Cancer Screening Companies Are Rapidly Proliferating: Are They Ready for Business?

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ABSTRACT

Cancer screening has been a major research front for decades. The classical circulating biomarkers for cancer (such as PSA, CEA, CA125, AFP, etc.) are neither sensitive nor specific and are not recommended for population screening. Recently, circulating tumor DNA (ctDNA) emerged as a new pan-cancer tumor marker, with much promise for clinical applicability. ctDNA released by tumor cells can be used as a proxy of the tumor burden and molecular composition. It has been hypothesized that if ctDNA is extracted from plasma and analyzed for genetic changes, it may form the basis for a non-invasive cancer detection test. Lately, there has been a proliferation of "for-profit" companies that will soon offer cancer screening services. Here, we comment on Grail, Thrive, Guardant, Delfi, and Freenome. Previously, we identified some fundamental difficulties associated with this new technology. In addition, clinical trials are exclusively case–control studies. The sensitivities/specificities/predictive values of the new screening tests have not been well-defined or, the literature-reported values are rather poor. Despite these deficiencies some of the aforementioned companies are already testing patients. We predict that the premature use of ctDNA as a cancer screening tool may add another disappointment in the long history of this field.

Introduction

More than 30 years ago, one of us (E. P. Diamandis) served as director of research and development of a small biotechnology company that had the task of developing *in vitro* diagnostic tests based on time resolved fluorometry, an innovative new technique at that time (1, 2). We made very significant progress in developing an instrument and its associated reagents/kits, and the company's administration was very keen to bring the system to the market, with or without the consent of the company's scientists. They had their reasons. For example, the CEO, whose performance bonus was linked to commercialization, was also under immense pressure from his investors, who were anxious to enter the market and start seeing sales and profits. This has proven to be a disastrous strategy. The product was "almost ready" for clinical use but its thorough evaluation in the field was lacking. When the system was launched, we started seeing problems that we could not see with our in-house evaluations and local small trials. This necessitated bringing the system in and out of the market. Potential customers, who initially believed in the technology and wanted to use it, were disappointed with the frequent failures and eventually gave up. The lesson learned was that if a new medical technology is not ready and thoroughly validated for clinical use, it is better to wait because prospective customers will almost never give you a second chance. We hope that the above mistakes will be avoided, as a number of cancer-screening diagnostic companies are starting to launch their tests to the public.

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Cancer Epidemiol Biomarkers Prev 2022;31:1146–50

doi: 10.1158/1055-9965.EPI-22-0102

Proliferation of cancer screening companies

Cancer screening has been in the forefront of cancer research for more than half a century. There is ample and robust data linking early cancer detection with better clinical outcomes (3). It is thus reasonable to assume that early detection should have a major impact on the global cancer burden. Over the last 20 years, significant improvements in screening have been introduced, including more standardized methods for sample collection, processing and storage, more robust analytics and prospective clinical trials examining the clinical benefit (such as cancer stage migration and improved survival; ref. 4). Yet, the United States Preventive Task Force (USPTF) recommends screening for only 4 cancers, cervical, colon, breast, and lung (5). The new modality for screening for cancer with a multi-cancer test (see below) is a novel approach that encompasses both advantages and disadvantages.

Despite intense efforts over many decades, the classical circulating protein biomarkers for cancer, mostly discovered over 40 years ago (such as PSA, CEA, CA125, AFP, CA19.9, to mention a few) have proven not to be either sensitive or specific biomarkers of cancer, and were deemed to be either not suitable, or controversial, for population screening (in the context of this paper population screening means testing of asymptomatic individuals to identify occult cancer). It is thus of no surprise that practice guidelines for use of tumor markers in the clinic do not recommend the current classical biomarkers for cancer screening (6). Other applications of these conventional biomarkers include patient monitoring and management of therapy. In these areas, they have undisputed clinical value (6).

Over the last 10 to 15 years, we have seen the emergence of a new tumor marker, circulating tumor DNA (ctDNA) (7–11). ctDNA represents a minute fraction of all circulating DNA (the latter is known as cell-free DNA, cfDNA). ctDNA released by tumor cells can be used as a proxy of the tumor burden, composition and molecular heterogeneity. The hypothesis was developed that if plasma ctDNA is isolated and analyzed for mutations, differential methylation patterns, size of fragments, gene copy number variations and other genetic changes associated with cancer pathobiology, it may form the basis for a non-invasive cancer detection test, based on the principle of the so called "liquid biopsy" (7, 10–12). Other, even stronger data, suggest that ctDNA is a valuable prognostic and predictive indicator of cancer (13–19). But is ctDNA ready for population screening for early

cancer detection? In 1968, Wilson and Jungner, formulated some rules which are applicable for any screening program and we briefly mention them here, in the context of ctDNA (ref. 20; **Table 1**).

On the basis of the emergence of ctDNA as a new and potentially powerful cancer biomarker, many companies were created, supported by unusually huge investments (in the hundreds of millions to even billions of dollars), aiming to characterize ctDNA and use it as a biomarker for cancer population screening. Surprisingly, a few fundamental difficulties that we identified earlier, associated with this goal, seem to have been ignored or underestimated (see below). In addition, the designed clinical trials to evaluate this technology, most of which are either completed or ongoing, are exclusively case-control studies. Sensitivities/specificities/predictive values of such screening tests have not been well-defined or they are reportedly rather poor (21–23). Despite these deficiencies, briefly highlighted further below, some of these companies are already testing patents and derive results that may indicate presence or absence of cancer. Such results could be true positives or true negatives, but also false positives or false negatives. We believe that routine patient testing with these new technologies is premature. Our prediction is that this hasty testing will expose the current weaknesses of ctDNA as a screening marker for almost all forms of cancer and will likely add another disappointment to the long history of this field.

Grail

Grail is a biotechnology company aiming to detect multiple types of cancer (>50) with a pan-cancer test, using ctDNA isolated from blood. Grail already optimized their analytical detection technology that is based on methylation differences between normal and cancer patients, in combination with artificial intelligence. They already reported very high specificity (99.5%) in case-control studies (22, 23). We have previously discussed in detail Grail's strengths and weaknesses, after careful calculation of the amount of ctDNA that exists in the circulation at certain tumor sizes, as a fraction of the total cfDNA (this is known as mutant allele fraction) (24, 25). We came to the conclusion that due to sampling error (no retrieval of ctDNA at mutant allele fraction levels of 0.01% or lower) this technology will not be able to effectively detect small, asymptomatic tumors (24). We have estimated the likely lowest detection limit to about 12 mm diameter tumors (24) but some variation of this estimate should be expected according to tumor location, heterogeneity, mitotic index and ability of the tumor to shed ctDNA in the circulation. Despite our criticism, which has not been addressed by the company, Grail continues to tout their test (now also known as the Galleri test) as a major breakthrough, despite the fact that their own, latest data show that the sensitivity of their test is relatively low and ranges from 17% at Stage I to 40% at stage II (and 28% for combined Stages I-II) (23). If it is considered that the trials conducted by Grail so far were all case-control studies (which do not emulate a hypothetical screening scenario) it is highly likely that their actual sensitivities under a screening scenario for early-stage, asymptomatic cancer will be less than 20%, and likely less than 10%, as they have shown for asymptomatic breast cancer detected by screening mammography (22). Based on this level of sensitivity, specificity and prevalence of cancer of various types in the candidate screening population, we calculated that the positive predictive value of their test will likely be in the 1% to 3% range in 12 cancer types (for details and data tabulation see Ref 25). This low positive predictive value is unlikely to support a successful cancer screening program. In their latest iteration (23), which included an analytical test with 99.5% specificity, the calculated positive predictive value (PPV) will still remain <10% for most cancers (see Supplementary Text).

Despite these presumed limitations, Grail has now entered into an agreement with the UK Government to screen approximately 165,000 Britons with Grail's Galleri test, aiming to identify more than 50 types of cancer (23). It will be interesting to see the outcome of this trial. Some of our predictions and associated consequences of this and similar screening programs are summarized in **Table 2**.

The results of patient testing may have legal ramifications although these are not clear and speculative at present. As a precedent, we mention a group lawsuit filed against the diagnostic company Theranos which included claims that the erroneous lab results prompted patients to change their lifestyle, make unnecessary medical appointments and undergo follow-up unnecessary procedures and took medications that they did not need. The outcome of the lawsuit is still pending (26).

More recently, Grail claimed that their test has strong prognostic value and that cancers missed by their tests may be indolent in nature and do not need to be detected (in other words, they claim no overdiagnosis) (27). This claim is speculative since it is not based on evidence. It remains to be seen how many cancers missed by the Galleri test represent mainly indolent or clinically important cancers.

One major complication of the Galleri and related ctDNA cancer diagnostic tests is that due to its multi cancer detection capability, there will be many situations (currently estimated at >20% of all positive tests) whereby the primary tumor will not be precisely located, even if the test detects it. This will make therapy decisions, and especially curative surgery, very problematic. This deficiency is inherent to all diagnostic tests that are based on ctDNA.

Thrive

Papadopoulos and colleagues (28) adopted another way of using ctDNA for early diagnosis and screening by combining ctDNA and conventional biomarkers, to improve sensitivity. Their so-called CancerSEEK tests demonstrated median sensitivities around 70% for eight cancer types, at specificity of 99% (28). The primary cancer could not be clearly located, which, as mentioned, is a major issue. Although the authors claim that most of the diagnostic information of their multiplexed test is derived from ctDNA, as opposed to the conventional tumor markers, this needs further verification. Also, the robustness of the reportedly high specificity needs to be verified since traditional tumor markers are sensitive, but notoriously non-specific indicators of malignancy (6). Maybe some newer and more specific cancer biomarkers could ameliorate this deficiency in the future (29). The CancerSEEK assay has now been licensed to create Thrive, a company which focuses on early cancer detection by screening asymptomatic patients. The published clinical trials showing promising results were case-control studies (28). Our mentioned shortcomings of Grail apply also to Thrive (24).

Guardant Health

Guardant Health is another company in the space of cancer screening, focusing specifically on colorectal cancer. The company is currently conducting a 10,000-person trial (known as the ECLIPSE trial (30)) to evaluate their technology and determine its actual sensitivity/specificity and positive and negative predictive value. From the information on their website, we assume that they are using multifactorial ctDNA molecular analysis, including the newest development, DNA fragmentomics (31), as a front-line test to detect colorectal cancer. Their positive screening results are then verified by colonoscopy as the accepted gold standard.

Guardant Health's strategy is intriguing since there are already highly sensitive (but not specific) front-line detection tests for

Table 1. Wilson and Jungner's principles of screening (20), showing those principles which, according to the authors, are partially or fully satisfied by the proposed pan-cancer screening test based on ctDNA.

Principle	Does cancer screening based on ctDNA meet the criterion?
The condition sought should be an important health problem.	Meets criterion for most cancers.
The natural history of the condition, including development from latent to declared disease, should be adequately understood.	Insufficient evidence that this criterion is met; Current knowledge is different between cancers.
There should be a recognizable latent or early symptomatic stage.	Insufficient evidence that this criterion is met; Current knowledge is different between cancers. ctDNA test may not be sensitive enough for latent or early symptomatic or asymptomatic disease.
There should be a suitable test or examination.	Insufficient evidence that the ctDNA test is suitable for cancer screening. According to criteria for suitability of the screening test based on evidence (36), the cancer screening method needs to be established to reduce the incidence of cancer of the target organ or to reduce mortality from cancer of the target organ. This information and the strength of such association (35) are currently unknown.
The test should be acceptable to the population.	Meets criterion, if liquid biopsy is performed (non-invasive test)
There should be an agreed policy on whom to treat as patients.	Meets criterion for at least some cancers.
There should be an accepted treatment for patients with recognized disease.	Meets criterion for at least some cancers.
Facilities for diagnosis and treatment should be available.	Meets criterion in most cases.
The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.	Probably meets criterion but more studies will be necessary, focusing on screening economics; overall costs may be different between cancers.
Case-finding should be a continuing process and not a "once and for all" project.	Meets criterion in most cases.

Table 2. Likely favorable and adverse outcomes of population screening with ctDNA[a].

Screening test characteristic	Anticipated results	Reason	Actions and Consequences	Legal ramifications
High sensitivity	Many true positives (>90%)	NA	Early interventions. Favorable clinical outcomes. Uncertain actions if no treatment available and patient is not given a clear choice for next step. Screening may lead to over-diagnosis (indolent disease) and over-treatment (34).	Possibility of lawsuits, if over-treatment proves to be harmful.
High specificity	Many true negatives (>98%)	NA	No action; reassurance for cancer absence.	NA
Poor sensitivity (e.g., <30%)	Many false negatives	Not enough ctDNA in plasma due to small tumor size. Test not analytically sensitive to reliably analyze ctDNA. Rare genetic changes are missed. Certain tumors shed less ctDNA in circulation (e.g., brain tumors).	Patients are falsely reassured of not having cancer, when in fact, they may do.	Possibility of lawsuits against screening company when cancer spreads and becomes symptomatic or threatens patient's life.
Relatively poor specificity (e.g., <98%)	Many false positives, especially for relatively rare cancers	Genetic alterations seen in normal/precancerous tissues. Indolent disease. Analytic test not absolutely specific.	Additional, unnecessary and probably harmful interventions (biopsies, exposure to radiation or toxic drugs).	Possibility of lawsuits against screening company for misleading information (cost/anxiety/possible harm).
Poor analytical performance	No interpretable result was obtained	Low or insufficient ctDNA amount. Genetic alteration of unknown significance.	Repeat analysis; delay in diagnosis and obtaining results. Heightened patient anxiety.	Possibility of lawsuits against screening company for not providing interpretable results.
Harms from testing or follow-up testing (34)	NA	NA	Exposure to radiation/anxiety/colon perforation; other harms.	Possibility of lawsuits against screening company for collateral damage.

Abbreviation: NA, not applicable.

[a]Many of these outcomes and actions are hypothetical, especially the legal ramifications; see text and Refs.34 for more discussion. A lawsuit against the diagnostic company Theranos for erroneous lab results can be found on https://amp.usatoday.com/amp/742008002.

colorectal carcinoma based on fecal immunochemical tests (FITs) for hemoglobin (Hb), which are widely used for colorectal cancer screening. FITs detect the majority of colorectal cancers, with summary estimates of sensitivity in meta-analyses in the order of 70% to 80% (32). The positive FIT tests are also verified by colonoscopy. Consequently, both Guardant and FIT testing, results in enrichment of patients for colorectal cancer and reduction of the number of colonoscopies needed to diagnose colorectal cancer. In our opinion, the substitution of one non-invasive test for colorectal cancer (FIT) with another, which is most likely less sensitive and more expensive (Guardant) seems to offer no additional advantage. However, we recognize that a blood test may be more convenient than a test that requires stool sampling and analysis.

Our published criticism of Grail and Thrive (24) as well as some of the concerns mentioned in **Tables 1** and **2** also applies to the Guardant test.

Delfi

Delfi is a start-up based on Victor Velculescu's pioneering work on fragmented ctDNA, as described by the inventors (31) and in our previous commentary (33). By examining cell-free DNA fragments across the genome, Delfi's machine learning system claims to analyze orders of magnitude more data on the presence or absence of cancer than is possible with alternative molecular technologies that look for changes in DNA sequences, methylation, or proteins. Delfi's assays are claimed not to be affected by confounding conditions such as clonal hematopoiesis of indeterminate potential (CHIP) or other diseases. A machine learning model that incorporated genome-wide fragmentation features had sensitivities of cancer detection ranging from 57% to more than 99% among seven cancer types, at 98% specificity. Fragmentation profiles could be used to identify the tissue of origin of the cancers to a limited number of sites in 75% of cases. Similarly to other ctDNA-based tests, the above encouraging data are based on case-control studies. Studies emulating cancer screening of asymptomatic individuals and calculation of actual positive and negative predictive values (PPV and NPV) have not as yet been published.

Freenome

Freenome is offering a multi-omics blood test that looks beyond mutations to detect the body's own early-warning signs for cancer, incorporating a multidimensional view of both tumor- and non-tumor-derived (e.g., immune) signatures that enable early detection (34). The process includes analyzing fragments of DNA, RNA, proteins, and other biomarkers circulating in blood plasma. This information is then fed into an artificial intelligence algorithm that is presumably able to decode billions of complex patterns associated with the body's response to specific tumor types. As a proof of concept, whole-genome sequencing was performed on cfDNA extracted from plasma samples (N = 546 colorectal cancer and 271 non-cancer controls). Reads aligning to protein-coding gene bodies were extracted, and read counts were normalized. Machine learning models were trained using k-fold cross-validation and confounder-based cross-validations to assess generalization of performance (35). In a colorectal cancer cohort heavily weighted towards early-stage cancer (80% stage I/II), the authors achieved a mean sensitivity of 85% at 85% specificity. Sensitivity generally increased with tumor stage and increasing tumor fraction. Prospective validation of this machine learning method and evaluation of a multi-analyte approach are underway. The deficiencies mentioned above for other technologies (24) apply also to this one. Under screening scenarios, the relatively low specificity of 85% will be detrimental to the PPV (for explanations see Supplementary Text and Table in Ref. 25).

Conclusions

The financial gains of companies dealing with early cancer detection are obvious, since millions of people will choose to get screened, if the test is non-invasive and highly effective (sensitive and specific for the intended clinical application). Illumina acquired Grail for $7.1 Billion! Cancer screening satisfies some, but not all, of Wilson and Jungner's criteria for screening (**Table 1**). Unfortunately, none of the tests marketed by the five mentioned companies, and other similar companies that are rapidly proliferating, has proven through clinical trials that are highly sensitive (even if some could be highly specific), and the anticipated positive predictive value will likely be <10% with unacceptable sensitivity (25). We suggest that patients who take these tests will likely have significant associated risks (**Table 2**) (36). It should also be emphasized that a cancer screening program includes a lot more than a good test. It is necessary to show that the cancer screening method has established value for reducing the incidence of cancer of the target organ or for reducing mortality from cancer of the target organ (37). We have no doubt that these additional requirements will be examined in due course.

The mission of the Early Detection Research Network (EDRN), a National Cancer Institute sister organization, is to discover, develop and validate biomarkers and imaging methods to detect early stage cancers and to assess risk for developing cancer, and to translate these biomarkers and imaging methods into clinical tests. Since its inception about 20 years ago, the EDRN significantly enhanced biomarker science as described (37–39). We recommend to all companies dealing with cancer screening to work with EDRN, to make sure that previously identified deficiencies in sample collection, storage, bioinformatic analysis and trial design are avoided.

Although ctDNA is a valuable biomarker for other clinical applications (7, 10, 11, 19) it seems that its value as an early cancer screening test is questionable due to its low sensitivity and PPV, despite seemingly adequate specificity of some assays. Application of the test in asymptomatic populations, which is work in progress, will demonstrate its actual value in cancer screening. Can sensitivity of ctDNA-based assays be improved without compromising specificity? One way includes obtaining much higher volumes of blood for testing (such as 100 mL instead of the usual 10 mL blood draw) but this will create other issues. We hope that additional ingenious new ways of cancer screening could be invented with the help of artificial intelligence.

Authors' Disclosures

E.P. Diamandis reports an advisory role with Abbott Diagnostics and a consultant role with Imaware Diagnostics. No disclosures were reported by the other authors.

Acknowledgments

O.D. Pons-Belda acknowledges the support from Fundación José Luis Castaño-SEQC and A. Fernandez-Uriarte acknowledges the support from the IFCC's Professional Scientific Exchange Programme (PSEP).

Received January 29, 2022; revised February 16, 2022; accepted March 14, 2022; published online June 1, 2022.

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