–224.e6 (2018).
36. Peng, X. et al. Molecular characterization and clinical relevance of metabolic expression subtypes in human cancers. *Cell Rep.* **23**, 255–269.e4 (2018).
37. Hoadley, K. A. et al. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. *Cell* **158**, 929–944 (2014).
38. Rodriguez, H., Zenklusen, J. C., Staudt, L. M., Doroshow, J. H. & Lowy, D. R. The next horizon in precision oncology: proteogenomics to inform cancer diagnosis and treatment. *Cell* **184**, 1661–1670 (2021).
39. Tweedie, S. et al. Genenames.org: the HGNC and VGNC resources in 2021. *Nucleic Acids Res.* **49**, D939–D946 (2021).
40. Wang, L. B. et al. Proteogenomic and metabolomic characterization of human glioblastoma. *Cancer Cell* **39**, 509–528.e20 (2021).
41. Rudnick, P. A. et al. A description of the clinical proteomic tumor analysis consortium (CPTAC) common data analysis pipeline. *J. Proteome Res.* **15**, 1023–1032 (2016).
42. Tariq, M. U. et al. Methods for proteogenomics data analysis, challenges, and scalability bottlenecks: a survey. *IEEE Access.* **9**, 5497–5516 (2021).
43. Eicher, T. et al. Challenges in proteogenomics: a comparison of analysis methods with the case study of the DREAM proteogenomics sub-challenge. *BMC Bioinforma.* **20**, 669 (2019).
44. Subramanian, I., Verma, S., Kumar, S., Jere, A. & Anamika, K. Multi-omics data integration, interpretation, and its application. *Bioinform. Biol. Insights* **14**, 1177932219899051 (2020).
45. Goble, C. et al. FAIR computational workflows under a creative commons attribution 4.0 international (CC BY 4.0) license. *Work. Data Intell.* **2**, 108–121 (2020).
46. Chambers, M. C. et al. An accessible proteogenomics informatics resource for cancer researchers. *Cancer Res.* **77**, e43–e46 (2017).
47. Srivastava, A. et al. Semantic workflows for benchmark challenges: enhancing comparability, reusability and reproducibility. *Pacific Symposium on Biocomputing* vol. 24 208–219 (World Scientific Publishing Co. Pte Ltd, 2019).
48. Huang, S., Chaudhary, K. & Garmire, L. X. More is better: recent progress in multi-omics data integration methods. *Front. Genet.* **8**, 84 (2017).
49. Manzoni, C. et al. Genome, transcriptome and proteome: The rise of omics data and their integration in biomedical sciences. *Brief. Bioinform.* **19**, 286–302 (2018).
50. Bratulic, S., Gatto, F. & Nielsen, J. The translational status of cancer liquid biopsies. *Regen. Eng. Transl. Med.* **7**, 312–352 (2021).
51. Ignatiadis, M., Sledge, G. W. & Jeffrey, S. S. Liquid biopsy enters the clinic — implementation issues and future challenges. *Nat. Rev. Clin. Oncol.* **18**, 297–312 (2021).
52. Li, J. et al. The growing impact of micro/nanomaterial-based systems in precision oncology: translating "multiomics" technologies. *Adv. Funct. Mater.* **30**, 1909306 (2020).
53. Martín-Gracia, B. et al. Nanoparticle-based biosensors for detection of extracellular vesicles in liquid biopsies. *J. Mater. Chem. B* **8**, 6710 (2020).
54. Viswambari Devi, R., Doble, M. & Verma, R. S. Nanomaterials for early detection of cancer biomarker with special emphasis on gold nanoparticles in immunoassays/sensors. *Biosens. Bioelectron.* **68**, 688–698 (2015).
55. Moro, L., Turemis, M., Marini, B., Ippodrino, R. & Giardi, M. T. Better together: strategies based on magnetic particles and quantum dots for improved biosensing. *Biotechnol. Adv.* **35**, 51–63 (2017).
56. Kelley, S. O. et al. Advancing the speed, sensitivity and accuracy of biomolecular detection using multi-length-scale engineering. *Nat. Nanotechnol.* **9**, 969–980 (2014).
57. Yaari, Z. et al. A perception-based nanosensor platform to detect cancer biomarkers. *Sci. Adv.* **7**, eabj0852 (2021).
58. Kim, M. et al. Detection of ovarian cancer via the spectral fingerprinting of quantum-defect-modified carbon nanotubes in serum by machine learning. *Nat. Biomed. Eng.* **6**, 267–275 (2022).
59. Hanash, S. M., Pitteri, S. J. & Faca, V. M. Mining the plasma proteome for cancer biomarkers. *Nature* **452**, 571–579 (2008).
60. Paul, J. & Veenstra, T. D. Separation of serum and plasma proteins for in-depth proteomic analysis. *Separations* **9**, 89 (2022).
61. Liotta, L. A., Ferrari, M. & Petricoin, E. Written in blood. *Nature* **425**, 905 (2003).
62. Fredolini, C. et al. Investigation of the ovarian and prostate cancer peptidome for candidate early detection markers using a novel nanoparticle biomarker capture technology. *AAPS J.* **12**, 504–518 (2010).
63. Longo, C. et al. Core-shell hydrogel particles harvest, concentrate and preserve labile low abundance biomarkers. *PLoS One* **4**, e4763 (2009).
64. Tamburro, D. et al. Multifunctional core-shell nanoparticles: discovery of previously invisible biomarkers. *J. Am. Chem. Soc.* **133**, 19178–19188 (2011).
65. Cedervall, T. et al. Understanding the nanoparticle-protein corona using methods to quntify exchange rates and affinities of proteins for nanoparticles. *Proc. Natl Acad. Sci. USA* **104**, 2050–2055 (2007).
66. Hadjidemetriou, M. & Kostarelos, K. Nanomedicine: evolution of the nanoparticle corona. *Nat. Nanotechnol.* **12**, 288–290 (2017).
67. García-Álvarez, R., Hadjidemetriou, M., Sánchez-Iglesias, A., Liz-Marzán, L. M. & Kostarelos, K. In vivo formation of protein corona on gold nanoparticles. the effect of their size and shape. *Nanoscale* **10**, 1256–1264 (2018).
68. Hadjidemetriou, M. et al. In vivo biomolecule corona around blood-circulating, clinically used and antibody-targeted lipid bilayer nanoscale vesicles. *ACS Nano* **9**, 8142–8156 (2015).
69. Kamaly, N., Farokhzad, O. C. & Corbo, C. Nanoparticle protein corona evolution: from biological impact to biomarker discovery. *Nanoscale* **14**, 1606–1620 (2022).
70. Hadjidemetriou, M. et al. The human in vivo biomolecule corona onto pegylated liposomes: a proof-of-concept clinical study. *Adv. Mater.* **31**, e1803335 (2019).
71. Hadjidemetriou, M., Al-ahmady, Z., Buggio, M., Swift, J. & Kostarelos, K. A novel scavenging tool for cancer

biomarker discovery based on the blood-circulating nanoparticle protein corona. *Biomaterials* **188**, 118–129 (2019).
72. Kostarlos, K. & Hadjidemetriou, M. Detection of cancer biomarkers using nanoparticles. Patent WO2018046542A1 (2018).
73. Hadjidemetriou, M. et al. Nano-scavengers for blood biomarker discovery in ovarian carcinoma. *Nano Today* **34**, 100901 (2020).
74. Di Domenico, M. et al. Nanoparticle-biomolecular corona: a new approach for the early detection of non-small-cell lung cancer. *J. Cell. Physiol.* **234**, 9378–9386 (2019).
75. Digiacomo, L. et al. A proteomic study on the personalized protein corona of liposomes. Relevance for early diagnosis of pancreatic DUCTAL adenocarcinoma and biomarker detection. *J. Nanotheranostics* **2**, 82–93 (2021).
76. Blume, J. E. et al. Rapid, deep and precise profiling of the plasma proteome with multi-nanoparticle protein corona. *Nat. Commun.* **11**, 3662 (2020).
77. Chantada-Vázquez, M. D. P. et al. Protein corona gold nanoparticles fingerprinting reveals a profile of blood coagulation proteins in the serum of her2-overexpressing breast cancer patients. *Int. J. Mol. Sci.* **21**, 8449 (2020).
78. Gómez, B. B. et al. Detection of circulating serum protein biomarkers of non-muscle invasive bladder cancer after protein corona-silver nanoparticles analysis by swath-ms. *Nanomaterials* **11**, 2384 (2021).
79. Gardner, L. et al. The biomolecule corona of lipid nanoparticles contains circulating cell-free DNA. *Nanoscale Horiz.* **5**, 1476–1486 (2020).
80. Lee, H. J. et al. Magnetic nanowire networks for dual-isolation and detection of tumor-associated circulating biomarkers. *Theranostics* **8**, 505–517 (2018).
81. Gwak, H. et al. On-chip isolation and enrichment of circulating cell-free DNA using microfluidic device. *Biomicrofluidics* **13**, 24113 (2019).
82. Jeon, S. H. et al. Efficient capture and isolation of tumor-related circulating cell-free dna from cancer patients using electroactive conducting polymer nanowire platforms. *Theranostics* **6**, 828–836 (2016).
83. Sina, A. A. I. et al. Epigenetically reprogrammed methylation landscape drives the DNA self-assembly and serves as a universal cancer biomarker. *Nat. Commun.* **9**, 4915 (2018).
84. Zhou, X. et al. Leukocyte-repelling biomimetic immunomagnetic nanoplatform for high-performance circulating tumor cells isolation. *Small* **15**, 1900558 (2019).
85. Hong, W., Lee, S., Chang, H. J., Lee, E. S. & Cho, Y. Multifunctional magnetic nanowires: a novel breakthrough for ultrasensitive detection and isolation of rare cancer cells from non-metastatic early breast cancer patients using small volumes of blood. *Biomaterials* **106**, 78–86 (2016).
86. Poudineh, M. et al. Tracking the dynamics of circulating tumour cell phenotypes using nanoparticle-mediated magnetic ranking. *Nat. Nanotechnol.* **12**, 274–281 (2017).
87. Mohamadi, R. M. et al. Nanoparticle-mediated binning and profiling of heterogeneous circulating tumor cell subpopulations. *Angew. Chem. Int. Ed.* **54**, 139–143 (2015).
88. Zhou, X. et al. Multifunctional luminescent immuno-magnetic nanoparticles: toward fast, efficient, cell-friendly capture and recovery of circulating tumor cells. *J. Mater. Chem. B* **7**, 393–400 (2019).
89. Lim, J. et al. Direct isolation and characterization of circulating exosomes from biological samples using magnetic nanowires. *J. Nanobiotechnol.* **17**, 1 (2019).
90. Park, M. H. et al. Enhanced isolation and release of circulating tumor cells using nanoparticle binding and ligand exchange in a microfluidic chip. *J. Am. Chem. Soc.* **139**, 2741–2749 (2017).
91. Ke, Z. et al. Programming thermoresponsiveness of nanovelcro substrates enables effective purification of circulating tumor cells in lung cancer patients. *ACS Nano* **9**, 62–70 (2015).
92. Wang, S. et al. Highly efficient capture of circulating tumor cells by using nanostructured silicon substrates with integrated chaotic micromixers. *Angew. Chem. Int. Ed.* **50**, 3084–3088 (2011).
93. Dong, J. et al. Bio-inspired NanoVilli chips for enhanced capture of tumor-derived extracellular vesicles: toward non-invasive detection of gene alterations in non-small cell lung cancer. *ACS Appl. Mater. Interfaces* **11**, 13973–13983 (2019).
94. Sun, N. et al. Purification of HCC-specific extracellular vesicles on nanosubstrates for early HCC detection by digital scoring. *Nat. Commun.* **11**, 4489 (2020).
95. Zhang, N. et al. Electrospun TiO2 nanofiber-based cell capture assay for detecting circulating tumor cells from colorectal and gastric cancer patients. *Adv. Mater.* **24**, 2756–2760 (2012).
96. Yoon, H. J. et al. Sensitive capture of circulating tumour cells by functionalized graphene oxide nanosheets. *Nat. Nanotechnol.* **8**, 735–741 (2013).
97. Loeian, M. S. et al. Liquid biopsy using the nanotube-CTC-chip: capture of invasive CTCs with high purity using preferential adherence in breast cancer patients. *Lab. Chip* **19**, 1899–1915 (2019).
98. Zhang, P., He, M. & Zeng, Y. Ultrasensitive microfluidic analysis of circulating exosomes using a nanostructured graphene oxide/polydopamine coating. *Lab. Chip* **16**, 3033–3042 (2016).
99. Schweiger, M. R., Kerick, M., Timmermann, B. & Isau, M. The power of NGS technologies to delineate the genome organization in cancer: from mutations to structural variations and epigenetic alterations. *Cancer Metastasis Rev.* **30**, 199–210 (2011).
100. Papafilippou, L., Claxton, A., Dark, P., Kostarelos, K. & Hadjidemetriou, M. Protein corona fingerprinting to differentiate sepsis from non-infectious systemic inflammation. *Nanoscale* **12**, 10240–10253 (2020).
101. Chetwynd, A. J. & Lynch, I. The rise of the nanomaterial metabolite corona, and emergence of the complete corona. *Environ. Sci. Nano* **7**, 1041–1060 (2020).
102. Chetwynd, A. J., Zhang, W., Thorn, J. A., Lynch, I. & Ramautar, R. The nanomaterial metabolite corona determined using a quantitative metabolomics approach: a pilot study. *Small* **16**, 2000295 (2020).
103. Raesch, S. S. et al. Proteomic and lipidomic analysis of nanoparticle corona upon contact with lung surfactant reveals differences in protein, but not lipid composition. *ACS Nano* **9**, 11872–11885 (2015).
104. Kapralov, A. A. et al. Adsorption of surfactant lipids by single-walled carbon nanotubes in mouse lung upon pharyngeal aspiration. *ACS Nano* **6**, 4147–4156 (2012).
105. Martel, J. et al. Fatty acids and small organic compounds bind to mineralo-organic nanoparticles derived from human body fluids as revealed by metabolomic analysis. *Nanoscale* **8**, 5537–5545 (2016).
106. Pink, M., Verma, N., Kersch, C. & Schmitz-Spanke, S. Identification and characterization of small organic compounds within the corona formed around engineered nanoparticles. *Environ. Sci. Nano* **5**, 1420 (2018).
107. Grintzalis, K., Lawson, T. N., Nasser, F., Lynch, I. & Viant, M. R. Metabolomic method to detect a metabolite corona on amino-functionalized polystyrene nanoparticles. *Nanotoxicology* **13**, 783–794 (2019).
108. La Barbera, G. et al. A comprehensive analysis of liposomal biomolecular corona upon human plasma incubation: the evolution towards the lipid corona. *Talanta* **209**, 120487 (2020).
109. Hellstrand, E. et al. Complete high-density lipoproteins in nanoparticle corona. *FEBS J.* **276**, 3372–3381 (2009).
110. Lee, J. Y. et al. Analysis of lipid adsorption on nanoparticles by nanoflow liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **410**, 6155–6164 (2018).
111. Hadjidemetriou, M. et al. Nanoparticle-enabled enrichment of longitudinal blood proteomic fingerprints in Alzheimer's disease. *ACS Nano* **15**, 7357–7369 (2021).
112. Lyons, N. et al. Early Detection and Diagnosis of Cancer: A roadmap to the future. *Cancer Research UK* https://www.cancerresearchuk.org/funding-for-researchers/research-opportunities-in-early-detection-and-diagnosis/early-detection-and-diagnosis-roadmap (2021).
113. Hou, S. et al. Polymer nanofiber-embedded microchips for detection, isolation, and molecular analysis of single circulating melanoma cells. *Angew. Chem. Int. Ed.* **52**, 3379–3383 (2013).
114. Shen, M. Y. et al. Glycan stimulation enables purification of prostate cancer circulating tumor cells on PEDOT NanoVelcro chips for RNA biomarker detection. *Adv. Healthc. Mater.* https://doi.org/10.1002/adhm.201700701 (2018).

Acknowledgements
The authors acknowledge funding support from the NIHR Manchester Biomedical Research Centre (BRC) and the Cancer Research UK International Alliance for Cancer Early Detection (ACED; EICEDAAP\100013).

Author contributions
L.G. researched data for the article; L.G., K.K. and M.H. made substantial contributions to the discussion of content; L.G., P.M. and M.H. wrote the manuscript; and L.G., K.K., C.D. and M.H. edited the manuscript before submission. M.H. took responsibility for revising the manuscript after submission.

Competing interests
The authors declare no competing interests.

Peer review information
Nature Reviews Clinical Oncology thanks D. Heller, J.X.J. Zhang and the other, anonymous, reviewers for their contribution to the peer review of this work.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.