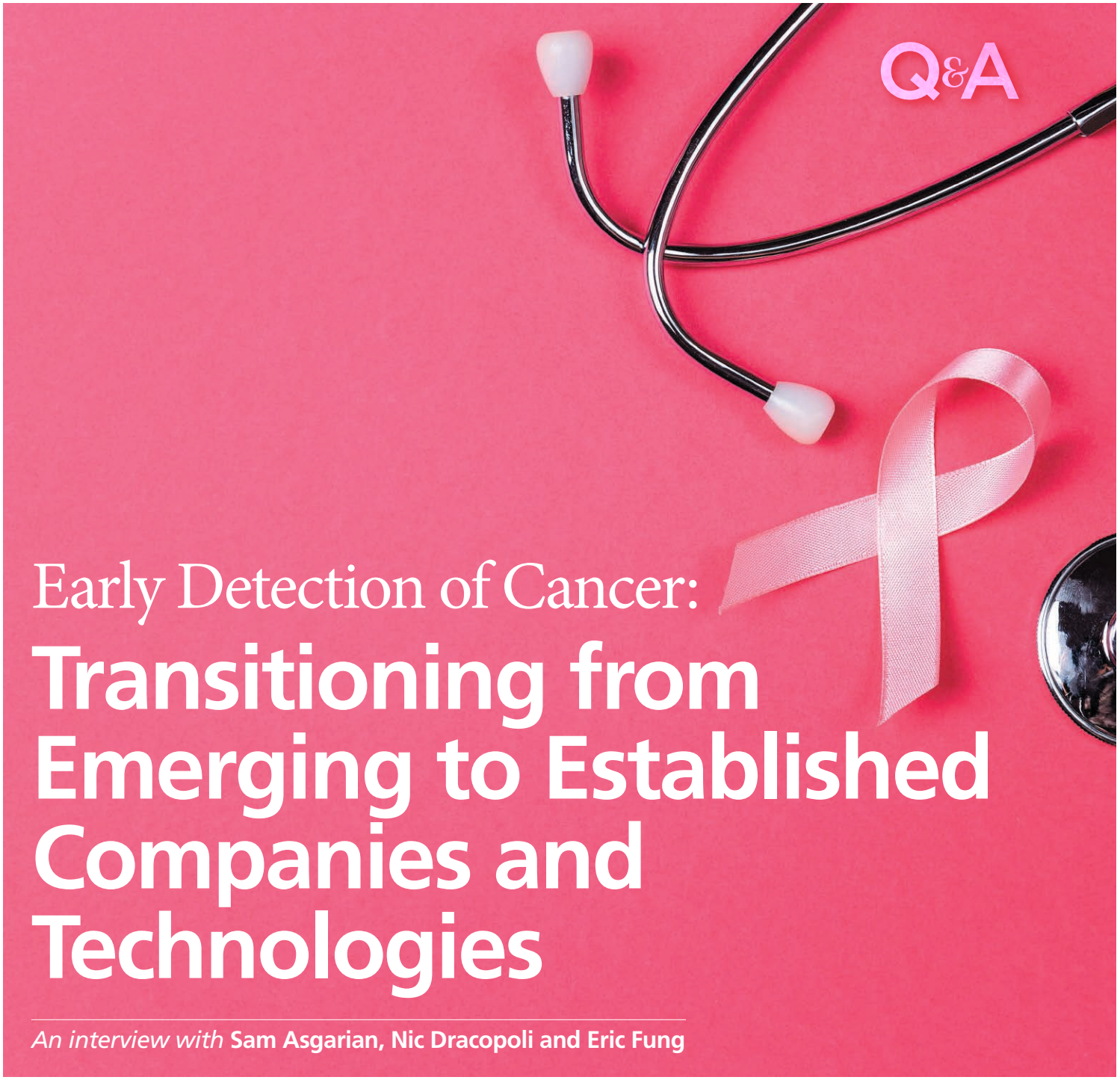

Q&A

Early Detection of Cancer: Transitioning from Emerging to Established Companies and Technologies

An interview with **Sam Asgarian, Nic Dracopoli and Eric Fung**

A PANEL OF EXPERTS was brought together at a recent Virtual Precision Medicine Leaders' Summit to discuss their respective company's efforts to roll out an emerging diagnostics technology platform.* The panel members, Sam Asgarian (Thrive), Nic Dracopoli (Delfi Diagnostics), and Eric Fung (Grail), all come with different experiences and insights; the companies they represent range from start-up to established, and two were recently acquired. The panelist first described their companies (see respective insets) and platforms, then answered questions on how the companies are addressing the various challenges they face introducing emerging platforms. We contacted the panelists to direct a few follow-up questions to them for deeper details.

Before getting to the questions, we note one case, cited by panelist Sam Asgarian, that is core to the shared mission of the companies: Rosemary, a healthy 71-year-old woman, presents with no signs or symptoms for cancer. She would likely have gone about her life normally, but her caregiver suggested she be tested with Thrive's cancer panel. Until now, no screening test has been available for *early-stage (pre-symptomatic)* detection of the cancers represented on the panel. In short, Rosemary was found to have Stage 1 ovarian cancer, an outcome that would likely have progressed to a late-stage case and an unfortunate outcome. Instead, a relatively simple surgical procedure was performed; she is now cancer-free and lives a much higher quality of life than she would have had otherwise.

The lessons from this case are two-fold: first, do

Delfi Diagnostics

Delfi Diagnostics is a Johns Hopkins spinoff focused on the non-invasive detection of cancer at earlier stages when it is most curable. Delfi uses artificial intelligence and whole genome sequencing to detect unique patterns of DNA fragmentation in the blood of patients with cancer. These analyses are performed through simultaneous examination of millions of DNA sequences using machine learning to identify tumor-specific abnormalities.

GRAIL

GRAIL is focused on saving lives and improving health by pioneering new technologies for early cancer detection. The company is using the power of next-generation sequencing, population-scale clinical studies, and state-of-the-art computer science and data science to overcome one of medicine's greatest challenges with Galleri, GRAIL's multi-cancer early detection test. Galleri uses circulating cell-free DNA to detect cancer signals early in disease onset, when treatment is more likely to be successful. GRAIL's proprietary technology analyzes DNA methylation patterns from the informative regions of the genome not only to detect the presence of a cancer signal, but also identify where the cancer is located in the body. GRAIL was acquired by Illumina in September 2020.

Thrive, An Exact Sciences Company

Thrive, An Exact Sciences Company, is focused on incorporating earlier cancer detection into routine medical care to extend and save lives. Thrive is developing CancerSEEK, a blood test that is designed to detect multiple types of cancer at earlier stages of disease. For mor information, please visit www.thrivedetect.com.

not lose sight of the patient in all the technology and, second, the need to show physicians how the platform fits within their workflows. We'll use these two lessons as the theme for this Q&A

Q1. Putting aside for now the cost to finance, build out, and market a product, what do you view as the highest obstacles that you encountered developing the respective platforms for the clinical space?

Nic Dracopoli (Delfi Diagnostics): One of the biggest challenges is how we train and validate assays in this space. For screening tests, the size of the clinical trials is an order of magnitude larger than you find in diagnostics. For example, only 1 to 3% of high-risk smokers would be expected to be diagnosed with cancer in a screening test, so the size of the study needs to reflect that rarity.

Another big challenge is that the cell-free DNA (cfDNA) for these tests can be shed by cancers derived from any tissue, so it's important to not only have a highly sensitive test for cancer, but also to be able to identify the tissue from which it arose to enable further interventions.

Eric Fung (GRAIL): Multi-cancer early detection is a relatively new technology, and as a result, will require significant education on its potential value and impact. We will need to define this new approach to cancer detection and ensure a broad understanding of what differentiates our test, Galleri – a test that can detect and predict the location for multiple cancers through a single blood draw – from single cancer, or multi-step tests.

Sam Asgarian (Thrive): We believe to enable broad adoption for these tests they must seamlessly integrate into a physician's workflow, and planning for that starts now. Our ultimate goal is to integrate a blood-based test to screen for many types of cancer during routine care and specifically, the end users are Primary Care Physicians (PCPs). PCPs see a multitude of different health issues throughout the day. While the vast majority of tests results will come back negative, it will be critically important to communicate what this means for the patient, the need to continue with other routine screenings like mammograms and colonoscopies, and to come back potentially the next year for another blood test. Likewise, and in the rare cases the result is positive for cancer, the PCP will need to have a clear and seamless path to confirmation and diagnosis. We think this handoff to radiology and potentially an oncologist is critically important, and we aim to help establish this workflow and continuity of care.

Q2. How did your platform achieve the sensitivity, specificity, and robustness to meet your performance requirements?

ND: The big advantage of using cfDNA fragmentation is that there are a very large number of differences between the cancer and normal genomes. These differences can be identified inexpensively by low coverage whole genome sequencing that would be unable to detect cancer mutations. We're in the process of further validating the test and locking in improved performance.

EF: GRAIL is conducting what we believe to be the largest clinical study program of its kind, with more than 134,000 participants enrolled to date. Through this rigorous effort, we developed a multi-cancer early detection blood test, Galleri.

GRAIL's early studies compared three comprehensive sequencing approaches to identify the most appropriate to move forward in development. More specifically, GRAIL compared whole-genome sequencing (to identify copy number variations), targeted sequencing (to identify single-nucleotide variations and indels), and whole-genome bisulfite sequencing (to identify whole-genome methylation patterns) in cancer detection and cancer signal origin accuracy at high specificity. We found that methylation patterns outperformed whole-genome sequencing and targeted sequencing methods. The performance advantage of DNA methylation is largely due to its biological characteristics, which make it more robust at low signal-to-noise ratios. Further development resulted in a targeted methylation approach, focusing on the most informative DNA methylation regions (~100,000 fragments covering ~1,000,000 CpGs). The large number and wide distribution of DNA methylation sites in the genome enable deeper sequencing of methylated regions that were identified as particularly informative for cancer detection and cancer signal origin accuracy.

We developed classifiers to distinguish cancer-specific signals from non-cancer signals, and to predict the cancer signal origin when a cancer signal is detected 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). 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.

This resulted in a multi-cancer early detection

test with high specificity (>99%) – which we believe to be appropriate for population-scale implementation – that detects a large number of cancer types, thus offering the potential to maximize population-scale cancer detection while attempting to minimize harms associated with false positives.

SA: A blood test being used to screen for multiple types of cancer in an asymptomatic population must have very high specificity of 99% or better in order to minimize false positives. This is imperative for patient safety and to avoid unnecessary or invasive follow-up procedures. There are inherent trade-offs to sensitivity in order to achieve such a high specificity but the value of adding a blood test as a complementary screening test to be used in addition to standard of care single organ tests is that in combination, we believe in the future the overwhelming majority of cancers will be detected by screening and earlier stages.

Q3. How have you been overcoming the adoption chasm – both take up by healthcare providers and clinical labs as well as raising funds for the company?

ND: Our goal is to ensure a low enough cost that enables population level screening that would be impossible for more expensive assays.

We also want to ensure our test provides clear results that lead to direct clinical interventions and have evidence of cost savings to the overall healthcare ecosystem.

SA: Our test is not commercially available at this time but we are starting the education and awareness process now as this will be a novel concept and meant to be incorporated into routine care and as part of the blood work-up during an annual check-up.

Q4. As part of the adoption strategy, companies need to make assays affordable, effective, and, preferably, covered by insurance. Colon, cervical, and breast cancer screening are reasonably-well established, but others are ad hoc. What do you see as the best route to introducing regular screening for cancer?

ND: What we're seeing right now is that there isn't a single unified path for screening tests for all cancers. Consequently, we'll likely need to take different approaches for different tumor types. Generating sufficient data for rarer cancers is much harder and will require different strategies for screening compared to more common cancers.

EF: We understand the importance of education to support adoption, particularly as multi-cancer early detection is a new category of healthcare and cancer screening. Galleri is currently available under investigational use in PATHFINDER, where it is being used to guide clinical care. Learnings from PATHFINDER include care pathways, clinical-decision-making, safety, patient-reported outcomes, and attitudes and adherence to guideline recommended screening. Together, these data should provide insight into actual implementation of a multi-cancer early detection test. As such, we plan to support the health care providers offering the test, which is prescription only, as well as their patients; we are also educating healthcare providers via standard channels, such as continuing medical education and outreach.

On September 21, 2020, Illumina and GRAIL announced an agreement under which Illumina will acquire GRAIL. Joining with Illumina will help us achieve this goal, enabling broader and faster adoption of our innovative, early multi-cancer detection blood test, Galleri, enhancing patient access and expanding our global reach.

We understand the importance of optimizing cancer detection through regular screening, which will require access to screening. As such, we are working on strategies to ensure access to multi-cancer early detection, and to educate healthcare providers, patients, and other audiences about the importance of regular screening for cancer.

GRAIL previously announced it expects to introduce Galleri in the second quarter of 2021. The blood test will be available initially through innovative health systems, medical practices, and self-insured employers. GRAIL is also pursuing insurance coverage pathways.

SA: Thrive's blood test has breakthrough designation with the FDA. We believe having FDA approval and Medicare reimbursement will be critical to ensure safety and efficacy of our test while also enabling broad adoption and access. In addition, an LDT is best utilized to develop real-world learnings to ensure these tests are effectively implemented.

We believe FDA approval and reimbursement coverage, including Medicare will be imperative to enabling broad adoption and access to blood tests that screen for multiple types of cancer. These tests also need to be affordable for an asymptomatic population and integrate seamlessly into routine care including through strong integration (beyond just the ordering mechanism) with the electronic medical record systems.

Q5. What are your companies' respective strategies with respect to regulatory oversight? For example, will these be positioned as laboratory-developed tests (LDTs) or submitted for in vitro diagnostic (IVD) review and approval?

ND: Currently we are committed to developing an IVD for use in the detection of early cancers, but we envision the possibility of developing LDTs for other non-screening applications of our technology.

EF: We plan to commercially launch Galleri, our multi-cancer early detection test, as a laboratory developed test (LDT) in the second quarter of this year. This will enable experience at scale and real-world evidence collection. To help support reimbursement and coverage for our tests, we also plan to seek U.S. Food and Drug Administration (FDA) approval for Galleri following its planned initial launch as an LDT.

FDA has granted us a Breakthrough Device Designation, which allows us more frequent communications with the FDA as we complete our clinical studies and work towards submitting a premarket approval application (PMA).

Q6. Not so long ago, capturing cancer DNA or fragments was a significant technical challenge. What technologies do your respective groups employ to extract, isolate, and purify samples for these assays?

ND: All the technologies basically capture, isolate, and purify cfDNA in a similar manner, but our technology takes advantage of the many cfDNA fragmentation differences that enable more sensitive detection than is possible with mutations or differential methylation.

EF: GRAIL leverages approaches as published in Liu *et al*, Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA.[1]

SA: Thrive's approach to date has been to leverage the biological signals – the actual circulating tumor DNA (ctDNA) and protein expression from the tumor at the core of its test. We use Safe-SeqS technology developed at Johns Hopkins to decipher cancer signals from noise and optimize our assay using a machine learning classifier. Several other techniques are under review but ultimately, our aim is to optimize sensitivity of our test to identify as many cancers as possible while also maintaining a very high specificity.

Q7. Early literature indicated that methylation or modification patterns

in cancer cells was significantly altered, and cancer genomes seemed to become more altered and noisier as a tumor progressed. One could imagine that DNA methylation patterns is more suited to early detection modalities.

1. What is the current thinking on the role of DNA methylation or histone modification in cancer?

2. How are these marker alterations used in your respective assays?

ND: We all agree that there are probably not enough mutations to enable sensitive detection in cfDNA. While methylation gives an alternative approach to differentiating cancer from normal DNA, we believe cfDNA fragmentation gives even more differences to explore.

EF: As noted above, our targeted methylation approach focuses on the most informative DNA methylation regions (~100,000 fragments covering ~1,000,000 CpGs), as identified from our earlier studies and from a large, proprietary database of methylation patterns from participants with and without cancer.

We developed classifiers to distinguish cancer-specific signals from non-cancer signals 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). 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. The performance advantage of DNA methylation is largely due to its biological characteristics, which make it extremely robust at low signal-to-noise ratios.

Robust training and independent validation datasets are critical to ensure real-world applicability of a test. CCGA, which enrolled 15,254 participants at 142 sites, provided a large sample set that could be divided into training and validation sets. Moreover, the composition of the study population included a large number of early-stage cancers in addition to a large variety of cancer types, while the non-cancer population included individuals with a range of benign, non-malignant conditions. As noted above, this resulted in Galleri, a multi-cancer early detection test with high specificity – appropriate for population-scale implementation – that detects a large number of cancer types, thus offering the potential to maximize population-scale cancer detection while minimizing harms from false positives. Our data also showed similar performance between training and validation sets, suggesting that the models were not overfit and would be generalizable.

SA: Regarding methlyation or histone modifications, see answer to previous question. Known biological signals (ctDNA and protein expression) versus patterns has been the core of our assay development.

Marker alterations, e.g., targeted methylation, is being studied; ultimately, if it improves sensitivity of our test without sacrificing specificity, we may consider it.

Q8. Tumors have been found to be heterogenous with a variety of cancer genomes in any blood and solid tumors – subclones, distinct clonal expansions, etc. How do your assays and analytical tools address such diverse DNA variations?

ND: Blood based tests not only look at the heterogeneity within a tumor, but also within all the different metastatic lesions as well. Consequently, the blood-based test gives a better representation of the disease than any single biopsy. This is especially true in hard to biopsy cancers like lung.

EF: Our early analyses interrogated a number of sequencing approaches (WGS, targeted sequencing, WGBS) to identify the highest performing assay with which to move forward. Methylation was selected for further development, as it outperformed WGS and targeted sequencing, and – as noted above – the WGBS assay was evolved into a more efficient targeted methylation approach that focuses on the most informative regions of the genome (~100,000 fragments with ~1,000,000 CgCs).

Methylation is more pervasive compared with canonical mutation sites typically interrogated in traditional liquid biopsy approaches. This targeted methylation approach allowed deeper sequencing of those informative regions compared with WGBS, and thus may overcome expected cost and efficiency limitations of WGS or WGBS approaches. Adding mutations or copy number variations to the methylation approach did not improve performance of the assay.

Additionally, the limit of detection was lower with methylation-based approaches compared to the other approaches. Importantly, epigenetic signals inherently reflect tissue differentiation and malignant cancer states; this likely contributes to the strong cancer detection and signal origin accuracy observed in our studies.

SA: We harness the most common genes that are mutated and proteins that are expressed across multiple types of cancer to develop the "right size test" to detect cancer in the blood. Ultimately, the assay design must be very deliberate in the number of genes and the regions to interrogate in order to maximize performance (sensitivity) while maintaining high specificity. Additionally, a machine-learning classifier helps calibrate the signal being processed to filter out 'noise' that would otherwise obscure positive results from the test.

Q9. In-process and post-assay quality has come to depend on high-powered analytic tools. Such tools provide computational and statistical precision, accuracy, and confidence. Can you briefly the tools used by your groups that lead to qualified data and subsequent reports for review?

ND: We use machine learning to identify recurrent patterns in cfDNA that differentiate between blood drawn from patients with cancer and that from healthy individuals. The algorithm uses data from low coverage whole genome sequencing to find evidence of epithelial cell DNA derived from the cancer in the blood.

SA: Our machine learning classifier which is used to optimize the biological signals coming from the blood must be rigorously trained as part of our overall clinical development program. The integration of software with the hardware allows for a more robust processing power and incorporation of additional data (clinical and social history, for example) into the determination process.

Q10. How are these data presented to the primary care providers to convey to patients? Do clinical decision support groups or patient advisors have a role here?

ND: For every screening test a balance between benefits and harms needs to be drawn. The test needs to be highly sensitive to avoid missing cancers but also needs to manage the number of individuals it sends for clinical follow up to avoid unnecessary procedures. Remember that screening is for people without known cancer, and that screening tests like these are critical parts of helping people to take control of their health and wellbeing.

EF: Galleri is not yet commercially available; we previously announced we expect to launch

the test in the second quarter of 2021. One of our clinical studies, PATHFINDER, is returning results to healthcare providers and patients and being used to guide clinical care so that we can understand how the test is implemented in the clinic. When we launch commercially, we have plans to support the healthcare providers offering the test, as well as their patients. In particular, we will provide educational resources to healthcare providers to help them understand the test performance demonstrated in our clinical studies, as well as to equip them to understand the Galleri test results and next steps. We will also provide patient education materials to use with patients in the office to help patients and providers have a productive discussion around cancer screening and Galleri.

Additionally, we understand the importance of transparency in data reporting, and as such have presented our data at major international medical meetings, s the second-leading cause of death, and that's in large part because we lack recommended screening tests for the majority of deadly cancers. A test that can detect multiple types of cancers, through a single blood draw, and identify where the cancer signal is located in the body with high accuracy, has the potential to save many lives. It is important that a test like Galleri detect as many cancer types as possible to maximize the number of cancer cases detected in the population; in this way, we can maximize the population-scale benefit of multi-cancer early detection, to bend the cancer mortality curve.

In fact, recent data published in *Cancer Epidemiology, Biomarkers & Prevention*[2] demonstrated that by adding Galleri to current guideline-recommended screening tests, we could detect nearly 70% of cancers resulting in death within five years at an earlier stage, potentially averting 39% of the deaths expected if not for earlier detection.

We are published in peer-reviewed journals, and leverage traditional medical education modalities (e.g., continuing medical education) to ensure HCPs and patients have access to our data.

SA: Thrive is working closely with PCPs, patients and other key members of the healthcare community to ensure that the design and content of our report will be clear and actionable for both positive and negative test results. At 99% specificity, it will be very rare to have a positive report, so when this does occur, we aim to ensure that the physician and patient have confidence in the next steps to confirm the presence of cancer and enable care coordination.

Q11. Do you have any final comments, follow-ups, or case studies worth recounting?

ND: Thank you for inviting us to participate in the panel discussion.

EF: Today, cancer remains the second-leading cause of death, and that's in large part because we lack recommended screening tests for the majority of deadly cancers. A test that can detect multiple types of cancers, through a single blood draw, and identify where the cancer signal is located in the body with high accuracy, has the potential to save many lives. It is important that a test like Galleri detect as many cancer types as possible to maximize the number of cancer cases detected in the population; in this way, we can maximize the population-scale benefit of multi-cancer early detection, to bend the cancer mortality curve. In fact, recent data published in Cancer Epidemiology, Biomarkers & Prevention demonstrated that by adding Galleri to current guideline-recommended screening tests, we could detect nearly 70% of cancers resulting in death within five years at an earlier stage, potentially averting 39% of the deaths expected if not for earlier detection.

We are excited by the progress made in our mission to detect cancer earlier and what's to come with the introduction of Galleri this year.

Thank you all for your contributions. JoPM

References

* Early Detection of Cance, https://www.engagez.net/pmls-precisiononcology#lct=conferencecenter--513976-calendar_187893_5ondemand

1. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA, M.C. Liu, G.R. Oxnard, E.A. Klein, C. Swanton, M.V. Seiden on behalf of the CCGA Consortium; Annals of Oncology, VOLUME 31, ISSUE 6, P745-759, JUNE 01, 2020, DOI:https://doi.org/10.1016/j.annonc.2020.02.011
2. Modeled reductions in late-stage cancer with a multi-cancer early detection test, Earl Hubbell, Christina A. Clarke, Alexander M. Aravanis and Christine D. Berg; Cancer Epidemiology, Biomarkers & Prevention, December 16, 2020 (online) https://cebp.aacrjournals.org/content/early/2020/12/12/1055-9965.EPI-20-1134

Dr. Eric Fung

Eric is Vice President, Clinical Development at GRAIL, where he leads several clinical development programs in support of the development of a blood-based multi-cancer detection test. Dr. Fung has previously held clinical development and R&D leadership roles at Affymetrix, Vermillion, Ciphergen, and Roche Molecular Diagnostics. Dr. Fung has led clinical trials leading to FDA clearance of multiple IVD products. Dr. Fung received his MD, PhD from the Johns Hopkins University School of Medicine.

Dr. Nicholas Dracopoli

Nicholas joined Delfi Diagnostics as Chief Scientific Officer (CSO) in February 2019 after spending one year as Senior Vice President of Translational Science at Personal Genome Diagnostics. Previously he was Vice President, Head Oncology Translational Research and Oncology Diagnostics at Janssen R&D. In these roles he was responsible for biomarker discovery, development and applications for oncology products. Previously, he was Vice President of Clinical Discovery Technologies at Bristol-Myers Squibb, and prior to that spent five years in the biotechnology industry at Sequana Therapeutics. Dr Dracopoli obtained his B.Sc. and Ph.D. degrees from the University of London, and completed post-doctoral fellowships at the Memorial Sloan-Kettering Cancer Center and the Massachusetts Institute of Technology. Subsequently, he served as an Assistant Director at the Whitehead/MIT Genome Center, and as a Section Chief at the National Center for Human Genome Research at the NIH before moving to the biotechnology industry. Dr Dracopoli has authored >80 scientific publications, and has extensive experience in the fields of genomics, molecular biology and cancer research.

Sam Asgarian, MD

Sam is the chief medical officer at Thrive, An Exact Sciences Company. As CMO, Dr. Asgarian oversees medical affairs, clinical development and clinical implementation of CancerSEEK, Thrive's blood test to screen for multiple types of cancer. Dr. Asgarian brings deep experience in healthcare strategy and operations, having led clinical product and sales teams at Aetna and CVS Health, where he successfully introduced new innovations into the healthcare system.

Prior to joining Thrive, CVS Health, Dr. Asgarian was vice president of CVS Health's Transformation Health Product organization, where he was responsible for the modernization of existing clinical products and implementation of newly defined solutions to improve consumer convenience and personalize health and wellness. He joined CVS in 2019 as part of the Aetna acquisition, where he was previously serving as the chief medical officer of the Clinical Services organization, overseeing medical management and clinical policy operations across Aetna's commercial and Medicare businesses. Prior to that, he was vice president of strategy and operations for Aetna's Consumer Health and Services division, where he was responsible for transitioning the organization from a business-to-business health plan to a business-to-consumer health and wellness company. Previously, Dr. Asgarian was the chief development and strategy officer for axialHealthcare, a startup company focused on mitigating adverse pain management, leading to better health care quality for patients across different segments and geographies.

Dr. Asgarian holds a B.A. in molecular and cell biology from the University of California at Berkeley, an M.A. in medical sciences from Loyola University Chicago, an M.D. from Tulane University, and an MBA from Cornell University.