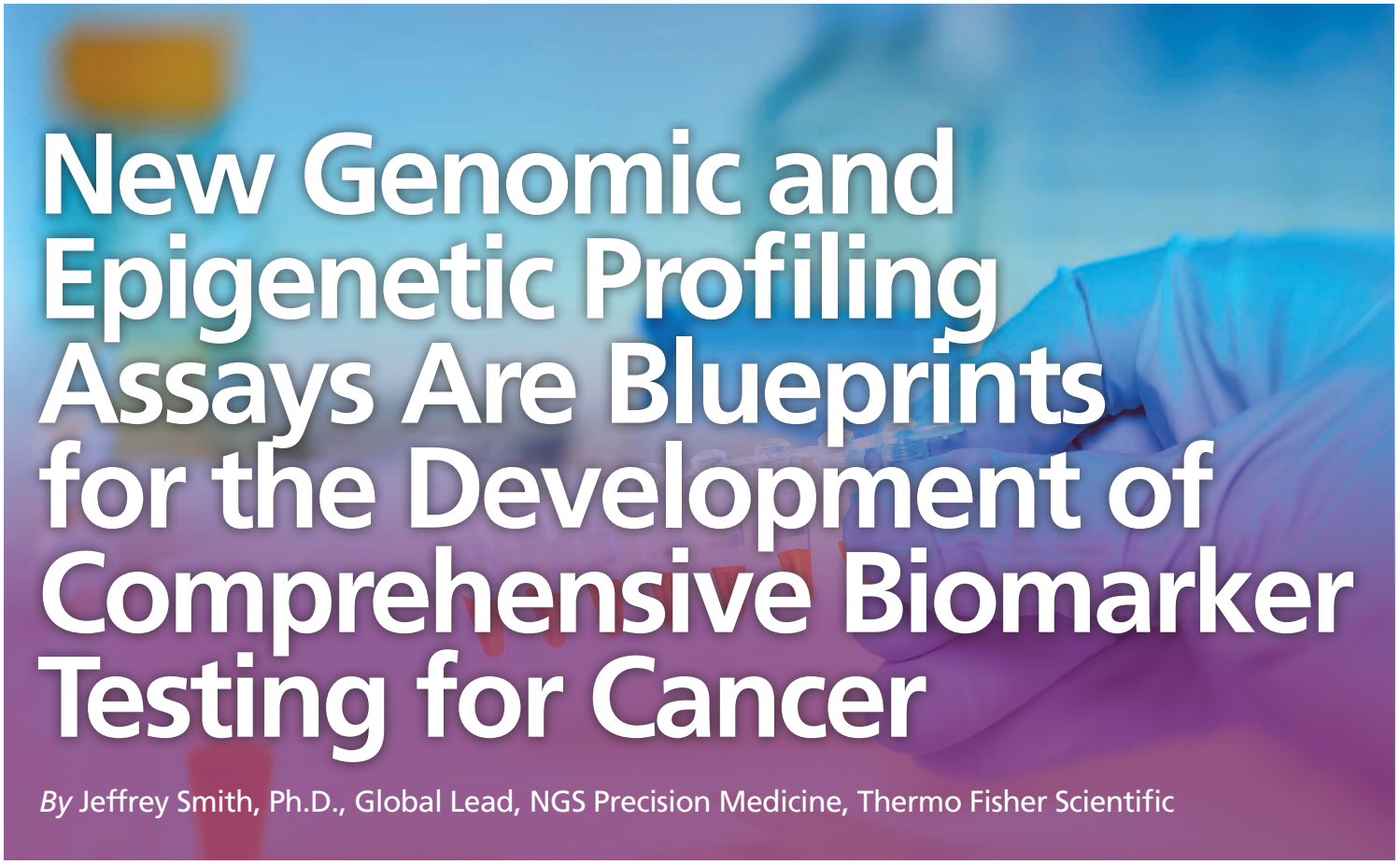

New Genomic and Epigenetic Profiling Assays Are Blueprints for the Development of Comprehensive Biomarker Testing for Cancer

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Introduction and rationale

Various underlying molecular events are now well-understood to trigger the onset, progression, and spread of cancer in the body. Among these underlying events are genomic changes associated with mutations or genomic instabilities. For example, the *erbB2* gene, which encodes a transmembrane glycoprotein belonging to the epidermal growth factor receptor (EGFR) family, is amplified or overexpressed in certain human breast cancers, as well as in other cancer types, including ovarian, stomach, bladder, salivary, and lung carcinomas.[1] In other cases, gene translocations and deletions can lead to new hybrid genes, such as the NTRK gene fusions that impact neurotrophin receptors and are known to be oncogenic drivers of various adult and pediatric tumor types.[2] Beyond genomic changes, researchers have also found that epigenetic events can play a role in how cancers begin, progress, and respond to treatment. As a result, researchers and clinicians are increasingly looking to layer genomics information with epigenetics to better understand cancer.

As more is learned about the complexity of the molecular events and the heterogeneity of cancerous tumors (solid or liquid), the more it is clear that traditional targeted (single-analyte) genetic testing has limited value for determining optimal treatment options for cancer patients. While the single analyte paradigm has historically been used to match biomarkers with clinically-useful drug therapies, research has shown that single-analyte testing is too limited in scope. Specifically, the reflexive nature of single-analyte tests often requires multiple, sequential patient testing – ultimately squandering time, tissue, and other valuable resources.

Most clinical research had focused on single-analyte tests for a particular cancer in a particular tissue type. To deliver on the promise of precision medicine in personalized medicine applications – that is, enabling care givers to match patients with the growing list of promising precision cancer therapies – multi-biomarker testing needs to become standard of care. To the point, a study published in *Current Oncology* recommends *both* comprehensive reflex molecular testing for all patients with non-squamous non-small cell lung cancer (NSCLC) at diagnosis to rapidly identify patients with EGFR-mutated NSCLC *and* molecular testing for resistance mutations during treatment.[3]

To that end, Thermo Fisher Scientific is working with patient groups, governments, researchers, pharmaceutical partners and other industry leaders to develop tests that can match patients with the growing list of promising therapies for cancer and provide earlier, faster, and more precise diagnosis that can save lives or prolong the quality of life.

New, comprehensive biomarker panels for cancer

Now, with next generation sequencing (NGS), we have the opportunity to expand testing to a simultaneous assessment of multiple genomic aspects that have been confirmed through the translational research process. In contrast to single-analyte genetic testing, NGS offers pathologists and clinicians a more comprehensive, efficient tool that delivers results quickly and makes the most of the precious sample available for testing in a timeframe necessary for aggressive cancers.

NGS also offers clinical researchers the ability to combine well-established genetic profiling assays with other approaches that take advantage of the latest -omics technologies, such as transcriptomics and methylation signatures. As our access to data types grows in the future, combining these sets of information will allow clinicians to take full advantage of a patient's biology to understand the best treatment as quickly as possible – and this time-savings matters. Experience shows that genomic results can point care teams toward an appropriate therapy if a biomarker matched with

a therapeutic option is found early in testing. In this regard, NGS testing can play an increasingly larger role to accelerate getting patients on an optimized therapy earlier in their care trajectory.

Recognizing the value of testing to match patients with targeted therapies as early as possible, the U.S. Food and Drug Administration (FDA) and other regulatory bodies encourage co-development of tests with new drugs. Given this impetus, pharmaceutical companies are increasingly turning to NGS-based companion diagnostics (CDx) to ensure as many patients as possible have access to their drugs as a first-line treatment. In 2017, the FDA approved Thermo Fisher's Oncomine Dx Target Test as the first CDx for use in testing NSCLC samples, paving the way for a new class of NGS-based CDx. Today, Thermo Fisher's globally distributed CDx is approved for 12 targeted therapies across 17 countries,* covering more than 550 million lives. We at Thermo Fisher continue to work closely with our pharmaceutical partners to extend its reach.

The power of NGS testing – genomic and epigenomic

NGS is not only a powerful tool for matching patients with promising emerging therapies, but also an effective way to identify new biomarkers, including next generation biomarkers such as tumor mutational burden (TMB) and the immune repertoire of a sample. TMB is a measure of the number of mutations in a cancer cell; this assay, along with other measures, is currently under evaluation for its clinical relevance to identify patients who are more likely to benefit from immune checkpoint inhibitor therapy.[4] Immune repertoire analysis is also gaining attention; recently, researchers found that a promising biomarker signature derived from T cell receptor repertoire analysis outperformed established biomarkers as an indicator that patients may benefit from chemoimmunotherapy.[5] As these new biomarkers are better understood, clinicians will be armed with increasing information and tools to guide patient treatment.

Clinical researchers will continue to study new genomic biomarkers; for example, researchers are also using epigenomics to help identify groups of patients with the same epigenetic changes to determine if such changes may serve as potential indicators of cancer growth or progression. One well-known example of an epigenetic marker is DNA methylation. With the identification of different methylation signatures across various disease indications, researchers have been able to funnel down to an addressable, smaller target set that could be deployed routinely in a clinical setting to monitor and predict cancer progression or response. For now, these biomarker panels are in use only for research, but the goal is to move them from translational studies to clinical use after demonstrating utility.

Potential for impact on clinical practice: Two brief case studies

While there are many exciting studies underway looking at promising emerging genomic and epigenomic biomarkers, I have personally had the opportunity to work closely with two recent studies that illustrate the great promise of a more comprehensive approach to biomarker panels for cancer over previous approaches.

In the first example, a research team from London Health Sciences Center, Western University sought to understand and validate how NGS can be used today without increasing the cost of testing. Through an analysis across hundreds of clinical specimens, they found clear benefits from using NGS as first-line profiling for myeloid cancer patients ahead of fluorescence in-situ hybridization and other traditional methods.

The second study demonstrates the potential to layer epigenetic analysis with genomic profiling. A team of researchers at the Ontario Institute for Cancer Research (OICR) is using an epigenomic profiling tool to investigate how ethnicity may impact early breast cancer through the analysis of abnormal patterns of DNA methylation. Looking at these two examples, one can readily see the value of more comprehensive biomarker panels and the benefits of outcome-oriented multi-omics collaborations.

[*Editor's Note:* see also *Journal of Precision Medicine* | Volume 7 | Issue 2 | June 2021, Clinical data and precision medicine: The urgent need for in-house NGS capabilities at community hospitals]

DNA/RNA-based next generation sequencing panel provides a more effective and economical approach to screenings for myeloid cancer patients

Myeloid samples can be challenging to profile because they are complex, heterogeneous, and are able to proliferate rapidly. Historically, cancers arising from mutations in hematopoietic stem or progenitor cells have been diagnosed using targeted single-analyte testing after initial prescreening by other diagnostic modalities. However, given the wide range of mutations associated with hematologic malignancies – and the fact that the list of relevant genes continues to grow – this kind of reflexive testing presents significant limitations, not least of which is that results are achieved one at a time. This type of serial testing requires more time, tissue, and other resources, whereas NGS allows labs to test for multiple alterations at once from a single, smaller amount of tissue.

Bekim Sadikovic (Head, Molecular Diagnostics Program at London Health Sciences Center, Western University) was the first to implement NGS as an initial, tier-1 screen for hematologic malignancies back in 2008. Since then, he and his team have been collecting data on the clinical impact of NGS in these cases. They recently published a study that showed clear benefits when it came to the use of NGS to evaluate patients' tumors, including the test's ability to: save costs by achieving a timely diagnosis, reduce turnaround time and the need for multiple tests, and minimize hands-on support during testing.[6]

In this published study, the Sadikovic laboratory used a cohort of 380 clinical specimens to perform clinical validation of a gene panel designed to assess 40 genes (DNA) and 29 fusion driver genes with over 600 gene fusion partners (RNA), including sample exchange data across three clinical laboratories, and correlation with cytogenetic testing results. They found that all fusion variants were detected down to 1% dilution, demonstrating 100% sensitivity for fusion detection. In addition, the Oncomine Myeloid Assay** NGS assay, demonstrated 100% specificity with no false-positive results observed, with 192 of the 380 specimens having at least one reportable (Tier I/II) variant for a diagnostic yield of 50.5%. The identification of both gene variants and gene fusions confirmed the opportunity to profile the multiple relevant driver genes in myeloid malignancies in a single test. Moreover, because clinical labs often perform multiple single-gene tests per myeloid patient, Sadikovic et al. reported that this approach yielded an overall cost savings per patient.

Taken altogether, these results led Sadikovic *et al.* to conclude that implementation of a DNA/RNA-based tier-1 NGS panel screening provides a "comprehensive alternative to targeted molecular testing in patients with suspected hematologic malignancies, with increased diagnostic yield, scalability, reproducibility, and cost effectiveness, making it ideally suited for implementation in clinical laboratories."

An epigenomic profiling tool to facilitate precision medicine in early breast cancer

While NGS panels are being clinically validated as a way to gain a more comprehensive view of genomic characteristics of patient specimens, other assays are being developed to help determine how other molecular events may combine with genetic alterations to impact the way cancer starts,

progresses, and responds to treatment. Many of these assays are focused on epigenetic factors, such as changes in chromatin composition and/or organization, disrupted patterns of histone post-translational modifications (PTMs), or abnormal patterns of DNA methylation. Of these, DNA methylation has been the most studied, and distinct and abnormal patterns of both hyper- and hypomethylation have been observed in certain cancers.[7] These epigenetic alterations, which may result from exogenous influences, may explain why what were once considered to be homogenous diseases of a tissue are now known to be heterogeneous, even within well-established clinical subtypes. In particular, DNA methylation has been associated with racial diversity observed in breast, prostate, colorectal and endometrial cancers.[8]

To further our understanding of how multi-omics may be used to improve cancer care, John Bartlett and a team of researchers at the Ontario Institute for Cancer Research set out to study the underlying biology of breast cancer, including the role that epigenomics may play in the regulation of breast cancer processes (e.g., DNA repair) and treatment response. Understanding the unique characteristics of an individual's breast cancer can help clinicians better link patients with treatments and clinical trials that can help them. More specifically, the research team's aim is to improve the delivery of targeted breast cancer treatment to Black and Asian women.

This kind of targeted approach is critical in the equitable delivery of healthcare to patients since a significant proportion of patients may not be adequately treated due to molecular processes influenced by the impact of exogenous factor on different ethnic groups. This project will develop and validate novel panel-based targeted approaches for the evaluation of epigenetic alterations in breast cancer and is designed to address two major needs: improved predictive and prognostic assays for all breast cancer patients and a focused study comparing methylation profiles between cancers in Black and Asian minority and other ethnic groups.

Advancing precision medicine depends on outcome-oriented multi-omics collaborations and the evolution of the healthcare ecosystem

By implementing integrated multi-omics solutions, we will be better able to understand the combined effects of genomic and epigenomic changes in driving cancer progression. It is this layered, multi-omics approach that will allow clinicians to better deliver on the promise of precision medicine.

The examples described above illustrate the benefits of a collaborative approach for the development of targeted therapies for cancer patients. Indeed, the best way to harness the power and potential of bio-innovation is to create outcome-oriented multi-omics collaborations across academic, industry, the not-for-profit, and public health sectors. Aligning the efforts of collaborative groups results in moving therapies with new diagnostics in parallel through trial, thus clearing pathways for the rapidly growing pipeline to clinical utility. The advancements of these partnerships will continue to accelerate innovation and spur the development of additional advanced comprehensive tools that bring together multi-omics for the greatest clinical impact.

That is the ultimate goal: to bring multi-omics testing for cancer into clinical use. Taking this one step further, this scientific innovation will only be effective if we can bring testing closer to patients at the local level, enabling broader, more equitable access to the most effective therapies. Advances to technology are facilitating this shift, allowing community hospitals to provide the same rapid, high quality genomic testing results available to patients as larger academic medical centers. In addition, as testing technology for cancer advances, the healthcare ecosystem, including payer reimbursement, will need to evolve as well. The promise of precision medicine is bright, but we must all work together to ensure comprehensive multi-omics cancer screens become the standard of care to improve patient outcomes.

Lessons learned

- As more is learned about the complexity of the molecular events underlying cancer, it is becoming increasingly clear that traditional targeted (single-analyte) genetic testing has limited value for determining optimal treatment options for cancer patients.
- To deliver on the promise of truly personalized medicine and match patients with the growing list of promising cancer therapies, multi-biomarker testing with next-generation sequencing (NGS) needs to become standard of care.
- NGS also offers clinical researchers the ability to combine well-established genetic profiling assays with other approaches that take advantage of the latest -omics technologies.
- The best way to harness the power and potential of bio-innovation is to create outcome-oriented multi-omics collaborations across academic, industry, the not-for-profit, and public health sectors.
- By bringing this testing closer to patients at the local level, we can enable broader, more equitable access to the most effective therapies and advance precision medicine. JoPM

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* Not all CDx claims are approved in all countries

** For Research Use Only. Not for use in diagnostic procedures

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Thermo Fisher Scientific Inc. is the world leader in serving science, with solutions that enable its customers to make the world healthier, cleaner and safer. Thermo Fisher offers a uniquely diverse portfolio of genomic solutions across a wide range of technologies, including next-generation sequencing (NGS), real-time PCR, Sanger sequencing, and microarrays. Thermo Fisher's clinical sequencing portfolio is focused on developing automated, accessible NGS-based technologies to help more patients benefit from precision medicine.