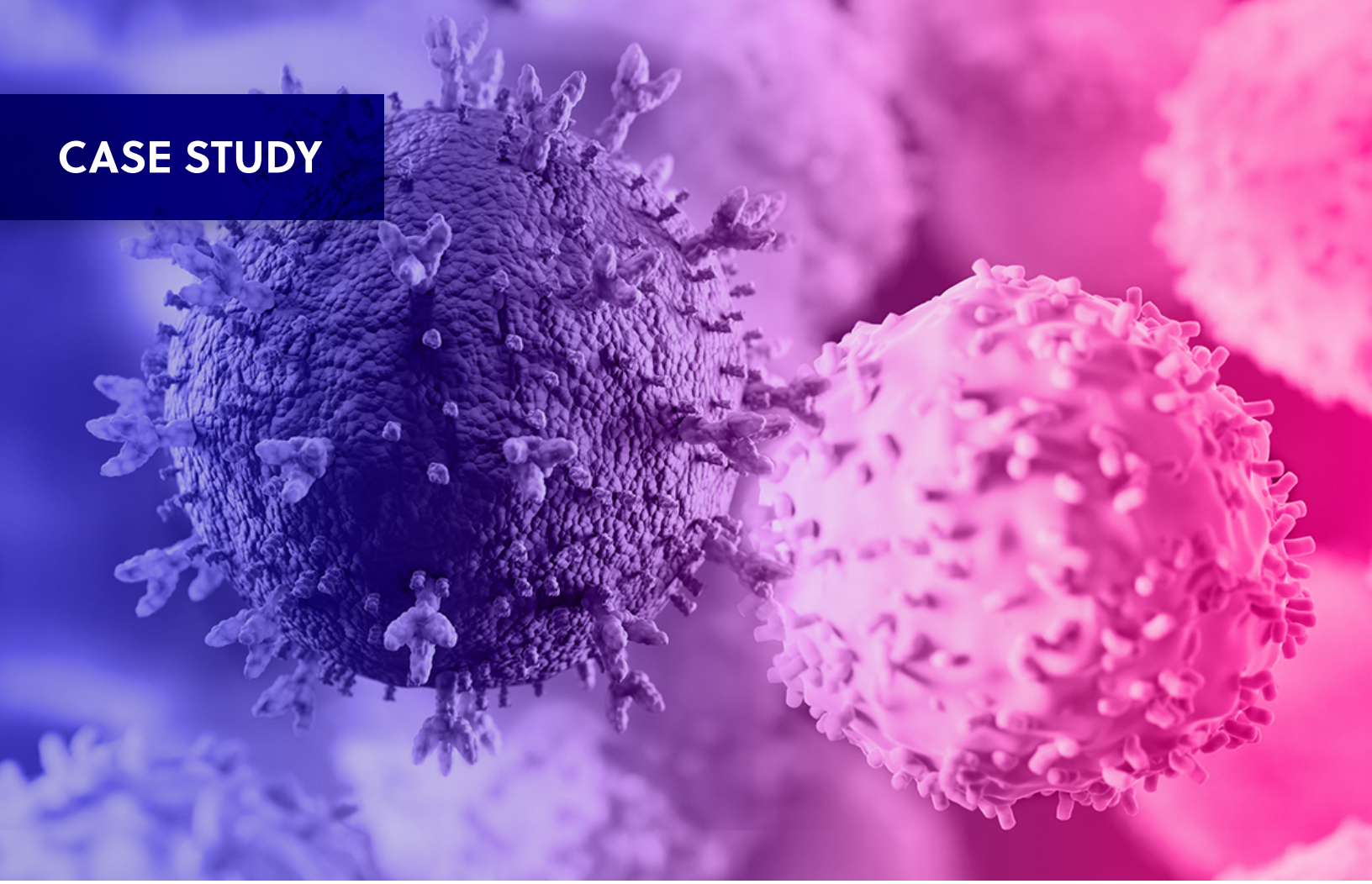

CASE STUDY

# Immune Repertoire Profiling to Assess Therapeutic Response in Autoimmune Disease

## INTRODUCTION

Autoimmune diseases affect nearly one in ten individuals and may be expressed as any one of 80 distinct conditions.[1] Autoimmunity results when the body's defense mechanisms mistakenly attack healthy cells. Depending on the specific condition, symptoms can either manifest throughout the body or remain localized to a specific organ.[2] The reasons for immune system malfunction are still not fully understood and are an active area of research.[2]

The adaptive immune response is largely conducted and regulated by B cells and T cells, and the study of their receptor chain profiles can help us better understand the immune response to disease and treatment. The adaptive immune receptor repertoire (AIRR) is composed of 7 receptor chains, encompassing four T cell receptor (α, β, δ, λ) and three B cell receptor

(heavy (H), κ, λ) chains. Several published studies have used immune repertoire sequencing to monitor changes in the B cell receptor (BCR) and T cell receptor (TCR) repertoires of patients undergoing treatment for autoimmune disorders.[2] Previous studies of BCR and TCR diversity have been limited to sequencing individual receptor chains. However, immune sequencing technologies can now amplify all 7 chains of the AIRR simultaneously, without introducing V-gene bias during multiplex PCR amplification. This enables quantitative and highly reproducible analysis of the full diversity of BCR and TCR repertoires, opening the potential to leverage the AIRR for endotyping complex disease pathobiology.

## AIRR CHANGES DETECTED IN AUTOIMMUNE DISEASE

Immune repertoire sequencing has provided critical information leading to a better understanding of the underlying mechanisms responsible for autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE or lupus) that result in chronic inflammation and organ damage.[3] Immunosuppression is the primary strategy for managing RA and SLE but lacks specificity for the disease being managed. Most patients do not respond to treatment and often suffer from debilitating side effects, so there is a great need for targeted therapies.[2]

Characterizing changes to the immune repertoires of RA and SLE patients, in response to immunosuppressive treatments, is the focus of several recent studies. Information from these studies may help in the development of personalized medicines for autoimmune disease management.[2]

## CHARACTERIZING THE IMMUNE REPERTOIRE RESPONSE TO THERAPY IN RHEUMATOID ARTHRITIS

A first-ever, exploratory, in-depth, quantitative analysis of the AIRR of RA patients and healthy controls was conducted by a team led by Dr. Adria Aterido. This study aimed to identify clonal populations important to RA's progression, define clinical phenotypes that may not have been recognized in previous studies limited to single receptor-chain analyses, and characterize the response to TNFα inhibition (TNFi) therapy.[4] To capture a maximum number of clonotypes within the expansive AIRR, unbiased, single-assay multiplex amplification and sequencing of all 7 receptor chains was conducted.

The immune repertoire of RA patients displayed distinct profiles compared to healthy controls. AIRR diversity was notably lower in RA patients across all 7 chains, particularly among B cells, with only moderate TCR diversity changes. This decline in diversity was mainly due to specifically expanded BCR H, κ, and λ chains, along with a decreased usage of the λ chain in blood. RA patients exhibited an isotype-specific signature, signifying differing immunoglobulin functionality in RA. Skewed V and J gene segment usage indicated disease-linked V-J pairs. Distinct IGL and IGK clones, meta-clones, and k-mers associated with RA and clinical phenotypes were detected in RA patients.

Interestingly, after a 12-week course of TNFi treatment, a common therapeutic approach for RA, the diversity of BCR H, κ, and λ chains exhibited a notable increase, resembling a similar diversity pattern observed in healthy individuals. Also, responders to TNFi therapy

had a different distribution of the complementarity determining region (CDR) 3 length within the BCR H chains compared to non-responders. Therefore, patients who will respond to therapy may have a different antigenic reactivity in the B cell compartment from those that will not show clinical improvement.

With this data, Dr. Aterido and colleagues successfully developed a highly accurate AIRR predictor for RA diagnostics (95.2%) and response to TNFi therapy (68.8%). This high accuracy was attributed to the incorporation of data from both BCR and TCR clonal types, underscoring the significance of the comprehensive 7-chain analysis. This predictor could improve early diagnosis and stratify patients that may benefit from TNFi therapy.

Biologic disease-modifying antirheumatic drugs (bDMARDs) are commonly used to treat RA by reducing inflammation and altering disease progression. A study conducted by Che-Mai Chang and colleagues, aimed to characterize the effects of different types of bDMARD treatments on the T cell receptor ß-chain (TCRB) repertoires of RA patients.5

A high throughput sequencing method was used to profile the TCRB repertoires of circulating T lymphocytes in eight RA patients that had achieved remission following treatment with adalimumab (anti-TNFα), with or without the use of either rituximab (anti-CD20) or tocilizumab (anti-IL6R) therapies. Sequencing analysis focused on the CDR3 regions of the TCRB gene to identify the unique clonotypes of TCRBs present among treated patients.

The study found that all groups of patients showed well-controlled disease activity scores after different treatment regimens of drugs, with no significant differences in TCRB repertoire diversity, distribution of CDR3 lengths, and usage of V and J genes of TCRB based on bDMARD treatment regimen. However, clinical association studies showed an observed trend between overall reduction in TCRB repertoire diversity and increased disease activity scores in all bDMARD-treated RA patients. Additional analysis, based on specific treatment regimens, revealed a strong negative trend between TCRB diversity and clinical disease activity index (CDAI) and simple disease activity index (SDAI) scores for patients treated with adalimumab followed by rituximab or tocilizumab, but only moderate increases in disease activity score of 28-joints (DAS28) scores (**Figure 1**).

**Figure 1**

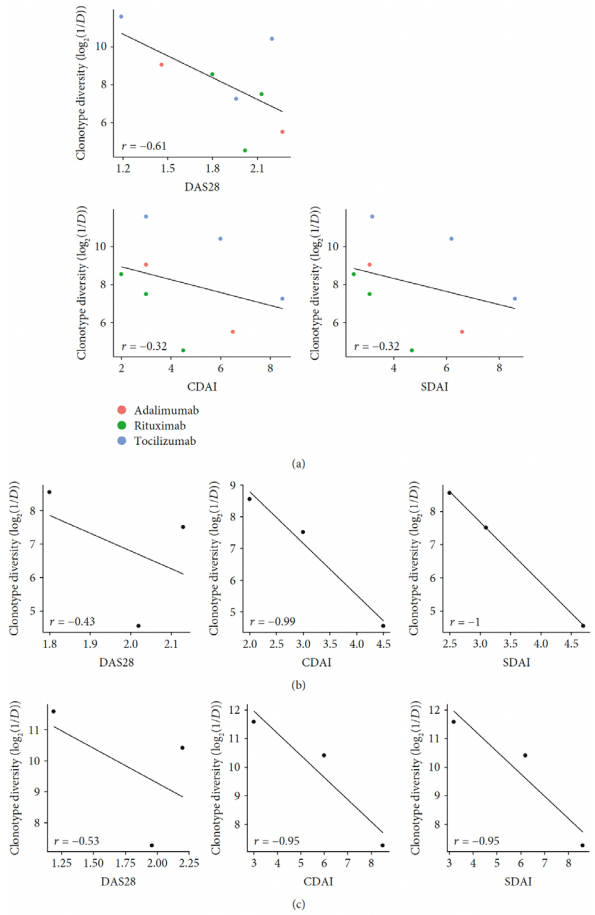


**Figure 1. Correlations in TCRB diversity and disease activity in bDMARD-treated RA patients. A negative trend was observed between TCRB repertoire diversity and DAS28, CDAI, and SDAI scores in (a) all treated RA patients, (b) patients treated with adalimumab plus rituximab, and (c) patients treated with adalimumab plus tocilizumab.[5]**

Age was also found to be associated with repertoire diversity in RA patients treated with bDMARDs. Results showed that TCRB repertoire diversity was significantly lower in patients over the age of 60 following bDMARD treatment.

Study findings showed that a reduction in TCRB repertoire diversity correlated with increased RA disease activity after biologic treatment and that older patients are more likely to have reduced TCRB repertoires.

Overall, these studies show that a decrease in diversity, either in BCR or TCR is associated with RA. Comprehensive analysis of all 7 chains of the AIRR and the measurement of global changes in diversity in response to therapy promises to unlock a new era of personalized treatment.

## CHARACTERIZING THE IMMUNE REPERTOIRE RESPONSE TO THERAPY IN LUPUS

Clinical benefits of immunosuppression in SLE have been demonstrated, yet our understanding of the mechanisms used by these drugs is not well characterized. Belimumab, a recombinant, fully human monoclonal antibody therapeutic targeting the B cell-activating factor (BAFF), used for the treatment of SLE, has shown clinical benefits, particularly for patients with high levels of anti-dsDNA antibodies.6 Belimumab treatment greatly reduces B cell populations (transitional, naïve, class-switched memory, B1) and plasmablasts, but it is not known whether changes to the immunoglobulin repertoire also occur in mature B cells or plasma cells. To answer this question, Weiquing Huang, Tam Quach, and colleagues profiled the immune repertoires of 15 patients with a history of continuous belimumab therapy, 17 patients as matched controls, and 5 patients evaluated before and after belimumab treatment.

Next-generation sequencing of the heavy chain revealed that belimumab treatment had no significant effect on usage of VDJH genes, no change in the frequency of the autoreactivity-associated VH4-34 gene, nor in CDR3 representation in unmutated IgM sequences. This finding suggested a minimal effect by belimumab on selection of the naïve B cell repertoire.

However, in the activated, antigen-selected B cell repertoire, a significant loss of VH4-34 was observed in mutated IgM and plasmablast sequences in belimumab-treated patients, indicating the promotion of negative selection of activated autoreactive B cells. Results suggest that belimumab benefits SLE patients by reducing activated autoreactive B cells and plasmablasts.

Meiyu Wu and colleagues evaluated the immune repertoires of ten SLE patients during immunosuppressive therapy to identify a potential biomarker for treatment response.[7] In this study, peripheral blood mononuclear cells (PBMCs) were collected from SLE patients undergoing treatment at 2 timepoints and separated for analysis into clinically sensitive and non-sensitive groups. Immune repertoires were profiled by sequencing all 7 immune chains. The expression of BCR repertoires was markedly decreased in sensitive patients post-treatment, while non-sensitive patients showed no significant changes (**Figure 2**).

**Figure 2**

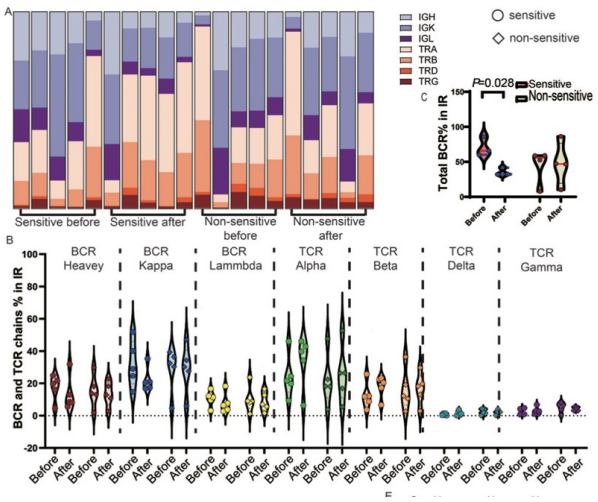


**Figure 2. Overall composition of the 7 chains of the BCR and TCR in sensitive and non-sensitive patients, before and after treatment. (A) Percentage of the 7 chains by read count. Each bar displays the composition ratio in a single sample. (B) Statistical percentage of each BCR and TCR chain by group and timepoint. (C) Total BCR percentage within the entire immune repertoire by group and time point.[7]**

IgM increased slightly in sensitive patients, but decreased in non-sensitive patients, while IgA demonstrated opposing results. TCR repertoire changes observed in sensitive patients consisted of shortened CDR3 regions in the TRB and TRG chains and significantly decreased lengths in IGK. The CDR3 of TCR δ/ λ also showed marked changes between timepoints in sensitive patients. In addition, results showed differential expression levels in six immune-related genes between clinically sensitive and non-sensitive patients, providing insights into the potential mechanisms of drug response.

This research highlights the importance of immune repertoire analysis as a potential biomarker for monitoring treatment response. It was demonstrated that it is the BCR repertoire that changes in response to immunosuppressive drug therapy in SLE patients, but that BCR repertoire sensitivity can vary among SLE patients, indicating a need for personalized therapeutic approaches. Data that indicate specific BCR changes may prove useful in disease management. For example, a patient with a low BCR ratio may benefit more from anti-inflammatory therapies than additional immunosuppression.

## CONCLUSION

Initial results from these studies indicate that immune repertoire sequencing can offer important insights into the response of the immune system to immunosuppressive therapy. Recent advances in immune sequencing technologies have enabled a comprehensive, unbiased, quantitative analysis of all seven immune chains in a single reaction. These advances are providing an unprecedented look at changes in BCR and TCR profiles in response to therapy that, may advance the development of targeted therapies for improved disease management. Further clinical trials are needed to provide confirmation of these observations.

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