GRAIL and the quest for earlier multi-cancer detection

GRAIL

AUTHORS

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Despite tremendous advances in treatment, cancer is still the second leading cause of death worldwide. In 2018, more than 9.5 million people died from cancer, roughly 18 per minute[1]. In addition to the number of lives lost and its devastating effects on patients and their families, cancer exacts a staggering economic toll on society. In the United States (US), an estimated US$94.4 billion in earnings were lost due to cancer mortality in 2019 and the projected total healthcare cost for cancer in 2020 is US$157 billion (2010 dollars)[2,3].

In large part, these losses are so great because cancer is diagnosed too late[4], usually after it has metastasized, or spread beyond a localised area to other parts of the body. Once this happens, treatment becomes much more difficult and costly, and survival is less likely (**Fig. 1**): 89% of patients who are diagnosed with any type of localised cancer survive 5 years, in contrast to only 21% of patients who are diagnosed with metastatic cancer[5]. In lung cancer, which was the leading cause of cancer death[4] in both men and women in the US in 2019, the difference in 5-year survival between early and late diagnosis is 56% versus 5%, respectively[6]. Furthermore, the total medical care costs of late-stage lung cancer are up to twice those for early-stage disease[7]. For
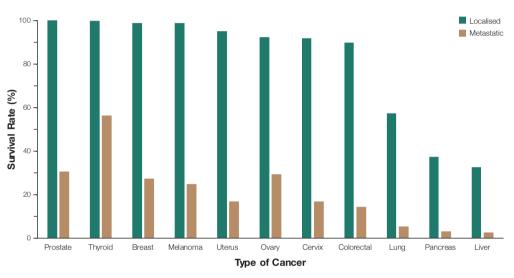

Figure 1. Five-year cancer survival rates in the United States (2009–2015) by extent of disease at the time of diagnosis. Individuals diagnosed with localised cancer (green bars) are more likely to be alive five years after diagnosis than those diagnosed with metastatic cancer (tan bars) that has spread to other parts of the body.

all cancers, even a 1% reduction in cancer mortality would translate into hundreds of billions of dollars of economic value[8].

Despite the one-in-three chance of being diagnosed with cancer (of any type) at some point during life in the US[4], only a handful of cancers – breast, cervical, colon, lung and prostate cancer – are screened for in the general population. More than 60% of cancer diagnoses and deaths are from cancers that lack screening paradigms, and in 2019, there were an estimated 1,762,450 new cases of cancer in the US, of which 614,010 were due to screened cancers[4]. The difference, 1,148,410, is the

number of new cases due to unscreened cancers, which is likely to increase with population growth in the future.

Most cancers are not screened because their prevalence in the general population is too low to justify screening programmes on an individual cancer basis[9]. However, the number of people that need to be screened to detect cancer goes down when prevalences are combined; that is, when multiple cancers are screened for in a single test. Because all cells in the body have access to the circulatory system (directly or indirectly), blood is an attractive analyte for a multi-cancer test (**Fig. 2**).

At GRAIL, we believe that a blood-based multi-cancer screening approach for deadly cancers could help overcome the limitations of organ-specific screening tests and detect cancer earlier, when treatment is more likely to be successful. Achieving this goal is one of the most ambitious undertakings in healthcare, and this is exactly what we are committed to doing.
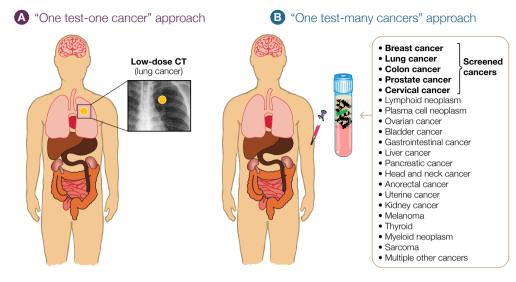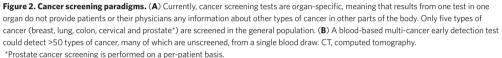
PAST SUCCESSES, CURRENT LIMITATIONS

The Pap screening test for cervical cancer is one of the best examples of the power of early detection to reduce the burden of cancer. Since 1950, the Pap test has reduced

the mortality of cervical cancer[10] in the US by approximately 70%. Other cancer screening tests, such as mammography, colonoscopy/faecal test and low-dose computed tomography (LDCT), have also helped reduce the mortality of breast, colorectal and lung cancer, respectively.

Despite these successes, recommendations for cancer screening continue to be debated due to the high false positive rates of existing tests and potential overdiagnosis of nonlethal cancers. If all Americans 50 to 79 years old were to follow current US Preventive Services Task Force screening recommendations at historical compliance rates, there would be approximately 9 million positive tests, with 151,000 actual cancers – or 60 times more false positives than true positives[11].

Prostate-specific antigen (PSA)-based screening for prostate cancer is an example of the potential consequences of high false positives and overdiagnosis. In 2018, the US Preventive Services Task Force on prostate cancer screening concluded that although PSA screening could benefit some men, many would experience harms, including psychological harm from false positive results and painful complications from subsequent biopsy and treatment, due to overdiagnosis and overtreatment. Large randomized clinical trials showed that 20% to 50% of men screened for prostate cancer had positive PSA test results when they did not actually have prostate cancer. Moreover, among men who do have prostate cancer, many are unnecessarily treated because most prostate cancers advance very slowly and may never be symptomatic[12].

The positive predictive value, or likelihood that a positive test result is a true positive, for most existing guideline-screening tests is also very low (generally <5%), with the exception of colonoscopy, which is the gold

A "One test-one cancer" approach

B "One test-many cancers" approach

Figure 2. Cancer screening paradigms. (**A**) Currently, cancer screening tests are organ-specific, meaning that results from one test in one organ do not provide patients or their physicians any information about other types of cancer in other parts of the body. Only five types of cancer (breast, lung, colon, cervical and prostate*) are screened in the general population. (**B**) A blood-based multi-cancer early detection test could detect >50 types of cancer, many of which are unscreened, from a single blood draw. CT, computed tomography.
*Prostate cancer screening is performed on a per-patient basis.

standard for colorectal cancer. As a result, most screening tests are only practical when they are used to test individuals who have a high risk of developing the screened cancer, and they have limited ability to detect cancers in the general population.

A NEW APPROACH

To overcome the limitations of individual organ-specific screening tests, GRAIL has developed an investigational multi-cancer early detection test that can detect cancer-derived signals in DNA from a single tube of blood. This test meets what we believe to be the key criteria for a safe and effective multi-cancer early detection test (**Table 1**), including maximal cancer detection (simultaneous detection of more than 50 different cancers[13]) in an elevated risk population (for example, >50 years of age); preferential detection of deadly cancers to avoid overdiagnosis of cancers that are not fatal if left untreated; an extremely low false positive rate; ease of use; and prediction of the tissue of origin (TOO), or the location in the body where the cancer started, which

clinicians need to perform further clinical and diagnostic evaluation and guide treatment[9].

The development of this test would not have been possible without many advances in cancer biology, DNA sequencing and computational data analysis. For example, the discovery that all cells, including tumour cells, release DNA fragments into blood is the basis of our ability to detect cancer from a blood sample[14]. Because these cell-free DNA (cfDNA) fragments are derived from the genome[15], which encodes instructions for all cellular functions, sequencing and analyzing these fragments can reveal whether cells in the body are normal or have become cancerous.

However, the cancer signal-to-noise ratio (tumour cfDNA fraction) in a blood sample is often very low – equivalent to one tumour cfDNA fragment among thousands of normal cfDNA fragments, which makes detection and analysis challenging[14]. Three problems limit clinical sensitivity: (1) there are more non-cancer cells than tumour cells in the body, resulting in many more

background cfDNA fragments than tumour cfDNA fragments; (2) most normal cfDNA is from blood cells, which accumulate common aging-related variations (due to clonal haematopoiesis) that can resemble cancer mutations[16], and (3) DNA sequencing is imperfect, causing errors[17]. Many chemistry and computational methods (for example, digital droplet polymerase chain reaction and molecular barcoding) have been developed to amplify signals and reduce errors in attempts to enable the clinical sensitivity needed for early cancer detection[14]. However, it has been challenging to achieve the clinical performance required for a multi-cancer test (for example, clinically useful sensitivity, high specificity and accurate TOO localization), because these have mostly been based on detection of tumour mutations. Tumour mutations are relatively rare per megabase of the human genome compared to non-cancer sources of mutations, such as those originating from clonal haematopoiesis, and few mutations identify the exact cancer type.

We achieved high specificity (low false positive rate) in our test by focusing on DNA methylation, a biologic mechanism that controls when and where different sets of genomic instructions are carried out in the body[14]. In contrast to typical cancer mutations that only affect a handful of genomic locations, there are nearly 30 million sites, known as CpG sites, across the human genome that can be methylated or unmethylated[18], making them a ubiquitous and rich signal for detecting cancer. By using highly efficient targeted bisulfite sequencing and machine learning, we can read methylated DNA sequences and identify those that are abnormally methylated. In our multi-cancer early detection test, we analyse more than 100,000 methylation regions (covering ~1 million CpG sites) in the genome to assess methylation patterns that indicate the presence or absence of cancer. In addition, we leverage the fact that different cell types in the body have unique DNA methylation patterns in their genomes, which are used to determine where cancer is in the body (**Fig. 3**)[14].

Our early studies showed that whole-genome bisulfite sequencing to analyse methylation patterns outperformed whole-genome sequencing and targeted mutation methods for cancer detection[19]. The performance advantage of DNA methylation is largely due to its biological characteristics, which make it more robust at low signal-to-noise ratios. Specifically, the large number and wide distribution of DNA methylation sites in the genome enable deeper sequencing of methylation regions that are identified as particularly informative for cancer detection and TOO localization. High sequencing depth, which is a measure of sequencing data quality (redundancy of coverage), is critical to detect tumour mutations at low cfDNA fractions

and to distinguish them from DNA sequencing errors. Additionally, the detection of tumour mutations using targeted mutation methods can also be confounded by natural aging-related clonal haematopoiesis variants that could be misinterpreted as cancer. To avoid these potential false positives and achieve high specificity, mutation-based approaches require parallel sequencing of white blood cells to filter out these non-cancer variants[16]. Because these variants rarely affect DNA methylation sites, a methylation-based cancer detection test should not require additional sequencing steps.

We developed computer models called classifiers to distinguish cancer-specific signals (abnormal methylation patterns) from non-cancer signals (normal methylation patterns) using machine learning. These classifiers were trained and validated using a proprietary database of DNA methylation patterns from thousands of individuals diagnosed with different types of cancer and individuals not known to have cancer (including healthy individuals and those with other medical conditions)[13,19]. This methylation database is, to our knowledge, the largest of its kind in the world and is key to the performance of the classifier used in our targeted methylation-based multi-cancer early detection test.

FROM LABORATORY TO CLINIC

We have designed a rigorous process to develop a blood-based multi-cancer early detection test for population-scale cancer screening (**Fig. 4**). This process includes one of the largest clinical genomics programmes to date, involving 4 clinical studies with a combined total of more than 180,000 participants in North America and the United Kingdom. These participants have diverse demographic characteristics

and include those with cancer (all types and stages) and those without cancer (healthy or with other medical conditions) to help ensure that our multi-cancer early detection test will be safe, effective and useful for as many people as possible.

The first of these 4 studies, the Circulating Cell-free Genome Atlas (CCGA) study (www.clinicaltrials.gov NCT02889978), has enrolled 15,254 participants from 142 sites. CCGA was divided into three pre-specified substudies to develop a machine-learning classifier for multi-cancer early detection and TOO identification: substudy 1 (discovery, previously reported) identified the highest performing assay(s) for further development; substudy 2 (training and validation) to train and validate a classifier for cancer detection and TOO localization based on an updated targeted methylation approach; and substudy 3 (final validation) to validate an optimised version of the targeted methylation approach (this analysis is ongoing). The second substudy[13] included nearly 4,500

cancer (more than 50 types across all stages) and non-cancer samples from CCGA, divided into a training set with 3,133 samples and an independent validation set with 1,354 samples. To optimise test specificity, this data set was combined with more than 2,200 samples (1,587 in training and 615 in validation) from participants without cancer in the STRIVE study (NCT03085888). The targeted methylation approach from the second substudy is being utilized in a clinical study (NCT04241796) that is returning results to physicians and patients.

In the validation data set, the multi-cancer early detection test had a false positive rate of 0.7% and an overall test sensitivity (true positive rate) of 54.9% (95% confidence interval [CI]: 51.0–58.8%). Sensitivity and 95% CIs by stage for all cancer samples with known tumour stage were: stage I (n = 185), 18% (13–25%); stage II (n = 166), 43% (35–51%); stage III (n = 134), 81% (73–87%); and stage IV (n = 148), 93% (87–96%). TOO was predicted for 96% of samples with cancer; of those, the

Table 1. Key criteria for evaluating a multi-cancer early detection test.

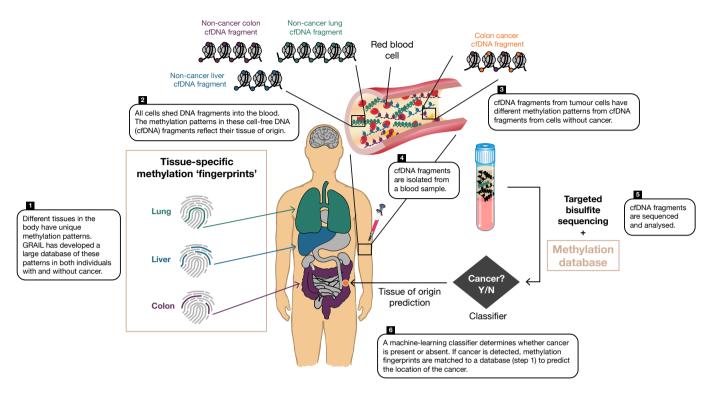| Criterion	GRAIL Multi-cancer early detection test
Elevated risk population	• Individuals with an elevated risk of cancer (for example, >50 years old).
Maximal cancer detection	• "Pan-cancer" detection of more than 50 cancer types. • 75% of cancers detected are not screened for[4,9].
Safety: Low false positive rate	• High positive predictive value, the optimal measure of safety. • Very low false positive rate, based on a specificity of >99%.
Safety: Limited overdiagnosis	• Preferential detection of deadly cancers, based on preliminary data[20].
Tissue of origin localization	• Highly accurate (93%) cancer localization to direct subsequent diagnostic workup[9].
Ease of use	• Simple and easy to use to maximize implementation and compliance.

Figure 3. Tracking down tumours using DNA methylation patterns in blood. A targeted methylation-based multi-cancer early detection test can recognize differences in DNA methylation patterns in cell-free DNA (cfDNA) fragments in a blood sample. These patterns are characteristic of different cell types and abnormally methylated cfDNA can be used to detect cancer and map its location in the body.

prediction was correct in 93%. TOO accuracy was not affected by cancer type or tumour stage. These results demonstrated that a targeted methylation-based multi-cancer early detection test using a machine-learning classifier can simultaneously detect more than 50 cancer types with a single, fixed, low false positive rate of less than 1%, and can accurately localise the TOO[13].

These early performance results, which approach those needed for population-level cancer screening, supported further clinical development of this multi-cancer early detection test, including a study that is returning results to patients and physicians to inform the broader, real-world implementation of this test.

We analytically validated the multi-cancer early detection test using five studies to characterize:
- Specificity in non-cancer samples;
- Sensitivity in cancer samples;
- Repeatability (agreement between results from the same test run) and reproducibility (agreement between results from different test runs) in both cancer and non-cancer samples across multiple reagent lots, instruments and test operators;
- Test performance as a function of different amounts of cfDNA;
- Effect(s) of four potential interfering substances (haemoglobin, bilirubin, triglycerides and white blood cell genomic DNA) on test performance.

In addition, our four prospective clinical trials to develop the multi-cancer early detection test and validate its performance include real-world, intended use populations:
- CCGA (NCT02889978) – a prospective, multi-centre, observational, case-control study with longitudinal follow-up. The purpose of the study is to develop and validate a blood-based multi-cancer early detection test using cfDNA methylation data from more than 15,000 participants with and without cancer in the US;
- STRIVE (NCT03085888) – a prospective, multi-centre, observational, cohort study with longitudinal follow-up. The study aims to validate the multi-cancer early detection test in a real-world, intended use population of approximately 100,000 women undergoing mammography screening in the US;
- SUMMIT (NCT03934866) – a prospective, multi-centre, observational, cohort study with longitudinal follow-up. The study aims to validate the multi-cancer early detection test in a second real-world, intended use population of approximately 50,000 individuals without cancer in the United Kingdom, half of whom are not high risk and half of whom are heavy smokers who are at high risk for lung cancer;
- PATHFINDER (NCT04241796) – a prospective, multi-centre study with longitudinal follow-up of approximately 6,200 individuals with different levels of cancer risk in the US. This study will be the first to return results from the multi-cancer early detection test to clinicians; if cancer is detected, the number and types of follow-up tests needed to reach a clinical diagnosis will be determined. Patient-reported experiences will also be assessed.
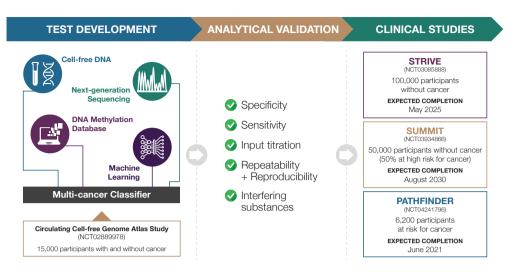
Together, these studies will enable us to develop a best-in-class multi-cancer early detection test to detect true cancer signals with high specificity and to accurately localise the TOO in population screening applications that complement existing guideline-recommended cancer screening.
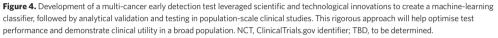
THE VALUE OF A MULTI-CANCER EARLY DETECTION TEST

Providing a multi-cancer early detection test that can simultaneously detect and localise many deadly cancers while minimizing false positive results is anticipated to be a high-value healthcare service. Analysis of the US National Cancer Institute Surveillance, Epidemiology and End Results (SEER) database and estimates of the natural history of cancers predict that a cancer screening programme incorporating a multi-cancer early detection test for individuals 50 to 79 years old could potentially avert approximately 110,000 deaths per year. Considering the potential benefit to individuals and society that would result from a shift in cancer diagnosis from stage IV to earlier stages, the cost per life-year associated with adding a multi-cancer early detection test to current guideline-recommended screening is expected to compare favourably with preventive and therapeutic interventions that are considered cost-effective in the US. Early detection compares especially well with the hundreds of thousands of dollars that are routinely spent on late-stage cancer treatments that often only extend survival by a few months. Moreover, a focus on early detection and early treatment of cancer is consistent with a changing paradigm for health services that favours prevention and health maintenance to reduce costs.

TRANSFORMING CANCER CARE THROUGH EARLY DETECTION

A blood-based multi-cancer early detection test could potentially reduce the enormous burden of cancer on patients, their families, healthcare systems and society. The addition of this test to existing cancer screening tests would broaden the detection net for

TEST DEVELOPMENT → **ANALYTICAL VALIDATION** → **CLINICAL STUDIES**

Figure 4. Development of a multi-cancer early detection test leveraged scientific and technological innovations to create a machine-learning classifier, followed by analytical validation and testing in population-scale clinical studies. This rigorous approach will help optimise test performance and demonstrate clinical utility in a broad population. NCT, ClinicalTrials.gov identifier; TBD, to be determined.

cancers that are not currently screened and enable more cancers to be identified more efficiently than currently possible.

On the basis of cancer incidence data from the SEER database[6] and test performance data from the second CCGA substudy, we predict that the addition of a multi-cancer early detection test to current US guideline-recommended screening tests for breast, lung, colon and cervical cancer in Americans 50 to 79 years old who are screening eligible could detect approximately 615,000 cancer cases versus 150,000 from standard screening tests alone. Of note, the true positive results from current screening tests would be accompanied by an estimated 9 million false positives, whereas the addition of a multi-cancer early detection test would only be expected to generate an additional 640,000 false positives. These numbers correspond to a signal-to-noise ratio (true positive-to-false positive ratio) of 1:60 for standard cancer screening tests versus 1:16 for screening tests plus a multi-cancer early detection test, a four-fold improvement. Thus, when added to current screening tests, a multi-cancer

early detection test could help detect four times more cancers, with only 7% more false alarms.

At GRAIL, our mission to improve and save lives through early cancer detection is closer to reality than ever before. There may be no greater opportunity in healthcare to make a significant impact to public health, and we are committed to changing the trajectory of cancer mortality and bringing stakeholders together to enable broad adoption of innovative, safe and effective technology that can transform cancer control and cancer care. The path may not be easy, but we believe it is the right one – for patients, providers, communities and healthcare systems around the world.

REFERENCES

1. World Health Organization. All Cancers Fact Sheet (2018). Available at: http://gco.iarc.fr/today/data/factsheets/cancers/39-All-cancers-fact-sheet.pdf [accessed 11 February 2020].
2. Islami, F. et al. JAMA Oncol. **5**, e191460 (2019).
3. Mariotto, A. B., Yabroff, K. R., Shao, Y., Feuer, E. J. & Brown, M. L. J. Natl Cancer Inst. **103**, 117–128 (2011).
4. Siegel, R. L., Miller, K. D. & Jemal, A. CA: Cancer J. Clin. **69**, 7–34 (2019).
5. Hawkes, N. BMJ **364**, l408 (2019).
6. Howlader, N. et al. SEER Cancer Statistics Review, 1975–2016. Available at: https://seer.cancer.gov/csr/1975_2016> (2019) [accessed 11 February 2020].
7. Banegas, M. P. et al. J. Natl Compr. Canc. Netw. **16**, 402–410 (2018).
8. Murphy, N. K. & Topel, R. H. The value of health and longevity (NBER Working Paper 11405). (National Bureau of Economic Research, Cambridge, MA, 2005).
9. Ahlquist, D. A. NPJ Precis Oncol. **2**, 23 (2018).
10. Wingo, P. A. et al. Cancer **97**, 3133–3275 (2003).
11. Ofman, J. J. in Patient-Centered Oncology Care 2019 (American Journal of Managed Care, Philadelphia, PA, 2019).
12. U. S. Preventive Services Task Force et al. JAMA **319**, 1901–1913 (2018).
13. Liu, M. C. et al. (in press).
14. Huang, C. C., Du, M. & Wang, L. Cancers (Basel) **11**, 805 (2019).
15. Thierry, A. R., El Messaoudi, S., Gahan, P. B., Anker, P. & Stroun, M. Cancer Metastasis Rev. **35**, 347–376 (2016).
16. Razavi, P. et al. Nat. Med. **25**, 1928–1937 (2019).
17. Pfeiffer, F. et al. Sci. Rep. **8**, 10950 (2018).
18. Soto, J., Rodriguez-Antolin, C., Vallespin, E., de Castro Carpeno, J. & Ibanez de Caceres, I. Transl Res. **169**, 1–18 e11 (2016).
19. Oxnard, G. R. et al. Ann. Oncol. **30**, v912 (2019).
20. Oxnard, G. R. et al. J. Clin. Oncol. **37**, 1545 (2019).