**Temporary Withholding of Immunosuppressant in Rheumatic diseases and Lupus (TWIRL) Study**

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# 1.0 Summary of proposal

Mycophenolate is an immunosuppressant that is widely used as a treatment for systemic lupus erythematosus (SLE). Its biological effects involve a reduction in immune cell functions, which in turn reduce systemic or organ related inflammation and damage. The benefits of using mycophenolate in SLE need to be carefully balanced against unintended side effects such as increased risk of infection or blunting of vaccine responses. One strategy to counteract these effects is the temporary withdrawal of the immunosuppressant, which allows for the immune system to recover and effectively respond to a vaccine or infection. Our study will examine the effects of withdrawing mycophenolate for a short period in SLE patients to improve their immune responses to the seasonal influenza vaccination while safely maintaining disease control.

# 2.0 Project Outline

## 2.1 Background

Mycophenolate mofetil (MMF) is an inhibitor of *de novo* guanosine nucleotide synthesis and has been used widely as an induction and maintenance immunosuppressant in patients with moderate to severe systemic lupus erythematosus (SLE). In the transplant setting, the suppression of the humoral (antibody) immune responses to influenza and SARS-CoV-2 vaccination by mycophenolate has been well described, raising concerns regarding the effectiveness of immune responses to vaccination in patients with rheumatic diseases such as SLE [1-3]. At the Monash Lupus Clinic, 42% of patients has received mycophenolate, with a higher proportion in those who had high disease activity [4].

In patients with rheumatic and musculoskeletal diseases, one strategy to improve immune responses to vaccination is to temporarily withhold the immunosuppressant. This has been done in several studies with regard to methotrexate use in the rheumatoid arthritis population, resulting in effectiveness in increasing antibody responses to influenza vaccination [5, 6]. However, temporary interruption of mycophenolate has only been studied in a small cohort (n=24) of mixed rheumatic diseases and was found to be associated with an increased antibody response to SARS-CoV-2 mRNA vaccination [7]. This study provides a basis to further evaluate the safety and efficacy of temporary and defined interruption of mycophenolate in SLE patients. There is limited data regarding the timing and duration of the temporary interruption. We propose to use the forthcoming seasonal influenza vaccination programs in 2023 and 2024 to assess the effects of temporary interruption of mycophenolate in SLE patients with stable disease. Based on the lesson learnt from withholding methotrexate in the rheumatoid arthritis population, we propose a study to compare the effects of a temporarily interruption of mycophenolate treatment for two weeks following vaccination, and to investigate if there is a benefit to such strategy in patients with systemic lupus erythematosus, following influenza vaccination.

## 2.2 Objective

2.2.1 To quantify antibody specific responses post-seasonal influenza vaccination in patients with systemic lupus erythematosus.

2.2.2 To compare the proportion of patients who have attained adequate antibody titres to the seasonal influenza vaccination with or without withholding immunosuppression.

2.2.3 To study the effects of temporary interruption of mycophenolate on flare rates.

## 2.3 Methodology

### 2.3.1 Study Design

This is a prospective randomised controlled study to evaluate immune responses following seasonal influenza vaccination in adult patients with systemic lupus erythematosus.

### 2.3.2 Recruitment

A total of 80 patients will be recruited from Monash Lupus Clinic at Monash Health, one of the largest subspecialty lupus clinics in Australia. The majority of the patients attending the clinic also participate in the Australian Lupus Registry and Biobank (ALRB), which is a prospective longitudinal cohort study of SLE patients. Patients have their demographics and longitudinal disease related data collected and agreed to have their health information shared with other researchers provided written approval is obtained for the study (refer to Generic Master ALR PISIC Version 10 2021-04-19). It is estimated about 150 patients from the clinic have had treatment with mycophenolate.

Screening procedure: We will go through the ALRB participants of Monash Health site and identify those patients who meet inclusion and exclusion criteria.

We will then send an email to provide information on the study and invite patients to take part in January/February of the calendar year (refer to Patient letter TWIRL study). We will send a reminder email four weeks later. Enrolment is anticipated to commence from February/March onwards.

ALRB participants will also be invited to partake in this study at their regular Monash Lupus Clinic appointments. Principal and associate investigators are clinicians or researchers who attend the Monash Lupus Clinic and will be able to provide information during patients’ clinic visit. It is also routine care for us to discuss vaccination schedule during patients’ visits. Patients will be provided opportunity to ask questions about the study, and clinicians can also provide any additional information for patients who may experience some vaccine hesitancy.

We will send a final email to those who have not yet been recruited by June to prompt patients’ participation. After that if during the clinic visits discussion on influenza vaccination is raised and the patients have not received the seasonal influenza vaccine, then we will provide information for patients (paper format). Recruitment will be closed by 31st August 2024.

### 2.3.3 Inclusion criteria

* Patients with systemic lupus erythematosus, who are currently enrolled in the Australian Lupus Registry & Biobank.
* Receiving stable doses of mycophenolate (either mycophenolate mofetil, mycophenolic acid or mycophenolate sodium) and have not experienced a flare in the last 3 months.
* Combination with hydroxychloroquine will be permitted
* Prednisolone must be at a dose equal or lower than 7.5mg/day.

### 2.3.4 Exclusion criteria

* Concomitant use of other biologics such as rituximab or belimumab in the last 6 months
* Other targeted synthetic disease modifying anti-rheumatic drugs such as JAK inhibitors

### 2.3.5 Study visit and assessment schedule

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Baseline | Week 0 | Week 2 | Week 4/5 | Week 8 | Week 12 |
| Patient visit | Y | Y |  | Y |  |  |
| Clinician review | Y |  |  | Y |  |  |
| Blood collection |  | Y |  | Y |  |  |
| Influenza vaccination |  | Y |  |  |  |  |
| RAPID3 |  | Y | Y | Y | Y | Y |
| Influenza checker |  |  |  | Y | Y | Y |

*Baseline visit* and *week 0* can be combined – depending on the recruitment and availability of the influenza vaccine.

Consent will be confirmed at the Baseline visit by one of the principal or associate investigators. This can be done at a face-to-face clinic visit, or a telehealth/telephone consultation when eligibility is confirmed, and the participant has had an opportunity to ask any questions. The Patient Information Sheet/Consent Form can be emailed to patients prior to the day of enrolment/day of consent so that potential participants have had an opportunity to read through. Where a patient’s consent is obtained verbally via telehealth or telephone consultation with a clinician who is a study investigator, this will be documented in SMR and the patient’s signed consent will be obtained by an investigator at their next face-to-face attendance (e.g. at their Week 0 visit). Recruitment is open until August 31st 2024.

*Week 0* is scheduled between March to August, when the seasonal influenza vaccine will be available. This will be administered by Monash Immunisation Service as part of the service offered to Monash Health immunocompromised patients.

Once a patient has consented to the study, provided baseline blood samples and received their influenza vaccination, they will be randomised into either Group 1 – temporary interruption of mycophenolate for 2 weeks following vaccination or Group 2 – continuation of mycophenolate as per usual care from *Week 0* onwards. All participants will be asked to complete a patient reported outcome measure, called RAPID3, currently approved as part of the Australian Lupus Registry & Biobank protocol as an optional clinical disease assessment.

*Week 2*: At the end of Week 2, participants in both groups will receive an email invitation to complete an online version of the RAPID3 via REDCap. Additionally, participants in Group 1 will receive an email to remind them that it is the end of the withholding period and to resume their usual dose of mycophenolate. They will also be provided with a date during week 4 to come in for a blood collection.

*Week 4/5*: Participants will provide a second blood sample at Week 4 alongside their routine Monash Lupus Clinic blood tests. They will be asked to complete the RAPID3 and Influenza checker. At Week 5, they will be reviewed by a clinician at Monash Lupus Clinic.

*Week 8*: Participant will be asked to complete the RAPID3 and Influenza checker.

*Week 12*: Participant will be asked to complete the RAPID3 and Influenza checker.

Scheduling may be affected by unforeseen circumstances, and we will allow deviation of +/- one week for each scheduled timepoint within our protocol. Participants can opt out or withdraw their consent at any time.

Examples of the email reminders can be found in the workbook.

### 2.3.6 Blood Sample processing

Blood samples will be collected by a study investigator or Monash Pathology. At each timepoint (Baseline/Week 0 and Week 4) we will collect:

1x 9ml EDTA tube for processing of plasma and cells

4x 9ml ACD tube for cells

Following collection, the blood samples will be sent to Biobanking Victoria for initial processing (spinning down of samples and isolation of PBMCs). The spun samples will be temporarily held at Biobanking Victoria prior to being sent to the Burnet Institute. The reason for temporary storage of the samples at Biobanking Victoria is because we do not expect all participants to have bloods collected on the same day, hence for transportation purposes it is easier to hold them at Biobanking Victoria so that they can be sent in batches to the Burnet Institute. Processing and analysis of the samples will be performed at CI-Khoury’s laboratory at the Burnet Institute. The absolute counts of CD4+ and CD8+ T cells, B cells, NK cells, in whole blood will be quantified by routine flow cytometry, allowing for the monitoring of changes in cell numbers before and after immunosuppressant withdrawal. Plasma and live peripheral blood mononuclear cells (PBMC) will be isolated and cryopreserved for evaluation of T-cell phenotype and influenza vaccine response assays. Following analysis of the samples at the Burnet Institute, any excess sample (serum or PBMCs) will be returned to the Australian Lupus Registry & Biobank for storage, as already approved under HREC 14262A, and future use may be subjected to written approval of specific HREC approval.

### 2.3.7 Influenza vaccine response measurements

Anti-Influenza antibody titres in plasma will be determined using a haemagglutination inhibition (HI) assay. T-cell responses will be measured in vitro through the stimulation of PBMC with influenza peptides and controls. These tests will be performed on samples collected at Baseline/Week 0 and at 4 weeks post-vaccination.

### 2.3.8 Immune profiling

To identify important biomarkers during immunosuppressant withdrawal which could indicate a potential future flare or an enhanced vaccine response, T-cell subset proportions and phenotypes will be measured at baseline and after MMF withdrawal using flow cytometry. CD4+ and CD8+ T-cell, naïve, memory and effector subsets, and functional CD4+ T-cell subsets. Soluble markers will be measured from stored EDTA plasma samples.

### 2.3.9 SLE disease activity

For each patient enrolled into the study, clinical parameters regarding their SLE disease activity will be obtained from the Australian Lupus Registry & Biobank Lupus Connect database. Their overall time adjusted mean SLE Disease Activity Index (SLEDAI), most recent SLEDAI before their vaccination date, rate of flares based on SLE Flare Index (SFI) in the preceding 12 months, and the most recent physician global assessment (PGA) will be collected. Disease severity will also be classified according to High Disease Activity Status (HDAS). There will be additional patient-reported outcome measures in the form of RAPID3 (score 0-30), to be recorded at five timepoints, at Baseline/Week 0, Week 2, 4, 8, and 12. Flares will be captured by SFI at week 4 (physician-reported). Flares will also be considered as a change in PGA ≥1 from Baseline (physician-reported). A score of >12 on the RAPID3 (patient-reported) will be used as indication of high disease activity [8].

### 2.3.10 Monitoring for influenza infection

Participants will be asked on week 4, 8 and 12 on their questionnaire, whether they have had symptoms that are suggestive of influenza infection, and whether they have had microbiological confirmation. The effects of influenza infection on work commitment and any hospitalisation will be recorded.

At enrolment, participants will be provided with a pathology request slip for a nasopharyngeal swab and symptom diary (paper and electronic form). Participants are encouraged to seek a nasopharyngeal swab to confirm the type of respiratory illness they may have during the study period. They are instructed to seek care from their usual doctor but let the study coordinator know if they have done a swab. It is standard of care to clarify the nature of the pathogen, where possible. Participants will be asked to fill out the symptom diary on a daily basis from the start of their symptoms until their resolution.

### 2.3.11 Immunisation record

Immunisation history will be sought regarding participants’ previous vaccination(s) to influenza, COVID-19, and pneumococcus.

2.3.12 Study extension

This study was initially intended to take place and be completed in 2023, however due to circumstances which unexpectedly delayed commencing of recruitment, we would like to extend this study into 2024. Participants who participated in the original study through the 2023 recruitment period, and who have completed all study-related procedures and tasks, will not be re-consented using the new PISIC (version 5 2023-10-30). However, if any patients who participated in the original study in 2023 wish to participate again in 2024, they will be re-consented with the new PISIC (version 5 2023-10-30). New participants who wish to participate in the 2024 study period will also be consented with the new PISIC (version 5 2023-10-30).

## 2.4 Data management

### 2.4.1 De-identification process

Eligible patients will be identified using the database (Lupus Connect) that contains longitudinal data of participants of the Australian Lupus Registry & Biobank. Identifiable information is only visible for clinicians who are directly involved in patient care. Lupus Connect generates a unique Registry ID and Biobank ID which are used to link clinical and laboratory data for analysis purposes.

Blood samples will be collected by a study investigator or Monash Pathology, and they will be labelled according to the following convention. A code will be generated based on 1) collection date YYYYMMDD; 2) Registry ID number; 3) type of tube used:

EDTA tube = EDTA

ACD tube = ACD1, ACD2, ACD3, ACD4

The coding list of participants’ UR and their corresponding Biobank ID will be provided to Monash Pathology and Biobanking Victoria in a password protected file and we will expect this to be deleted at the conclusion of the study.

### 2.4.2 Data sharing

Clinical data will be collected using REDCap, which is a web application that is provided free by Helix team at Monash University to allow a researcher to collect health data, create surveys, randomise participants, and setup longitudinal study in a secure environment.

Laboratory data will be stored in LabArchive, and transferred for analysis with participant’s Biobank ID as linkage.

All data, analysis and results will be stored at Monash University secured drive, and retained for at least 7 years following completion of the study.

### 2.4.3 Analysis

Satisfactory vaccine response to seasonal influenza vaccination will be defined by greater than or equal to four-fold increase in antibody titre in ≥2 of four influenza vaccine antigens compared to baseline responses prior to immunization [6]. The proportion of participants who achieve a satisfactory vaccine response from each group will be reported and compared using χ2 tests or Fisher’s exact test, as appropriate. Other continuous variables are analysed by using a t-test or Mann Whitney U test as appropriate. Binary secondary efficacy variables (such as frequency of disease flare and incidence of infection) will be compared by using χ2 tests or Fisher’s exact test, as appropriate. Logistic regression will be used to assess association of clinical variables such as absolute B-cell and/or T-cells numbers at baseline, study group assignment (temporary interruption vs continuation), and other disease related parameters with adequate vaccine response. P<0.05 is considered to indicate statistical significance.

### 2.4.4 Project closure

At completion of the study, the Monash Health Research Support Services unit will be informed, and a project final report will be submitted via email to research@monashhealth.org.

The final progress report will cover the entire period of the grant and may include a listing of any research outputs such as journal articles or conference presentations.

A scientific and a plain language reports will be separately prepared for the funder Arthritis Australia in March 2024. Annual and final reports will be provided to Monash Health HREC at the completion of the study in March 2025.

# 3.0 Expected outcomes and future directions

Immunogenicity and safety of vaccination in SLE patients has been of significant interest to clinicians, but limited data are available. We anticipate that SLE patients who have had temporary interruption of immunosuppression will have improved antibody responses, with minimal flare risk. The results from these studies will help us to develop guidelines around withholding immunosuppression for clinicians to give practical and evidence-based advice to patients. We will plan to study the effect of withholding mycophenolate on T-cell phenotype, function and response to influenza vaccination in an extension study from the cell samples collected. T cell vaccine responses may provide a more accurate measure of protection, in additional to antibody responses, and it may be differentially affected by different immunosuppressant. Results from our study may have broader implications for mycophenolate users in other rheumatic diseases.

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