

# Brave new world of cfDNA-omics for early cancer detection

Christian Rolfo ,<sup>1</sup> Alessandro Russo <sup>2</sup>

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Early cancer detection still represents a mirage for most individuals with solid tumors, as effective and approved screening programs are available only for patients with breast, cervical, prostate, and colorectal cancers, as well as lung cancer in high-risk individuals. In addition to the limited set of diseases covered, adherence to currently approved screening tests is in some cases low due to invasive procedures and/or costs.

Liquid biopsy has recently entered clinical practice in solid tumors as a minimally invasive method for cancer genotyping through the analysis of cell-free DNA (cfDNA) either at baseline or after acquired resistance to targeted therapies, with the most prominent example represented by non-small cell lung cancer.<sup>1</sup> The term liquid biopsy encompasses a wide variety of components that can be isolated and analyzed from biofluids, including not only cfDNA, but also circulating tumor cells, tumor educated platelets, microRNAs, cell-free RNA, tumor-derived metabolites, and extracellular vesicles. Among these, cfDNA is the most widely used and the most extensively studied for early cancer detection, promising to revolutionize cancer screening. However, the use of this biomarker has been hampered by technical and biological limitations, due to difficulties of intercepting very low quantities of tumor-derived DNA, which can be in some cases, especially early-stage tumors, at a tumor fraction far below the limits of detection of currently available technologies for cfDNA analysis.<sup>2</sup> Two different approaches have been used to date for studying cfDNA analysis as potential biomarker for early cancer detection, depending on the need of knowing in advance the tumor mutation profile through whole exome sequencing of tissue (tumor-informed approach) or not (tumor-uninformed approach) (figure 1).<sup>3</sup> In order to avoid the risk of false positive detection, due to the identification of non-tumor-derived mutations, such as those associated with clonal hematopoiesis of indeterminate

potential (CHIP), different technical expedients have been used for filtering CHIP mutations, as for example concurrent leukocyte DNA analysis.

In order to overcome some of the limitations with traditional cfDNA-based analyses, two promising approaches have been described for early cancer detection exploiting two peculiar characteristics of circulating tumor DNA: DNA fragment length (fragmentomics)<sup>4</sup> and methylation patterns (methylomics).<sup>5</sup>

The first approach relies on the notion that cancer patients have altered fragmentation profiles as compared with healthy individuals. A machine learning model called 'DNA evaluation of fragments for early interception' (DELFI) was developed to detect a large number of abnormalities in cfDNA examined by whole-genome sequencing (WGS) to determine genome-wide copy number profiles, sequence motifs, and fragmentation profiles between patients with cancer and healthy individuals.<sup>4</sup> The initial results on 236 cancer and 245 healthy individuals showed that the DELFI model was associated with sensitivities of detection ranging from 57% to more than 99% among the seven cancer types included in the study at 98% specificity, potentially identifying the cancer tissue of origin (TOO) in 75% of the cases. Combining DELFI with mutation detection in cfDNA increased the sensitivity to 91% (115 out of 126 patients) with a specificity of 98%.<sup>4</sup> A prospective study in 365 individuals at high risk of developing lung cancer was recently reported (LUCAS cohort) and validated in an independent cohort of 385 non-cancer individuals and 46 patients with lung cancer. Combining carcinoembryonic antigen (CEA) levels, age, smoking history, and presence of chronic obstructive pulmonary disease (COPD) in a multimodal model (DELFI<sub>multi</sub>) followed by low-dose CT (LDCT) was associated with a sensitivity of 94% (stage I=87%, stage II=100%, stage III=97%, and stage IV=96%) and 52% reduction of unnecessary



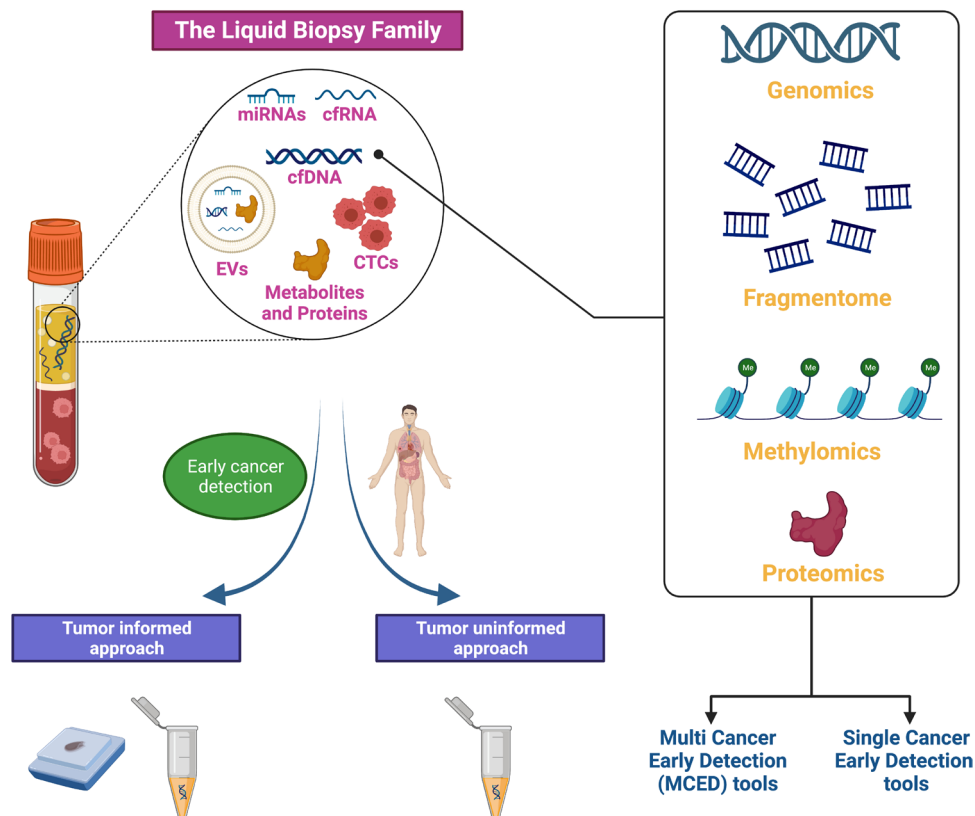
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<sup>1</sup>Center for Thoracic Oncology, Tisch Cancer Institute, Mount Sinai Medical System & Icahn School of Medicine, Mount Sinai, New York, New York, USA

<sup>2</sup>Department of Onco-Hematology, Papardo Hospital, Messina, Italy

## Correspondence to

Dr Christian Rolfo;  
christian.rolfo@mssm.edu



**Figure 1** An overview of the potential role of cfDNA analyses for early cancer detection. cfDNA, cell-free DNA.

procedures with LDCT alone.<sup>6</sup> Two large prospective case–control studies are currently ongoing (DELFI-L201/NCT05306288 and DELFI-L101/NCT04825834) for early lung cancer detection.

Another promising cfDNA-based approach for early cancer detection evaluates the different methylation patterns between cancer patients and healthy individuals. After a discovery phase in which three independent methods were evaluated (targeted sequencing, WGS, and whole genome methylation), the Circulating Cell-free Genome Atlas study developed a whole genome methylation assay and classifier for cancer detection and TOO localization. This multicancer early detection (MCED) assay, named Galleri, was validated in a large-scale clinical study (CGCA substudy 3), enrolling 5309 participants with a 5-year longitudinal follow-up. This MCED assay showed a specificity of 99.5% with a low false-positive rate (0.5%) and a sensitivity across cancer classes and stages of 51.5% overall and 76.3% in the prespecified group of 12 cancer classes. Sensitivity of cancer signal detection increased with stage.<sup>5</sup> The feasibility of MCED screening in healthy individuals with and without additional cancer risk was recently reported in the prospective PATHFINDER study<sup>7</sup> and further large-scale studies are currently underway (NCT05611632, NCT05205967, NCT03085888, NCT03934866). However, sensitivity of this assay for cancer signal detection in potentially curable disease (stage I: 16.8%; stage II: 40.4%)<sup>5</sup> is still quite low and, although this assay demonstrated efficacy among >50 different cancer types, sensitivity was lower outside

the 12 prespecified cancer types. For these reasons, at the moment, this test might represent a valid complement to currently approved single-cancer screening tests, but cannot be used as a stand-alone screening strategy. Further technological developments and implementations to the first version of Galleri are awaited.

Another promising approach is to combine genomics (mutations in 2001 genomic positions) and proteomics (levels of 8 proteins), called CancerSEEK.<sup>8</sup> This MCED assay was evaluated in 1005 patients with non-metastatic (stage I–III), clinically detected cancers of eight different types with a median sensitivity of 70% and a specificity >99% (only 7 of 812 healthy controls scored positive). Accuracy of predictor varied between the different tumor types (higher for colorectal cancer and lower for hepatocellular carcinoma and lung cancer) and stages (43% for stage I cancers vs 73% for stage II and 78% for stage III). The clinical utility of this test is currently under evaluation in large prospective studies (NCT04213326).

Collectively, several cfDNA-omics approaches are under active evaluation to validate and demonstrate the clinical utility of a liquid biopsy-based cancer screening test for early detection. At the moment, all these assays have not reached sufficient sensitivity for potentially curable cancers (stages I–II) to clinically recommend liquid biopsy for cancer screening, at least as stand-alone tests. Combinatorial approaches with currently approved cancer screening tests/procedures are likely to increase the sensitivity and specificity of these blood assays, which might serve as prescreening procedures and/or stratify

high-risk individuals for further diagnostic procedures. In addition, the concurrent use of different liquid biopsy approaches might increase the sensitivity of MCED assays. For instance, a novel method for the early detection of 14 cancer types based on the glycosaminoglycan profiles (GAGomes) has been recently reported. The structures and levels of these polysaccharides are altered by tumors. Using plasma and urine samples from 1260 participants, Bratulic *et al* found that the detection method had a 41.6%–62.3% sensitivity to stage 1 cancer at 95% specificity,<sup>9</sup> suggesting that this novel methodology might have a complementary role with other MCED approaches, due to the peculiarity of detecting cancer types that are usually poor cfDNA-shedders and might increase the sensitivity of identifying cancer signals in stage I tumors. Finally, coupled approaches between liquid biopsy approaches and conventional radiological imaging, such as CT/PET,<sup>10</sup> should be further explored, as it can significantly increase the chances of detecting cancers at a potentially curable stage.

In this special series titled ‘*Liquid Biopsies Coming of Age: Biology, Emerging Technologies, and Clinical Translation*’ in the *Journal for ImmunoTherapy of Cancer*, Velculescu *et al* provide a comprehensive overview of cfDNA approaches for cancer early detection and interception, deciphering the challenges and opportunities for clinical implementation of minimally invasive blood-based screening tests.

**Twitter** Christian Rolfo @ChristianRolfo and Alessandro Russo @Al3ssandroRusso

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#### ORCID iDs

Christian Rolfo <http://orcid.org/0000-0002-7860-8417>

Alessandro Russo <http://orcid.org/0000-0002-3365-1972>

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