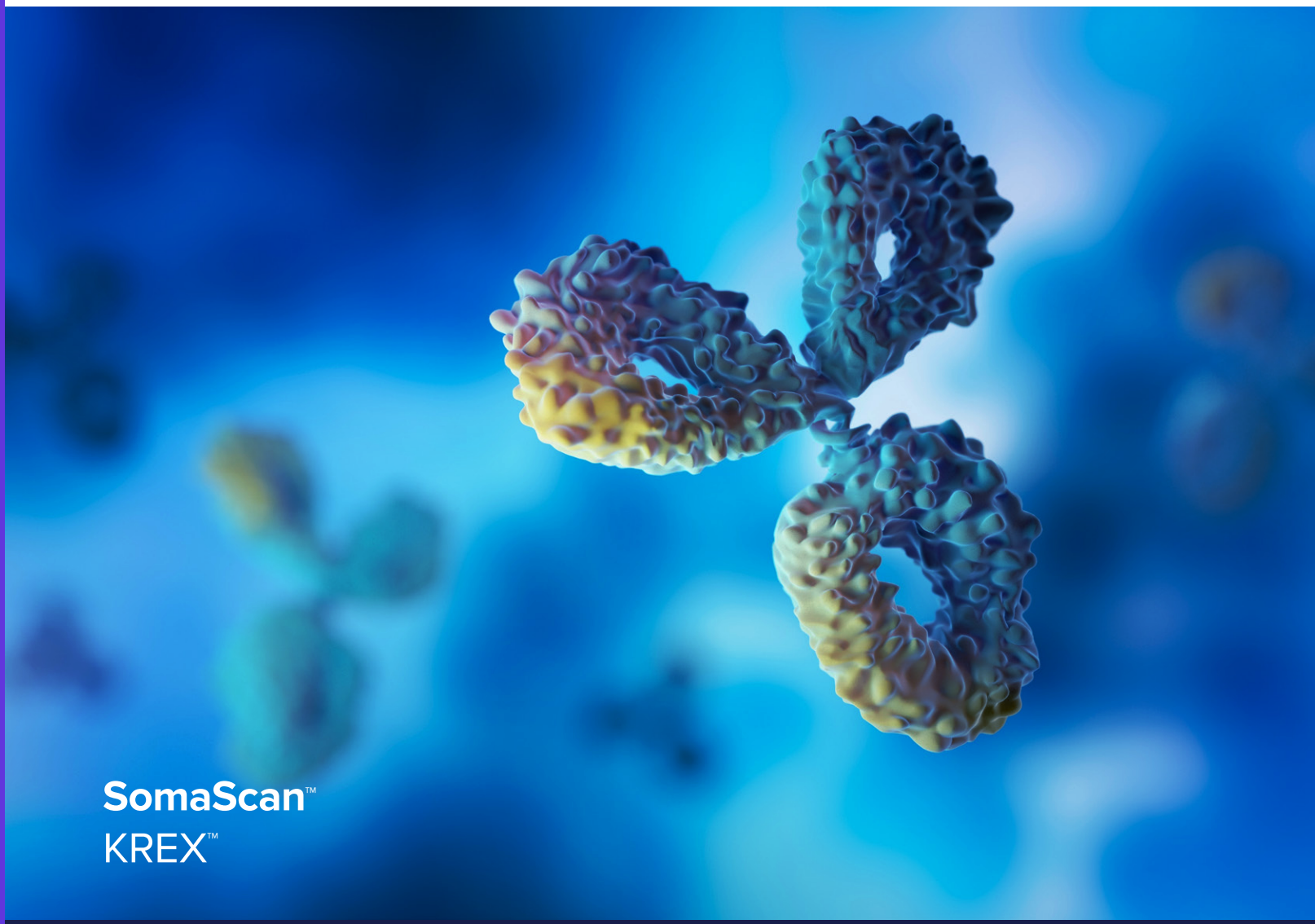
Beyond Genetics: Autoantibodies as Biomarkers



SomaScan™
KREX™

Table of Contents

Introduction

Genetic biomarkers: Limited by static information

Genetic biomarkers, such as DNA mutations, polymorphisms and other inherited genetic variations, have long been used to assess disease risk, susceptibility and inherited traits. While these biomarkers provide valuable insights into a person's genetic predisposition to certain diseases, they offer only a static snapshot of an individual's biology. Genetic markers reveal what might happen based on a person's genetic code but don't capture the real-time changes occurring in the body as it responds to disease, lifestyle factors, environmental influences or treatments.

This limitation means that **genetic biomarkers alone cannot account for the dynamic nature of diseases** – especially complex, heterogeneous conditions like cancer, autoimmune disorders and neurodegenerative diseases, where disease progression is influenced by a constantly shifting biological environment. Because genetic biomarkers are fixed from birth and remain constant throughout life, they offer limited value in monitoring disease progression or treatment response in real time.

Autoantibodies: Dynamic, real-time and historical indicators of disease

Autoantibodies, one of the most underexplored classes of biomarkers, are produced by the immune system in response to disease-driven changes, targeting the body's own proteins. Unlike genetic biomarkers, they provide real-time insights into the body's current biological state, capturing the immune system's response to active disease processes.

This eBook explores the expanding role of autoantibodies in precision medicine, particularly as biomarkers for complex diseases like cancer, autoimmune disorders, neurodegenerative conditions and chronic inflammation. Through in-depth explanations and real-world case studies, each chapter reveals how autoantibody profiling enables earlier diagnosis, more personalized treatment and the discovery of new therapeutic targets.

By harnessing autoantibodies as dynamic biomarkers, we can move beyond genetic predisposition and gain a real-time view of disease progression, opening up new possibilities for targeted, individualized care.

In the following chapters, we invite you to delve into the science, applications and future potential of autoantibodies as biomarkers in precision medicine. Join us on this journey into the immune system's hidden messages and discover how they hold the key to unlocking a healthier, more personalized future for all.

Autoantibodies: Transforming Precision Medicine

Introduction

Autoantibodies (AAbs) are antibodies produced by the immune system that mistakenly target the body's own proteins, often as a response to cellular changes caused by disease. Traditionally, AAbs were primarily associated with autoimmune diseases, where the immune system attacks healthy tissues, as seen in conditions like rheumatoid arthritis and lupus. In these contexts, AAbs served as key diagnostic markers, helping clinicians identify and monitor autoimmune disorders.

However, recent research has shown that AAbs are not limited to autoimmune diseases; they are also found in various other conditions, including cancer, neurodegenerative disorders and chronic inflammatory diseases. Their presence across such a wide range of diseases has revealed their potential as valuable biomarkers for early detection, patient stratification and monitoring treatment response.

AAbs reflect pathological states

Most antibodies are elicited through a limited set of mechanisms, all of which are associated with abnormal or disease states rather than health. These mechanisms that cause self-proteins to become autoantigenic include:

- **Altered protein levels:** Significant and rapid changes in protein concentration
- **Protein sequence variation:** Genetic mutations or abnormal mRNA processing
- **Ectopic expression:** Proteins appearing in the wrong cellular compartment or at an inappropriate time
- **Aberrant post-translational modifications:** Changes caused by abnormal enzyme activation or oxidative damage
- **Molecular mimicry:** An AAb initially produced in response to a foreign antigen that also recognizes a self-protein

AAb biomarkers in disease

- Antibody generation is linked to the formation of TDP-43 protein aggregates that occurs in amyotrophic lateral sclerosis (ALS)[1]
- Tumor-associated proteins in cancer elicit an autoimmune response[2,3]
- Antibody biomarkers of lupus in the blood samples of military personnel were detected up to nine years before their diagnosis[4]
- Antibodies have shown significant potential in predicting disease outcomes[5–7]
- Researchers identified and validated a signature of 13 antibodies predictive of a lower five-year survival in non-small-cell lung cancer[8]

- **Abnormal protein folding or aggregation:** Misfolding or aggregation during cellular stress or toxicity that overwhelms normal protein degradation processes
- **Aberrant proteolysis:** Abnormal activity of proteolytic enzymes in inflammatory microenvironments

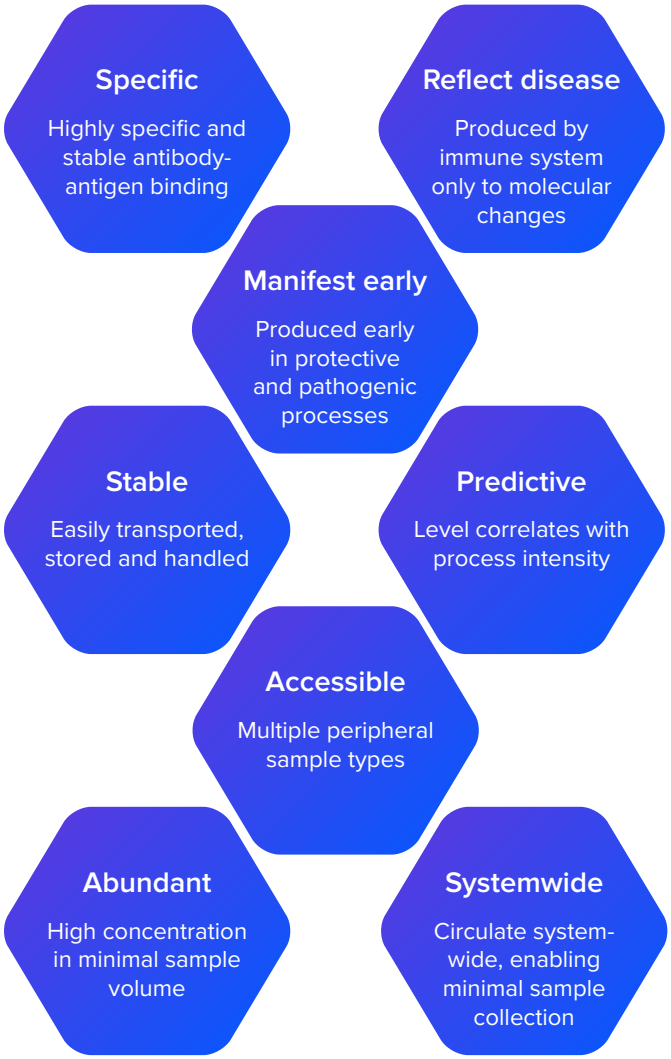
Various mechanisms can lead to the formation or exposure of new antibody binding sites, known as neoepitopes, that are typically not accessible for binding under normal *in vivo* conditions (Figure 1).

Examples of AAbs associated with various diseases can be found in the light purple box on the previous page.

AAbs have ideal biomarker characteristics

Antibodies – including AAbs – are direct indicators of disease, often appearing before symptoms and remaining detectable throughout the course of illness. This makes antibody profiling a powerful tool for identifying disease-associated proteins. In contrast, protein profiling alone cannot distinguish between proteins directly linked to disease or those indirectly affected.

Key characteristics of antibodies are listed in the hexagons to the right.



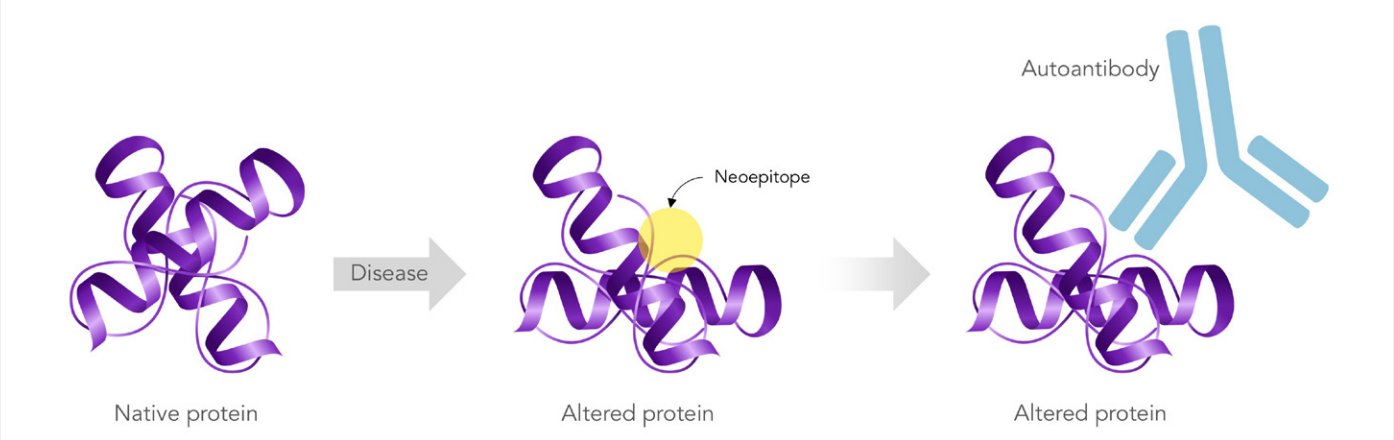


Figure 1. Neoepitopes formed during pathological processes can elicit the production of AAbs.

Concluding remarks

AAb biomarkers hold the potential to transform patient care. They can reveal disease-associated proteins and protein pathways, providing valuable insights for unraveling the complexities of diseases and aiding in the development of new medications. They can help facilitate early detection, subtype diseases more accurately, predict who will (or won't) respond to treatments, predict the likelihood of immune-related adverse events and improve patient stratification for clinical trials.

By analyzing the vast array of AAbs through a process called immunoprofiling, scientists can gain a clear understanding of a patient's disease state, offering guidance in the complex landscape of chronic illnesses.

> In many disorders and pathologies affecting tissue structure, neoepitopes trigger the production of AAbs, contributing to disease pathology directly or systemically.
>
> Profiling these AAbs is vital for detecting and monitoring disease activity and treatment outcomes.

Allan Stensballe, PhD
Associate Professor
Aalborg University

ON-DEMAND WEBINAR

Serum Autoantibodies Differentiate Rheumatoid Arthritis Subgroups

Patients with rheumatoid arthritis (RA) can be categorized as either anti-citrullinated protein antibody-positive (ACPA+) or negative (ACPA–). In this webinar, Jaeyun Sung, PhD, presents his research exploring a broad range of serological autoantibodies to uncover immunological differences between these RA subgroups using data from ACPA+ RA patients, ACPA– RA patients and matched healthy controls.

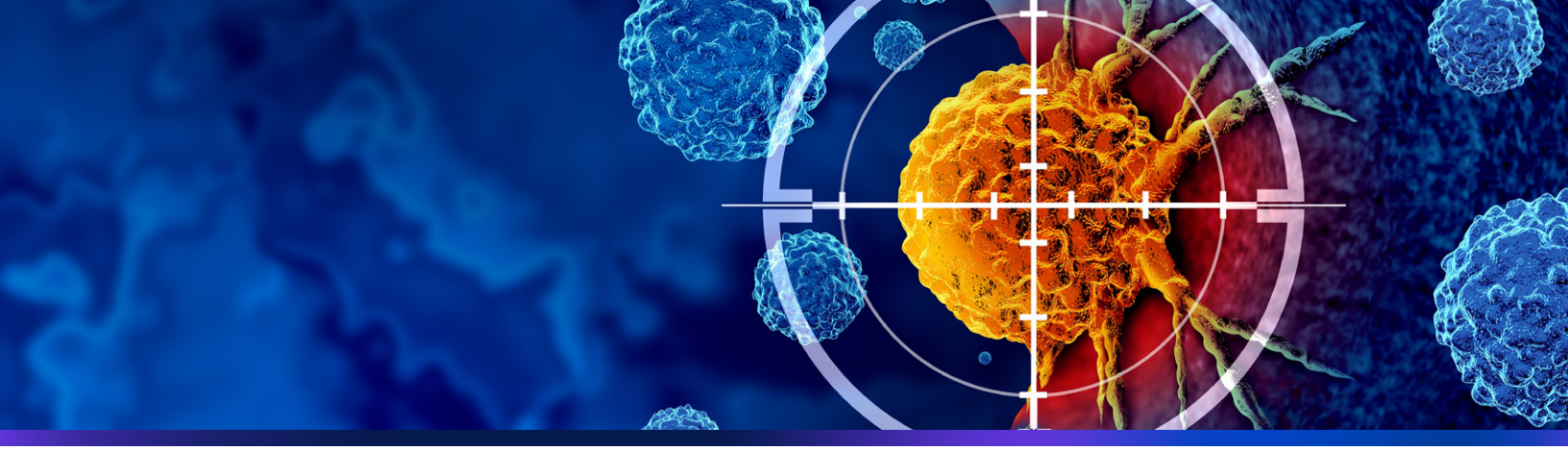
See also **Citrullination in Disease: The Role of Autoantibodies**.

Jaeyun Sung, PhD
Senior Associate Consultant
and Assistant Professor
Mayo Clinic

Watch now

References

1. Conti, E. et al. “Serum naturally occurring anti-TDP-43 auto-antibodies are increased in amyotrophic lateral sclerosis.” *Scientific Reports* 11 (2021): 1978.
2. Aziz, F. et al. “Autoantibody-based diagnostic biomarkers: technological approaches to discovery and validation.” *Autoantibodies and Cytokines*. IntechOpen (2019).
3. Sexauer, D. et al. “Tumour-associated autoantibodies as prognostic cancer biomarkers – a review.” *Autoimmunity Reviews* 21 (2022): 103041.
4. Arbuckle, M.R. et al. “Development of autoantibodies before the clinical onset of systemic lupus erythematosus.” *The New England Journal of Medicine* 16 (2003): 1,526–1,533.
5. Bizzaro, N. “Autoantibodies as predictors of disease: The clinical and experimental evidence.” *Autoimmunity Reviews* 6 (2007): 325–333.
6. Kathrikolly, T. et al. “Can serum autoantibodies be a potential early detection biomarker for breast cancer in women? A diagnostic test accuracy review and meta-analysis.” *Systemic Reviews* 1 (2022): 215.
7. Zaenker, P. et al. “Serologic autoantibodies as diagnostic cancer biomarkers – a review.” *Cancer Epidemiology, Biomarkers & Prevention* 12 (2013): 2,161–2,181.
8. Patel, A.J. et al. “A highly predictive autoantibody-based biomarker panel for prognosis in early-stage NSCLC with potential therapeutic implications.” *British Journal of Cancer* 2 (2022): 238–246.



Autoantibodies in Cancer

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 AAbs, or antibodies that target self-molecules, can serve as precise and reliable biomarkers in cancer[1]. This chapter 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.

AAbs: 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 (Figure 1). 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, AAbs may have a pathogenic or protective role in cancer progression.

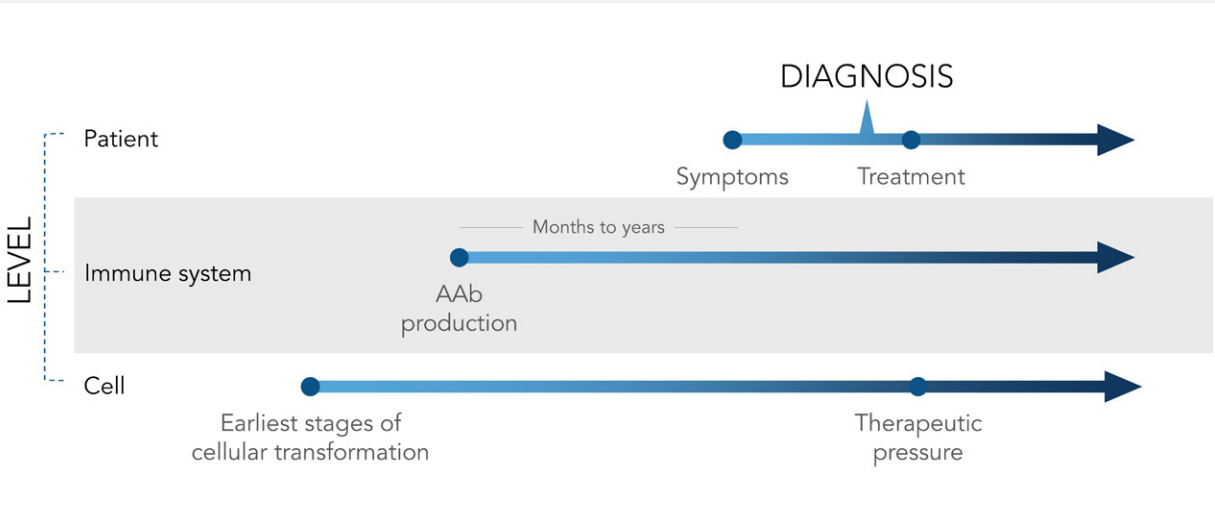


Figure 2. Timeline of AAb production before and after cancer diagnosis

Early detection and predictive power of AAbs in cancer

AAbs generated during carcinogenesis provide a valuable opportunity for earlier diagnosis, enabling intervention before symptoms appear (Table 1, Figure 2)[2]. For example, AAbs targeting p53, the most commonly mutated protein in cancer, were detected on average three-and-a-half 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 five years prior to diagnosis[4]. In fact, an AAb-based assay, EarlyCDT-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.

> To my surprise, AAbs can also predict response to treatment.

Iman Osman, MD
Associate Dean for Clinical Research Strategy,
Rudolf L. Baer Professor of Dermatology,
Professor of Departments of Medicine (Oncology) and Urology,
Director of the NYU Melanoma SPORE,
Director of the Interdisciplinary Melanoma Cooperative Group (IMCG)
New York University (NYU),
Grossman School of Medicine

> AAbs in cancer provide critical insights into how strongly and extensively the immune system recognizes and responds to the disease. Additionally, they reveal specific proteins that the immune system targets, offering personalized insights that could lead to new treatment options.

Jessica Da Gama Duarte, PhD
Laboratory Head,
Senior Research Fellow
Monash University

Impact of cancer therapies on AAb levels

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

Role of AAbs 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* 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.

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.

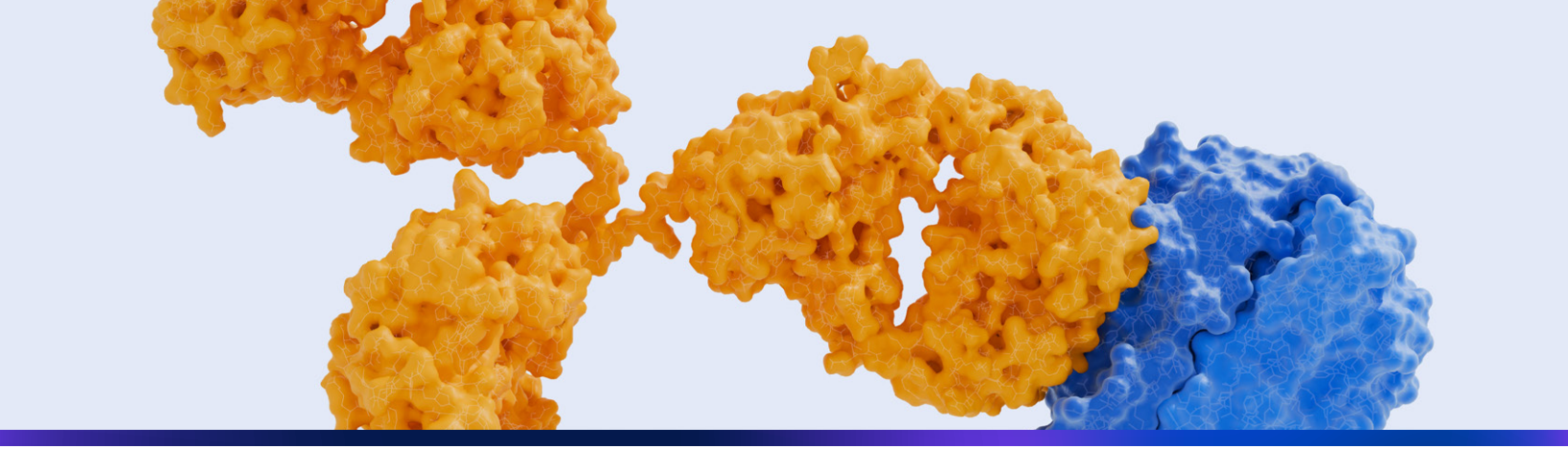
AAb profiling can map the immune-targeted diversification elicited by vaccines and drugs. For instance, the HER-2/neu peptide vaccine elicits the generation of AAbs that target endogenous HER-2/neu. Through a process called epitope spreading, a patient's immunoreactivity can spread to the p53 protein.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

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.

References

1. Wu, J. et al. “The roles and applications of autoantibodies in progression, diagnosis, treatment and prognosis of human malignant tumours.” *Autoimmunity Reviews* 16 (2017): 1,270–1,281.
2. De Jonge, H. et al. “Anti-cancer auto-antibodies: Roles, applications and open issues.” *Cancers* 13 (2021): 813.
3. Li, Y. et al. “Autoantibodies predict subsequent development of cancer.” *International Journal of Cancer* 114 (2005): 157–160.
4. Zhong, L. et al. “Profiling tumor-associated antibodies for early detection of non-small cell lung cancer.” *Journal of Thoracic Oncology* 1 (2006): 513–519.
5. Chapman, C.J. et al. “EarlyCDT-Lung test: Improved clinical utility through additional autoantibody assays.” *Tumour Biology* 33 (2012): 1,319–1,326.
6. Anderson, K.S. et al. “Protein microarray signature of autoantibody biomarkers for the early detection of breast cancer.” *Journal of Proteome Research* 10 (2011): 85–96.
7. Patel, A.J. et al. “A highly predictive autoantibody-based biomarker panel for prognosis in early-stage NSCLC with potential therapeutic implications.” *British Journal of Cancer* 126 (2021): 238–246.
8. Ibrahim, M. et al. “Determinants of racial disparities in immune-related adverse events (irAE) with checkpoint inhibition (ICI) in melanoma.” *Journal of Clinical Oncology* 41 (2023): 9549.
9. Wang, B. et al. “Recent advances in characterization of citrullination and its implication in human disease research: From method development to network integration.” *Proteomics*, 23 (2023): 21–22.
10. Yuzhalin, A.E. “Citrullination in cancer.” *Cancer Research* 1 (2019): 1274–1284.



Anti-Cytokine Autoantibodies

Introduction

Anti-cytokine autoantibodies (ACAAs) are naturally occurring or elicited antibodies that target cytokines – key proteins that mediate and regulate immune responses (Table 2). While cytokines generally direct and modulate immune activity, ACAAs often inhibit or potentiate these effects, influencing immune regulation in both health and disease. Although less common, ACAAs can also directly drive disease processes. This chapter delves into the role of ACAAs across various disease contexts and explores their potential use in predicting treatment outcomes, classifying disease severity, guiding vaccine development and other therapeutic applications.

ACAAs in health

Low levels of ACAAs are relatively common in the general population. A Danish study of nearly 9,000 healthy blood donors found that 86% of participants had at least one detectable ACAA, although the prevalence varied significantly depending on the cytokine target[2]. Anti-IL-6 ACAAs were the most frequent, present in 65% of participants, while anti-GM-CSF ACAAs were found in just 10%. Interestingly, the study also found that the cumulative presence of multiple ACAAs correlated with several indicators of immune function, including self-reported health scores and the frequency of antibiotic prescriptions, supporting the belief that ACAAs influence overall immune health and resilience[3].

ACAAs could function as natural regulators, balancing the immune response and reducing the risk of excessive inflammation without compromising the body's ability to fight infections. By neutralizing pro-inflammatory cytokines, they may help prevent harmful immune overactivation like cytokine storms. These storms trigger excessive cytokine production, leading to severe inflammation across multiple organs and systems, which can potentially result in multi-organ failure and death.

ACAAs in disease

ACAAs were initially discovered in patients with thymoma-associated autoimmune diseases but are now known to be present in healthy individuals and a variety of other conditions[4]. These include autoimmune diseases like rheumatoid arthritis, systemic lupus erythematosus and psoriasis; immunodeficiencies; and infectious diseases. In these contexts, ACAAs may play a causative or associative role, potentially contributing to disease by inhibiting cytokines crucial for immune defense. They can also be linked to specific subtypes within disease categories.

For instance, ACAAs can increase susceptibility to **infections**[5]. Anti-IFNγ antibodies are strongly associated with disseminated non-tuberculous mycobacterial (NTM) infections. Anti-IL-6 ACAAs have been linked to severe staphylococcal and streptococcal infections while anti-IL-17/IL-22 antibodies are tied to chronic mucosal candidiasis.

Anti-GM-CSF antibodies have been implicated in opportunistic infections such as those caused by *Nocardia spp.* and *Cryptococcus spp.*, as they impair macrophage function, which is essential for combating these pathogens. In at least one patient, anti-GM-CSF autoantibodies were detected 10 years prior to developing *Nocardia spp.* infection[6].

Cytokine-based therapies

The crucial role of cytokines in disease is evident through the numerous cytokine-based therapies approved by the US Food and Drug Administration (FDA). To date, at least 39 therapies that mimic or target cytokines or their receptors have been approved for clinical use (Table 2)[1].

Table 2. Cytokine-based therapies approved by the US FDA

Cytokine	Drug Type	Brand Name	Disease Area
G-CSF	Protein	Neupogen, Neulasta	Neutropenia
GM-CSF	Protein	Leukine	Neutropenia
IFNα	Protein	Intron A, Roferon-A	Chronic hepatitis B, chronic hepatitis C, hairy cell leukemia, Kaposi's sarcoma, malignant melanoma, follicular lymphoma, chronic myelogenous leukemia (CML)
IFNβ	Protein	Avonex, Betaseron, Rebif, Plegridy	Multiple sclerosis (MS)
IFNγ	Protein	Actimmune	Chronic granulomatous disease (CGD), severe malignant osteopetrosis
IL-1R	Small molecule	Kineret	Rheumatoid arthritis, neonatal-onset multisystem inflammatory disease (NOMID)
IL-2	Protein	Proleukin	Metastatic renal cell carcinoma, metastatic melanoma
IL-5	Antibody	Nucala, Cinqair, Fasenra	Severe eosinophilic asthma, eosinophilic granulomatosis with polyangiitis (EGPA)
IL-6R	Antibody	Actemra, Kevzara	Rheumatoid arthritis, juvenile idiopathic arthritis, giant cell arteritis, cytokine release syndrome
IL-11	Protein	Neumega	Severe thrombocytopenia
IL-12/23[1]	Antibody	Stelara, Tremfya, Omvoh	Psoriasis, psoriatic arthritis, Crohn's disease, ulcerative colitis
IL-13	Antibody	Adbry	Atopic dermatitis
IL-17A	Antibody	Cosentyx, Taltz, Siliq	Psoriasis, psoriatic arthritis, ankylosing spondylitis, axial spondyloarthritis (axSpA), hidradenitis suppurativa (HS)
IL-17A/IL-17F[2]	Antibody	Bimzelx	Psoriatic arthritis, axSpA, ankylosing spondylitis
IL-23	Antibody	Tremfya, Ilumya, Skyrizi	Psoriasis, psoriatic arthritis, Crohn's disease
TNF	Antibody	Remicade, Humira, Cimzia, Simponi	Rheumatoid arthritis, Crohn's disease, ulcerative colitis, ankylosing spondylitis, psoriasis, psoriatic arthritis
TNFR	Receptor fusion protein	Enbrel	Rheumatoid arthritis, psoriasis, ankylosing spondylitis, juvenile idiopathic arthritis

[1] Drugs target a shared subunit between IL-12 and IL-23.
[2] Drug targets IL-17A and IL-17F separately or as a heterodimer.

GM-CSF also plays a key role in the pathophysiology of **pulmonary alveolar proteinosis** (PAP), which is a lung condition characterized by the buildup of surfactant in the alveoli. The titer of circulating anti-GM-CSF autoantibodies may help predict how well a patient will response to PAP treatment with subcutaneous recombinant human GM-CSF[7].

During the **COVID-19** pandemic, the discovery of anti-IFNα and IFNω ACAAs in some patients offered critical insights into why certain individuals experienced more severe outcomes[8]. These antibodies impair the body's antiviral defense by neutralizing key interferons essential for early viral response, thereby increasing susceptibility to severe COVID-19. Interestingly, the frequency of these ACAAs was associated with increased age and male sex in patients with critical COVID-19 ($p = 3x10^{-6}$ and $p = 0.003$, respectively)[9].

Another study found that recovered COVID-19 patients with higher autoantibody titers targeting a subset of cytokines known as chemokines – specifically CCL21, CXCL13 and CXCL16 – were less likely to experience long COVID-19 symptoms one year after infection[10]. This suggests that certain ACAAs may be linked to the progression and outcome of the disease.

In **systemic lupus erythematosus** (SLE), disease severity is linked to elevated levels of IFNα and ACAAs targeting BAFF, a cytokine involved in B cell activation[5]. On the other hand, ACAAs that neutralize IFNα and TNF have been associated with reduced lupus severity, suggesting that ACAAs may have therapeutic potential in diseases driven by cytokine activity.

Beyond these established associations, emerging research suggests that ACAAs may be more widespread, with implications for conditions like cancer, cardiovascular disease and neurological disorders.

It is also worth mentioning that ACAAs may also arise as unintended consequences of severe infections or tissue damage, contributing to immune dysregulation. As such, they could serve as early indicators of immune imbalance and dysfunction.

Expanding role of anti-cytokine antibodies in disease treatment and therapeutics

The therapeutic potential of anti-cytokine antibodies (ACAs) is being explored in various disease contexts (Table 2). In **oncology**, ACAs have garnered attention for their possible roles in treating cancer-related cachexia (CRC) and improving the efficacy of monoclonal antibody (mAb) therapies. Among the most promising approaches for treatment of CRC-associated cachexia is a combination of anti-IL-1A antibodies and thalidomide, reducing the inflammatory burden associated with the condition[11].

Additionally, researchers are investigating a new class of therapies called immunocytokines[12–14]. Immunocytokines are antibody-cytokine fusion proteins or therapeutic ACAs (such as those listed in Table 2) that are coupled to antibodies targeting cancer-specific or cancer-associated tumor antigens. These are designed to address the challenges posed by the tumor microenvironment in solid tumors, which often suppresses immune activity and diminishes mAb efficacy, yet remains sensitive to pro-inflammatory cytokines.

Traditional cytokine therapies have been limited by off-target toxicity and the short half-life of cytokines when administered alone. Immunocytokines offer a solution by delivering cytokines directly to the tumor site, extending their half-life and reducing systemic toxicity. These fusion proteins can also bridge local cytotoxic immune cells, such as macrophages and natural killer (NK) cells, with tumor cells, enhancing the immune system's ability to target and destroy cancer cells.

Several immunocytokines are currently in clinical trials (Table 3), demonstrating promise for future cancer treatment strategies by optimizing immune activation at the tumor site without the widespread side effects of systemic cytokine therapy[13,14].

Experimental models suggest that ACAAs may play a protective role in certain **cardiovascular diseases** by modulating inflammatory responses. For instance, anti-IL-17 antibodies have been shown to reduce inflammation in murine models of autoimmune myocarditis, a condition driven by an autoimmune response against cardiac myosin[15]. In Kawasaki disease, a meta-analysis of mAb studies revealed that while anti-TNF did not lower the incidence of coronary artery aneurysms, it did reduce resistance to treatment with intravenous immune globulin[16].

Additionally, romilkimab, a bispecific ACA that neutralizes IL-4 and IL-13, has shown promise in treating patients with systemic sclerosis[17]. Tocilizumab (TCZ), an mAb targeting IL-6R and blocking its interaction with IL-6, is used to treat Takayasu arteritis, giant cell arteritis and other inflammatory diseases, including Castleman disease, idiopathic juvenile arthritis and rheumatoid arthritis.

In **neurological disorders**, murine models have demonstrated that ACAs can reduce the severity of experimental autoimmune encephalitis (EAE), which is a model for multiple sclerosis (MS)[18]. Additionally, anti-cytokine mAbs targeting IL-1β and IL-6 have shown promise in reducing brain inflammation and preventing blood-brain barrier permeability in fetal ischemia-reperfusion injury ovine models[19]. These

Table 3. Clinical trials using immunocytokines

Cytokine	Target Antigen	Name	Cancer Type	Phase
IFN-α2B	CD38	Modakafusp alfa (TAK-573)	Multiple myeloma	1, 2
IL-2	GD2	Hu14.18-IL-2	Neuroblastoma, melanoma, sarcoma, solid childhood tumors	1, 2
IL-2	EpCAM	huKS-IL2	SCLC, prostate, ovarian, breast, bladder, kidney, lung, solid tumors	1, 2
IL-2	CD20	DI-Leu16-IL2	B cell lymphoma	1, 2
IL-2	Tenascin-C	F16-IL2	Breast, AML, solid tumors, MCC	2
IL-2 variant	CEA	CEA-IL2v (RG7813)	Solid tumors	1
IL-2 variant	FAP	FAP-IL12v	Solid tumors, RCC, melanoma, pancreatic, breast, HNC, esophageal, cervical	1, 2
IL-2 variant	PD-1	RG6279, IBI363, IAP0971	Solid tumors	1
IL-2, IL-12, TNF	EDB	L19-IL2, L19-TNF, BC1-IL-12 (AS1409), L19-IL-12	Melanoma, RCC, NSCLC, solid tumors, pancreatic, colorectal, DLBCL, glioblastoma, sarcoma, glioma	1, 2, 3
IL-2LT, IL-12	Histone/DNA structures	NHS-IL12	NSCLC, solid tumors, pancreatic, urogenital, bladder, NHL, Kaposi sarcoma, melanoma	1, 2
IL-15	PD-L1	KD033, SIM0237, IGM-7354	Solid tumors	1
IL-21	PD-1	AMG256	Solid tumors	1

AML = acute myeloid leukemia; DLBCL = diffuse large B cell lymphoma; HNC = head and neck cancer; MCC = Merkel cell carcinoma; NSCLC = non-small-cell lung cancer; RCC = renal cell carcinoma

findings suggest a potential therapeutic role for ACAs in neuroinflammatory and neurodegenerative conditions.

ACAs that target TNF, IL-12 and IL-23 are in clinical use to treat **inflammatory bowel disease** (IBD), such as Crohn's disease and ulcerative colitis[1]. These mAbs bind to their respective pro-inflammatory cytokine, inhibiting their function, which helps reduce inflammation and alleviate pain.

Additional conditions treated with FDA-approved mAbs targeting cytokines or cytokine receptors are listed in Table 2.

ACAAs in disease prevention

Leveraging ACAAs through anti-cytokine vaccination before disease onset has demonstrated promise in preclinical animal studies. For instance, vaccines targeting IL-17 have been effective in reducing the severity of collagen-induced arthritis (CIA) and EAE[20]. Similarly, an anti-IL-6 vaccine protected mice from CIA, while an anti-IL-18 vaccine helped reduce the severity of SLE and prevented renal damage[21]. Anti-cytokine vaccination also shows potential in other conditions, including cachexia, antibody-induced arthritis, Leishmaniasis, atherosclerosis and collagen antibody-induced arthritis (CAIA).

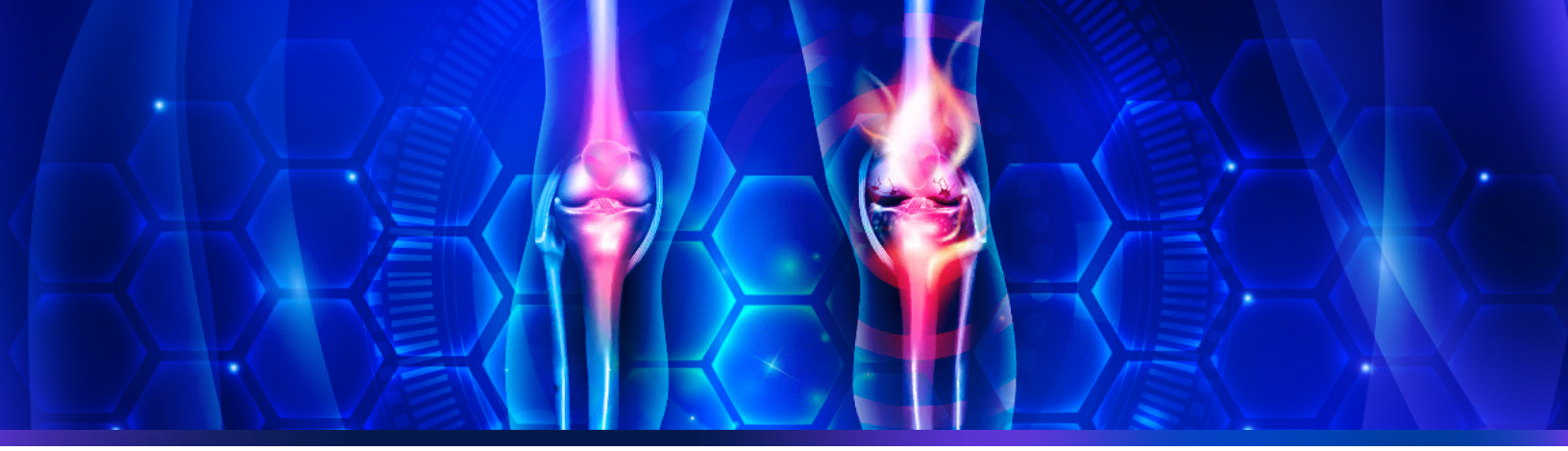
However, clinical trials are needed to evaluate the long-term safety, efficacy and potential side effects of anti-cytokine vaccination in humans. If successful, these vaccines could provide a new, targeted approach for managing autoimmune diseases and reducing the need for lifelong immunosuppressive therapies.

Conclusion

ACAAs play a dual role, acting as natural immune regulators while also contributing to pathology by impairing critical cytokine functions. Numerous studies highlight their potential as biomarkers for predicting disease severity, progression and treatment response. Monoclonal ACAs are being explored as therapeutic tools in diseases like cancer, cardiovascular disease and neurological disorders, offering targeted immune modulation. Continued research into ACAAs and ACAs, especially through precision antibody profiling with KREX protein microarrays from Standard BioTools, holds transformative potential for treating immune-mediated diseases and paving the way for personalized, more precise interventions.

References

1. Drugs@FDA: FDA-Approved Drugs. U.S. Food and Drug Administration (2024): accessdata.fda.gov/scripts/cder/daf/index.cfm.
2. Von Stemann, J.H. et al. "Prevalence and correlation of cytokine-specific autoantibodies with epidemiological factors and C-reactive protein in 8,972 healthy individuals: Results from the Danish Blood Donor Study." *PLoS One* 12 (2017): e0179981.
3. Von Stemann, J.H. et al. "IL-6 autoantibodies predict lower platelet counts and altered plasma cytokine profiles in healthy blood donors: Results from the Danish Blood Donor Study." *Frontiers in Medicine* 9 (2022): 914262.
4. Arts, R.J.W. et al. "Anticytokine autoantibodies in infectious diseases: A practical overview." International *Journal of Molecular Sciences* 25 (2023): 515.
5. Vincent, T. et al. "Emerging clinical phenotypes associated with anti-cytokine autoantibodies." *Autoimmunity Reviews* 14 (2015): 528–535.
6. Rosen, L.B. et al. "Nocardia-induced granulocyte macrophage colony-stimulating factor is neutralized by autoantibodies in disseminated/extrapulmonary nocardiosis." *Clinical Infectious Diseases* 60 (2015): 1,017–1,025.
7. Bonfield, T.L. et al. "Anti-GM-CSF titer predicts response to GM-CSF therapy in pulmonary alveolar proteinosis." *Clinical Immunology* 105 (2002): 342–350.
8. Wang, X. et al. (2023). "Autoantibodies against type I interferons in COVID-19 infection: A systematic review and meta-analysis." *International Journal of Infectious Diseases* 130 (2023): 147–152.
9. Bastard, P. et al. "Autoantibodies neutralizing type I IFNs are present in ~4% of uninfected individuals over 70 years old and account for ~20% of COVID-19 deaths." *Science Immunology* 6 (2021): eabl4340.
10. Muri, J. et al. "Autoantibodies against chemokines post-SARS-CoV-2 infection correlate with disease course." *Nature Immunology* 24 (2023): 604–611.
11. Prado, B.L. et al. "Anti-cytokines in the treatment of cancer cachexia." *Annals of Palliative Medicine* 8 (2019): 67–79.
12. Gout, D.Y. et al. "The present and future of immunocytokines for cancer treatment." *Cellular and Molecular Life Sciences* 79 (2022): 509.
13. Shi, W. et al. "Advancements and challenges in immunocytokines: A new arsenal against cancer." *Acta Pharmaceutica Sinica B* 14 (2024): 4,649–4,664.
14. Runbeck, E. et al. "Utilizing immunocytokines for cancer therapy." *Antibodies* 10 (2021): 10.
15. Fan, Y. et al. "Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of coxsackievirus b3-induced viral myocarditis reduces myocardium inflammation." *Virology Journal* 8 (2011): 17.
16. Nomura, O. et al. "Monoclonal antibody and anti-cytokine biologics for Kawasaki disease: A systematic review and meta-analysis." *Seminars in Arthritis and Rheumatism* 51 (2021): 1,045–1,056.
17. Shima Y. "Cytokines involved in the pathogenesis of SSc and problems in the development of anti-cytokine therapy." *Cells* 10 (2021): 1104.
18. Palle, P. et al. "Cytokine signaling in multiple sclerosis and its therapeutic applications." *Medical Sciences* 5 (2017): 23.
19. Patra, A. et al. "Neutralizing anti-interleukin-1β antibodies reduce ischemia-related interleukin-1β transport across the blood-brain barrier in fetal sheep." *Neuroscience* 346 (2017): 113–125.
20. Wraith D.C. "Anti-cytokine vaccines and the immunotherapy of autoimmune diseases." *European Journal of Immunology* 36 (2006): 2,844–2,848.
21. Delavallée, L. et al. "Anti-cytokine vaccination in autoimmune diseases." *Swiss Medical Weekly* 140 (2010): w13108.



Citrullination in Disease: The Role of Autoantibodies

Introduction

The dynamic landscape of proteomics is profoundly influenced by post-translational modifications (PTMs), which modulate protein structure, function, stability, localization and enzymatic activity. Among these modifications, citrullination has attracted significant research interest across various diseases due to its involvement in processes related to chronic inflammation and its diagnostic relevance in autoimmune disorders[1].

Citrullination, a specific PTM, entails the enzymatic conversion of arginine residues into citrulline by the family of peptidylarginine deiminases (PADs) (Figure 3). This process, which is still not fully understood in the context of standard cellular activities, predominantly transpires under conditions of cellular stress. These conditions are often accompanied by inflammation, autophagy, and biological processes that increase calcium levels required for citrullination, such as apoptosis, necrosis and oxidative stress.

Profiling anti-citrullinated protein autoantibodies (ACPAs) adds valuable insight into the role of citrullination in disease. ACPAs are well-established biomarkers in autoimmune diseases like rheumatoid arthritis, where they are detected years before clinical symptoms appear and correlate with disease severity and progression. Beyond autoimmune disorders, recent studies have suggested that citrullinated proteins may also play a role in cancer, neurodegenerative diseases and chronic inflammatory conditions, making ACPAs a potential tool for early diagnosis and patient stratification in these contexts as well.

By incorporating ACPA profiling, researchers can not only deepen their understanding of citrullination in various disease mechanisms but also identify new diagnostic and therapeutic targets across a range of conditions. This approach highlights the broader value of autoantibody profiling in precision medicine, particularly for complex diseases driven by inflammatory and stress-related pathways.

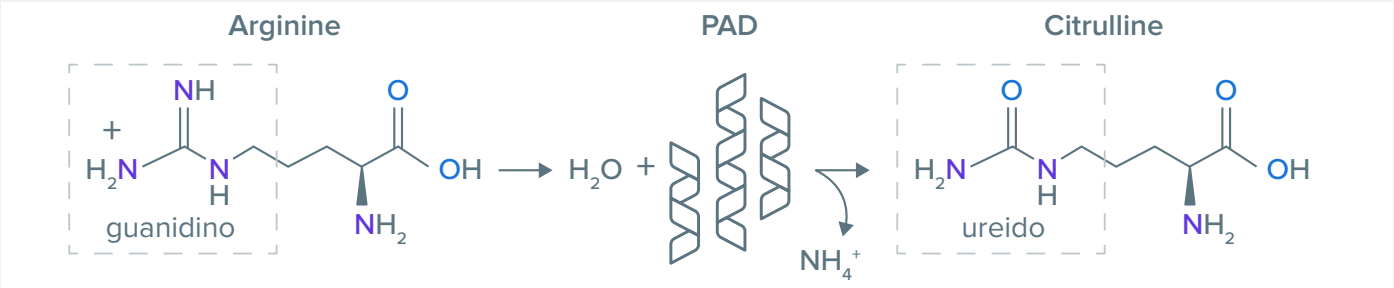


Figure 3. The citrullination process

Citrullination in the context of complex diseases

Recent investigations have shed light on the extensive role of citrullination within the etiology of complex diseases and its association with the innate immune system (Table 4). In rheumatoid arthritis (RA), ACPAs are detectable years before symptoms appear, serving as diagnostic markers in 70% of cases[2–4]. They also correlate with disease prognosis[1].

The elevated levels of PAD enzymes observed in various carcinomas hint at the potential diagnostic utility of citrullinated proteins in oncology. PAD4, for example, has been detected in the blood of patients with breast, lung, colon, ovarian and prostate cancers[5,6].

Additionally, links between citrullination and neurodegenerative diseases have been explored. In patients with Alzheimer's disease, researchers have discovered citrullinated beta-amyloid protein in the brain[7]. A meta-analysis of blood metabolites from dementia patients showed a significant increase in citrulline levels[8]. Additionally, a recent structural analysis identified a potential citrullination site on an arginine residue in TDP-43 protein from patients with frontotemporal lobar degeneration[9]. Proteins with disordered tertiary structures, such as arginine, are known to undergo citrullination readily[1]. The presence of citrullinated proteins in neurological conditions opens avenues for novel diagnostic approaches. The opportunity to develop new diagnostic methods using autoantibodies against citrullinated proteins has yet to be fully investigated.

Challenges and advances in citrullination research

The study of citrullination, characterized by its low abundance as a PTM, necessitates highly sensitive detection techniques. While traditional methods like ELISA and western blotting offer insights into the distribution of citrullinated proteins and PADs, they are limited by low throughput and semi-quantitative analysis.

Mass spectrometry (MS) and protein microarrays have emerged as powerful tools for the high-throughput, sensitive profiling of citrullination, each presenting distinct advantages and limitations in the context of sample processing and analytical specificity.

MS offers diverse methodologies to detect citrullination, tailored to specific sample requirements and investigative goals. Predominantly, a bottom-up proteomic approach using tandem MS is utilized for the direct identification of citrullinated proteins.

Table 4. Proteins commonly citrullinated in disease

Tissue	PAD	Proteins	Disease
Connective	PAD2, PAD4	Fibrinogen, vimentin, fibrin collagen type II, enolase	Rheumatoid arthritis
Tumorous	PAD2, PAD4	p53, p21, p300, ETS like-1, histone	Cancer
White matter	PAD2	Myelin basic protein	Multiple sclerosis
Central nervous system	PAD2, PAD4	Vimentin, myelin basic protein, glial fibrillary acidic protein	Alzheimer's disease
Skin	PAD1, PAD3	Filaggrin	Psoriasis
Eye	PAD2	Myelin basic protein	Glaucoma

While a very sensitive approach, MS demands skilled technicians, involves multiple procedural steps susceptible to errors and necessitates the use of expensive high-resolution mass spectrometers[10,11]. A notable challenge with MS is that it is difficult to distinguish between citrullination and the deamidation of glutamine or asparagine.

Protein microarrays present an alternative for the high-throughput and sensitive detection of citrullination, employed via direct or indirect methods. The direct method involves the application of labeled PAD enzymes to an array, enabling the screening of hundreds to thousands of immobilized proteins to identify those undergoing citrullination. This approach has led to the discovery of several new citrullinated protein substrates, predominantly involved in glycolysis, although these findings have yet to be directly linked to disease-specific citrullination[12].

Indirectly, protein arrays are used to detect autoantibodies against citrullinated proteins. This method has broad applications, including early disease detection, disease subtyping, patient stratification, and the identification of new therapeutic targets and pathways. It also provides insights into the immune system's response to this PTM.

As an example of studying citrullination using the indirect approach, sera from both anti-cyclic citrullinated peptide (CCP) positive and negative patients with diverse pathologies were analyzed using a high-density protein array. Researchers identified 844 autoantibodies, many previously undiscovered, differentiating between patient groups. This indicates potential for further stratification of RA patients and suggests that anti-CCP negative patients might be incorrectly diagnosed through conventional methods[13].

The analysis of autoantibody biomarkers via protein arrays offers significant advantages in studying citrullination's role in disease. Antibodies, typically analyzed in serum, represent a complete spectrum of circulating antibodies, are stable and can be present long before disease symptoms manifest. This technique requires minimal sample preparation and training. Additionally, analyzing multiple antibody isotypes concurrently (for example, IgG and IgA) can provide comprehensive information on the immune response, enhancing the accuracy of disease detection and monitoring treatment efficacy[14].

Implications for disease diagnosis and therapeutic development

Citrullinated antigens are increasingly recognized for their potential in therapeutic applications. The specificity of citrullination to diseased and autophagic tissues makes citrullinated proteins attractive targets for therapies aimed at reducing inflammation while preserving healthy cells. This approach is particularly relevant for conditions like cancer and RA.

For instance, vimentin, which becomes highly citrullinated in metastatic epithelial tumors but remains unmodified in normal tissue, has been explored as a therapeutic target. Studies in mice with melanoma have demonstrated that immunization with citrullinated vimentin significantly enhances survival rates compared with controls receiving placebo. Importantly, this strategy inflicts minimal damage on healthy tissues, underscoring the precision of targeting citrullinated proteins for disease treatment[15].

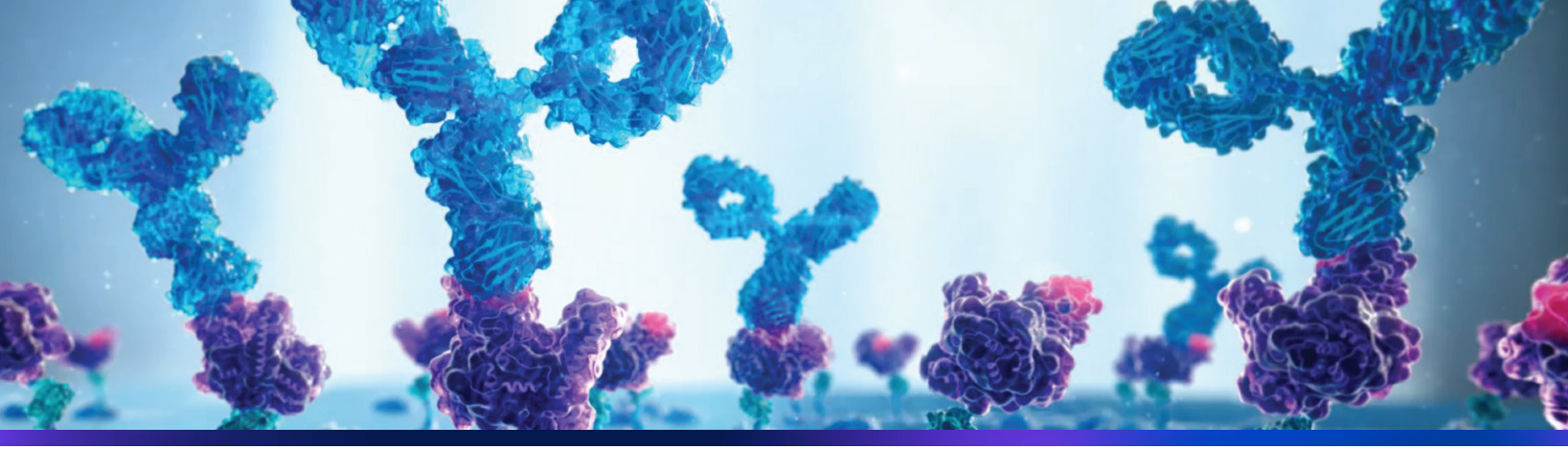
Sonoma Biotherapeutics recently shared promising preclinical findings for SBT-77-7101, a CAR T cell therapy designed to identify and target citrullinated proteins in patients with RA, aiming to alleviate pain and inflammation. These preclinical studies successfully demonstrated the CAR T cells' ability to recognize citrullinated proteins, directly addressing the inflammation at its source. With clinical trials in progress[16], this innovative approach highlights the potential of using CAR T cells against citrullinated proteins as a more targeted and potentially less side-effect-prone treatment compared with existing monoclonal antibody and CAR T cell therapies. This strategy opens up new possibilities for creating highly specific treatments by leveraging the identification of citrullinated proteins.

Conclusion

The exploration of citrullination has unveiled its close association with a broad spectrum of diseases, particularly in conditions characterized by chronic inflammation and immune dysregulation. The sensitivity of current methodologies, including mass spectrometry and protein microarrays, has significantly advanced our ability to detect and analyze citrullinated proteins, thereby enhancing our understanding of their role in disease. The potential diagnostic and therapeutic applications arising from this research highlight a promising approach for the development of targeted treatments and early detection strategies. Moreover, the specificity of citrullination to diseased tissues offers a unique biomarker for distinguishing diseased from healthy states, promising more precise and less invasive diagnostic tools.

References

1. Gudmann, N.S. et al. “Biological relevance of citrullinations: Diagnostic, prognostic and therapeutic options.” *Autoimmunity* 48 (2015): 73–79.
2. Rantapää-Dahlqvist, S. et al. “Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis.” *Arthritis & Rheumatology* 48 (2003): 2,741–2,749.
3. Harada, K. et al. “Citrullination and the protein code: Crosstalk between post-translational modifications in cancer.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 378 (2023).
4. Schellekens, G.A. et al. “Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies.” *Journal of Clinical Investigation* 101 (1998): 273–281.
5. Chang, X. et al. “Expression of peptidylarginine deiminase type 4 (PAD4) in various tumors.” *Molecular Carcinogenesis* 45 (2006): 183–196.
6. Lastwika, K.J. “Posttranslational modifications induce autoantibodies with risk prediction capability in patients with small cell lung cancer.” *Science Translational Medicice* 15 (2023).
7. Mukherjee, S. et al. “Citrullination of amyloid-β peptides in Alzheimer’s disease.” *ACS Chemical Neuroscience* 12 (2021): 3,719–3,732.
8. Zinellu, A. et al. Circulating arginine metabolites in Alzheimer’s disease and vascular dementia: A systematic review and meta-analysis.” *Ageing Research Reviews* 92 (2023): 102139.
9. Arseni, D. et al. “TDP-43 forms amyloid filaments with a distinct fold in type A FTLD-TDP.” *Nature* 620 (2023): 898–903.
10. De Ceuleneer, M. et al. “*In vivo* relevance of citrullinated proteins and the challenges in their detection.” *Proteomics* 12 (2012): 752–760.
11. Rebak, A.S. et al. “Characterizing citrullination by mass spectrometry-based proteomics.” *Philosophical Transactions of the Royal Society* B 378 (2023): 20220237.
12. Thomas, M.A. et al. “An unbiased proteomic analysis of PAD4 in human monocytes: Novel substrates, binding partners and subcellular localizations.” *Philosophical Transactions of the Royal Society* B 378 (2023): 20220477.
13. Poulsen, T.B.G. et al. “Identification of potential autoantigens in anti-CCP-positive and anti-CCP-negative rheumatoid arthritis using citrulline-specific protein arrays.” *Scientific Reports* 11 (2021): 17300.
14. Sieghart, D. et al. “The diagnostic and prognostic value of IgG and IgA anti-citrullinated protein antibodies in patients with early rheumatoid arthritis.” *Frontiers in Immunology* 13 (2023): 1096866
15. Brentville, V.A. “Citrullinated vimentin presented on MHC-II in tumor cells is a target for CD4+ T-cell-mediated antitumor immunity.” *Cancer Research* 76 (2016): 548–560.
16. Charmsaz, S. et al. “Detection of citrullinated proteins recognized by a novel chimeric antigen receptor T reg therapy in both synovial fluid and serum from patients with rheumatoid arthritis.” American College of Rheumatology Convergence (2023).



Profiling Autoantibodies with Precision

Introduction

Protein microarrays provide a fast and cost-effective way to profile AAbs against hundreds to thousands of antigens at once. This makes them a powerful tool for studying the immune system, advancing both biomedical research and diagnostic capabilities.

Profiling antibodies and AAbs enables the discovery of targetable and exploitable biomarkers: protein antigens, pathogenic antibodies and protective antibodies. These insights advance precision medicine for improved patient outcomes. Here are some applications of protein microarrays:

- **Vaccine and drug development:** Validate target specificity and efficacy while mapping immune-targeted diversification to reveal how the immune system responds to different antigens over time
- **Patient stratification and subtyping:** Identify subtypes within heterogeneous diseases and select patients for clinical trials who are most likely to benefit, reducing variability and increasing statistical power
- **Response prediction:** Predict patient outcomes to treatments, enabling personalized plans and minimizing the risk of immune-related adverse reactions
- **Understanding disease mechanisms:** Discover disease-associated proteins and AAbs to uncover therapeutic targets, reveal early diagnostic biomarkers and track disease progression

Most protein microarrays use peptides or proteins that are misfolded, denatured or fragmented, resulting in the loss of conformational epitopes – specific three-dimensional (3D) structures recognized by 90% of AAbs[1,2]. In addition, new, non-native epitopes that are not typically present *in vivo* are exposed, leading to nonspecific binding and increased false positives and negatives.

To ensure precise antibody-antigen interactions and obtain reliable data, it is crucial to use correctly folded proteins. Selecting the right protein microarray is therefore key to achieving high-quality results.

WHITE PAPER

Address Key Challenges in Vaccine Development with Antibody Profiling

Explore how antibody profiling can guide the development of safer, more effective vaccines.

Read now

Visualizing immune interactions with protein microarrays

Protein microarrays are miniaturized immunoassays that display a large number of antigens – such as proteins, peptides or fragments – on a solid surface, usually a glass slide, in an organized, addressable format. To perform an assay, a test sample, often serum-rich in host antibodies, is applied to the array (Figure 4). Antibodies in the sample bind specifically to target antigens on the array, creating antibody-antigen complexes.

Following antibody binding, a fluorescently tagged secondary antibody specific to the host species of the sample is added. This secondary antibody binds to the primary antibodies attached to the proteins, enabling indirect visualization and detailed immunoprofiling.

The fluorescence intensity is proportional to the quantity of antibodies, whereas the fluorescence location on the array facilitates the identification of the targeted antigen.

Depending on the array, multiple antibody isotypes (i.e., IgG, IgM, IgA, IgE) with unique effector functions may be analyzed simultaneously (Table 5). This capability is made possible with the use of secondary antibodies that bind to specific isotypes and are labeled with different fluorophores. Measuring various isotypes offers a more comprehensive view of disease, delivering valuable insights into the timing and localization of the immune response[3].

Table 5. Antibody isotypes: Primary functions and serum reference ranges in adults

Antibody	Subclasses	Primary Function	Serum Level (g/L)
IgA	IgA1-2	Pathogen neutralization, anti-inflammatory, mucosal	0.6–4
IgD	None	Upper aerodigestive immunity, B cell development, immune regulation	0–0.14
IgE	None	Tumor surveillance, anti-venom defense, anti-parasitic defense, type 1 hypersensitivity	Trace
IgG	IgG1-4	Circulating and tissue immunity	7–15
IgM	None	Immune surveillance, acute response (for example, agglutination, complement activation)	Psoriasis

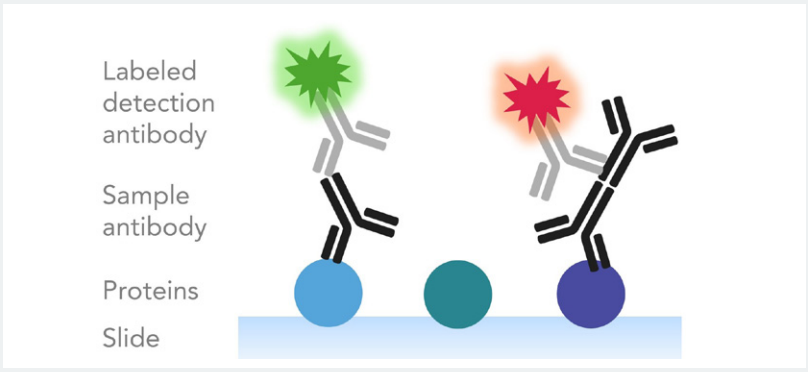


Figure 4. Some protein microarrays can simultaneously detect two antibody isotypes using fluorescently labeled antibodies.

Correct protein folding with KREX technology

Standard BioTools proprietary KREX technology ensures that only correctly folded proteins are used for AAb profiling (Figure 5). Each protein is tagged with a small 10 kDa subunit of biotin carboxyl carrier protein (BCCP), which acts as a folding marker. If a protein is misfolded or fragmented, the BCCP misfolds as well, concealing its biotinylation site and preventing it from attaching to the streptavidin-coated array surface. This allows only properly folded proteins to be immobilized for AAb profiling, while misfolded proteins are washed away, removing them from further analysis.

Unlike other immobilization tags, such as glutathione S-transferase (GST) or maltose-binding protein (MBP), BCCP uniquely supports the retention of correctly folded proteins. Additionally, the non-denaturing surface of KREX microarrays preserves the three-dimensional protein structures essential for accurate AAb binding[4].

Finally, recombinant human proteins on KREX arrays are expressed in insect cells, which more closely replicate protein processing of mammals compared with bacteria or yeast[4,5]. For instance, protein expression in the commonly employed bacterial system, *Escherichia coli*, frequently results in insoluble, poorly folded proteins. Yeast expression systems, such as *Saccharomyces cerevisiae* and *Pichia pastoris*, do not mimic glycosylation patterns of mammalian cells well, and the harsh cell lysis conditions often lead to denatured or fragmented proteins. Therefore, the use of insect cells aligns more closely with mammalian systems, significantly improving the functional expression of human proteins.

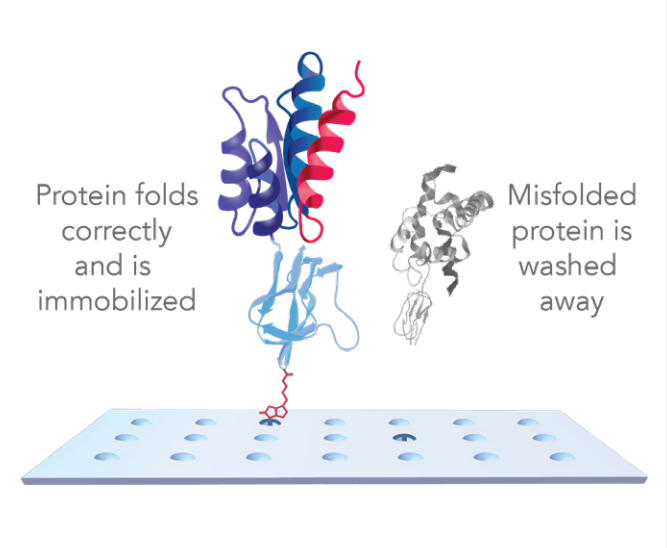


Figure 5. KREX technology for precise antibody profiling

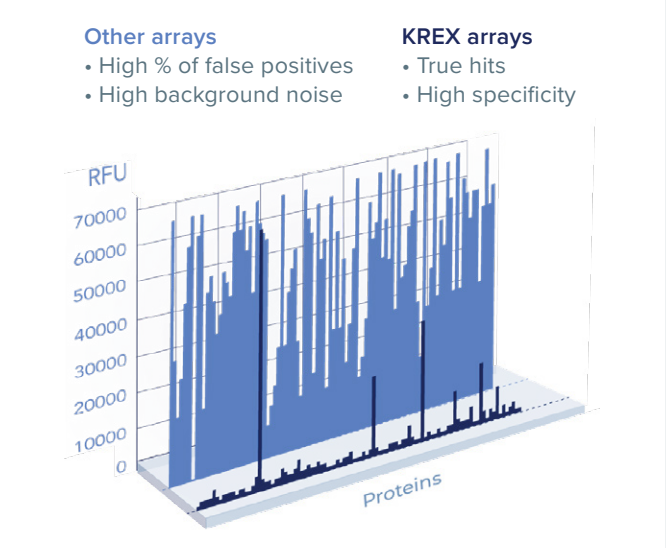


Figure 6. KREX microarrays reveal distinct and specific hits while data from other arrays are characterized by a high number of nonspecific hits contributing to elevated background noise.
RFU = relative fluorescence units

Key features of KREX technology

- **Native protein structure:** Full-length proteins with intact epitopes, ensuring exceptional specificity and a low false discovery rate (FDR) <1% (Figure 6)
- **High sensitivity and wide linear range:** Picomolar (pM) sensitivity and a linear range spanning over four logs
- **Highly reproducible:** Mean intra-array CV <10% and an inter-batch Pearson correlation (R2) >0.95 (Figure 7)
- **Versatile and scalable:** Ideal for various applications and study sizes, consuming minimal sample (<50 µL) without compromising on quality
- **Dual isotype analysis:** Simultaneous insights into two antibody isotypes (for example, IgG and IgA or IgM)
- **Multiple measurements:** Three or four replicates per antigen enable more robust and reliable data

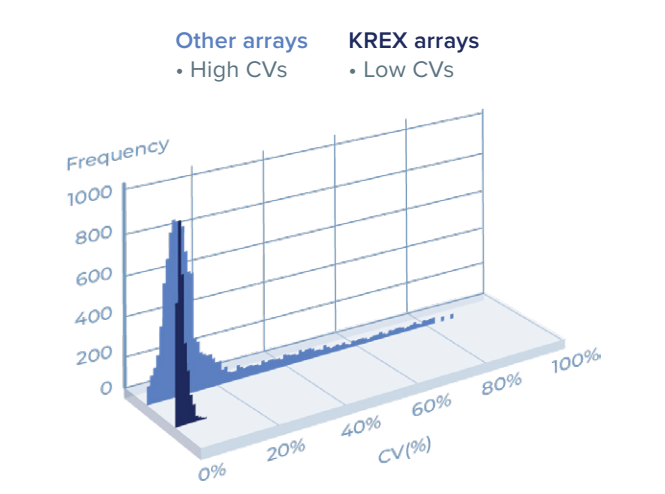


Figure 7. KREX microarrays have a lower per-protein coefficient of variation percentage (CV%) than other arrays. Data represents all proteins and the same 50 serum samples.

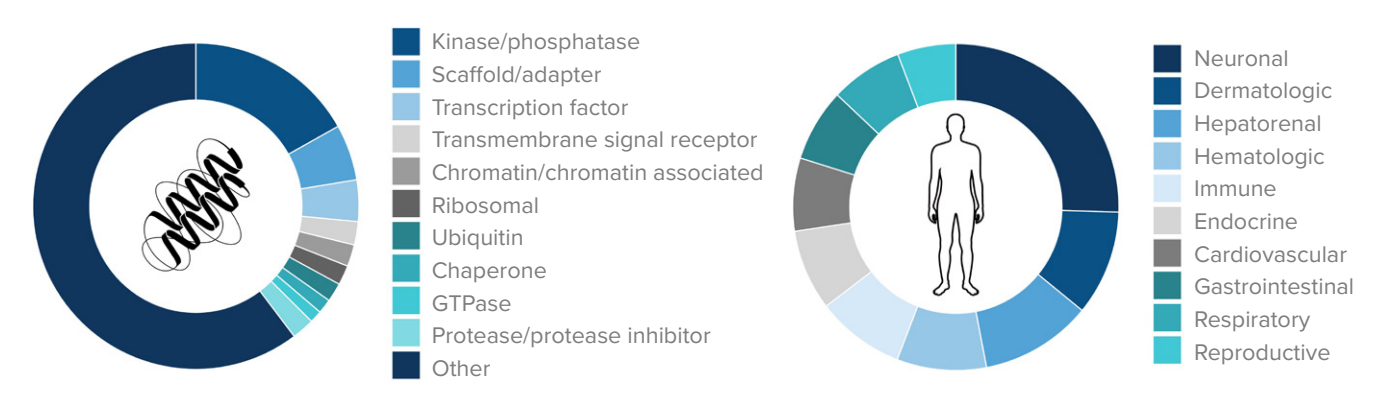


Figure 8. Protein functional classes and disease categories represented by protein antigens in the Standard BioTools™ library

Expertly curated library

Standard BioTools extensive library of over 2,000 human proteins has been carefully curated by immunologists for their role as established and potential autoantigens. In selecting these proteins, considerations included their relevance in various diseases, biological functions, expression in specific tissues and compartments, and interactions with immune cells from both the innate and adaptive immune systems.

This library covers a wide range of protein functional classes and subcellular locations, providing comprehensive representation of critical disease-related pathways and potential drug targets (Figure 8). It includes essential immune regulators and modulators such as cytokines, chemokines and their receptors (Table 6; see also **Anti-Cytokine Autoantibodies**).

Importantly, human antigens are also highly relevant in infectious disease research due to mechanisms like molecular mimicry and epitope spreading, where pathogens trigger immune responses that cross-react with human proteins. Additionally, tissue damage and inflammation from infections can directly elicit AAbs. These factors broaden the platform's applications, making it valuable not only for autoimmune and cancer research but also for understanding host-pathogen interactions and immune responses in infectious diseases.

Table 6. Cytokines, chemokines and their receptors included in the Standard BioTools protein library

Cytokines	Chemokines	Receptors
Activin A (INHBA)	CCL1	**Cytokine Receptors**
Amphiregulin (AREG)	CCL2	
APRIL (TNFSF13)	CCL3	ACVR2A
G-CSF (CSF3)	CCL7	ACVR2B
IFNA2	CCL8	CSF3R
IFNB2	CCL13	CXCR2
IFNW1	CCL16	IL13RA1
IFNG	CCL17	IL13RA2
IL1A	CCL19	IL21R
IL1β	CCL20	IL6ST
IL3	CCL21	
IL5	CCL22	**Chemokine Receptors**
IL6	CCL25	
IL7	CCL27	CCR5
IL8 (CXCL8)	CCL28	CXCR2
IL10	CXCL6	CXCR4
IL11	CXCL8	CXCR6
IL12A	CXCL9	
IL12B	CXCL10	
IL13	CXCL11	
IL15	CXCL12	
IL17A	CXCL13	
IL18	CXCL16	
IL19	CXCL17	
IL20	CX3CL1	
IL21		
IL22		
IL23 (IL23A+IL12B)		
IL24		
IL26		
IL27		
IL31		
IL32		
IL34		
IL35		
IL36		
IL37		
IL39 (IL23A+EBI3)		
IL40 (C17orf99)		
M-CSF (CSF2)		
TNF		
TSLP		

Flexible, end-to-end solutions

Standard BioTools offers both ready-to-use panels and fully customizable options for antibody and AAb profiling, tailored to specific protein targets (Table 7). This flexibility allows researchers to select or create arrays that best meet their research objectives, whether they are investigating specific diseases, identifying biomarkers or studying immune responses. Sample analysis is supported by a global network of certified service providers, ensuring accessibility and consistent quality regardless of location.

At every stage, Standard BioTools provides comprehensive support – from initial experimental design to advanced bioinformatics analysis – helping researchers maximize the value of their data. Detailed data reports, complete with figures and statistical analyses, are easily accessible through i-Ome AI, a user-friendly, open source data analysis platform from Standard BioTools. This platform streamlines data interpretation, enabling researchers to quickly extract actionable insights.

Conclusion

Protein microarrays are a powerful tool for profiling antibodies and AAbs, with applications that span biomarker discovery, disease diagnostics and therapeutic development. However, to generate high-quality, reliable data, it is essential to preserve the conformational epitopes recognized by the vast majority of humoral antibodies. Standard BioTools addresses this need through its proprietary KREX microarrays, ensuring that only correctly folded, functional proteins are displayed for precise antibody-antigen interactions.

By combining cutting-edge protein folding technology, a comprehensive selection of disease-relevant protein antigens and robust analytical support, KREX microarrays empower researchers to achieve deeper insights into disease initiation and progression, ultimately driving advancements in personalized medicine and targeted treatments.

Table 7. KREX protein microarrays

Microarray	Protein Number	Protein Panel
i-Ome Discovery	1,800-plus	Protein antigens representing a myriad of protein functional classes and disease categories for comprehensive biological and immunoproteomic insights
i-Ome Cancer	500-plus	Cancer-associated proteins, encompassing tissue and pathway relevance, therapeutic targets, cytokines and chemokines, cancer-driver proteins, prognostic indicators, cancer-testis antigens, B cell and AAb targets, and ectopic expression
Autoimmune Disease Autoantigen Panel	100-plus	Clinically relevant protein antigens linked to a wide array of autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's and diabetes
OncoRex p53 Cancer	100-plus variants	p53 wild-type and mutant variants, enabling precise screening of therapeutic AAbs and protein-binding compounds for cancer
Custom	Project-specific	Select from over 2,000 proteins in our library or request your own

References

1. Barlow, D.J. et al. “Continuous and discontinuous protein antigenic determinants.” *Nature* 27 (1986): 747–748.
2. Van Regenmortel, M.H.V. “Mapping epitope structure and activity: From one-dimensional prediction to four-dimensional description of antigenic specificity.” *Methods* 9 (1996): 465–472.
3. Janeway Jr., C.A. et al. Immunobiology: *The Immune System in Health and Disease.* Garland Science (2001).
4. Aziz, F. et al. “Autoantibody-based diagnostic biomarkers: technological approaches to discovery and validation.” *Autoantibodies and Cytokines.* IntechOpen (2019).

For more information,
visit **standardbio.com**

LAB-00016 Rev 1 032025
Beyond Genetics Autoantibodies as Biomarkers eBook
For Research Use Only. Not for use in diagnostic procedures.