Genialis

RNA:
the Future of Predictive Biomarkers

Genialis is the RNA biomarker company.

We model fundamental biology using human data and machine learning to ensure successful development of new drugs, and inform targeted treatment decisions for better outcomes.

We are building the next generation of cancer biomarkers based on gene expression data. They incorporate dozens to hundreds of genes into machine learning classifiers capable of capturing therapeutically meaningful relationships between genes and pathways.

Providing patients with better outcomes is at the core of everything we do at Genialis. We value collaboration with like-minded partners to build better biomarkers.

To learn more about collaboration opportunities with Genialis, please reach out.

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The Current State of Biomarker Applications

Biomarkers may have prognostic, diagnostic, or predictive value, and have impacted the field of oncology in:

- **screening,** e.g. CA-125 in ovarian cancer;
- **diagnosis,** e.g. KRAS G12C alteration in non-small cell lung cancer;
- **choice of treatment,** e.g. a tyrosine kinase inhibitor for an EGFR exon 19 deletion in non-small cell lung cancer as a first-line treatment versus chemotherapy; and
- **prediction,** e.g. BRCA1, BRCA2 status in inherited breast cancer.

The inclusion of a biomarker in the design of clinical trials has been found to increase the odds of meeting the trial endpoint and advancing to the next stage of development by **5-12x** in the top five most prevalent cancers[1].

The Need for Better Biomarkers

Despite early promise the success of biomarkers in predicting drug efficacy still falls short of the industry's hopes and expectations. As of 2020, roughly 27% of cancer patients were eligible for genome-informed therapy, while only 11% showed a clinically beneficial response to such treatment. Furthermore, the durability of response has been stagnant at ~18 months for nearly the past two decades[2]. For example, PD-L1 is a valuable prognostic biomarker for overall survival across a variety of cancers[3] and has been approved by the FDA as a companion diagnostic for immune checkpoint therapy. However, a retrospective study (of all clinical trials between 2011-2019 prompting FDA approval of immune checkpoint inhibitors) identified PD-L1 as a predictive biomarker in **only about 30%** of cases[4]. **Better biomarkers are required.**

First, Choose a Better Analyte

The first choice to make is what to measure as a readout of the disease. DNA and RNA are molecules that form the basis of every cell and are precursors to the formation of proteins that make up the human body. DNA, RNA, and proteins all may be analytes that serve as biomarkers associated with various aspects of health and disease. Before we explore the potential of our preferred RNA-based biomarkers, let's examine the state of the art for the other two analytes.

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[1] Parker JL, Kuzulugil SS, Pereverzev K, et al. Does biomarker use in oncology improve clinical trial failure risk? A large-scale analysis. Cancer Med. 2021;10(6):1955-1963. doi:10.1002/cam4.3732

[2] Haslam A, Kim MS, Prasad V. Updated estimates of eligibility for and response to genome-targeted oncology drugs among US cancer patients, 2006-2020. Ann Oncol. 2021;32(7):926-932. doi:10.1016/j.annonc.2021.04.003

[3] Mok TSK, Wu YL, Kudaba I, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet. 2019;393(10183):1819-1830. doi:10.1016/S0140-6736(18)32409-7

[4] Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. J Immunother Cancer. 2019;7(1):278. Published 2019 Oct 26. doi:10.1186/s40425-019-0768-9

DNA-Based Biomarkers

Among DNA, RNA, and proteins, DNA is most commonly used for predictive biomarker discovery and companion diagnostic development.

One such example is the measurement of EGFR T790M mutations in metastatic NSCLC, which guides the use of Osimertinib when patients are resistant to first-generation tyrosine kinase inhibitors like Erlotinib[5].

Another DNA biomarker is the analysis of the oncogene KRAS, specifically forG12C mutations, for which targeted therapies have only recently earned approval.

DNA can tell us the genetic background and provides the mutational status, however, DNA does not report on changes in cell biology associated with disease onset or progression.

Protein-Based Biomarkers

Protein biomarkers were among the first to be used in cancer diagnostics. Often the proteins themselves are the therapeutic targets (e.g. HER2 levels in breast cancer[6], and the use of Trastuzumab in HER2-positive breast cancer[7]).

A protein biomarker may also consist of cancer antigens (e.g., CEA), enzymes, or hormones, or changes in protein modifications such as distinct glycosylation profiles found in tumors[8]. Protein biomarkers rely on techniques such as enzyme-linked immunoassay (ELISA), mass spectrometry, or immunohistochemistry which may be limited by poor reproducibility (due to inter-lab variability[9] of the antibody-based methods) or prohibitive complexity and cost (mass spectrometry). In addition, for biomarkers in plasma, various normal proteins that are dominantly expressed can mask the low expression of cancer proteins or modifications of proteins[10].

These present challenges for provenance and preclude the consistent use of protein-based biomarkers.

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[5] www.nccn.org/professionals/physician_gls/pdf/nscl.pdf

[6] Burstein HJ. The distinctive nature of HER2-positive breast cancers. N Engl J Med. 2005;353(16):1652-1654. doi:10.1056/NEJMp058197

[7] Hudis CA. Trastuzumab--mechanism of action and use in clinical practice. N Engl J Med. 2007;357(1):39-51. doi:10.1056/NEJMra043186

[8] Stowell SR, Ju T, Cummings RD. Protein glycosylation in cancer. Annu Rev Pathol. 2015;10:473-510. doi:10.1146/annurev-pathol-012414-040438

[9] Barker AD, Alba MM, Mallick P, Agus DB, Lee JSH. An Inflection Point in Cancer Protein Biomarkers: What was and What's Next. Mol Cell Proteomics. 2023;22(7):100569. doi:10.1016/j.mcpro.2023.100569

[10] Sarhadi VK, Armengol G. Molecular Biomarkers in Cancer. Biomolecules. 2022;12(8):1021. Published 2022 Jul 23. doi:10.3390/biom12081021

The Advantages of RNA-Based Biomarkers

Genialis focuses on RNA because we have found that gene expression-based biomarkers provide dynamic insights into cellular states and regulatory processes when compared with DNA or protein biomarkers. These aspects of disease biology are especially relevant to predicting treatment outcomes of complex diseases. We prefer RNA biomarkers for several reasons:

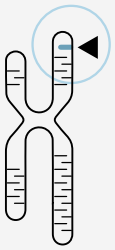
- RNA is closer to the phenotype than DNA. DNA represents potential biological states, providing information on what a cell could do. In contrast, RNA represents the **actuation of biological states**, a snapshot of the cellular or tissue physiology. Assessment of RNA reveals differential changes in gene expression associated with varying biological function due to disease progression, mutation, drug response, chemical perturbation, etc.
- RNA can be used to infer mutation status in the same manner that DNA can (in expressed genes)
- The data from total RNA sequencing (bulk RNA-Seq) includes all species of RNA including protein coding (mRNA) and non protein coding RNA (e.g. miRNA and lncRNA in structural interactions, transcriptional and translational regulation, etc). Thus RNA contains high-dimensional information which can be leveraged to understand the biology of a given cancer with far more nuance than DNA alone.

RNA Biomakers in Practice

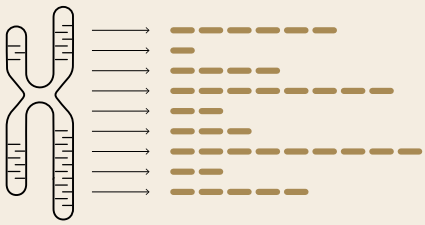
Common concerns regarding RNA-Seq include its cost, access to bio-specimen material, and data reproducibility. Given advancements in the state of the art, RNA Seq has become more attractive as a clinical analyte as some of the above objections are satisfied. The standardization of laboratory methods for sample preservation, nucleic acid extraction, and sequencing preparation has led to demonstrably high reproducibility from lab to lab, even from small amounts of archival samples.

The wide availability of sequencing services means RNA-seq is **routinely integrated** into clinical workflows and can be **reliably extracted** and quantified from FFPE slides without the requirement for a fresh biopsy or frozen tissue. The ability to multiplex many different tests from the same analyte combined with the ever-decreasing costs of sequencing makes transcriptomic biomarkers cost-effective for complex disease diagnosis, and clinical R&D.

How biomarkers predict response to targeted treatment

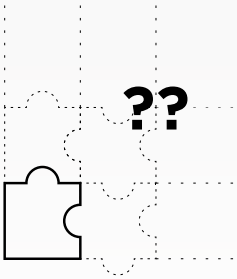


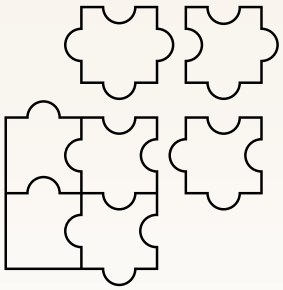
DNA biomarker



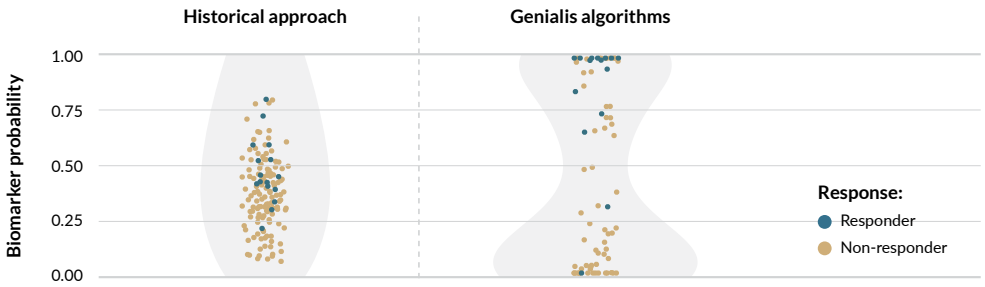
RNA biomarker

	DNA biomarker	RNA biomarker
BIOLOGY	Driver gene disruption, mutational burden	Pathway disruption, dysregulation, activation, suppression, etc **PLUS:** Driver gene disruption, mutational burden
FEATURE	One or just a few DNA variants	Quantitative signatures comprising the expression and variants of dozens of genes
ALGORITHM	Binary mutation status	Machine learning classifier
CAPABILITY	**Necessary but insufficient:** **Describes the status of a drug target or disease driver, but does not describe the biological state (or phenotype) of the disease.**	**Predictive and explanatory:** **Captures complex interactions to define the biological state and treatment susceptibility of the disease.**





The Genialis Approach



Genialis has successfully developed RNA-based biomarkers for drugs of different mechanisms of action (MOA) and cancers of various tissues of origin. In one such well-publicized example, the **Xerna TME Panel** was developed to predict response to virtually any drug targeting the tumor microenvironment (TME), and has been shown to enrich for clinical benefit to numerous approved and investigational drugs across a dozen solid tumors[11,12]. While historically, RNA has been underutilized as an analyte for diagnostic devices, regulatory bodies have demonstrated a keen interest in this class of biomarkers. The Xerna TME Panel is currently being developed for clinical applications as a clinical trial assay with FDA IDE acceptance; an LDT for clinical research; and as a CDx for a novel anti-angiogenic drug.

Key to the above success is Genialis' innovation of novel machine learning techniques to ensure biomarker models work across different clinical contexts and in the real world. For example, we employ methods that select only those genes that are consistently and robustly expressed across different bias modalities, such as tissue types and platforms, to be included in the predictive model. The Xerna TME panel comprises approximately 100 genes that performed consistently in data derived from numerous cancer types generated by various sequencing providers, each with a different protocol and platform. The depth of this signature also provided redundancy in signal, mitigating the influence of technical and biological variability on model performance. The end product is a reproducible and robust RNA-based biomarker that predicts clinical benefit in the real world.

Genialis' biomarker discovery framework **ResponderID** and the next-generation biomarker models we have built to date are advancing the leading edge of clinical development of potentially life-saving therapies.

[11] Fu S, Corr BR, Culm-Merdek K, et al. Phase Ib Study of Navicixizumab Plus Paclitaxel in Patients With Platinum-Resistant Ovarian, Primary Peritoneal, or Fallopian Tube Cancer. J Clin Oncol. 2022;40(23):2568-2577. doi:10.1200/JCO.21.01801

[12] Uhlik M, Pointing D, Iyer S, et al. Xerna™ TME Panel is a machine learning-based transcriptomic biomarker designed to predict therapeutic response in multiple cancers. Front Oncol. 2023;13:1158345. Published 2023 May 12. doi:10.3389/fonc.2023.1158345