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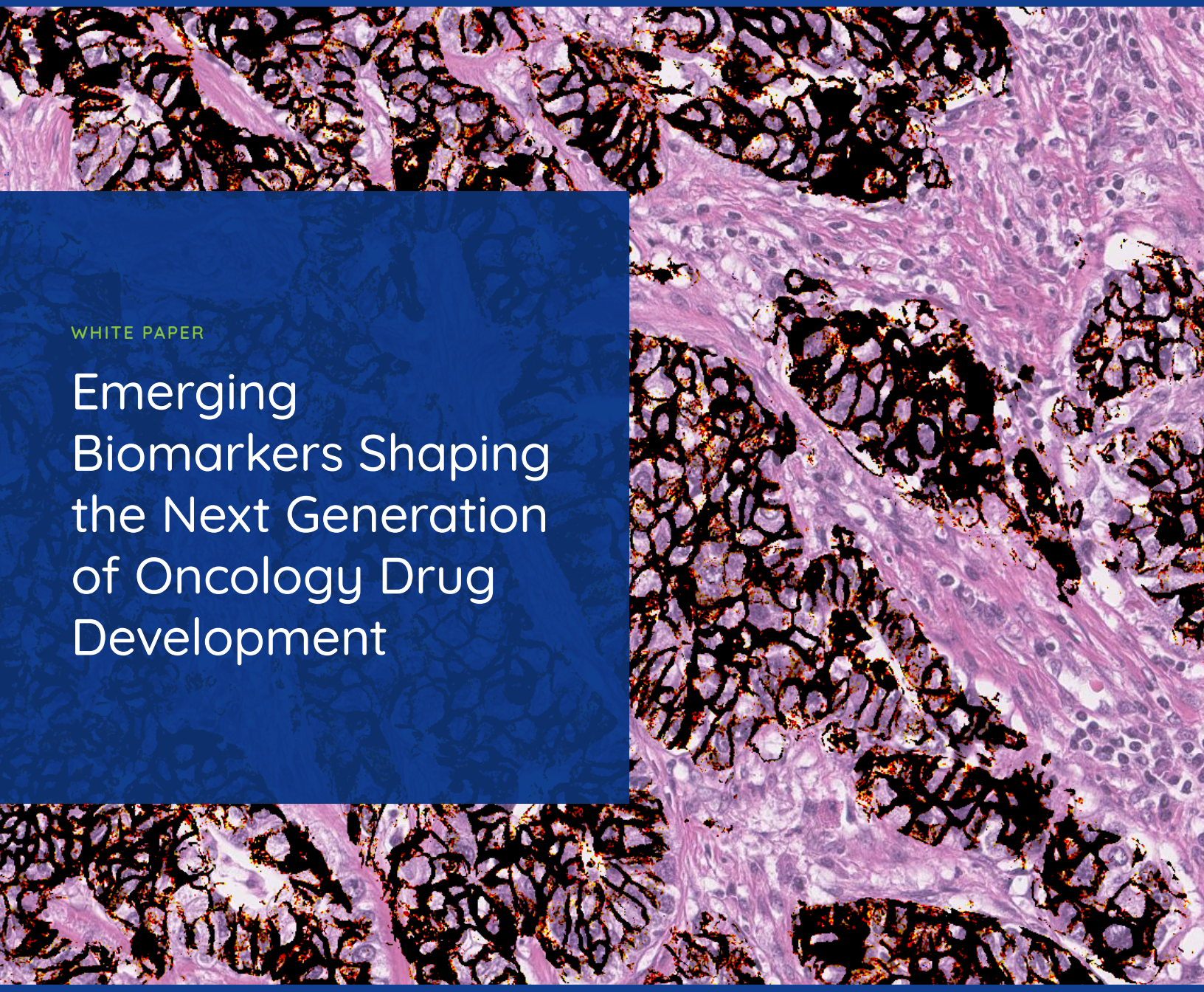


WHITE PAPER

Emerging Biomarkers Shaping the Next Generation of Oncology Drug Development

NeoGenomics Laboratories

Precision oncology has made significant contributions to the efficacy of cancer treatment. The paradigm of precision oncology is undergoing a fundamental biophysical and structural shift. For the past two decades, translational medicine has largely relied on bulk multi-omic approaches, in which heterogeneous tumor samples are averaged to produce aggregate genomic and proteomic readouts.

While these whole-tissue approaches excel at identifying what molecular aberrancies exist within a specimen at a surface level, they strip away the vital topological coordinates of the tissue. As a result, these methods fail to capture spatial relationships such as cell positioning, molecular distributions, and microenvironmental gradients within intact tissue. In oncology clinical trials, understanding this spatial context is crucial to tease out the root cause of late-stage pipeline attrition (which could be up to 58% in Phase III).

Tumors function as highly organized, compartmentalized tissues, rather than as independent entities. Therapeutic failure often arises not from lack of target expression, but from restricted accessibility driven by stromal barriers, heterogeneous distribution, or localized cellular reprogramming. To systematically deconstruct these resistance mechanisms, clinical development must move beyond crude binary biomarker scoring and adopt a synergistic, multi-tiered tissue phenotyping framework. Traditional single-plex Immunohistochemistry (IHC) remains the indispensable, regulatory-validated foundation of anatomic pathology —offering high-throughput quantification, robust enzyme-linked signal amplification, and essential baseline diagnostic screening (Figure 1). Furthermore, it provides clinically valuable spatial context by preserving tissue architecture and enabling visualization of biomarker expression within specific cell types and regions of the tumor microenvironment. This enables pathologists to distinguish tumor cells from stromal and immune components, while also assessing subcellular localization (e.g., membrane HER2 or nuclear ER), which is critical for accurate diagnosis and therapeutic decision-making. Importantly, IHC captures intratumoral heterogeneity by revealing regional variability in biomarker expression, immune infiltration, and proliferative activity, helping to identify sub-clonal populations and heterogeneous target expression. Together, these spatial and functional insights improve biomarker interpretation, guide treatment selection, and provide insight into treatment efficacy, ultimately being instrumental to the success of the clinical trial.

With advances in the understanding of tumor biology, IHC target identification has evolved beyond differential expression to incorporate functional relevance, resistance mechanisms, and tumor microenvironmental context (Figure 2).

Reflecting this shift, a new wave of high-priority oncology targets is emerging across three key domains: surface antigens suitable for antibody-drug conjugates (ADCs), metabolic pathways, and immune-modulating molecules.

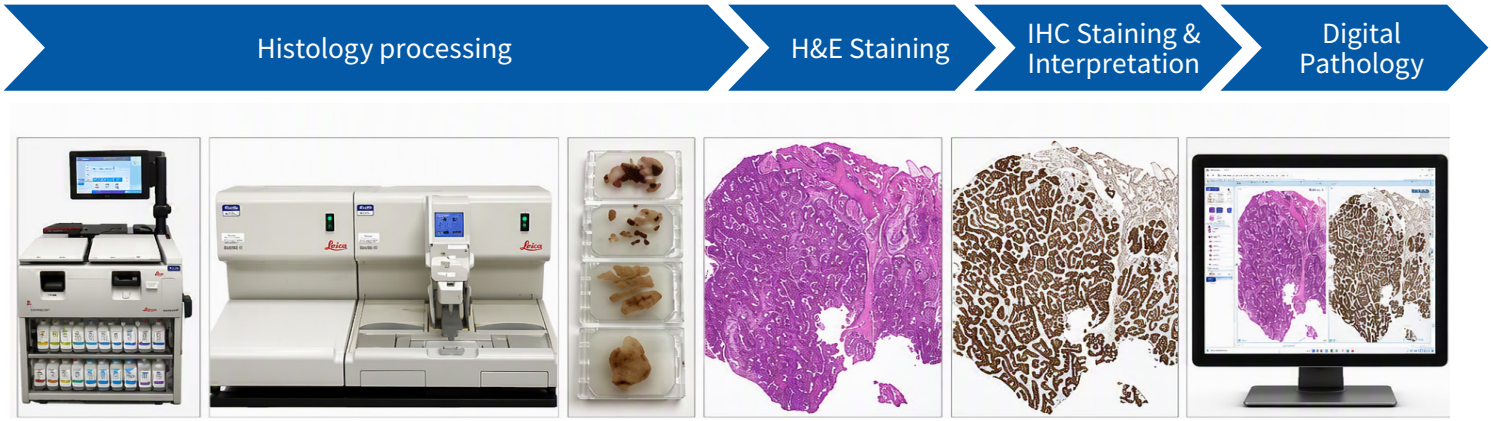


Figure 1: *IHC workflow.*

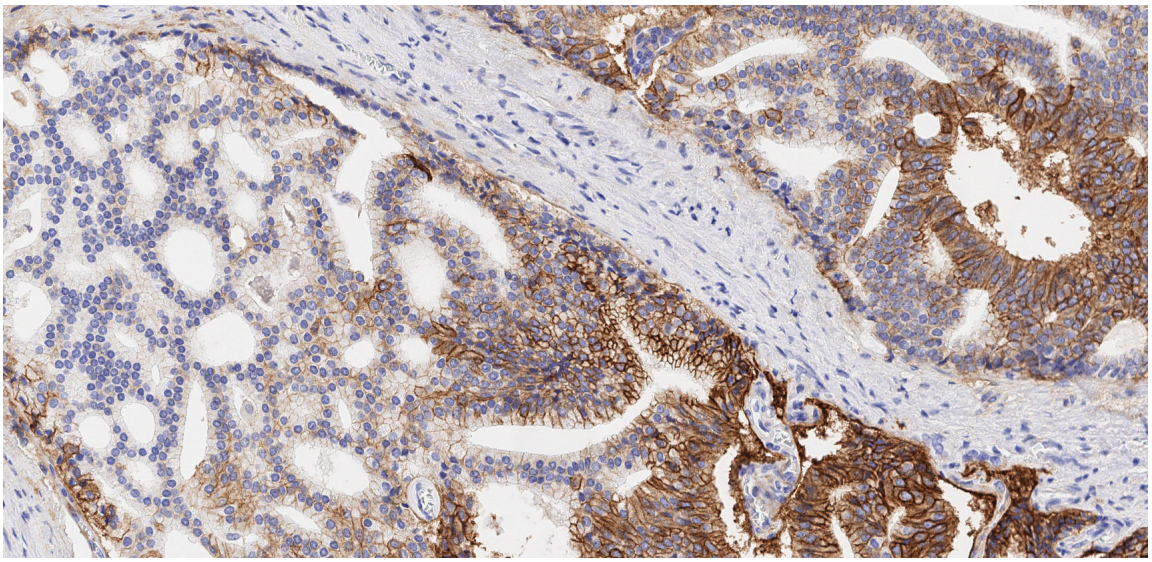


Figure 2: *B7-H3 expression in prostate carcinoma demonstrating intratumoral heterogeneity.*

Targeting Tumor Surface Antigens for Antibody-Drug Conjugates

ADCs have become a highly promising therapeutic modality in oncology, demonstrating significant clinical and commercial impact in recent years. By early 2026, fifteen ADCs had been approved by the U.S. Food and Drug Administration (FDA), and a strong, growing pipeline across the industry is expected to yield additional approvals in the near term. The global ADC market is projected to reach approximately $64.7 billion by 2030. Immunohistochemistry (IHC) plays a critical role in ADC development programs by enabling accurate and quantitative assessment of target expression within the tumor microenvironment. However, the effectiveness of IHC assays in this context is closely linked to the specific ADC target being evaluated.

ADCs target specific transmembrane proteins that are overexpressed on the surface of tumor cells while minimally expressed in healthy tissues. Following antigen binding at the cell surface, the ADC–target complex undergoes internalization, enabling intracellular release of the cytotoxic payload. Additionally, cytotoxic payloads released from ADCs can diffuse across cell membranes, leading to the killing of adjacent cancer cells (the so-called bystander effect) (Figure 3).

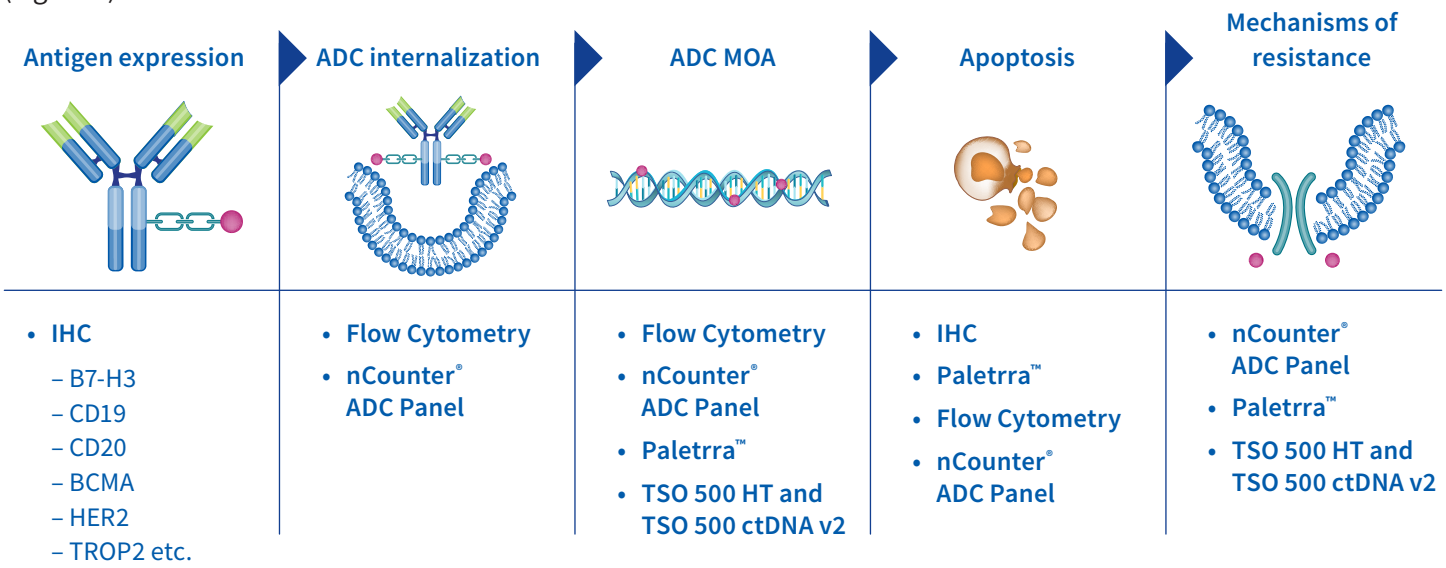


Figure 3: *ADC mode of action. Neogenomics provides an end-to-end multimodal ADCs solution.*

To qualify as a successful ADC transmembrane target, the protein must possess the following three properties:

1. **Strong tumor-selective expression:** To reduce off-target toxicity, the transmembrane target must be highly overexpressed on the surface of the tumor cells compared to normal tissue.
2. **Efficient Internalization:** For the successful transport of the ADC-antigen complex into the lysosome and subsequent release of the cytotoxic payload, the transmembrane protein must be capable of rapid endocytosis.
3. **High surface abundance:** A high density of exposed extracellular domains is needed to optimize ADC binding.

In recent years, there has been increasing interest in two such transmembrane proteins, namely, B7-H3 and TROP2.

B7/CD28 family proteins are critical regulators of immune function, delivering both inhibitory and stimulatory signals that help preserve immunological balance[1]. During tumor development, these molecules can contribute to the formation of an immunosuppressive tumor microenvironment, facilitating immune evasion by cancer cells. Among the B7 family members, PD-1/PD-L1 (B7-H1, CD274), PD-L2 (B7-DC, CD273), and VISTA (B7-H5) have been the most widely investigated for their clinical relevance.[2,3] While initially described as a co-stimulatory immune regulator, B7-H3 is now more frequently associated with suppression of anti-tumor immune responses.[4,5] Although its expression is limited in normal tissues, B7-H3 is highly expressed across a variety of malignancies, including lung, esophageal squamous cell, gastric, pancreatic, colorectal, liver, breast, brain, and prostate cancers.[6] Importantly, increased B7-H3 expression has been associated with enhanced tumor cell proliferation, metastasis, and drug resistance, ultimately correlating with poorer patient outcomes.[7,8] The importance of this target is highlighted by the fact that there are over 15 B7-H3 ADCs that are currently under clinical development.

Trophoblast cell surface antigen 2 (TROP2), encoded by the TACSTD2 gene, is a type I transmembrane glycoprotein that plays an important role in embryonic development and is expressed at low levels in normal tissues. On the other hand, TROP2 is prominently overexpressed in multiple solid tumors, including triple-negative breast cancer (TNBC), non-small cell lung cancer (NSCLC), prostate cancer, colorectal cancer, ovarian and cervical cancers.[9] Mechanistically, TROP2 has been shown to mediate its oncogenic effects via dysregulation of oncogenic signaling pathways including MAPK/ERK, JAK/STAT, Wnt/β-catenin signaling networks among others.[10] Clinically, overexpression of TROP2 has been associated with poor prognosis in patients and may serve as an independent prognostic biomarker.[11,12] It is of interest to note that TROP2-targeted therapies are most commonly developed in the form of ADCs, and currently, there are over 70 active clinical trials involving TROP2 ADC therapeutics based on NCI data.

In clinical studies of ADCs targeting such overexpressed transmembrane proteins, IHC is routinely used to measure antigen expression levels. These measurements support patient selection and stratification strategies. Longitudinal monitoring is particularly important, as changes in target expression, including reduction or loss, are well-established mechanisms of resistance to ADC therapies. Additionally, heterogeneous expression of TROP2 within tumors has been associated with disease outcomes, and accurately characterizing this variability through IHC can aid in distinguishing responders from non-responders.

Metabolic and Epigenetic Dependencies in Cancer

Another major frontier in oncology innovation involves targeting cancer-specific metabolic dependencies. This strategy focuses on disrupting the bioenergetic and biosynthetic processes that enable tumor growth, immune evasion, and therapeutic resistance.

Cellular protein structure and function are precisely regulated by post-translational modifications (PTMs) via covalent alterations.[13] It is well known that PTMs such as phosphorylation, acetylation, methylation, and ubiquitination play essential roles in cellular signaling, epigenetic regulation, and tumorigenesis. Aberrant epigenetic regulation is a fundamental driver of oncogenesis, with arginine methylation increasingly recognized as a clinically relevant post-translational modification. This process, mediated by protein arginine methyltransferases (PRMTs), controls a range of processes integral to tumor biology, such as proliferation, differentiation, transcriptional regulation, and DNA damage response.[14] Among this enzyme class, PRMT5 has emerged as a key therapeutic target owing to its consistent overexpression across a broad range of solid tumors and hematologic malignancies, as well as its link to poor prognosis in patients. Importantly, tumors harboring loss of methylthioadenosine phosphorylase (MTAP) demonstrate a selective dependency on PRMT5 activity, providing a biologically defined context for targeted therapeutic intervention.

Yet another key epigenetic regulator, Enhancer of Zeste Homolog 2 (EZH2), is known for its role in global gene silencing and is involved in a variety of cellular processes, including cell survival, proliferation, invasion, and self-renewal.[15] As a core component of the Polycomb Repressive Complex 2 (PRC2), EZH2 catalyzes the trimethylation of histone H3 at lysine 27 (H3K27me3), resulting in chromatin compaction and transcriptional repression.[16] Dysregulated EZH2 expression is observed in a wide range of solid tumors and hematological malignancies and is frequently associated with increased metastatic potential and poor clinical outcomes.[17] While EZH2 primarily mediates gene silencing through its canonical PRC2-dependent activity, it also exerts oncogenic effects via non-canonical mechanisms. In its non-canonical role, EZH2 acts independently of PRC2, interacting with other signaling molecules as a transcriptional activator or co-activator, thereby promoting the activation of oncogenic pathways.[18] Through both canonical and non-canonical mechanisms, EZH2 significantly contributes to tumor initiation and its subsequent progression. Given its critical role in oncogenesis and cancer progression, EZH2 is under investigation as a potential biomarker for cancer diagnosis and prognosis.

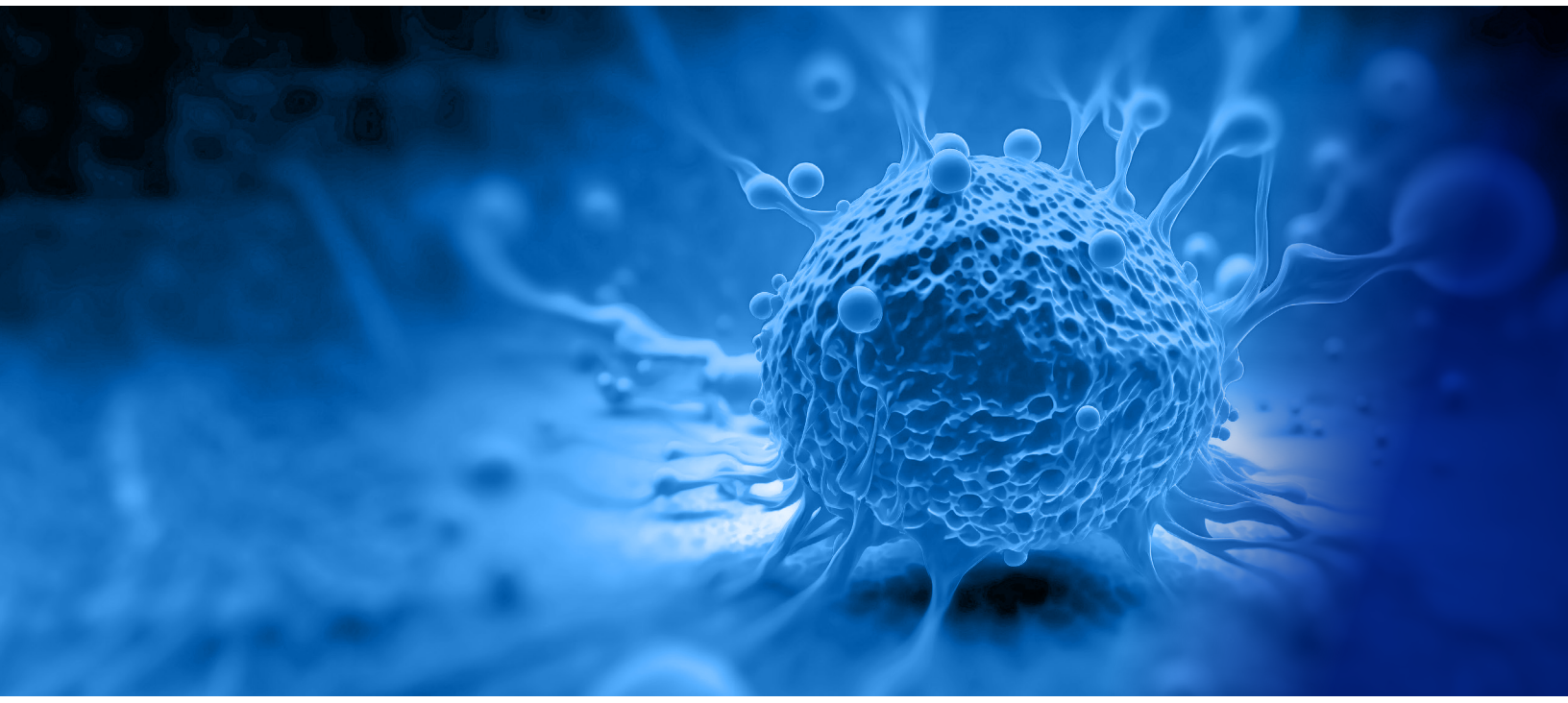
For these intracellular epigenetic regulators, IHC facilitates biomarker-driven patient stratification by enabling the evaluation of nuclear protein expression, downstream pathway activity—including markers such as H3K27me3 as an indicator of EZH2 function—and the distinction between tumor and stromal compartments. These factors can play a critical role in informing rational combination approaches and guiding therapeutic positioning.

Immune-Modulatory Targets in the Tumor Microenvironment

A third category of emerging targets includes immune-modulatory molecules such as 4-1BB (CD137; TNFRSF9), a tumor necrosis factor receptor superfamily member, which is a key costimulatory molecule that has emerged as a promising therapeutic target for enhancing antitumor immunity, supported by extensive preclinical and clinical evidence over the past two decades.[19] Targets such as these exert their effects by enhancing immune cell activation rather than directly targeting tumor cells, making a detailed understanding of their cellular context within the tumor microenvironment essential. IHC is particularly valuable in this setting, as it allows for the characterization of the distribution, density, and spatial localization of immune cell populations expressing the target, including T cells and natural killer (NK) cells. These insights help inform both anticipated efficacy and potential safety risks.

Within the landscape of precision oncology therapeutics, IHC also supports pharmacodynamic evaluations, reinforcing its role as a key translational tool linking target biology with clinical response. Importantly, the utility of IHC extends beyond measuring individual protein expression levels. The spatial organization of immune cells within the tumor microenvironment—specifically their presence within the tumor core versus the surrounding stroma or invasive margin—can have a significant impact on the effectiveness of immunotherapies, including 4-1BB/CD137 agonists. For example, a highly immunosuppressive peri-tumoral environment characterized by elevated levels of PD-1, TGF-beta, and FAP-alpha may reduce the effectiveness of 4-1BB-mediated immune activation. Therefore, incorporating spatial context into biomarker analysis is essential for understanding mechanisms of immune resistance and improving therapeutic strategies.

Soluble CD137 isoforms generated through alternative splicing have been shown to act as decoy receptors that bind CD137 ligand and inhibit T cell co-stimulation, representing a potential mechanism of tumor immune escape and therapeutic resistance.[20] Such acquired resistance mechanisms can be elucidated through an integrated approach combining longitudinal IHC to monitor target antigen expression with ELISA-based detection of soluble, truncated target variants released into circulation.



To address the growing complexity of oncology drug development, NeoGenomics continues to expand its offerings with a comprehensive portfolio of next-generation IHC assays and has recently launched B7-H3, CD137, EZH2, PRMT5, and TROP2, providing access to high-priority targets aligned with leading therapeutic approaches, including ADCs, immune-modulating agents, and epigenetic therapies. In addition, the recent launch of the Paletrra™ spatial biology platform integrates whole-slide imaging with AI-based analytical tools. This approach preserves tissue architecture while enabling high-dimensional, spatially resolved analysis of tumor–immune interactions, cellular phenotypes, and microenvironmental dynamics. Together, these capabilities form a fully integrated biomarker ecosystem designed to reduce risk and support oncology programs from early discovery through late-stage clinical development. However, the successful translation of biomarker discoveries into clinical and commercial impact requires more than advanced testing technologies alone. As precision medicine continues to further evolve, it becomes increasingly imperative to not only identify the best biomarker candidates, but also to understand how a biomarker is utilized in routine clinical practice.

Real-world data insights into provider ordering behaviors, testing adoption patterns, geographic variation, and patient access can reveal critical disconnects between biomarker development strategies and real-world clinical practice. Understanding these dynamics, along with the real-world prevalence of target patient populations, where and when the patients are being tested, as well as predominant specialties, can help sponsors not only quantify market opportunity but also improve commercial success. As an end-to-end provider of biomarker testing capabilities and real-world oncology insights, NeoGenomics is uniquely positioned to help pharma partners bridge the gap between biomarker discovery and real-world clinical adoption to improve patient care and accelerate commercial success.

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NeoGenomics, Inc. is a premier cancer diagnostics company specializing in cancer genetics testing and information services. We offer one of the most comprehensive oncology-focused testing menus across the cancer continuum, serving oncologists, pathologists, hospital systems, academic centers, and pharmaceutical firms with innovative diagnostic and predictive testing to deliver timely, actionable insights that guide personalized care decisions. Headquartered in Fort Myers, FL, NeoGenomics operates a network of CAP-accredited and CLIA-certified laboratories for full-service sample processing and analysis services throughout the US and a CAP-accredited full-service sample-processing laboratory in Cambridge, England.

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CORP-MRKT-0454 08-26