News Release

”Can a Blood Test Predict Whether Rituximab Will Work for Hard-to-Treat Nephrotic Syndrome?”
— Predicting Treatment Response Based on T-cell Status

Key Points

・Nephrotic syndrome, a disease that causes large amounts of protein to leak into the urine, is usually treated with steroids. However, many patients cannot discontinue steroids because the disease relapses every time the dose is lowered. For these patients, rituximab (RTX)—a drug that removes B cells, a type of immune cell—can be effective, but there has been no way to tell whether a given patient will respond.

・By analyzing gene expression in individual blood T cells before and one month after RTX treatment using single-cell RNA sequencing, the researchers found that patients who responded well to the drug showed changes in the expression of about 18 times as many genes as those who did not respond, with signs of increased mitochondrial energy production, especially in a subset of T cells called CD4-positive cytotoxic T cells (CD4+ CTLs). This suggests that changes in T-cell activity after treatment—even though RTX does not directly target T cells—are linked to how well the drug ultimately works.

・In patients who responded to RTX, levels of reactive oxygen species (ROS) in T cells, a marker of oxidative stress, fell after treatment, whereas ROS levels rose in patients who did not respond. These findings suggest that measuring how T-cell metabolism and ROS levels change shortly after the first RTX infusion could help clinicians identify, well before the clinical outcome is otherwise known, which patients are likely to benefit.

Summary

A research group led by Assistant Professor Eri Koshi-Ito (Department of Nephrology, and Research Center of Health, Physical Fitness and Sports, Nagoya University Graduate School of Medicine), Assistant Professor Yu Watanabe (Division of Molecular Oncology, Nagoya University), Associate Professor Kazuhiro Furuhashi (Department of Nephrology), and Professor Shoichi Maruyama (Department of Nephrology), together with Professor Hiroshi I. Suzuki (Division of Biochemistry and Integrative Cancer Research, The Institute of Medical Science, The University of Tokyo, and Division of Molecular Oncology, Nagoya University), has found that changes in T cells detected in blood samples shortly after starting rituximab (RTX) treatment may help predict whether a patient with hard-to-treat nephrotic syndrome is likely to respond.

The key lies in how gene expression in T cells, a type of immune cell, changes after treatment begins.

Minimal change disease (MCD), the most common cause of nephrotic syndrome, is usually treated first with steroids. However, some patients relapse every time their steroid dose is reduced, leading to a difficult-to-treat form of the disease in which steroids cannot be stopped. RTX, which removes B cells, is effective for many of these patients, but there was previously no way to tell early on—without waiting many months to see whether the disease returns—who would respond and who would not.

The research group studied adult patients with MCD who had been unable to stop steroids for nearly ten years. Blood samples were collected before and one month after the patients' first RTX infusion, and T cells were analyzed (single-cell analysis). Comparing patients who went on to respond well to RTX with those who did not, the researchers found that gene expression changed far more dramatically in the responders after treatment—about 18 times as many genes showed changes in expression—with evidence pointing to increased mitochondrial energy production (oxidative phosphorylation), especially in $CD4^+$ cytotoxic T cells. This metabolic change was also reflected in measurements of reactive oxygen species (ROS), a marker of oxidative stress in cells: ROS levels fell after treatment in responders but rose in non-responders.

These results suggest that RTX's benefits may not come only from directly removing B cells, but also from ripple effects on T cells that become apparent soon after treatment starts. If these early changes in T-cell metabolism and ROS levels can be used as biomarkers, clinicians may be able to identify likely responders much earlier than currently possible, helping to optimize treatment for this difficult-to-treat form of nephrotic syndrome.

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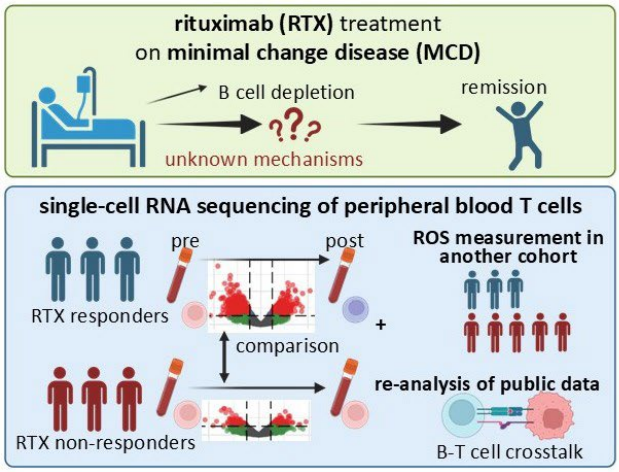
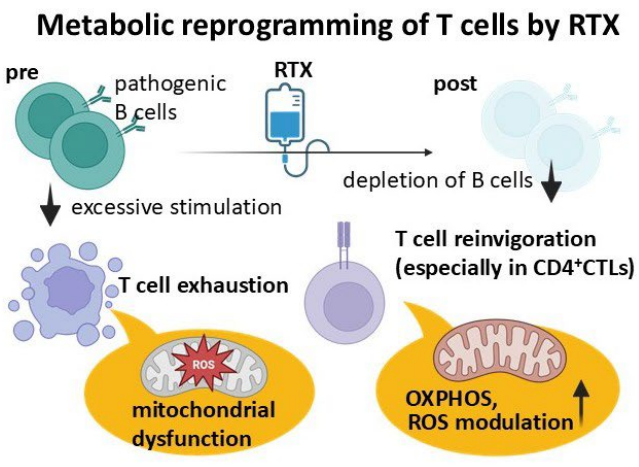




Research Background

Nephrotic syndrome occurs when the kidney's filtering units (glomeruli) are damaged, causing large amounts of protein to leak from the blood into the urine. The most common cause is minimal change disease (MCD), which is usually treated first with steroids. While most patients respond well, 10-30% develop a difficult-to-treat form in which the disease relapses every time the steroid dose is reduced ("frequently relapsing" or "steroid-dependent" nephrotic syndrome). Because long-term steroid use can cause side effects such as osteoporosis, infections, and diabetes, the inability to stop steroids places a heavy burden on patients.

For these difficult cases, rituximab (RTX)—an antibody drug that removes B cells, a type of immune cell—is often effective. RTX has been covered by Japanese health insurance for children since 2014, but for a long time adult-onset cases were not covered. This changed in June 2026, when insurance coverage was extended to adult-onset cases. The present study focuses on adult patients treated at Nagoya University between 2018 and 2022, before this insurance approval, when RTX was used off-label under an approved ethical protocol.

Now that adults also have wider access to this treatment, an important question remains: which patients should receive RTX, and when? After RTX infusion, B cells nearly disappear from the blood and typically recover after about six months. Some patients stay in remission for over a year even after B cells return, while others relapse while B cells are still absent. Whether a patient will respond usually only becomes clear months later, once the disease either stays in remission or relapses. This means that B-cell removal alone cannot explain how RTX works, and no reliable biomarker has been established to predict treatment response. Identifying likely responders soon after the first infusion could lead to earlier remission and fewer complications, while also avoiding unnecessary repeat infusions in likely non-responders, reducing associated infection risks and unnecessary use of medical resources. The research team therefore focused on T cells—cells that RTX does not directly target. Because B cells and T cells communicate with each other to regulate immune responses, the team compared blood samples from before and after RTX administration, at single-cell resolution, to examine how B-cell removal might indirectly affect T cells and relate to later treatment outcomes.

Research Results

The researchers studied adult MCD patients treated at Nagoya University Hospital who had been steroid-dependent for nearly ten years. T cells were collected from blood samples taken before and one month after the

first RTX infusion, and the T cells were analyzed using single-cell RNA sequencing together with T-cell receptor (TCR) analysis. Patients were classified, based on their eventual clinical course, into a ”responder” group, who were able to stop steroids and stay in remission after RTX, and a ”non-responder” group, who could not stop steroids or relapsed within six months of treatment.

(1) T cells in responders show much greater changes in gene expression between the pre- and post-treatment samples

Comparing gene expression before and one month after RTX infusion, only 77 genes showed changes in expression in non-responders, compared with 1,415 genes in responders—an approximately 18-fold difference (Figure 1). In responders, genes involved in OXPHOS, as well as genes related to T-cell activation through interferon-alpha/gamma and IL-2–STAT5 signaling, showed increased expression after treatment (Figure 2).

Figure 1 Comparison of number of differentially expressed genes

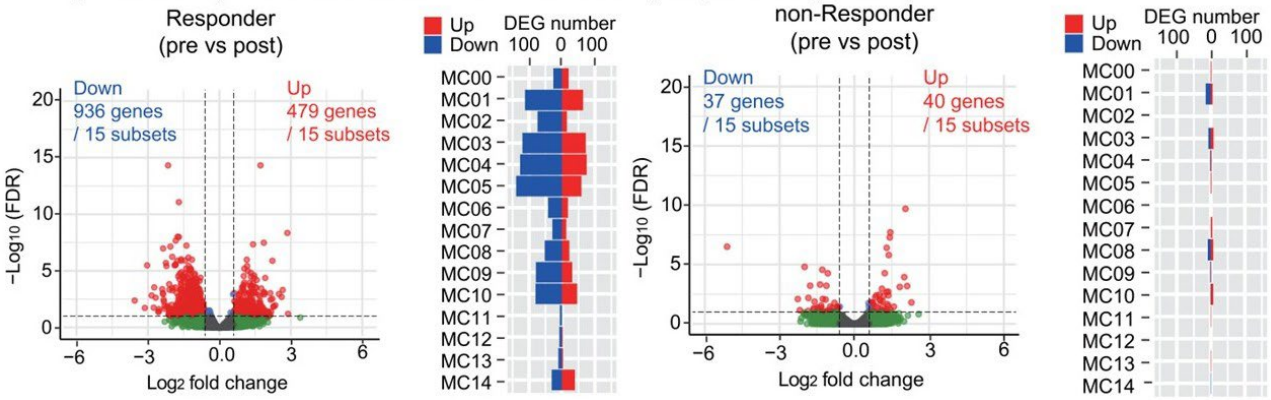
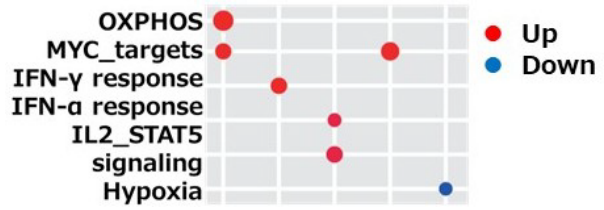


Figure 2
T-cell activation–related genes are upregulated after rituximab treatment in responders.

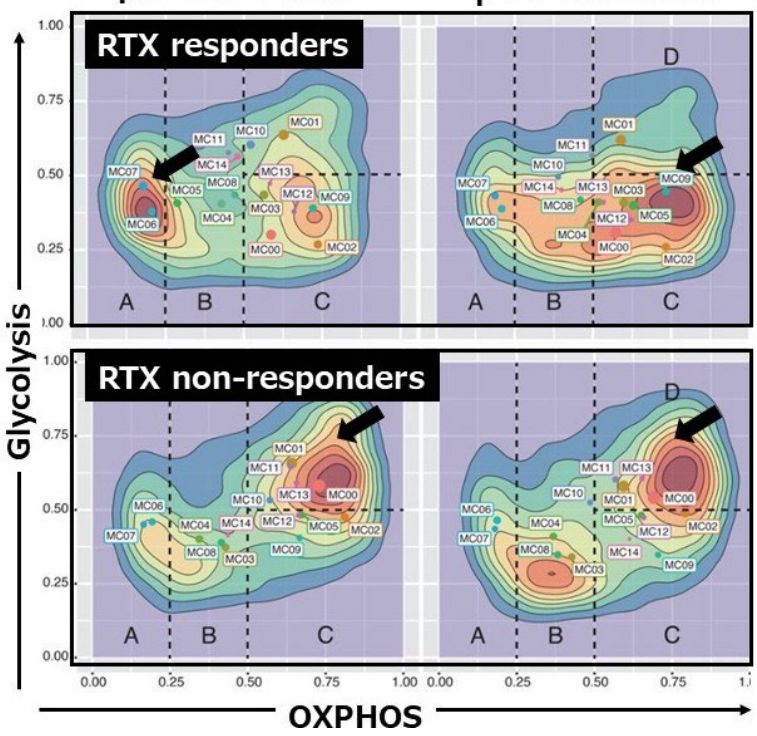


(2) Responders show fewer exhausted T cells and increased proliferation of CD4⁺ cytotoxic T cells (CD4⁺ CTLs) after treatment

In responders, so-called ”exhausted T cells”—T cells whose function has declined due to prolonged stimulation—were significantly less common one month after RTX treatment than before. The increase in OXPHOS after treatment was most prominent in CD4+ CTLs (Figure 3), and TCR analysis showed that cells within this subset with increased energy metabolism tended to proliferate more.

The team also used gene regulatory network analysis, which estimates the activity of transcription factors, to identify potential upstream drivers of these changes.

Figure 3
Activation of oxidative phosphorylation after rituximab treatment



(3) Opposite changes in oxidative stress confirmed in an independent cohort; pediatric data support the underlying mechanism

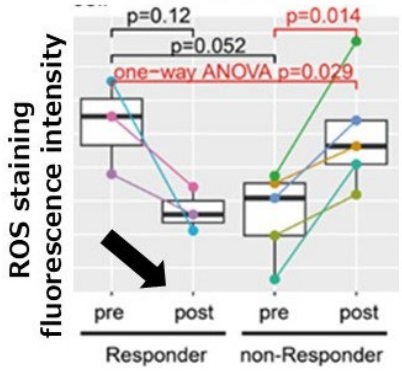
In a separate group of patients, flow cytometry was used to measure ROS inside T cells before and after treatment. ROS levels, which were elevated in responders before treatment, fell after RTX administration, while in non-responders they rose instead (Figure 4). This opposite direction of change suggests that RTX's effectiveness is linked to improved mitochondrial function and reduced oxidative stress soon after treatment begins.

In addition, by reanalyzing publicly available data from pediatric nephrotic syndrome patients, the team found that T-cell metabolism in nephrotic syndrome is shifted away from OXPHOS toward a different energy pathway called glycolysis, and that abnormal communication (crosstalk) occurs between disease-associated B cells and T cells, particularly CD4+ CTLs.

Together, these findings suggest that in nephrotic syndrome, disease-related B cells influence T cells, and that in patients who go on to respond, RTX's B-cell depletion appears to trigger a ripple effect on T cells within about a month—including increased OXPHOS, falling ROS levels, and increased CD4+

CTL proliferation, all evident when comparing pre- and post-treatment samples. In non-responders, by contrast, T cells show little such change, or ROS levels even rise, suggesting that their disease may be less dependent on B cells. In other words, comparing T-cell status and oxidative stress before and shortly after the first RTX infusion may make it possible to tell, well before the clinical outcome is otherwise apparent, whether a patient is likely to respond.

Figure 4 Decrease in ROS after RTX



Research Summary and Future Perspective

RTX has recently become covered by insurance in Japan for adult-onset, difficult-to-treat nephrotic syndrome, making it possible to use the drug promptly when steroid treatment alone is insufficient. At the same time, new questions have emerged: which patients respond best to RTX, when should treatment be considered, and how long should it be continued?

This study is the world's first single-cell analysis comparing blood samples from before and after treatment in adult patients who had been steroid-dependent for nearly a decade, collected before early RTX use became common practice. Because the study focused specifically on truly difficult-to-treat cases—patients for whom steroids alone could not control the disease—and because RTX was used cautiously at the time, often with infusion intervals of six months or more, it was possible to identify a clearly defined non-responder group (patients who relapsed within six months). Now that early and repeated RTX use has become standard, such clear-cut non-responders are harder to find in everyday clinical practice, making the samples used in this study especially valuable for directly comparing responders and non-responders. However, because this is an exploratory study with a limited number of patients, larger studies will be needed to confirm whether the observed changes in T-cell status and ROS levels can truly predict response to rituximab.

Now that RTX is covered by insurance for adult-onset, frequently relapsing or steroid-dependent nephrotic syndrome, identifying likely

responders before treatment has become an important part of clinical decision-making. If a blood test analyzing B cells and T cells before treatment can be developed to predict response to RTX, this could help achieve earlier remission and reduce complications in patients likely to benefit, while avoiding ineffective treatment in those unlikely to respond—paving the way toward personalized medicine for difficult-to-treat nephrotic syndrome.

Publication
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Title: Single-cell transcriptomic signatures of T cells associated with Rituximab responsiveness in Idiopathic Nephrotic Syndrome
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