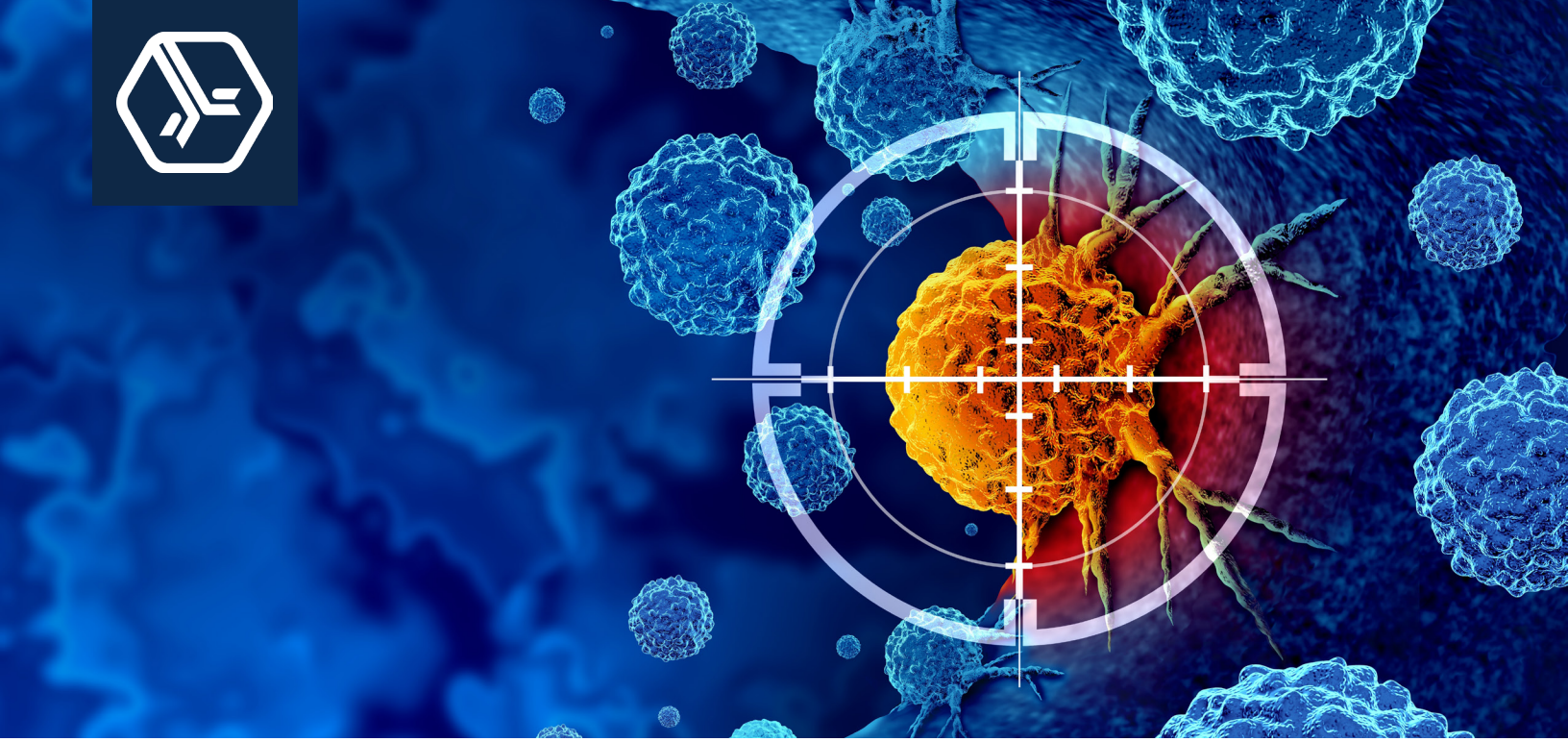

WHITE PAPER
CANCER AUTOANTIBODIES

Next-Generation Biomarkers in Cancer Precision Medicine

- Understand how cancer and cancer treatment can induce autoantibody production
- Explore the critical role of autoantibodies in early cancer diagnosis and predicting patient outcomes
- Discover how autoantibody profiling guides cancer drug development

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Introduction

Biomarkers are essential for diagnosing, monitoring, and treating diseases. However, developing biomarker signatures with high sensitivity and specificity is particularly challenging for heterogeneous diseases like cancer.

Recent advancements in immunology suggest that autoantibodies (AAbs), or antibodies that target self-molecules, can serve as precise and reliable biomarkers in cancer (1). This white paper explores how cancer can induce AAb production and how AAbs provide valuable insights into disease mechanisms and therapeutic targets, aiding in early diagnosis, predicting treatment response, and guiding drug development.

Autoantibodies: A Paradigm Shift in Cancer Biomarker Discovery

Cancer processes can induce AAb production by forming or exposing new binding sites on proteins, known as neoepitopes, through cellular changes or therapeutic pressures (Figures 1 and 2). Changes in AAb profiles can reflect both the malignant transformation continuum and subsequent disease progression, offering detailed insights into the disease's location, nature, and timing. Importantly, the AAbs may have a pathogenic or protective role in cancer progression.

AAbs possess several ideal biomarker characteristics, including but not limited to:

- Stability: Less prone to degradation compared to mRNA and proteins within their native matrix.
- Accessibility: Easily obtained from peripheral blood samples.
- Detectability: Profile AAbs with high specificity and sensitivity using multiplexed, high-throughput assays.

Their stability and accessibility make AAbs suitable for retrospective and longitudinal studies, even in resource-limited settings.

Early Detection and Predictive Power of Autoantibodies in Cancer

AAbs generated during carcinogenesis provide a valuable opportunity for earlier diagnosis, enabling intervention before symptoms appear (Table 1, Figure

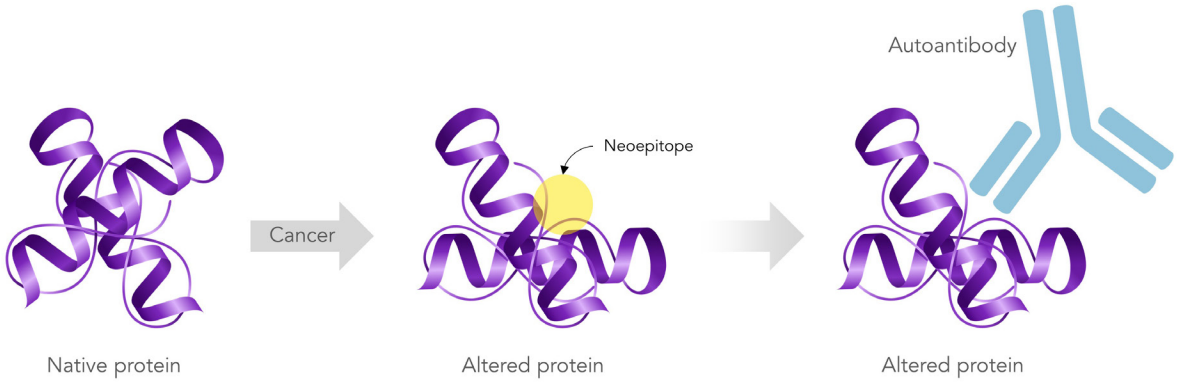

Figure 1. Neoepitopes formed during cancer can elicit the production of AAbs

2) (2). For example, AAbs targeting p53, the most commonly mutated protein in cancer, were detected on average 3.5 years prior to diagnosis, with a positive predictive value of 0.76 for subsequent malignancy (3). AAbs in lung cancer patients are present up to 5 years prior to diagnosis (4). In fact, an AAb-based assay, *Early*CDT®-Lung test, has been approved for clinical use as a complementary diagnostic method (5). AAb profiling also helped guide the protein signature that is now utilized in Videssa® Breast, a CLIA-certified blood-based assay to help diagnose early-stage breast cancer following an abnormal mammogram (6).

Earlier cancer detection provides a window of opportunity during which interventions are more able to effectively modify disease to improve survival rates. For public health systems, early diagnosis can reduce the long-term burden of cancer treatment, both in terms of healthcare costs and patient quality of life. Additionally, early-stage treatments are generally less resource-intensive, leading to better allocation of healthcare resources and increased accessibility to care.

AAb profiling also provides valuable information beyond their use as early diagnostic biomarkers. For instance, a study discovered a signature of 13 AAbs predictive of poor survival rates in patients with resected non-small cell lung cancer (7). This signature was validated in an independent cohort, achieving a sensitivity of 84% and specificity of 74%. Another study identified AAb signatures predictive of outcomes of melanoma patients treated with immune checkpoint inhibitors (8). Interestingly, different AAb profiles were observed for toxicity (i.e., immune-related adverse events) and response between non-Hispanic whites and underrepresented minorities.

Impact of Cancer Therapies on Autoantibody Levels

Common cancer treatments like chemotherapy and radiotherapy induce massive cell death and release tumor-associated proteins, which can trigger AAb production (Figure 2). AAb levels also frequently increase in response to next-generation cancer therapies that stimulate the immune system, such as immune checkpoint inhibitors.

Role of Autoantibodies in Cancer Vaccine and Drug Development

AAbs play a significant role in rational cancer vaccine and drug development (Table 1). They reveal which cancer-associated autoantigens are targeted *in vivo*

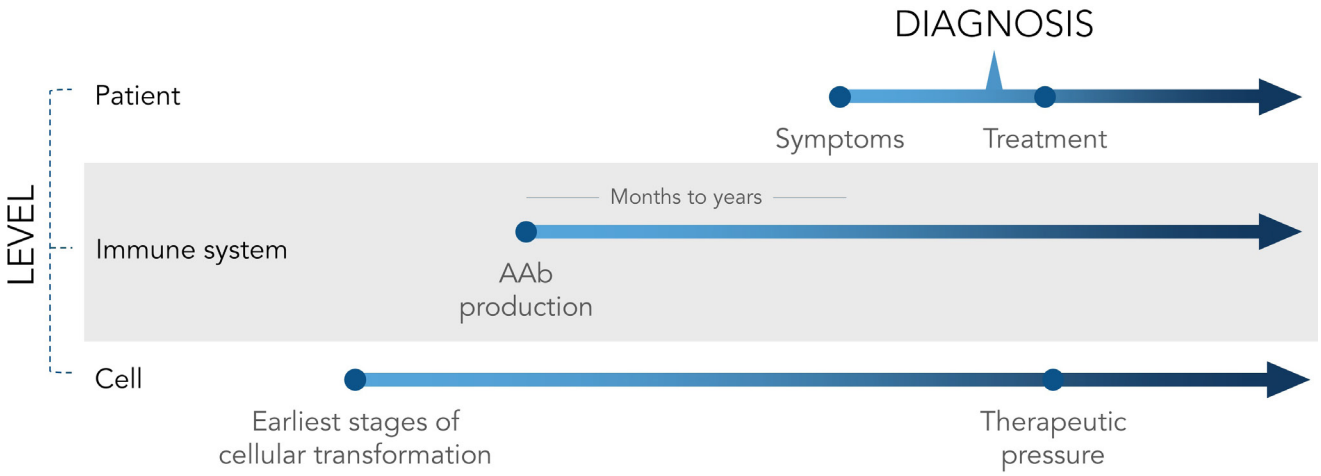

Figure 2. Timeline of AAb production before and after cancer diagnosis

Table 1. Examples of AAb profiling applications in cancer

Application	Description
Understand disease mechanisms	Discover AAb targets that are often associated with disease
Identify biomarkers	Diagnose, stratify patients, and subtype disease. Predict patient prognosis and treatment outcomes.
Guide vaccine and drug development	Identify potential therapeutic targets. Map epitope spreading. Determine B-cell specificities.

by the patient's immune system. In other words, AAb profiling can pinpoint which autoantigens are immunodominant, elicit B-cell memory, contribute to paraneoplastic syndromes, or stimulate the production of protective antibodies that slow disease progression. Such autoantigens could be explored as targets for vaccines, chimeric antigen receptor T-cell (CAR-T) therapy, and antibody-drug conjugates. Moreover, AAb profiling aids in identifying B-cell specificities for chimeric autoantibody receptor T-cell (CAAR-T) therapy, useful in managing cancer-related immune-related adverse events (irAEs) or paraneoplastic disease.

AAb profiling can map the immune-targeted diversification elicited by vaccines and drugs. For instance, the HER-2/neu peptide vaccine elicits the generation of AAbs that target endogenous HER-2/neu. Through a process called epitope spreading, a patient's immunoreactivity can spread to the p53 protein. Epitope spreading is also relevant in certain therapies, enhancing efficacy by stimulating the immune system to target more than just the original protein target.

Advancements in Antibody Profiling: From Traditional Methods to Cutting-Edge Protein Microarrays

Classical antibody profiling methods, such as western blotting and ELISA, are typically limited in their capacity for multiplexing and throughput. In contrast, newer techniques like protein microarrays provide a rapid, cost-effective approach to screen samples for hundreds to thousands of antibodies simultaneously, yielding more comprehensive insights.

However, not every protein microarray necessarily guarantees high-quality data. Since 90% of AAbs bind to three-dimensional binding sites called conformational epitopes, it is essential that correctly folded proteins are employed for accurate and meaningful results. Incorrectly folded or denatured proteins result in the loss of biologically relevant data, non-specific binding, and inaccurate results.

Sengenics has developed proprietary KREX® technology for precise AAb profiling. This unique technology ensures that samples are screened against correctly folded, full-length, and functional human proteins. Moreover, the proteins are immobilized onto a modified, non-denaturing surface, enabling them to remain accessible for AAb binding, just as they would *in vivo*.

Sengenics offers the largest cancer-focused protein microarray on the market, i-Ome® Cancer, designed to profile autoantibodies against over 500 antigens simultaneously. These antigens were meticulously selected for their significance in cancer, encompassing tissue and pathway relevance, therapeutic targets, cytokines and chemokines, cancer-driver proteins, prognostic indicators, cancer-testis antigens, B-cell and autoantibody targets, and ectopic expression.

A subset of proteins included in i-Ome Cancer represents known autoantigens in various types of cancer, including breast, colorectal, gastric, lung, melanoma, ovarian, and pancreatic cancers.

Sengenics has a comprehensive library of over 2000 human proteins, which represent diverse protein functions and disease areas. Researchers can utilize ready-to-use panels or customize their own by selecting from this extensive library or requesting specific proteins of interest.

Additionally, Sengenics provides a unique citrullination assay to detect AAbs to citrullination, a protein post-translational modification linked to inflammation and cellular stress (9). Citrullination is highly relevant in multiple diseases, including cancer (9,10).

Conclusion

Genetic testing alone does not accurately reflect the dynamic, real-time biological changes and molecular heterogeneity that occur during cancer. AAb profiling bridges this gap by providing a direct view of the immune system's ongoing response to each individual's evolving molecular landscape. Highly complementary to other omics datasets, AAb profiling is a key component in the precision medicine toolkit, transforming cancer profiles into actionable clinical insights and ultimately improving patient outcomes.

Contact us

Sengenics develops key solutions for biological research, specializing in the discovery and validation of autoantibody biomarker signatures. For more information on how Sengenics can advance your research, email us at enquiries@sengenics.com or visit our website at sengenics.com.

Resources

Learn about citrullination, a post-translational modification associated with inflammation and cellular stress [White Paper]: https://sengenics.com/wp-content/uploads/2024/04/White-Paper-Citrullination-in-Disease-Emerging-Insights-and-Implications-vs1pt1.pdf

Find out why protein shape matters in antibody binding [White Paper]: https://sengenics.com/wp-content/uploads/2024/04/White-Paper-Antibody-Antigen-Binding-Shape-Matters-vs1pt0.pdf

Watch a 1-minute video to discover why protein shape matters in antibody-antigen binding [Video]: https://www.youtube.com/watch?v=lBEYLlk5Yws

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