Plain language summary (Lay report)

a. Give a brief overview of the research you have undertaken, what you hoped to achieve and what you have achieved. Your answer should be no more than two or three paragraphs.

Glucocorticoids (GC or 'steroids') have been used for over 60 years as the mainstay treatment for lupus. Despite their widespread use, glucocorticoids cause debilitating side effects including osteoporosis, diabetes, organ damage and heart disease and the discovery of a safe and effective replacement for steroids represents a major unmet need for medicine. Our group has discovered that a protein called the Glucocorticoid Induced Leucine Zipper (GILZ) is a powerful steroid mimic that does not cause the harmful side effects of steroid. Therefore, targeting GILZ therapeutically represents an alternative to steroids, and has already gathered deep interest from future potential venture capital and pharmaceutical industry partners. ***This research project sought to identify mechanisms by which the GILZ gene is regulated, with the goal of future drug development efforts targeting mediators of GILZ regulation.*** Identifying the pathways of GILZ regulation is essential to discover GC independent GILZ enhancing drugs, and we have strong support for this strategy from pharma advisors.

I analysed a very large dataset that had been previously generated by our laboratory to identify a list of genes that regulated GILZ. Then I performed bioinformatic analysis to group those genes into biological pathways. From this analysis I discovered several biological pathways that switch GILZ on or off. Some of these pathways were known to us from our own previous studies or other researchers work, and some revealed new biology. I selected a small group of candidate proteins from the list which I studied further by looking at their levels in blood samples from lupus patients. Here I found that there were much higher levels of these proteins in lupus patients compared to healthy people which increased the clinical relevance of us studying these candidate proteins.

I further investigated the candidate proteins using experimental methods to delete them in immune cells and then measure the amount of GILZ present. This confirmed that they are key regulators of GILZ abundance and are good targets to characterise in future work for therapeutic potential.

b. What questions did the grant set out to answer? What problems did you try to solve, or gaps in knowledge did you try to fill? Why is this important? Your answer should be at least 100 words.

Increasing GILZ abundance without the use of steroids is a compelling future therapeutic strategy offering the possibility of a broadly therapy without steroid adverse effects. The ultimate goal of our group is to find a way to therapeutically restore GILZ abundance without the use of steroids. Having validated GILZ at the level of biology, the next critical step from both the scientific and translational point of view was to comprehensively map the mechanisms which regulate GILZ. This was important because it would provide advanced knowledge of GILZ regulatory mechanisms and would identify key mediators of this regulation. Identifying the pathways of GILZ regulation is essential to subsequent work to discover GILZ-enhancing drugs.

c. What did you discover during the course of the grant? Your answer should be at least 200 words.

A previous study in our lab generated a list of GILZ regulators. During the course of this grant, I carefully studied this list using bioinformatic tools to discover which proteins were relevant in lupus and would be good candidates to study further as possible therapeutic targets. I grouped the list of candidates into relevant biological pathways which provided important information as to what role they might

be exerting in lupus. Next, to understand the relevance of the selected proteins in lupus I measured their levels in patient samples. This revealed that the candidate proteins were present at high levels in lupus, making them very clinically relevant. I also assessed their levels in response to an inflammatory stimulus that drives inflammation in lupus and found that this profoundly increased their levels, again demonstrating their strong clinical relevance. Finally, I confirmed that the candidate proteins do regulate GILZ levels through a set of experiments in immune cells.

d. Have the findings of the research already benefitted people with musculoskeletal disease? How might the findings inform further research to help sufferers in the future?

Finding a steroid alternative is an urgent unmet clinical need in lupus and other muscoskeletal diseases where steroids are the mainstay treatment. Our research group has a spin out company called GILZRx which aims to develop a steroid alternative by targeting GILZ. The findings from this study provides crucial pre-clinical data on possible candidates to progress further for therapeutic outcomes.

e. Are you planning to continue the research? Please provide details.

Yes, our lab is continuing the research on the proteins identified in this study. The next step is to further explore their effects in other types of immune cells and in mouse models of lupus.

Scientific summary (Scientific report)

a. What were the main scientific objectives of the grant?

This project aimed to understand how GILZ expression is regulated in immune cells by mapping the GILZ transcriptional regulatory network by:

1. Examining the results generated from a whole genome CRISPR screen and identify transcription factors that result in GILZ repression.
2. Map the mechanism by which the identified transcription factors repress GILZ

b. What were the main scientific achievements of the grant? Your answer should be at least 200 words.

In this study, I bioinformatically analysed the results of a genome wide CRISPR screen to identify possibly transcriptional regulators of GILZ that are part of relevant biological pathways in autoimmune disease such as lupus. STAT3 and CREB1 demonstrate clinical relevance for their downstream action in cytokine signaling pathways. In contrast the E3 ligases E3-Y, E3-W and E3-Z (de-identified for commercial reasons) regulate protein abundance via post translation modifications, which could be targeting GILZ directly, or indirectly by targeting transcription factors that regulate GILZ transcription. I assessed the clinical relevance of these candidate proteins by analyzing their gene expression in the peripheral blood of SLE patients, and in response to type 1 IFN signaling which is a pathological pathway in SLE. Finally, I deleted one of the candidate genes (encoding E3-Z) in the SUDHL4 cell line and assessed the effect on GILZ gene and GILZ protein expression. In future work I will delete the candidate genes using CRISPR in mice and assess the effects on GILZ abundance across immune cell subsets and the association with protection against various models of inflammation including skin application of imiquimod for psoriasis, intraperitoneal injection of imiquimod for sepsis and crossing CRISPR mice onto Lyn knock out mice for lupus.

I also sought to address the mechanism by which the identified transcription factors repress GILZ. I focused on STAT3, because of its clinical relevance identified by gene expression in lupus and because the cytokines that act upstream of STAT3, such as IL-6 and IL-21, were also hits in the CRISPR screen.

Thus, I investigated the role of IL-6 and IL-21 inhibiting GILZ transcription and found that IL-6 negatively correlated with TSC22D3 expression in the peripheral blood from SLE patients. Additionally, IL-21 was found to repress GILZ mRNA in vitro, which was lost in IL-21 deficient mice. Next, I assessed STAT3 binding to the GILZ promoter following *in vitro* IL-21 treatment. Indeed, IL-21 signaling resulted in enrichment of STAT3 directly to the GILZ locus. Together, these data reveal a novel mechanism by which inflammatory signals that are active in SLE, repress the anti-inflammatory molecule GILZ. Future work will assess whether this novel mechanism can be inhibited to achieve a desirable therapeutic effect.

c. Have you disseminated, or plan to disseminate, the results of this research? Please tell us about:

Conference presentation:

I presented the work reported here at the annual scientific meeting of the Australian Rheumatology Association (ARA) in Hobart 2023. My abstract was accepted for a poster presentation.

Taylah Bennett *et al*. "A cellular map of regulation of GILZ, a key determinant of immunological responses and autoimmunity". ARA Annual Meeting, Hobart 2023

Manuscript in preparation:

The work presented in this report is part of a manuscript in preparation which we plan to submit for publication in 2024-early 2025.

Taylah Bennett *et al.* "A cellular map of regulation of GILZ, a key determinant of immunological responses and autoimmunity"