MAPLE study

Assessing residual inflammation and **ma**crophage **p**resence in **l**upus n**e**phritis after 3 months of intensified treatment with prednisolone, mycofenolate mofetil and voclosporin as compared to mycofenolate mofetil and prednisolone, an open label randomized controlled trial

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DOCUMENT HISTORY

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Original protocol (1.0)	*01-05-2025*	Not applicable
Protocol V 2.0	*09-11-2025*	- Adjusted clinical response to secondary endpoint - Removed IMP label from section 8.7 - MMF capsule dosage has been corrected in section 8.4.1 & 8.4.3 - Referenced the most recent version of Declaration of Helsinki in section 15.1 - Definition end of trial was revised in section 12.1 - Leftover sentence from template in section 16.3.3 has been removed - Added “at least” to section 16.3.1 - Description of mitigation added to section 16.3.1 - All references to a legal representative were removed

		- A structured risk analysis has been included in chapter 4 of the protocol - Sentence about Dutch Personal Data Protection Act has been removed in section 16.3.1 - Prolongation of the QT-interval, bradycardia, abnormal potassium levels and untreated hypertension, co-administration of voclosporin with strong CYP3A4 inhibitors (e.g. ketoconazole, itraconazole, clarithromycin etc) were listed in the exclusion criteria. - Referred to version/date of KDIGO/EULAR guidelines and referenced specific passages. - Reference list in section 17 has been updated - Added extra explanation to aims in synopsis - Added detailed description of power calculation in section 14.5 - Description of WOCBP and additional paragraph regarding contraception was added in section 9.2.1
Protocol V 3.0	*05-01-2026*	Additions to the protocol as per conditions stated in the letter of approval 15-12-2025 and informal conversation on 18-12-2025: - Exact content of treatment guidelines have been cited in

		addition to section references - Rationale on randomization, repeat biopsy and early rescue therapy possibility have been elaborated in section 6.1 - Clarification on specific primary endpoints has been added in section 5 - An additional dichotomous sample size calculation has been added to section 14.5 - Aim of study has been rephrased to clarify immunological endpoints in section 2 and 5 - Added biobank option in section 16.3.4
Protocol V 4.0	*09-01-2026*	- Date has been adjusted
Protocol V 5.0	*17-02-2026*	- Substantiation on randomization has been added in section 6.1

CONFIDENTIALITY STATEMENT

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigative team, regulatory authorities, and members of the Research Ethics Committee.

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1. ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event of Special Interest
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AR	Adverse Reaction
AST	Aspartate Aminotransferase
ATMP	Advanced Therapy Medicinal Product
AxMP	Auxiliary Medicinal Product
BID	Twice Daily (or 2 Times a Day)
BMI	Body Mass Index
bpm	Beats per Minute
BUN	Blood Urea Nitrogen
CA	Competent Authority
CCI	Company Confidential Information
CCMO	Centrale Commissie Mensgebonden Onderzoek (Central Committee on Research Involving Human Subjects)
CL	Total Body Clearance of Drug from Plasma
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CSR	Clinical Study Report
CT	Clinical Trial

CTA	Clinical Trial Authorisation
DEI	Diversity, Equity, and Inclusion
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
e-CRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
eICF	Electronic Informed Consent Form
EMA	European Medicines Agency
EPD	Electronic Patient Record
ET	Early Termination
EudraCT	European Clinical Trial Data Base
EU	European Union
FBR	Future Biospecimen Research
FDA	United States Food and Drug Administration
GCD	Global Clinical Development
GCP	Good Clinical Practice
GGT	Gamma Glutamyl Transferase
GDPR	General Data Protection Regulation
HDL	High-Density Lipoprotein
HEOR	Health Economics and Outcomes Research
IB	Investigator's Brochure
IC	Informed Consent
ICF	Informed Consent Form
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
IRB	Institutional Review Board
IRE	Immediately Reportable Event
IUD	Intrauterine Device
IUS	Intrauterine Hormone-Releasing System
KDIGO	Kidney Disease Improving Global Outcomes
LAM	Lactational Amenorrhea Method
LDH	Lactic Dehydrogenase
LN	Lupus Nephritis
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MS	Member State
MTD	Maximum Tolerated Dose

PBMC	Peripheral Blood Mononuclear Cells
PE	Physical Examination
PI	Principal Investigator
PO	Oral
POCBP	Participants of Childbearing Potential
PORP	Participants of Reproductive Potential
QD	Once Daily
QID	Four Times Daily
RBC	Red Blood Cell
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SLE	Systemic Lupus Erythematodes
SmPC	Summary of Product Characteristics
SoA	Schedule of Assessments
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Event
TID	Three Times Daily
WBC	White Blood Cell
WOCBP	Women of childbearing potential
WHO	World Health Organization

2. SYNOPSIS

EU trial number: 2025-522187-32-00
Full trial title: Assessing residual inflammation and **ma**crophage **p**resence in **l**upus n**e**phritis after 3 months of intensified treatment with prednisolone, mycofenolate mofetil and voclosporin as compared to mycofenolate mofetil and prednisolone, an open label randomized controlled trial (**MAPLE study**)

Systemic lupus erythematosus (SLE), a severe autoimmune disease with unknown etiopathogenesis, predominantly affects female patients of child-bearing age, often of non-caucasian descent. Kidney involvement – lupus nephritis (LN) – affects ~60% of SLE patients, severely impacts quality of life and increases morbidity with up to 30% of patients developing end-stage kidney disease (ESKD) despite the previous standard-of-care (SOC) regimen, necessitating dialysis and/or kidney transplantation, mainly due to persistent LN activity.
LN, characterized by deposition of circulating immune complexes, leads to large-scale immune cell infiltration and inflammation involving both the adaptive and the innate immune system. Kidney biopsy studies in LN demonstrated large numbers of infiltrated macrophages.
Previous SOC consisted of prednisolone (and induction with 3 days of iv methylprednisolone) paired with mycophenolate mofetil (MMF) or cyclophosphamide. Less than half of patients reach complete kidney remission after one year with this SOC. Recent data showed that patients taking voclosporin

(a calcineurin inhibitor (CNI) without the need for monitoring and having less side-effects than comparable medication) on top of MMF and prednisolone, reach complete remission more often but importantly, faster. Recently, international guidelines (KDIGO 2024) have acknowledged the added value of this “triple therapy” for the treatment of lupus nephritis, however the choice of therapy (dual vs triple) is dependent on expert opinion of the treating physician (section 6.1 of the protocol) . The exact cellular and/or humoral effects of intensified CNI treatment in LN remain unknown but previous studies showed macrophage suppressing features of CNI with decreased antigen presentation and less macrophage-mediated T cell activity.

This trial focuses on two hypotheses:
(1) monocyte-derived macrophage subtypes substantially contribute to LN by driving persistent inflammation and subtypes are skewed to anti-inflammatory types by intensified treatment
(2) rapid early immunological remission of LN as reflected by kidney histology results in better clinical outcome at 2 years follow-up.

Aim 1: Understanding early immunological changes in LN patients (class III/IV+/-V) on a cellular and histopathological level, comparing the CNI voclosporin on top of MMF and prednisolone (triple therapy) to MMF and prednisolone (dual therapy).
Objective 1: determine differences in macrophage clusters in kidney tissue per treatment arm
Objective 2: assess histological response of intensified treatment (triple therapy) compared to dual therapy
Objective 3: assess if early histological response is associated with clinical remission after two years and identify innovative early response determinants (including circulating monocyte phenotyping)

New-onset or flaring LN patients from the Amsterdam region (n=30;class III or IV+/-V) will be randomized to two treatment groups: arm 1 (n=15) will receive MMF+prednisolone with voclosporin and arm 2 (n=15) will only receive MMF+prednisolone. Included patients undergo a diagnostic kidney biopsy to assess LN class and to perform spatial scRNA-seq of kidney tissue. Results will be associated with disease activity, patient-reported and physician observed outcome measures and biochemical (erythrocyturia/proteinuria, cystatin C measured kidney function, anti-dsDNA levels, urinary biomarkers) parameters.

After 3 months, all included LN patients undergo a repeat kidney biopsy for assessment of early histological response and for scRNAseq. Included patients are followed for a period of 2 years with regular checkups. Non-invasive response markers (aim 2) including circulating cell phenotyping, besides traditional biomarkers, will be associated with histological response.

Aim 2: assess phenotype of circulating monocytes in LN patients compared to SLE patients and healthy subjects, to be associated with clinical/biochemical parameters and scRNA-seq data

Objective 4: identify differences between LN, SLE and healthy subjects in peripheral monocyte phenotype, to be associated with scRNAseq data and clinical/biochemical/histological parameters.

Circulating monocytes at different timepoints will be analyzed using blood counts and bulk RNA-seq, validated by flow cytometry. SLE patients (without LN; n=15) and healthy subjects (n=10) will be included to identify differences in monocyte phenotypes. Results will be associated with clinical/biochemical and scRNA-seq parameters.

Aim 3: identify potential biomarkers of disease activity in the scRNA-seq data to validate in urine and/or serum
Objective 5: attempt to render kidney biopsy in LN obsolete by identifying serum and/or urinary biomarkers

Rationale

Lupus nephritis (LN), i.e., kidney involvement of systemic lupus erythematodes (SLE), is a major complication. LN may result in end-stage kidney failure in up to 30% of patients, necessitating dialysis and/or kidney transplantation. Current immunosuppressive treatment with cyclophosphamide or mycofenolate mofetil (MMF) is insufficient to reach (complete) renal remission. We hypothesize that rapid immunological remission of LN with intensified immunosuppressive treatment results in less kidney damage assessed by histology. This improved response may be driven by macrophages present in LN lesions.

Objective

- Assess whether rapid early immunological remission of LN as reflected by kidney histology at 3 months results in better clinical outcome at 2 years follow-up.
- Determine that kidney macrophage subtypes substantially contribute to LN by driving persistent inflammation and that macrophage subtypes are skewed to anti-inflammatory types by intensified treatment

Main trial endpoints

Main endpoints:

1. To determine scRNA-seq differences within the full macrophage spectrum between arm 1 vs arm 2; the macrophage spectrum will be compared between baseline kidney biopsy and kidney biopsy at 3 months treatment

Secondary trial endpoints

Secondary endpoints:

1. Numerically distinguish residential macrophages and monocyte-derived macrophages in kidney tissue with spatial scRNAseq, comparing arm 1 vs arm 2;
2. To analyze rapid clinical remission of intensified treatment by proteinuria levels
3. To assess histological response of intensified treatment (arm 1) compared to dual therapy (arm 2);
4. To assess if early histological response is associated with clinical remission after two years and identify innovative early response determinants (including tissue monocyte/macrophage phenotyping)
5. To identify differences between LN, SLE and healthy subjects in peripheral monocyte phenotype by using bulk RNA sequencing and flowcytometric analyses, to be associated with scRNAseq data and clinical/biochemical parameters
6. To identify potential non-invasive urinary biomarkers of disease activity in the scRNA-seq data, to be tested in sequentially stored urine and/or serum from patients
7. Attempt to render (repeat) kidney biopsy in LN obsolete by identifying reliable serum and/or urinary biomarkers that reflect tissue damage and treatment response;

8. Confirm scRNA-seq identified macrophage markers immunohistochemically, identify spatial distribution (glomerular vs interstitial) and assess their predictive and prognostic use.

Trial design

This is a single-center open label randomized controlled trial. Patients will be randomized into one of two arms and will be followed for the duration of 2 years.
Inclusion starts at the time of kidney biopsy, when the diagnosis of a class III or IV LN is certain, with or without a component of class V.

Trial population

New-onset or flaring LN patients from the Amsterdam region (envisioned N=30;NIH class III or IV+/-V) will be recruited and allocated to one of two treatment groups: arm 1 (n=15; intervention) will receive an intensified regimen of prednisolone, mycophenolate mofetil (MMF) and voclosporin and arm 2 (n=15) will receive prednisolone and MMF, both arms following current guidelines (standard of care, SOC).

The diagnosis of SLE and/or LN will be based on current EULAR/KDIGO guidelines (2019 EULAR/ACR Classification Criteria for Systemic Lupus Erythematosus / KDIGO 2024 LN guideline, section 10.1) (1). It is expected based on current epidemiology that 80-90% of LN participants will be female. All included LN patients will undergo a diagnostic kidney biopsy to assess LN class and to perform single cell RNA sequencing (scRNA-seq) of kidney tissue infiltrated immune cells. Fifteen SLE patients without clinical LN (n=15) will be included as disease controls for disease activity and peripheral monocyte phenotype. In these patients no kidney biopsy will be performed. Ten healthy subjects will be included (n=10) as controls for peripheral monocyte phenotyping. In these healthy subjects no kidney biopsy will be performed. These healthy subjects may serve as controls for biomarker analysis in serum and/or urine.

Key Inclusion/Exclusion Criteria:

<u>Lupus nephritis patients</u>

i. Inclusion criteria
   - Patients with de novo or flaring SLE according to the EULAR/ACR criteria and a suspicion of class III or IV LN with a clinical indication to perform a kidney biopsy
   - Age 16-70
   - eGFR as measured by cystatin C >20 mL/min

ii. Exclusion criteria
   - LN class I, II or pure class V upon kidney biopsy
   - eGFR as measured by cystatin C <20 mL/min
   - Histological chronicity score (NIH) of 8 or higher in the kidney biopsy
   - Active infection of any kind as evidenced by cultures (blood, urine or otherwise)
   - History of hepatitis B, hepatitis C, tuberculosis and/or HIV
   - Treatment with any of the following agents within one month before screening: tacrolimus, belimumab, anifrolumab
   - Treatment with any of the following agents within 6 months before screening: rituximab, daratumumab, eculizumab

- Prolongation of QT-interval (QTc >470ms) and/or bradycardia (resting heart rate <50bpm) measured on two separate occasions
- Hyperkalaemia (serum potassium >6.0 mmol/L)
- Hypertension (blood pressure > 165/105 mmHg, with symptoms of hypertension)*
- Co-administration of voclosporin with strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin)Pregnancy

* A single elevated blood pressure measurement will not lead to immediate exclusion from the trial. Antihypertensive therapy may be initiated as appropriate. Dose adjustments of voclosporin should be made in accordance with Section 11.3.2 .

SLE patients without LN (disease control group)

i. Inclusion criteria
   - Diagnosis of SLE according to EULAR/ACR guidelines
   - Age 16-70

ii. Exclusion criteria
   - Suspicion of LN
   - Signs of active infection

Healthy subjects (control group)

i. Inclusion criteria
   - Blank medical history
   - Age 16-70
   - A majority (75%) of female healthy subjects will be sought

ii. Exclusion criteria
   - Signs of active infection

Interventions

The indication of treatment for this trial is proliferative lupus nephritis class III or IV with or without a class V. Both treatment arms are approved treatment for proliferative LN and recommended in current KDIGO 2024 guidelines, with treatment choice typically highly dependent on the physician' expert opinion.

Arm 1: corticosteroid induction (3 days 500mg methylprednisolone intravenously per day), followed by prednisolone 1mg/kg with tapering to reach 5mg or less in 3 months; MMF 1000mg BID (or 500mg QID) with the addition of voclosporin 23.7mg BID

Arm 2: corticosteroid induction (3 days 500mg methylprednisolone intravenously per day), followed by prednisolone 1mg/kg with tapering to reach 5mg or less in 3 months; MMF 1000mg BID (or 500mg QID)

In both arms, oral corticosteroids will be tapered to below 5mg within 3 months where the tapering speed is at the discretion of the treating physician.

If patients are not in (partial) clinical remission (based on kidney function and proteinuria recovery) at any point after the second kidney biopsy (at three months), a decision for treatment adjustment may be made by the treating physician independent of treatment arm at randomization as is in line with current guidelines.

Pretreatment investigations

After the signing of informed consent, the screening period will start. During the screening period (~2 weeks before renal biopsy) additional inclusion and exclusion criteria will be checked by inspection of the EPD. As part of routine clinical care, all patients that start with treatment for LN will be screened for the presence of infection through blood analyses (CRP, leukocyte count), urine culture and a chest X-ray. Relevant lab results from <4 weeks will be analyzed and screened for recent kidney function and electrolyte abnormalities (potassium).

To prepare for possible randomization, an EKG will be made in order to rule out prolongation of the QT-interval and/or bradycardia. This will be done during the regular outpatient clinic visit. Vital functions as noted down in de EPD by the treating physician will be checked for hypertension and if not treated, the physician will be notified.

The histological result of the first kidney biopsy as well as eGFR, infection screen, potassium, QT-interval duration and vital functions will need to be known (and if applicable sufficiently treated by the physician) before the patient can be randomized and definitively included.

Interventions during follow-up

During the follow-up period, all randomized patients will attend routine outpatient clinic visits with their own treating physician. Notes and additional information (vital functions, lab results etc) will be collected from the EPD during the follow-up period.

At the following timepoints specific study interventions* will be done:

Start of treatment (day 0)	Week 4	Month 3	Month 6	Month 12	Month 18	Month 24
- SLEDAI-2K questionnaire filled in by physician - LupusPRO questionnaire - PBMC, EDTA, serum and PAX gene blood samples - Urine portion collection - feces sample collection	- SLEDAI-2K questionnaire filled in by physician - LupusPRO questionnaire	- Renal biopsy - SLEDAI-2K questionnaire filled in by physician, - LupusPRO questionnaire - PBMC, EDTA and serum blood samples - Urine portion collection	- SLEDAI-2K questionnaire filled in by physician, - LupusPRO questionnaire - Urine portion collection	- SLEDAI-2K questionnaire filled in by physician - LupusPRO questionnaire - PBMC, EDTA and serum blood samples - Urine portion collection	- SLEDAI-2K questionnaire filled in by physician - LupusPRO questionnaire	- SLEDAI-2K questionnaire filled in by physician - LupusPRO questionnaire - PBMC, EDTA and serum blood samples - Urine portion collection - Feces sample collection

*Since blood draw and urine analysis before every outpatient clinic visit is standard clinical care during follow-up of LN patients during treatment, all study interventions will be merged with regular checkups and all collected samples (extra blood tubes and/or collection of urine and feces) will be combined with regular lab visits in order to limit patient burden.

During follow-up after starting treatment (regardless of treatment arm) the physician will evaluate medication compliance and side effects of medication, as well as measure mycophenolic acid drug levels and additional lab values (eGFR, electrolytes, blood cell counts, etc) at set timepoints after starting treatment (as is usual during regular follow-up). The physician may alter treatment dosage based on these results and on their own account as stated in the EULAR/KDIGO 2024 guidelines and MMF/voclosporin SmPC. This includes an evaluation of efficacy of treatment at 24 weeks (based on clinical kidney response as reflected by eGFR improvement and proteinuria response) in the triple therapy arm to decide on continuation of therapy. Information about therapy effects and dosage will be extracted from the EPD.

Ethical considerations relating to the clinical trial including the expected benefit to the individual subject or group of patients represented by the trial subjects as well as the nature and extent of burden and risks

The main benefit of this study is a group benefit: it aims to demonstrate that adding voclosporin to MMF and prednisolone results into a stronger decrease of macrophages in kidney tissues and peripheral circulating monocytes, and therefore reflecting the specific macrophage-focused effect of voclosporin.
Since LN is a disease with mostly unknown pathophysiology, knowledge about macrophage counts and macrophage skewing before and after treatment could aid in optimizing personalized treatment and assessing reliable biomarkers.

The addition of voclosporin has the important identified risk of serious infections (including opportunistic infections). Important potential risks include major adverse cardiovascular events (MACEs), neurotoxicity, nephrotoxicity (acute and chronic) and malignancies (including lymphomas) associated with long term use. Routine risk minimization measures are described in the SmPc and include advice to avoid direct UV light and regular monitoring for risk of infection, eGFR, risk of pure red cell aplasia (PRCA), serum potassium levels and blood pressure. Since the chance of potential risks is considered low, participants are closely monitored during follow-up visits and vital functions and lab results are frequently checked (as would also be standard in regular care), adding voclosporin in the intervention group was deemed justified.

Native kidney biopsies are complicated by bleeding necessitating an intervention in 0.1% of patients and have an extremely low reported mortality. The second kidney biopsy after three months carries the same risk as the first one.

The risk of blood draws is negligible and will be merged with routine clinical care blood draws. Apart from routine clinical care, no additional site visits are necessary. Participants are expected to bring in extra samples of urine and feces, as well as fill in a questionnaire at different timepoints when already visiting the hospital to minimize discomfort.

3. INTRODUCTION AND RATIONALE

3.1 Therapeutic condition and current treatment status

Loss of self-tolerance is central to SLE. Auto-antibodies targeting self-antigens, especially anti-double stranded DNA antibodies result in immune complex formation and deposition in tissues with consequent systemic inflammation and complement factor depletion. In SLE the entire immune system, innate and adaptive, is involved and activated (2).
Current treatment consists of corticosteroids (targeting all immune cells), MMF (targeting T- and B-cells), cyclosphosphamide (targeting T-cells), calcineurin inhibitors (CNI, e.g., tacrolimus or voclosporin; targeting T-cells), rituximab (targeting B-cells), belimumab (targeting B-cell activating factor, BAFF) and anifrolumab (anti-IFNAR) (3). With the exception of corticosteroids and probably CNI, all treatments target the adaptive immune system, not the innate immune system. Despite some progress, the treatment of SLE and especially LN is disappointing, with around 50-60% of patients not reaching complete remission after one year (as measured by proteinuria, erythrocyturia and/or kidney function), necessitating intensified immunosuppressive treatment or treatment switch ('second-line treatment'). Due to insufficiently predictive specific follow-up parameters, treatment usually is continued for three to five years to maintain remission, often resulting in large cumulative amounts of corticosteroids: most likely in many patients this is unnecessary. Consequently, overtreatment is highly prevalent, with toxic side-effects and infectious complications, while undertreatment in those with persistent inflammation remains undetected, resulting in organ damage, ESKD and increased cardiovascular risk. There is an unmet need for both disease monitoring and treatments that bring LN into rapid and sustained remission. The addition of voclosporin to MMF and prednisolone has shown promising results in the AURORA trials (1 and 2)(3, 4)). LN patients receiving voclosporin on top of MMF and prednisolone reached remission more often with a faster decrease in proteinuria, compared to MMF and prednisolone.
In LN, macrophages are present in large numbers especially in active lesions (e.g., fibrinoid necrosis, crescent formation, interstitial inflammation), both in the glomeruli and in the interstitium (5). It is unclear what the role of these macrophages is: based on immunohistochemical findings of M2-like macrophages many researchers believe these macrophages clear immune complexes and/or cellular debris. Urinary markers such as monocyte chemoattractant protein 1 (MCP1) and colony-stimulating factor 1 (CSF1) that are found in urine of patients with active LN may rather indicate proinflammatory features of kidney macrophages. The value of MCP1 (a classic macrophage product) reflecting active disease in the kidney was demonstrated in multiple studies but is not used as a routine clinical follow-up marker.

3.2 Clinical trial rationale

LN results in increased morbidity and mortality despite the previous standard of care consisting of MMF or cyclophosphamide. The addition of voclosporin on top of MMF results in more rapid remission of LN class III or IV (with or without class V) but it is unknown what the effect is on the 'tissue level', i.e. inside the kidneys. This study will investigate the histological changes after 3 months of intensified immunosuppressive treatment versus MMF+prednisolone by performing a repeat kidney biopsy.
A single-cell RNA-sequencing study of kidney biopsies in active LN demonstrated the presence of a macrophage spectrum, ranging from phagocytosing, profibrotic to proinflammatory macrophages

with overlapping features (6). To date, the exact role of macrophage populations in LN remains unknown. The question therefore is: are these macrophages clearing immune complexes and cellular debris, or are they the culprit and contributor of the loss of self-tolerance in SLE, presenting self-antigen to T-cells, resulting in antibody production and immune complex formation? It is also unknown how these macrophages are under the influence of previous standard of care therapeutics (dual therapy) and whether intensified immunosuppressive treatment (triple therapy) changes their numbers and/or subtype distribution.
In LN murine models, removing monocytes attenuates the disease with less crescent formation and less proteinuria. Targeting chemokines and cytokines originating from pro-inflammatory macrophages is a promising therapeutic venue, presumably because it stops peripheral cells migrating towards the areas where these chemokines are made, the lesions, and thus reducing inflammation. Recently, belimumab, targeting BAFF, and anifrolumab (anti-IFNAR), were shown to be of additive therapeutic value in LN (7). Importantly, macrophages carry the BAFF receptor and IFNAR so both belimumab's and anifrolumab's mechanisms of action may be macrophage mediated. Importantly, older studies have shown immunosuppressive effects of CNI specifically on macrophages resulting in less antigen presentation and less T cell activation by macrophages. Besides investigating short-term effects of intensified treatment, this study also aims to assess the effects on the long term by serum and urine markers.

3.3 Mechanism of action, Drug class

Voclosporin is a calcineurin-inhibitor (CNI) immunosuppressant that inhibits calcineurin in a dose-dependent manner up to a maximum dose of 1.0 mg/kg. Activation of lymphocytes involves an increase in intracellular calcium concentrations. Calcineurin is a calcium/calmodulin-dependent phosphatase whose activity is required for the induction of T-cell lymphokine production and proliferation. The immunosuppressant activity results in inhibition of lymphocyte proliferation, T-cell cytokine production, and expression of T-cell activation surface antigens.

3.4 Rationale for Dose Regimen/Dose Justification

Medications used in this trial are (methyl)prednisolone, mycofenolate mofetil (MMF) and voclosporin. Dosages are based on earlier studies that have been taken up in international guidelines such as the KDIGO guideline (January 2024)(8).

Methylprednisolone: maximum 500mg per day intravenously for a maximum of 3 days.

Prednisolone: maximum 1 mg/kg/day orally during the first week after treatment start; dosage will be tapered to maximum 5mg per day at three months after start of treatment with a preference to have it stopped completely before that timepoint.

MMF: 1000mg BID orally during the complete trial duration, unless side-effects are present

Voclosporin: 23,7mg BID orally according to the manufacturer's instructions based on the AURORA trials and earlier dose-finding studies, and approved by the European Medicines Agency (EMA). Dosage reduction might be necessary based on kidney function and/or hepatic impairment.

4. STRUCTURED RISK ANALYSIS

4.1 Potential issues of concern

The investigational product is voclosporin, which is a registered medicine for the indication of LN.

4.1.1 Level of knowledge about mechanism of action

Voclosporin is a second generation calcineurin-inhibitor immunosuppressant that inhibits calcineurin in a dose-dependent manner up to a maximum dose of 1.0 mg/kg. Activation of lymphocytes involves an increase in intracellular calcium concentrations. Calcineurin is a calcium/calmodulin-dependent phosphatase whose activity is required for the induction of T-cell lymphokine production and proliferation. The immunosuppressant activity results in inhibition of lymphocyte proliferation, T-cell cytokine production, and expression of T-cell activation surface antigens.

4.1.2 Previous exposure of human beings

The safety and efficacy of voclosporin were investigated in two placebo-controlled clinical trials (AURORA 1 and AURA-LV) in patients with LN of Class III or IV (alone or in combination with Class V) or pure Class V.

A detailed overview of the structure and results of these clinical studies is provided in section 8.1.2.

4.1.3 Induction of the mechanism in animals and/or *ex-vivo*

Voclosporin can attenuate the severity of disease in rodent models of autoimmune uveitis and corneal inflammation (9). Experimental data on animal models of autoimmune glomerulonephritis, LN, or other proteinuric kidney diseases, have not been reported in the public domain. However, he mechanism of action of CNI is well established. Mouse models of SLE respond to the traditional CNI, especially in terms of proteinuria control (10, 11).

4.1.4 Selectivity of the mechanism

Voclosporin is a CNI, whose molecular target (calcineurin) is broadly expressed in immune and non-immune cells; thus it does not have an exclusive receptor selectivity for particular tissue. Its functional selectivity in LN arises from dual actions: immunosuppression (T-cell inhibition)and a renal-cell effect (podocyte cytoskeleton stabilization) (12) .Comparative pharmacokinetic/distribution data suggest voclosporin has a more systemic calcineurin inhibition than older CNIs and relatively lower cardiovascular and renal adverse effects, which may contribute to its safety/efficacy profile(13).

4.1.5 Analysis of potential effect

Individuals will receive the recommended dose of 23.7mg, twice daily.

Voclosporin mainly affects the immune system, and immunosurpessants, including voclosporin may increase the risk of developing bacterial, viral, fungal, and protozoal infections, including opportunistic infections which may be serious or fatal. Additionally, immunosuppressants increase the risk of developing lymphomas and other malignancies, particularly of the skin, and patients are at an increased risk of neurotoxicity.

As with other calcineurin-inhibitors, adverse reactions of acute worsening of renal function or eGFR decreases have been seen in patients treated with voclosporin. In the first four weeks of treatment with voclosporin, haemodynamic reductions in eGFR have been observed. This can be managed by dose adjustments. Cases of pure red cell aplasia (PRCA) have been reported in patients treated with a different calcineurin inhibitor. Hyperkalaemia, which may be serious and require treatment, has been reported with calcineurin inhibitors, including voclosporin. Concomitant use of medicinal products associated with hyperkalaemia (e.g., potassium-sparing diuretics, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs)) may increase the risk of hyperkalaemia. Voclosporin can cause or worsen systemic hypertension. The use of voclosporin in combination with other medicinal products that are known to prolong QTc may result in clinically significant QT prolongation. Voclosporin has not been studied in patients with severe hepatic impairment (Child-Pugh Class C) and, therefore, it is not recommended for use in this patient population.

Voclosporin is metabolised by CYP3A4. When co-administering voclosporin with moderate cytochrome P450 (CYP)3A4 inhibitors (e.g., verapamil, fluconazole, diltiazem), daily dose must be reduced.

4.1.6 Pharmacokinetic considerations

Absorption

Following oral administration (voclosporin 23.7 mg twice daily) the median time to reach maximum whole blood concentrations (Cmax) is 1.5 hours (range: 0.75 hour to 2 hours). With a twice daily dosing regimen, voclosporin steady state is achieved after 6 days and voclosporin accumulates approximately 2-fold relative to a single dose. At steady state, the whole blood mean Cmax and pre-dose trough values for voclosporin were 120 ng/mL (32 % CV) and 15.0 ng/mL (49 % CV), respectively. In vitro data investigating if voclosporin is a substrate of the efflux transporters P-gp or BCRP are inconclusive, but clinically relevant effects of P-gp/BCRP inhibitors are not expected. Co-administration of voclosporin with food decreased both the rate and extent of absorption. Cmax and AUC of voclosporin were reduced by 53 % and 25 % when given with high-fat food and by 29 % and 15 % when given with low-fat food. These changes were not considered to be clinically relevant. Therefore, voclosporin can be taken with or without food.

Distribution

Voclosporin is 97 % bound to plasma proteins. Voclosporin partitions extensively into red blood cells and distribution between whole blood and plasma is concentration- and temperature-dependent. A population pharmacokinetic analysis in patients resulted in an apparent volume of distribution (Vss/F) of 2,154 L.

Biotransformation

Voclosporin is extensively metabolised, predominantly by CYP3A4 to form oxidative metabolites. Voclosporin is the major circulating component following a single dose of [14C]-voclosporin. One

major metabolite was observed in human whole blood and represented 16.7 % of total exposure. The major metabolite is not expected to contribute to the pharmacological activity of voclosporin since it was reported as about 8-fold less potent in a lymphocyte proliferation assay and has lower exposure than voclosporin.

Elimination
The mean apparent clearance at steady state (CLss/F) after voclosporin 23.7 mg twice daily is 63.6 L/h (37.5 % CV). The mean terminal half-life (t½) at steady state is approximately 30 hours (range: 24.9 hours to 36.5 hours). Following single oral administration of 70 mg [14C]-voclosporin, 94.8 % of the radioactivity was recovered by 168 hours post dose: 92.7 % was recovered in faeces (including 5 % as unchanged voclosporin), and 2.1 % was recovered in urine (including 0.25 % as unchanged voclosporin).

Linearity/non-linearity
In healthy volunteers, a non-linearity between dose and exposure was observed at the lower end of the dose range studied (0.25 mg/kg to 1.5 mg/kg twice daily), which had a relatively minor effect on the pharmacokinetics. The dose-proportionality factor was always less than 1.5. This non-linearity has not been detected over the dose range studied in LN patients.

Pharmacokinetics in special populations
Renal impairment
In clinical studies, kidney function was monitored by eGFR and doses were adjusted based on a predefined dose adjustment protocol. Enrolled LN patients had a baseline eGFR > 45 mL/min/1.73 m2 . Dosing adjustments have to follow the recommendations outlined in table 1. A dedicated renal impairment study revealed that after single and multiple doses of voclosporin, Cmax and AUC were similar in volunteers with mild (creatinine clearance (CLCr) 60 mL/min to 89 mL/min as estimated by Cockcroft Gault) and moderate (CLCr 30 mL/min to 59 mL/min) renal impairment compared to volunteers with normal renal function (CLCr ≥ 90 mL/min). After a single dose of voclosporin in volunteers with severe renal impairment (CLCr < 30 mL/min), Cmax and AUC increased 1.5-fold and 1.7-fold, respectively. The effect of end-stage renal disease (ESRD) with or without haemodialysis on the pharmacokinetics of voclosporin is unknown (see section 4.2).

Hepatic impairment
A dedicated hepatic impairment study compared systemic exposure of voclosporin in patients with mild or moderate hepatic impairment (Child-Pugh A and B, respectively) vs. healthy controls with normal hepatic function. In patients with mild and moderate hepatic impairment, voclosporin Cmax and AUC0-48 increased by 1.5-fold and approximately 2-fold, respectively (see section 4.2). Voclosporin has not been evaluated in patients with severe hepatic impairment (Child-Pugh C) and its use in these patients is not recommended (see section 4.4).

Age, sex, race and body weight
A population pharmacokinetic analysis assessing the effects of age, sex, race and body weight did not suggest any clinically significant impact of these covariates on voclosporin exposures.

4.1.7 Predictability of effect

The pharmacokinetics of other legacy CNIs used (off-label) in the treatment of LN (cyclosporine, tacrolimus) are complex and unpredictable because of characteristics such as a narrow therapeutic index and variable absorption, distribution, and elimination, together with pharmacogenetic factors; the resulting intra-individual and inter-individual pharmacokinetic variability means that therapeutic drug monitoring is required to optimize treatment with these agents, to ensure adequate dosing and minimize toxicity (14).

In contrast, population pharmacokinetic analysis in patients with LN showed voclosporin to have predictable pharmacokinetics, with no clinically meaningful influence of sex, body weight, age, serum albumin, total bilirubin or eGFR (>45 mL/min/1.73 m^2; patients with eGFR ≤45 mL/min/1.73 m^2 were excluded from voclosporin clinical trials). Dose adjustment is not required based on these covariates. Likewise, age (18–66 years), race (White, Black, Asian, other), and body weight (32–133 kg) had no clinically significant impact on voclosporin pharmacokinetics. The predictable pharmacokinetic profile of voclosporin in patients with LN, together with the strong correlation between voclosporin whole blood concentration and calcineurin inhibition, eliminates the need for therapeutic drug monitoring, and enables a pharmacodynamic rather than pharmacokinetic approach to dosing, with dose adjusted in response to decreases in eGFR. Therapeutic drug monitoring of voclosporin is unlikely to be of added benefit to patient management (12).

4.1.8 Interaction with other products

Potential for other medicinal products to affect voclosporin exposure

Voclosporin is metabolised by CYP3A4. Concomitant use of medicinal products or herbal remedies known to inhibit or induce CYP3A4 may affect the metabolism of voclosporin and thereby increase or decrease voclosporin blood levels.

<u>CYP3A4 inhibitors</u>
Voclosporin exposure was 18.6-fold higher in the presence of the strong CYP3A4 inhibitor ketoconazole compared to voclosporin administered alone. Co-administration of voclosporin with strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin) is contraindicated. Voclosporin exposure was 2.71-fold higher in the presence of the moderate CYP3A4 inhibitor verapamil compared to voclosporin administration alone. Reduce the dose to 15.8 mg in the morning and 7.9 mg in the evening when voclosporin is co-administered with moderate CYP3A4 inhibitors (e.g., verapamil, fluconazole, erythromycin, diltiazem, grapefruit and grapefruit juice, see section 4.2). Mild CYP3A4 inhibitors may increase voclosporin exposure, but no in vivo study has been performed. No dose adjustment is required when voclosporin is co-administered with mild CYP3A4 inhibitors but additional monitoring of eGFR is recommended when initiating treatment with a mild CYP3A4 inhibitor.

<u>CYP3A4 inducers</u>
Voclosporin exposure was 87 % lower and maximum concentration (Cmax) was 68 % lower in the presence of the strong CYP3A4 inducer rifampicin (600 mg once daily for 10 consecutive days) compared to voclosporin administration alone. Co-administration of multiple doses of moderate CYP3A4 inducers are also expected to result in clinically relevant decreases of voclosporin exposure. Strong and moderate CYP3A4 inducers (e.g., carbamazepine, phenobarbital, rifampicin, St John's Wort, efavirenz) are not recommended to be dosed concomitantly with voclosporin (see section 4.4).

Mild inducers of CYP3A4 may also result in decreased exposure and possibly a decreased effect, but the clinical relevance is unknown.

Potential for voclosporin to affect exposure to other medicinal products
P-gp substrates Voclosporin is an inhibitor of P-glycoprotein (P-gp). Concomitant administration of voclosporin with multiple doses of digoxin increased digoxin Cmax and area under the curve (AUC) by 1.51-fold and 1.25-fold, respectively. Caution must be exercised in case of co-administration of voclosporin with sensitive P-gp substrates, especially those with narrow therapeutic index (e.g., digoxin, dabigatran etexilate, fexofenadine) where patients should be appropriately monitored as outlined in respective product labelling.

OATP1B1/OATP1B3 substrates
Voclosporin is an inhibitor of OATP1B1 and OATP1B3 transporters. In one clinical study the concomitant administration of a single 40 mg dose of simvastatin with 23.7 mg BID voclosporin increased Cmax and AUC of the active metabolite simvastatin acid (a sensitive OATP1B1/OATP1B3 7 substrate) by 3.1-fold and 1.8-fold, respectively. In the same study, exposure of the parent drug simvastatin (which is also a BCRP substrate) was unaffected in terms of AUC while its Cmax increased by 1.6-fold, which could potentially be attributed to an interaction between intestinal BCRP and voclosporin. Patients should be monitored for adverse events such as myopathy and rhabdomyolysis when OATP1B1/OATP1B3 substrates (e.g., simvastatin, atorvastatin, pravastatin, rosuvastatin) are used concomitantly with voclosporin.

BCRP substrates
Voclosporin inhibits breast cancer resistance protein (BCRP) in vitro. A clinically relevant inhibition of intestinal BCRP cannot be excluded and voclosporin may increase the concentration of these substrates in vivo. Monitor use of BCRP substrates where small concentration changes may lead to serious toxicity (e.g., rosuvastatin) when used concomitantly with voclosporin.

4.1.9 Managing of effects

Negative effects will be monitored and managed by frequent medical controls in the outpatient clinic. Voclosporin dosing can be reduced or stopped based on the occurrence of events. Patients are always instructed to contact their phycisian when experiencing complaints.

4.1.10 Study population

The study population consists of patients with severe proliferative lupus nephritis (LN), defined as class III or IV, with or without a concomitant class V component. If left untreated, these patients are at high risk of progressing to end-stage kidney disease (ESKD) and requiring renal replacement therapy. Treatment with immunosuppressive agents is therefore always indicated in this population. Current clinical guidelines recommend a range of immunosuppressive regimens, with the final treatment choice largely based on expert consensus.

Women of childbearing potential are included in the study, as lupus nephritis predominantly affects individuals within this demographic.

4.2 Overall synthesis of the direct risks for the research subjects

Voclosporin is a registered product for the intended indication and is not administered in combination with other products, except for mycophenolate mofetil (MMF) and prednisolone, which are part of the recommended treatment regimen.

The addition of voclosporin has the important identified risk of serious infections (including opportunistic infections). Major adverse cardiovascular events (MACEs), neurotoxicity, nephrotoxicity (acute and chronic) and malignancies (including lymphomas) associated with long term use have been identified as important potential risks. Routine risk minimization measures have been described in the SmPc and include advice to avoid direct UV light and regular monitoring for risk of infection, eGFR, risk of pure red cell aplasia (PRCA), serum potassium levels and blood pressure. Since the chance of potential risks is considered low and participants are closely monitored during regular follow-up visits (during which vital functions and lab results are consistently checked as is standard), adding voclosporin in the intervention group is deemed justified and poses no additional risks for patients participating in this study.

An additional biopsy will be performed 3 months after initiation of treatment to evaluate histological response and to conduct spatial single-cell RNA sequencing (scRNAseq) analysis. While renal biopsy is associated with a recognized risk of bleeding (estimated at approximately 1 in 1,000 procedures), this risk is influenced by factors such as concomitant anticoagulant use, anemia, and impaired renal function with elevated urea levels. Importantly, repeat biopsies are often a routine part of clinical management in lupus nephritis, particularly in cases of new active urinary sediment, rising serum creatinine, or worsening proteinuria during treatment, as no reliable biomarker currently exists to monitor therapeutic response or disease progression.

5. OBJECTIVES AND ENDPOINTS

Table 3-1 Trial Objectives and Endpoint(s)

Objectives	Endpoint(s)
Primary: Understanding early immunological changes in LN patients (class III/IV+/-V) on a cellular and histo-pathological level, comparing the CNI voclosporin on top of MMF and prednisolone (triple therapy) to MMF and prednisolone (dual therapy).	**Objective 1a**: to determine scRNA-seq differences within the full macrophage spectrum between arm 1 vs arm 2; the macrophage spectrum will be compared between baseline kidney biopsy and kidney biopsy at 3 months treatment; - Achievement of 'healthy' myeloid cell proportions at 3 months - Assessment of differences in immune cell proportions and gene expression levels at 3 months
Secondary: - To assess the association of tissue remission/changes with improved long term (2 year) outcome - To assess specific differences in residential and monocyte-derived macrophages. - To assess phenotype of circulating monocytes in LN patients compared to SLE patients without clinical kidney involvement and healthy subjects, guided by initial scRNAseq data, to be associated with clinical and biochemical parameters - To identify potential non-invasive urinary biomarkers of disease activity in the scRNA-seq data, to be tested in sequentially stored urine and/or serum from patients	**Objective 1b**: analyze rapid clinical remission of intensified treatment by proteinuria levels for relation to tissue changes **Objective 2a.** numerically distinguish residential macrophages and monocyte-derived macrophages in kidney tissue with spatial scRNAseq, comparing arm 1 vs arm 2; **Objective 2b**: to assess histological response of intensified treatment (arm 1) compared to dual therapy (arm 2); **Objective 3**: to assess if early histological response is associated with clinical remission after two years and identify innovative early response determinants (including tissue monocyte/macrophage phenotyping) **Objective 4**: to identify differences between LN, SLE and healthy subjects in peripheral monocyte phenotype by using bulk RNA sequencing and flowcytometric analyses, to be associated with scRNAseq data and clinical/biochemical parameters **Objective 5:** To identify potential non-invasive urinary biomarkers of disease

	activity in the scRNA-seq data, to be tested in sequentially stored urine and/or serum from patients **Objective 6**: attempt to render (repeat) kidney biopsy in LN obsolete by identifying reliable serum and/or urinary biomarkers that reflect tissue damage and treatment response; **Objective 7**: confirm scRNA-seq identified macrophage markers immunohistochemically, identify spatial distribution (glomerular vs interstitial) and assess their predictive and prognostic use.

6. STUDY PLAN AND DESIGN

6.1 Trial Design

This is a single-center open label randomized controlled trial. Patients will be randomized into one of two arms and will be followed for the duration of 2 years.
Inclusion starts at the time of kidney biopsy, when the diagnosis of a class III or IV LN is certain, with or without a component of class V.

The study team deliberately chose a randomized trial design, since EULAR/KDIGO guidelines (however acknowledging the added effect of triple therapy as shown in clinical trials like AURORA 1 and 2) still entrust the choice of therapy upon the treating physician.

KDIGO 2024, section 10.2.3.1
"Recommendation 10.2.3.1.1: We recommend that patients with active Class III or IV LN, with or without a membranous component, be treated initially with glucocorticoids plus any one of the following:

i. mycophenolic acid analogs (MPAAs) (1B); or
ii. low-dose intravenous cyclophosphamide (1B); or
iii. belimumab and either MPAA or low-dose intravenous cyclophosphamide (1B); or
iv. MPAA and a calcineurin inhibitor (CNI) when kidney function is not severely impaired (i.e., estimated glomerular filtration rate [eGFR] ≤45 ml/min per 1.73 m2) (1B)."

"This recommendation places a high value on results from clinical trials demonstrating clinical efficacy with combined immunosuppressive regimens that include glucocorticoids and either low-dose intravenous cyclophosphamide or MPAA (dual immunosuppressive therapy), as well as triple immunosuppressive regimens that include belimumab or voclosporin added to the above dual

immunosuppression … The Work Group acknowledges the considerable variation of characteristics among patients. Readers are advised to refer to the practice points for discussions on the preferred choice of therapy according to patient characteristics. Also, regarding the choice of therapy and the dose of specific medications, the Work Group emphasizes the importance of preventing potential adverse effects that could result from highdose glucocorticoid, high-dose cyclophosphamide, and CNI nephrotoxicity. In this regard, patients with CKD G3b–G5 were often excluded from the respective clinical trials."

EULAR 2023 update, Table 1, recommendation 8
"Patients with active proliferative lupus nephritis should receive low-dose (EuroLupus) intravenous cyclophosphamide (1a/A) or mycophenolate (1a/A) and glucocorticoids (pulses of intravenous methylprednisolone followed by lower oral doses); combination therapy with belimumab (either with cyclophosphamide or mycophenolate (1b/A)) or calcineurin inhibitors (especially voclosporin or tacrolimus, combined with mycophenolate, 1b/A) should be considered."

Since the primary aim of this study is to compare the immunological effects of two treatment regimens by assessing immune cell composition and gene expression patterns with a focus on the added effect of voclosporin, the study is not designed to compare clinical efficacy since clinical parameters may not reflect tissue-level immunological changes.

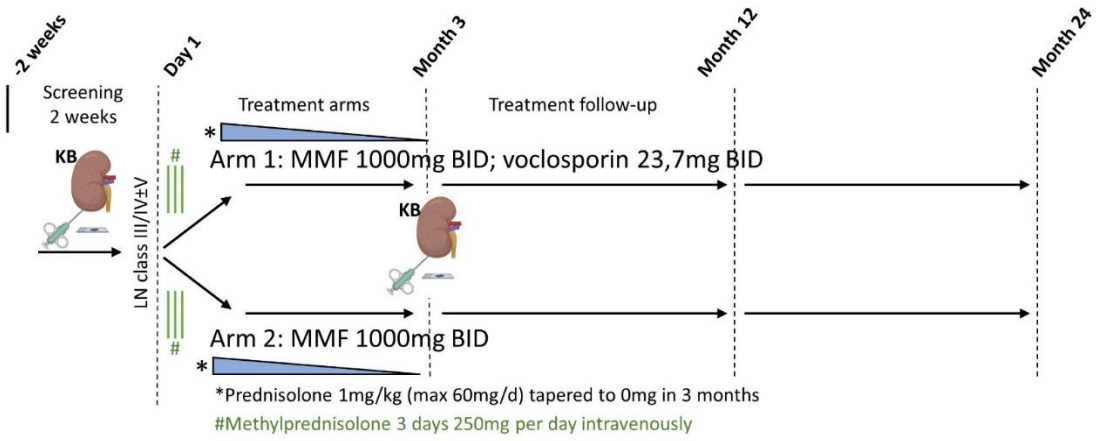
Randomization is necessary because treatment choice in current clinical practice is largely based on physician judgment, influenced by factors such as physician experience, regional drug availability and patient characteristics, for which clear cut-off points are lacking. Both dual and triple therapy are equally recommended in current LN guidelines. Without randomisation, treatment allocation would be prone to substantial bias related to physician-specific preferences.

In routine practice, physicians with more experience or access to voclosporin may preferentially prescribe triple therapy to patients with more proteinuria, higher inflammatory activity, or specific histopathological characteristics, while patients treated by a different physician may receive dual therapy. These clinical features are associated with differences in immune cell composition and gene expression profiles. As the addition of voclosporin is expected to have a specific additional effect on myeloid cell populations, immunological response parameters such as immune cell proportions and (damage and tissue-repair associated) gene expression could therefore be influenced both by therapy choice and by underlying differences in disease severity. Consequently, treatment allocation based on physician experience or drug availability could systematically correlate with baseline immunological status, introducing confounding when comparing immune cell populations and gene expression profiles between groups.
A randomized design therefore ensures an unbiased comparison of immunological outcomes and may provide tissue-specific insights to inform future treatment guidance.

In addition to the equal treatment recommendations as stated above, the study team considers it unlikely that randomisation will result in undertreatment. The primary endpoint is assessed after a relatively short period of three months following treatment initiation and the standard glucocorticoid tapering point. If tapering is not feasible due to an unfavourable treatment response, rescue therapy may be initiated. Regardless of treatment allocation or response, patients will not be left untreated, as all receive concomitant corticosteroids with strong immunosuppressive effects.

A second kidney biopsy is required to assess short-term immunological responses relative to the baseline biopsy. Immunological response will be evaluated by changes in immune cell-type proportions and gene expression patterns. Longitudinal clinical data collected over a two-year follow-up period will be related to these immunological changes to link favourable short-term tissue-level responses to established clinical markers (e.g. eGFR, proteinuria) as well as to the development of novel biomarkers.

The open label design is based on the decision that the clinical effect of the addition of voclosporin has already been proved by other clinical trials and is not the focus of this trial. The study team concludes that based on the objectives of the trial, namely; attaining rapid clinical/histological remission with triple therapy and assessing macrophage/monocyte clusters in kidney tissue and peripheral blood (and therefore having a primary focus on histological and cell specific outcomes), a placebo controlled setup is not necessary and bias due to referring from placebo use is minimal.



6.2 Number of Patients

A total of 55 participants will be included:

- 30 LN patients will be randomized
- 15 SLE without LN patients as control group
- 10 healthy participants as control group

6.3 Overall study duration and follow-up

The expected duration of participation per participant (randomized LN patients) will be 24 months.

For both control groups blood and urine samples will be collected at 2 different timepoints with an interval of 3 to 6 months between collections.

6.4 Patient participation

Patients were involved in the design of the study, and evaluated and approved the study protocol as part of the evaluation for co-funding by the Dutch Kidney Foundation, which was granted.

7. STUDY POPULATION

7.1 Population

Randomized research population: Patients (age 16-70) with histologically proven LN class III or IV with or without class V

Control group research population:
- SLE patients (age 16-70) without LN
- Healthy volunteers (age 16-70)

7.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

Lupus nephritis patients
- Patients with de novo or flaring SLE according to the EULAR/ACR criteria and a suspicion of class III or IV LN with a clinical indication to perform a kidney biopsy
- Age 16-70
- eGFR as measured by cystatin C >20 mL/min

SLE patients (disease control group)
- Diagnosis of SLE according to EULAR/ACR guidelines
- Age 16-70

Healthy subjects (control group)
- Blank medical history
- Age 16-70

7.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

Lupus nephritis patients
- LN class I, II or pure class V upon kidney biopsy (in the case of a class I, II or pure V, the patient will be asked consent for analysis of the scRNA-seq data from the kidney biopsy but the patient will not be randomized)
  eGFR as measured by cystatin C <20 mL/min
- Histological chronicity score (NIH) of 8 or higher in the baseline kidney biopsy
- Active infection of any kind as evidenced by cultures (blood, urine or otherwise)
- History of hepatitis B, hepatitis C, tuberculosis and/or HIV
- Treatment with any of the following agents within one month before screening: tacrolimus, belimumab, anifrolumab

- Treatment with any of the following agents within 6 months before screening: rituximab, daratumumab, eculizumab
- Prolongation of QT-interval (QTc >470ms) and/or bradycardia (resting heart rate <50bpm) measured on two separate occasions
- Hyperkalaemia (serum potassium >6.0 mmol/L)
- Hypertension (blood pressure > 165/105 mmHg, with symptoms of hypertension)*
- Co-administration of voclosporin with strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin)
- Pregnancy

*A single elevated blood pressure measurement will not lead to immediate exclusion from the trial. Antihypertensive therapy may be initiated as appropriate. Dose adjustments of voclosporin should be made in accordance with Section 11.3.2.

SLE patients (disease control group)

- Suspicion of LN
- Signs of active infection

Healthy subjects (control group)

- Signs of active infection

7.4 Vulnerable populations and clinical trials in emergency situations

Not applicable

8. STUDY TREATMENTS

8.1 Investigational Medicinal Product(s) (IMP(s))

8.1.1 Name and description of the IMP

The investigated product is voclosporin, soft capsule. Voclosporin has been approved for use under the trade name Lupkynis. ATC code: L04AD03.

8.1.2 Status of development of the IMP

The safety and efficacy of voclosporin were investigated in two placebo-controlled clinical trials (AURORA 1 (3) and AURA-LV (15)) in patients with LN of Class III or IV (alone or in combination with Class V) or pure Class V. All patients received background therapy of MMF (2 g/day) and corticosteroids (up to a total of 1 g of intravenous (IV) methylprednisolone over days 1 and 2 followed by a starting dose of oral corticosteroids of 25 mg/day (or 20 mg/day if body weight was < 45 kg), tapered down to 2.5 mg/day by week 16. 11 Patients that completed the AURORA 1 study could continue in a 2-year continuation study (AURORA 2).

The AURORA 1 study was a phase 3, prospective, randomised, double-blind, study comparing 23.7 mg (corresponding to a 0.37 mg/kg dose) twice daily of voclosporin (n = 179) vs. placebo (n = 178) over a 52-week treatment period. The demographic characteristics of patients in the study were well balanced across the two treatment arms. The mean age was 33 years (range 18 years to 72 years) and the majority of patients were female (87.7 %), of which 81.8 % were of childbearing potential. Most patients were White (36.1 %) or Asian (30.5 %), and approximately one third of the study population was Hispanic or Latino. The mean weight was 66.5 kg (range 36 kg to 142 kg). The median time since systemic lupus erythematosus (SLE) diagnosis was 5.0 years and the median time since LN diagnosis was 2.0 years. Before entering the AURORA 1 study, most patients (98 %) had received treatment for LN in the past, with approximately 55 % of patients taking MMF at screening. The proportion of LN treatment naïve patients was very low (2 %). More patients in the voclosporin arm than the placebo arm achieved the primary endpoint of renal response.

The overall proportion of patients that achieved each of the components assessed for the primary endpoint at 52 weeks in the voclosporin vs placebo arm were:
- urine protein to creatinine ratio (UPCR) ≤ 0.5 mg/mg: 45.3 % vs 23.0 %
- with normal, stable renal function (defined as eGFR ≥ 60 mL/min/1.73 m2 or no confirmed decrease from baseline in eGFR of > 20 %): 82.1 % vs. 75.8 %
- in the presence of sustained, low-dose steroids (not more than 10 mg for ≥ 3 consecutive days or for ≥ 7 days in total during weeks 44 to 52): 87.2 % vs. 85.4 %
- and received no rescue medication for LN: 91.1 % vs. 86.5 %

More patients in the voclosporin arm than the placebo arm achieved UPCR ≤ 0.5 mg/mg (64.8 % vs. 43.8 %) and the time to UPCR ≤ 0.5 mg/mg was significantly shorter for voclosporin treatment (median time: 169 days vs. 372 days for placebo treatment; hazard ratio (HR) 2.02; 95 % CI: 1.51, 2.70; $p < 0.001$).

The time taken to reach a 50 % reduction in UPCR was significantly shorter for the voclosporin arm than the placebo arm (HR 2.05; 95 % CI: 1.62, 2.60; $p < 0.001$). Median time to 50 % reduction in UPCR was 29 days for voclosporin vs. 63 days for placebo.

Over 80 % of patients in the AURORA 1 study achieved a reduction in dose of oral corticosteroid to ≤ 2.5 mg/day at week 24 and this dose was maintained by over 75 % of patients at week 52.

The AURORA 2 study (4) was a continuation study to evaluate the long-term safety and efficacy of voclosporin in patients that completed treatment in the AURORA 1 study. Patients stayed on the same treatment and dose of voclosporin (n = 116) or placebo (n = 100) as at the end of AURORA 1 and continued treatment for up to a further 2 years. Over 85 % of patients completed the study (voclosporin: 87.1 %, placebo 85.0 %); 79.3 % of voclosporin patients and 73 % of placebo patients were still on study treatment at the end of study. The proportion of patients in renal response at month 36 was 33 % (59/179) in the voclosporin group and 22 % (39/178) in the placebo group (ITT, AURORA 1) and 51 % (59/116) in the voclosporin group and 39 % (39/100) in the placebo group (ITT, AURORA 2).

8.1.3 Description and justification of dosage and route of administration

Route of administration: soft capsule for oral intake
Dosage: 23.7 mg (three 7.9 mg soft capsules), twice daily
Justification:

The AURA-LV study tested the novel calcineurin inhibitor voclosporin for efficacy and safety in active LN. AURA-LV was a Phase 2, multicenter, randomized, double-blind, placebo-controlled trial of two doses of voclosporin (23.7 mg or 39.5 mg, each twice daily) versus placebo in combination with mycophenolate mofetil (2 g/d) and rapidly tapered low-dose oral corticosteroids for induction of remission in LN. The primary endpoint was CRR at 24 weeks; the secondary endpoint was CRR at 48 weeks. Two hundred sixty-five subjects from 79 centers in 20 countries were recruited and randomized to treatment for 48 weeks. CRR at week 24 was achieved by 29 (32.6%) subjects in the low-dose voclosporin group, 24 (27.3%) subjects in the high-dose voclosporin group, and 17 (19.3%) subjects in the placebo group (OR=2.03 for low-dose voclosporin versus placebo). The significantly greater CRR rate in the low-dose voclosporin group persisted at 48 weeks, and CRRs were also significantly more common in the high-dose voclosporin group compared to placebo at 48 weeks. There were more serious adverse events in both voclosporin groups, and more deaths in the low-dose group compared to placebo and high-dose voclosporin groups (11.2%, 1.1%, and 2.3%, respectively). These results suggest that the addition of low-dose voclosporin to mycophenolate mofetil and corticosteroids for induction therapy of active LN results in a superior renal response compared to mycophenolate mofetil and corticosteroids alone, but higher rates of adverse events including death were observed.

Dose modifications:

- Dose adjustment based on eGFR:
    - Limited data are available on the use of Lupkynis in patients with baseline eGFR 30 to < 45 mL/min/1.73 m2 . It is recommended to use Lupkynis in these patients, only if the benefit outweighs the risk, and at a starting dose of 23.7 mg twice daily. Lupkynis has not been studied in patients with severe renal impairment (eGFR < 30 mL/min/1.73 m2) and is not recommended in these patients unless the benefit outweighs the risk. If used, the recommended starting dose is 15.8 mg twice daily.
    - It is recommended to establish a baseline estimated glomerular filtration rate (eGFR) before starting treatment with voclosporin, and assess every two weeks for the first month, and every four weeks 3 thereafter. Dose adjustments are required for those individuals whose eGFR is confirmed to be reduced (i.e., two consecutive assessments within 48 hours) and below 60 mL/min/1.73 m2 . If eGFR remains ≥ 60 mL/min/1.73 m2 no dose modification is required (see table 1). It is recommended that patients requiring a reduction in dose are reassessed for eGFR recovery within two weeks. For patients that had a decrease in dose due to eGFR reduction, increasing the dose by 7.9 mg twice a day for each eGFR measurement that is ≥ 80 % of baseline should be considered; the starting dose should not be exceeded.
- Co-administration with moderate CYP3A4 inhibitors: When co-administering Lupkynis with moderate cytochrome P450 (CYP)3A4 inhibitors (e.g., verapamil, fluconazole, diltiazem), daily dose must be reduced to 15.8 mg in the morning and 7.9 mg in the evening.
- Hepatic impairment: In patients with mild and moderate hepatic impairment (Child-Pugh Class A and B, respectively), the recommended starting dose is 15.8 mg twice daily. The effect of

voclosporin in patients with severe hepatic impairment (Child-Pugh Class C) has not been assessed and voclosporin is not recommended in this patient population.

Table 1: Recommended dose adjustments based on eGFR

Confirmed eGFR decrease from baseline[1]	Recommendation
≥ 30 % reduction	Stop administration of voclosporin. Restart treatment upon eGFR recovery at 7.9 mg (1 capsule) twice daily and increase as tolerated based on renal function.
> 20 % and < 30 % reduction	Reduce dose of voclosporin by 7.9 mg (1 capsule) twice daily. Retest within two weeks; if eGFR decrease has not recovered, reduce dose by further 7.9 mg (one capsule) twice daily.
≤ 20 % reduction	Maintain current dose and monitor.

[1] If eGFR remains ≥ 60 mL/min/1.73 m^2 no action is required

The study team concludes that in patients with an eGFR >20 mL/min/1.73 m2, the addition of voclosporin (and therefore benefit of therapy) outweighs the risk based on the severity of a rapidly progressive glomerulonephritis (RPGN) and risk of ESKD when not treated. This conclusion is based on expert opinion and experience with previously treated (clinical) patients, since evidence for this specific group of patients is lacking due to the rarity of the disease and novelty of this specific CNI. The KDIGO 2024 guideline (Practice Point 10.2.3.1.4) states that *"The use of a CNI in patients with severe CKD (eGFR <45 mL/min/1.73 m2) requires careful individualized consideration of risk versus potential benefit, and should be done with caution and careful monitoring, and at reduced drug exposure."* , and the study team concludes that dose reduction and intensive follow-up (as is standard in this patient group) is sufficient.

<u>Potential for voclosporin to affect exposure to other medicinal products</u>

- P-gp substrates: Voclosporin is an inhibitor of P-glycoprotein (P-gp). Concomitant administration of voclosporin with multiple doses of digoxin increased digoxin Cmax and area under the curve (AUC) by 1.51-fold and 1.25-fold, respectively. Caution must be exercised in case of co-administration of voclosporin with sensitive P-gp substrates, especially those with narrow therapeutic index (e.g., digoxin, dabigatran etexilate, fexofenadine) where patients should be appropriately monitored as outlined in respective product labelling.
- OATP1B1/OATP1B3 substrates: Voclosporin is an inhibitor of OATP1B1 and OATP1B3 transporters. In one clinical study the concomitant administration of a single 40 mg dose of simvastatin with 23.7 mg BID voclosporin increased Cmax and AUC of the active metabolite simvastatin acid (a sensitive OATP1B1/OATP1B3 7 substrate) by 3.1-fold and 1.8-fold, respectively. In the same study, exposure of the parent drug simvastatin (which is also a BCRP substrate) was unaffected in terms of AUC while its Cmax increased by 1.6-fold, which could potentially be attributed to an interaction between intestinal BCRP and voclosporin. Patients should be monitored for adverse events such as myopathy and rhabdomyolysis when OATP1B1/OATP1B3 substrates (e.g., simvastatin, atorvastatin, pravastatin, rosuvastatin) are used concomitantly with voclosporin.
- BCRP substrates: Voclosporin inhibits breast cancer resistance protein (BCRP) in vitro. A clinically relevant inhibition of intestinal BCRP cannot be excluded and voclosporin may increase the concentration of these substrates in vivo. Monitor use of BCRP substrates where small concentration changes may lead to serious toxicity (e.g., rosuvastatin) when used concomitantly with voclosporin. MMF Co-administration of voclosporin with mycophenolate mofetil (MMF) had no clinically significant impact on mycophenolic acid (MPA) blood concentrations.

- CYP3A4 substrates Multiple administrations of voclosporin orally (0.4 mg/kg twice daily) had

Treatment duration

The treatment duration is equal to the duration of the follow-up time (24 months). Medication may be continued after the trial and patients will attend regular follow-up appointments at the outpatient clinic for an undetermined period.

8.2 Comparator IMP(s)

Not applicable

8.3 Placebo

Not applicable

8.4 Auxiliary Medicinal Product(s) (AxMP(s))

8.4.1 Name and description of the AxMP

1. Mycophenoplate mofentil (Cellcept) 500mg, tablet
2. Glucocorticoïds
    -) Methylprednisolone (Depo-Medrol) 500mg, suspension for intravenous use
    -) Prednisolone (generic) 5-20mg, tablet

8.4.2 Statement on authorisation and justification unauthorised AxMP (if applicable)

Not applicable

8.4.3 Description and justification of dosage and route of administration

All doses/dose steps, dose rationale, route and mode of administration, schedule, treatment duration and dose modifications of AxMP's are derived from the EMA SPmC and KDIGO 2024 Lupus Nephritis Guideline (section 10.2.3, Figure 5) (8).

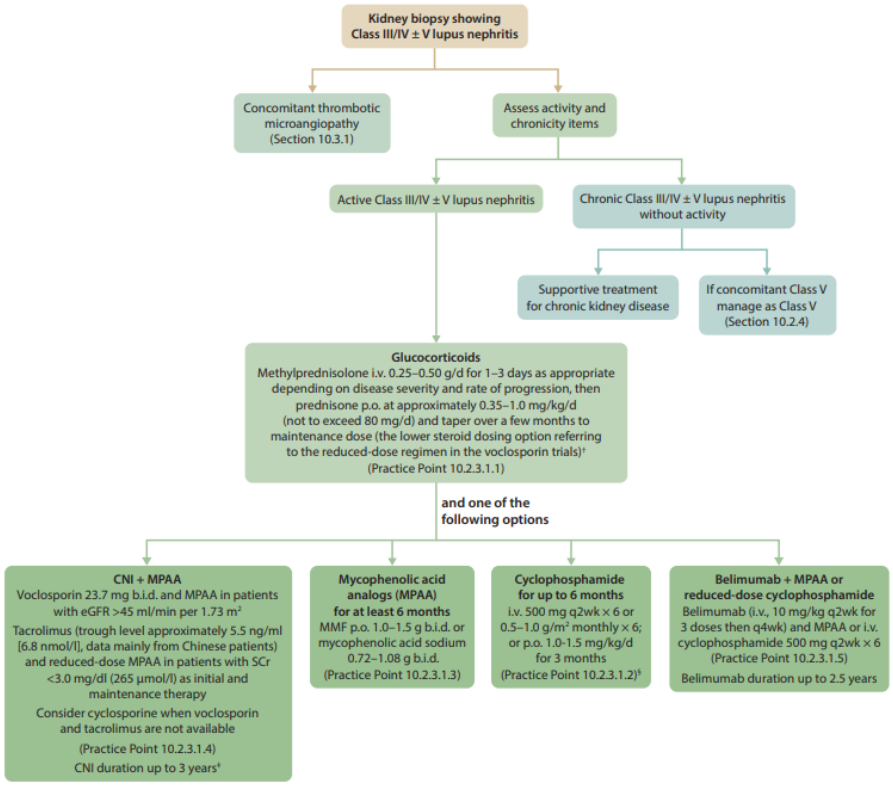


Figure 5 | Recommended approach for initial therapy of active Class III/IV lupus nephritis. Caution is warranted when calcineurin inhibitors (CNI) are used in patients with significantly impaired kidney function, in view of increased susceptibility for severe consequences due to CNI nephrotoxicity. The estimated glomerular filtration rate (eGFR) and serum creatinine (SCr) levels stated in the figure were patient selection criteria adopted in the respective clinical trials. †Refer to Figure 7 for examples of glucocorticoid treatment regimens. ‡Refer to Figure 9 for durations of CNI or belimumab treatment in clinical trials. §Refer to Figure 6 for comments on cyclophosphamide regimens. Note: Chapter 5 refers to Chapter 5 of the KDIGO Guideline on Glomerular Diseases; b.i.d., twice daily; i.v., intravenous; MMF, mycophenolate mofetil; p.o., oral; q2wk, every 2 weeks; q4wk, every 4 weeks; s.c., subcutaneous.

1. Mycophenolate mofentil (MMF)

   Route of administration: hard capsule for oral intake
   Dosage: 2000 mg (2 tablets of 500mg bidaily) daily
   Justification:
   The dose of mycophenolate mofetil (MMF) in the early maintenance phase is approximately 750–1000 mg twice daily. The suggested dosages are largely based on data from the ALMS (16) and MAINTAIN (17) trials.

   MAINTAIN: A total of 105 patients with lupus with proliferative LN were included. All received three daily intravenous pulses of 750 mg methylprednisolone, followed by oral glucocorticoids and six fortnightly cyclophosphamide intravenous pulses of 500 mg. Based on randomisation performed at baseline, AZA (target dose: 2 mg/kg/day) or MMF (target dose: 2 g/day) was given at week 12. The baseline clinical, biological and pathological characteristics of patients allocated to AZA or MMF did not differ. Renal flares were observed in 13 (25%) AZA-treated and 10 (19%) MMF-treated patients. Time to renal flare, to severe systemic flare, to benign flare and to renal remission did not statistically differ. Over a 3-year period, 24 h proteinuria, serum creatinine, serum albumin, serum C3, haemoglobin and global disease activity scores improved similarly in both groups. Doubling of serum creatinine occurred in four AZA-treated and three MMF-treated patients. Adverse events did not differ between the groups except for haematological cytopenias, which were statistically more frequent in the AZA group (p=0.03) but led only one patient to drop out.

ALMS: We carried out a 36-month, randomized, double-blind, double-dummy, phase 3 study comparing oral mycophenolate mofetil (2 g per day) and oral azathioprine (2 mg per kilogram of body weight per day), plus placebo in each group, in patients who met response criteria during a 6-month induction trial. The study group underwent repeat randomization in a 1:1 ratio.
A total of 227 patients were randomly assigned to maintenance treatment (116 to mycophenolate mofetil and 111 to azathioprine). Mycophenolate mofetil was superior to azathioprine with respect to the primary end point, time to treatment failure (hazard ratio, 0.44; 95% confidence interval, 0.25 to 0.77; P=0.003), and with respect to time to renal flare and time to rescue therapy (hazard ratio, <1.00; P<0.05). Observed rates of treatment failure were 16.4% (19 of 116 patients) in the mycophenolate mofetil group and 32.4% (36 of 111) in the azathioprine group. Mycophenolate mofetil was superior to azathioprine in maintaining a renal response to treatment and in preventing relapse in patients with lupus nephritis who had a response to induction therapy.

The KDIGO Work Group (KDIGO LN guideline 2024, Practice Point 10.2.3.2.3) recommends "*maintenance of these doses until achievement of complete response, and then tapering. Due to pharmacogenetic differences, the level of MPA (mycophenolic acid) exposure varies considerably among patients receiving the same dose of MPAA (mycophenolic acid analogs).*"

Dose modification
Due to pharmacogenetic differences, the level of MPA exposure varies considerably among patients receiving the same dose of MPAA. The dose of MPAA may need to be reduced when kidney function is significantly impaired, as patients with CKD are more susceptible to the adverse effects of MPA. Although there are insufficient data to date to provide recommendations on therapeutic drug monitoring, measurement of MPA exposure may be helpful in patients with unsatisfactory treatment response or patients who manifest or are at increased risk of drug toxicities. There are preliminary data associating disease flares with low MPA exposure, but the optimal drug level at different phases of clinical management remains to be determined.

2. Glucocorticoïds
    a. Methylprednisolone

    Route of administration: suspension for intravenous use
    Dosage: 500 mg daily, up to 3 days as initial treatment
    Justification:
    The role of i.v. methylprednisolone pulses at the start of treatment has not been well-studied, but i.v. glucocorticoid is commonly given as up to 3 daily doses of 500 mg each (range 250–1000 mg/d). There is general agreement to start treatment with i.v. pulse methylprednisolone in patients who present with a clinical syndrome of rapidly progressive glomerulonephritis (RPGN)—acute and severe deterioration of kidney function often accompanied by a high proportion of crescents or vascular lesions in the kidney biopsy–or when there are severe extrarenal manifestations, such as central nervous system or lung involvement.

    b. Prednisolone

    Route of administration: tablet
    Dosage: 1mg/kg daily (max 60mg), followed by tapering schema
    Justification:

Glucocorticoids are used in all current treatment regimens for LN. These drugs have both immunosuppressive and anti-inflammatory effects and provide immediate treatment for the often-extensive intrarenal inflammation that is seen in patients with Class III and Class IV LN. This regimen is necessary because there is a lag before the immunosuppressive effects of cyclophosphamide, MPAA, CNIs, or B cell–directed therapies are seen. The dose, tapering regimen, and duration of glucocorticoid schemes vary considerably among clinicians and are largely opinion-based. In view of the established efficacy associated with combined immunosuppression, there is a move towards reducing glucocorticoid exposure.

Dose modification

The dose, tapering regimen, and duration of glucocorticoid schemes vary considerably among clinicians and are largely opinion-based. Examples as proposed by the KDIGO 2024 guidelines (Practice Point 10.2.3.1.1, Figure 7) are given below:

	High-dose scheme	Moderate-dose scheme	Reduced-dose scheme
Methylprednisolone intravenous pulses	Nil or 0.25–0.5 g/day up to 3 days as initial treatment	0.25–0.5 g/day up to 3 days often included as initial treatment	0.25–0.5 g/day up to 3 days usually included as initial treatment
Oral prednisone equivalent (/day)			
Week 0–2	0.8–1.0 mg/kg (max 80 mg)	0.6–0.7 mg/kg (max 50 mg)	0.5–0.6 mg/kg (max 40 mg)
Week 3–4	0.6–0.7 mg/kg	0.5–0.6 mg/kg	0.3–0.4 mg/kg
Week 5–6	30 mg	20 mg	15 mg
Week 7–8	25 mg	15 mg	10 mg
Week 9–10	20 mg	12.5 mg	7.5 mg
Week 11–12	15 mg	10 mg	5 mg
Week 13–14	12.5 mg	7.5 mg	2.5 mg
Week 15–16	10 mg	7.5 mg	2.5 mg
Week 17–18	7.5 mg	5 mg	2.5 mg
Week 19–20	7.5 mg	5 mg	2.5 mg
Week 21–24	5 mg	<5 mg	2.5 mg
Week >25	<5 mg	<5 mg	<2.5 mg

8.5 Additional considerations for trials involving a medical device

Not applicable

8.6 Additional considerations for trials involving an in-vitro diagnostic or companion diagnostic

Not applicable

8.7 Preparation and labelling of the study treatment(s)

Labelling of IMP and AxMP will be provided through the clinical (methylprednisolone) and outpatient (prednisolone and voclosporin) pharmacy.

Following the flow diagram on the CCMO website, no additional labelling is required since the IMP and AxMP's are registered, without additional specific circumstances.

9. OTHER TREATMENTS AND RESTRICTIONS

9.1 Concomitant therapy

9.1.1 Permitted medication(s)

Not applicable

9.1.2 Prohibited medication(s)

Patients randomized to voclosporin:

- strong CYP3A4 inducers (e.g., ketoconazole, itraconazole, clarithromycin)

When co-administering Lupkynis with moderate cytochrome P450 (CYP)3A4 inhibitors (e.g., verapamil, fluconazole, diltiazem, grapefruit and grapefruit juice), daily dose must be reduced to 15.8 mg in the morning and 7.9 mg in the evening. The concomitant use of voclosporin and grapefruit/grapefruit juice is therefore not prohibited but will be checked by the treating physician.

All patients:

- Before the trial: Eculizumab (1 month), Rituximab (3 months), Belimumab (1 month)
- During the trial: Belimumab, Cyclophosphamide (unless for rescue), Complement inhibitors, NSAID's (first 3 months)

9.2 Lifestyle restrictions

9.2.1 Contraception measures

<u>Definition WOCBP</u>

Women of childbearing potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over age 45 in the absence of other biological or physiological causes.

<u>WOCBP</u>

The AxMP mycophenolate is a powerful human teratogen. Spontaneous abortion (rate of 45% to 49%) and congenital malformations (estimated rate of 23% to 27%) have been reported following MMF exposure during pregnancy. Therefore, Mycophenolate mofetil Teva is contraindicated in pregnancy. Women of childbearing potential must use at least one form of reliable contraception before starting Mycophenolate mofetil Teva therapy, during therapy, and for six weeks after stopping the therapy, unless abstinence is the chosen method of contraception. Two complementary forms of contraception simultaneously are preferred. Birth control methods as proposed in the CTCG clinical trials coordination group *'CTFG recommendations related to contraception and pregnancy testing in clinical trials'* will be proposed by the treating physician.

Before starting Mycophenolate mofetil Teva treatment, women of childbearing potential must have a negative serum or urine pregnancy tests with a sensitivity of at least 25 mIU/mL in order to exclude unintended exposure of an embryo to mycophenolate.

<u>Contraception</u>

During the trial, a highly effective method of contraception will be used. Effective contraceptive measures should be discussed with the patient by weighing the individual risk factors, including general (hypertension, obesity, tobacco use, family history of hormonal-dependent cancers) and disease-related risk factors, particularly disease activity and thrombotic risk (emphasis on antiphospholipid antibodies (aPLs)). Copper IUD can be used in any patient, while levonorgestrel-containing IUD should be considered only if the benefits of the released hormone (such as the reduction of excessive menstrual bleeding due to anticoagulation) outweigh the risk of thrombosis.
The safety of the combined (oestrogen plus progestin) and progestin-only pill in SLE patients with inactive or stable active SLE and negative aPL has been demonstrated in randomised controlled trials (RCTs). In women with *positive aPL* (*with or without definite APS*), contraception with combined hormones (oral pill, vaginal ring, transdermal patch) should be discouraged (18).

Breastfeeding
Limited data show that mycophenolic acid is excreted in human milk. Because of the potential for serious adverse reactions to mycophenolic acid in breast-fed infants, Mycophenolate mofetil Teva is contraindicated in breast-feeding mothers

Men with WOBCP partner
MPA is a powerful teratogen. It is not known if MPA is present in semen. Calculations based on animal data show that the maximum amount of MPA that could potentially be transferred to woman is so low that it would be unlikely to have an effect. Mycophenolate has been shown to be genotoxic in animal studies at concentrations exceeding the human therapeutic exposures by small margins, such that the risk of genotoxic effects on sperm cells cannot completely be excluded. Therefore, the following precautionary measures are recommended: sexually active male patients or their female partners are recommended to use reliable contraception during treatment of the male patient and for at least 90 days after cessation of mycophenolate mofetil. Male patients of reproductive potential should be made aware of and discuss with a qualified health-care professional the potential risks of fathering a child.

If pregnancy does occur, study medication will be stopped as soon as possible and the participant will be excluded from further participation in the study.

9.2.2 Other requirements

Not applicable

10. TRACEABLILITY, STORAGE, ACCOUNTABLILITY AND COMPLIANCE

10.1 Traceability and storage of the study treatment(s)?

Since this trial is a low-intervention clinical trial, routinely maintained pharmacy documentation on receipt storage and handling is ought to be sufficient.

Voclosporin: Keep this medicine out of the sight and reach of children. Do not use this medicine after the expiry date which is stated on the carton and blister after "EXP". The expiry date refers to the last day of that month. Store in the original blister in order to protect from moisture. Do not throw away any medicines via wastewater or household waste. Ask your pharmacist how to throw away medicines you no longer use. These measures will help protect the environment.

10.2 Accountability of the study treatment(s) and compliance

The medication (voclosporin, MMF and prednisolone) will be provided by the clinical and outpatient pharmacy. The pharmacy will be responsible for storage and issuing upon study prescription by the treating physician. Since this trial is open label, patient, investigator and treating physician are aware of treatment allocation. Treatment compliance is monitored by the treating physician by asking the patient during regular follow-up visits and therapeutical drug monitoring of MMF through blood samples if necessary (as is usual when administering MMF). (suspected) Non-compliance should be mentioned in the EPD but is not an exclusion criterium for this trial.

11. STUDY ASSESSMENTS AND PROCEDURES

11.1 Screening procedure

Screening will be conducted in the outpatient clinic of the Amsterdam UMC. Eligible patients (age 16-70) should be diagnosed with de novo or flaring SLE (according to the EULAR/ACR criteria) and there must be a suspicion of LN with a clinical indication to perform a kidney biopsy. eGFR as measured by cystatin C must be >20 mL/min. If the patient wants to participate, informed consent will be signed and the "screening period" of the trial will start, in order to prepare for the admission of medication.

The duration of the screening period is approximately 2 weeks and lasts from the day of the informed consent signing to the day of randomization and start of treatment. The duration of the screening period may fluctuate based on the timing of the planned outpatient clinic visits, but informed consent must be signed before the diagnostic kidney biopsy takes place. During the screening period the treating physician will conduct an infection screen, EKG, lab analysis and pregnancy tests to prepare for treatment as is standard clinical care before starting immunosuppressants for LN, and results will be extracted from the EPD.

When the definitive biopsy result is known and class III or IV (with or without class V) is diagnosed, randomization can commence. The day of randomization is the same day as start of treatment. All screening tests (as mentioned above) must be completed before randomization, this will be checked by the study team. If no LN class III or IV is found upon diagnostic kidney biopsy patients will be excluded from the study. If patients fail to concur with the other in- and exclusion criteria during the screening period they will be excluded as well.

11.2 Randomisation, blinding and treatment allocation

For this study, Castor EDC will be used to assign participants to treatment groups, minimize selection bias and maintain data integrity. Castor EDC is a web based tool used for clinical data management and randomization in clinical trials. The system is cloud based and allows individual users to log in, randomize patients and receive treatment allocation information. All study related events are logged in an audit trail. Randomization cannot be started without filling in and passing all in- and exclusion criteria during the screening period.

Randomization strategy is carried out by Castor EDC and consists of variable block randomization with block sizes 2, 4, 6 based on treatment arm group size. No stratification will be applied. Since this trial is open label, randomization results are openly accessible in the system and will be noted down in the EPD.

11.3 Study procedures and assessments

LN patients

During the follow-up period, all randomized patients will attend routine outpatient clinic visits with their own treating physician. Notes and additional information (vital functions, lab results etc) will be collected and extracted from the EPD after every visit during the follow-up period.

At the following timepoints specific study interventions* will be done:

Start of treatment (day 0)	Week 4	Month 3	Month 6	Month 12	Month 18	Month 24
- SLEDAI-2K questionnaire filled in by physician - LupusPRO questionnaire - PBMC, EDTA, serum and PAX gene blood samples - Urine portion collection - feces sample collection	- SLEDAI-2K questionnaire filled in by physician - LupusPRO questionnaire	- Renal biopsy - SLEDAI-2K questionnaire filled in by physician, - LupusPRO questionnaire - PBMC, EDTA and serum blood samples - Urine portion collection	- SLEDAI-2K questionnaire filled in by physician, - LupusPRO questionnaire - Urine portion collection	- SLEDAI-2K questionnaire filled in by physician - LupusPRO questionnaire - PBMC, EDTA and serum blood samples - Urine portion collection	- SLEDAI-2K questionnaire filled in by physician - LupusPRO questionnaire	- SLEDAI-2K questionnaire filled in by physician - LupusPRO questionnaire - PBMC, EDTA and serum blood samples - Urine portion collection - Feces sample collection

*Since blood draw and urine analysis before every outpatient clinic visit is standard clinical care during follow-up of LN patients during treatment, all study interventions will be merged with regular checkups and all collected samples (extra blood tubes and/or collection of urine and feces) will be combined with regular lab visits in order to limit patient burden.

SLEDAI-2K questionnaire

The SLEDAI-2K (Systemic Lupus Erythematosus Disease Activity Index 2000) (19) is a point based questionnaire to be filled in by the physician for stratifying severity of SLE. Scores will be noted down in the EPD.

LupusPRO questionnaire

The lupusPRO v1.7 questionnaire (20) is a 43-question patient reported outcome survey tool to follow the impact of lupus or its treatment on the patient's quality of life. The questionnaire takes 7-

8 minutes to complete and is validated and available in multiple languages. Patients will be asked to either fill in the questionnaire at home (and bring it on their next outpatient clinic visit) or while visiting the hospital. The questionnaires are filled in on paper.

PAX gene blood samples
At start of treatment 2x4mL PAXgene tubes will be collected during regular blood draw appointments by venapunction. The samples will be stored until kidney tissue single-cell data is processed and used to relate RNA expression of circulating cells to kidney tissue.

PBMC blood samples
At 4 instances (day 0, month 3, month 12, month 24), 50mL PBMC tubes will be collected during regular blood draw appointments by venapunction. PBMC samples will be stored and used to perform flow-cytometric typing and clustering. When kidney tissue single-cell data is processed, flow-cytometry data can be associated with kidney tissue data in order to extract possible disease biomarkers.

EDTA and serum blood samples
At 4 instances (day 0, month 3, month 12, month 24), 9mL EDTA (4mL) and serum (5 mL) blood samples will be collected during regular blood draw appointments by venapunction. These samples will be stored and used to measure circulating cytokines and relate this to kidney tissue single-cell data.

Portion urine samples
Urine samples (portion urine) will be collected at 5 timepoints during follow-up. During regular clinical care, patients will bring in urine for urinalysis, and urine specimen for study storage will be taken from these samples. The samples are stored and spun down for leukocyte isolation and to be related to PBMC flow-cytometry and kidney tissue single cell data to extract possible disease biomarkers.

Feces samples
Participants will be asked to bring in a feces sample at day 0 and at 12 months after starting treatment. This is not part of regular care. Feces samples will be stored to relate to single-cell kidney tissue and urine leukocyte findings.

<u>SLE without LN patients</u>
From the SLE without LN patients (disease control), samples below are collected at two timepoints (PAXgene tubes only once), with an interval of (min) 3 and (max) 6 months between collection. All blood draw instances will be synchronized with regular blood draws during outpatient clinic visits to limit patient burden.

- PAXgene tubes 2x4mL
- PBMC blood samples 50mL
- EDTA and serum blood samples 9mL
- Urine portion

<u>Healthy participants</u>

From the healthy participants (healthy control), samples below are collected at two timepoints (PAXgene tubes only once), with an interval of (min) 3 and (max) 6 months between collection.

- PAXgene tubes 2x4mL
- PBMC blood samples 50mL
- EDTA and serum blood samples 9mL
- Urine portion

11.3.1 Efficacy assessments

During regular care follow-up, the treating physician will evaluate the treatment effect of both treatment arms. Voclosporin SpMC recommends effect evaluation to take place at 24 weeks (6 months). However, treatment effect time varies and patients show great dissimilarities in clinical remission signs, which makes evaluation of LN treatment a dynamic process. Therapy effect is therefore assessed during every outpatient clinic visit and not bound to a specific timepoint.
The treating physician uses eGFR and proteinuria levels to determine (partial) clinical remission or insufficient therapy effect. The guidelines state that there is lack of consensus on the appropriate time for assessing response, and even though large clinical trials often evaluate response at 6-12 months, choosing a specific timepoint for assessment is not possible. In the figure below, definitions for response are proposed, and in the case of unsatisfactory response to treatment, the treating physician may consider switching to an alternative recommended treatment regimen when there is persistent active disease (after verifying adherence to treatment).

Criteria	Definition
Complete response*	• Reduction in proteinuria <0.5 g/g (50 mg/mmol) measured as the PCR from a 24-h urine collection • Stabilization or improvement in kidney function (±10%–15% of baseline) • Within 6–12 mo of starting therapy, but could take more than 12 mo
Primary efficacy renal response	• PCR ≤0.7 g/g (70 mg/mmol) • eGFR that was no worse than 20% below the pre-flare value or ≥60 ml/min per 1.73 m^2 • No use of rescue therapy for treatment failure
Partial response	• Reduction in proteinuria by at least 50% and to <3 g/g (300 mg/mmol) measured as the PCR from a 24-h urine collection • Stabilization or improvement in kidney function (±10%–15% of baseline) • Within 6–12 mo of starting therapy
No kidney response	• Failure to achieve a partial or complete response within 6–12 mo of starting therapy

Figure 11 | Definitions of response commonly used in clinical trials of lupus nephritis. *For children <18 years old, complete response is defined as proteinuria <0.5 g/1.73 m^2 per day or <300 mg/m^2 per day based on a 24-hour urine specimen. eGFR, estimated glomerular filtration rate; PCR, protein–creatinine ratio.

The study team therefore concludes that the assessment of efficacy of treatment is a variable process and highly dependent on the patient and treating physician. Based on earlier clinical trials, the efficacy assessment is proposed from 6 months onwards after starting therapy, and carried out during the outpatient clinic visits by the own treating physician, aided by results from blood draws and urinalysis. The criteria above will be used to classify definitions of response.

The decision to switch or intensify treatment (however allowed during follow-up) is solely based on the opinion of the treating physician and may be done from 6 months after starting treatment and onward.

11.3.2 Safety assessments

To ensure the safety and well-being of all study participants, several safety parameters and assessments have been established:

Safety assessments included in regular clinical care (however related to IMP and AxMP's)

Vital Signs
During each outpatient clinic visit, the treating physician will comprehensively monitor vital signs including blood pressure, heart rate, respiratory rate, and body temperature. Any deviations from the baseline or abnormal readings will be further investigated and if necessary, subsequent intervention will take place.
Blood pressure management is standard during the treatment of LN, but in case of hypertension in the voclosporin arm, a dose reduction or temporary interruption of therapy admission might have to be made based on blood pressure levels.

Blood pressure	**Recommendation**
Systolic pressure > 130 and ≤ 165 mmHg and Diastolic pressure > 80 and ≤ 105 mmHg	Antihypertensive therapy may be initiated/adjusted
Blood pressure > 165/105 mmHg, with symptoms of hypertension	Stop administration of voclosporin and initiate/adjust antihypertensive therapy

Physical Examination
Participants will undergo physical examination during all outpatient clinic visits. This examination will particularly entail:

- Auscultation of the heart to identify any irregularities or murmurs
- Auscultation of the lungs to assess for signs of infection or fluid retention
- Inspection of joints and/or skin
- Specific examination based on patient complaints

Laboratory Assessments
Blood and urine samples will be collected and analyzed to monitor any internal changes or complications. Specifically eGFR, potassium/magnesium, liver parameters and blood cell counts (MMF) will be closely observed and measured. Examples of interventions for abnormal lab values include (but are not limited to):

- Dose reduction of voclosporin when observing a >20% and <30% reduction in eGFR
- Repeating EKG for QT prolongation assessment when finding hypokalemia and or hypomagnesaemia
- Reporting an SAE and notifying the study team (for withdrawal from trial) when finding severe liver impairment as stated in section 13.3.1

- Reducing MMF dose (e.g. to 1500mg/day) when patients are experiencing side effects or when finding high plasma levels

Specific lab assessments for every outpatient clinic visit are not further elaborated in this protocol since they are part of regular clinical care, might vary among patients and visit and are conducted at the discretion of the treating physician.

Study specific safety assessments

Screening period
During the screening period, abovementioned assessments will be conducted in order to prepare for medication admission, as is standard. In addition to the treating physician, the study team will also check the EPD for the following information as an extra safety check before randomization can take place:

- Significant QT prolongation (defined as QTc>470ms)
- Hypertension (blood pressure > 165/105 mmHg, with symptoms of hypertension)
- If applicable: pregnancy (negative serum or urine pregnancy test)
- Remaining exclusion criteria (section 7.3)

In the case of abnormal or missing observations, the physician will be notified and randomization will be postponed.

Adverse Event Monitoring
Any adverse events (AEs) reported or observed will be diligently documented. This will include the nature, severity, onset, duration, and outcome of the AE. Necessary action will be taken based on the severity of the event.

12. STUDY DISCONTINUATION AND COMPLETION

12.1 Definition End of Trial

End of Trial is defined as the last visit of the last subject (at 24 months).

12.2 Criteria for temporary halt and early termination of the clinical trial

Since the IMP and AxMP's are shown to be safe it is unexpected that the study will need to be terminated prematurely. In the hypothetical case of an unacceptable rate of SAEs (i.e., in >20% of participants after including at least 10 patients), or otherwise as deemed justified by the study team, subsequent actions will be discussed among members of the research team. In such situation, according to art 37 of the CTR, the sponsor will report the temporary halt of the study in CTIS within 15 days. In case the temporary halt is for reasons of subject safety, then the sponsor will report this in CTIS without undue delay but not later than in 15 days. The reasons for such action will be included as well as the follow-up measures. If the reason for the unexpectedly high SAE rate cannot be found or solved, the trial will be terminated early.

Reasons for non-feasibility of the trial are 1) insufficient (<50%) participant inclusion by January 2029 and 2) an excessive percentage of participants (>75%) that cannot be randomized after the screening period in combination with low inclusion rate (<1 Inclusion in the running in phase per 3 months). Feasibility of the trial will be discussed at least annually in consortium progress meetings. In the case of suspected non-feasibility of the clinical trial, early termination of the trial will be discussed among members of the research team.

12.3 Discontinuation/withdrawal of individual subjects

In the event of pregnancy, end-stage kidney failure (defined as need for renal replacement therapy), serious hepatotoxicity (an elevation in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) that is ≥ 3 times the upper limit of normal (ULN) and total bilirubin is ≥ 2 times the ULN) or a serious allergic reaction, participants will discontinue the study.

Included patients that do not meet all of the inclusion criteria during the screening period will not be randomized and are excluded from further study participation.

Subjects can leave the study at any time for any reason if they wish to do so without any consequences.

12.4 Arrangements for subjects after their participation in the clinical trial ended

Not applicable

13. SAFETY REPORTING

13.1 Definitions

13.1.1 Adverse events (AEs)

Adverse events are defined as any untoward medical occurrence in a subject to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment.

13.1.2 Serious adverse events (SAEs)

Serious adverse event is any untoward medical occurrence in a patient or trial subject that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect

13.1.3 Suspected unexpected serious adverse reactions (SUSARs)

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. The event must be serious;
2. There must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. The adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in the reference safety information (RSI).

13.2 Recording of AEs/SAEs/SUSARS

(S)AEs

The investigators will record and document all adverse events and report all SAEs to the sponsor. The reporting of SAEs to the sponsor will be done without undue delay but not later than within 24 hours of obtaining knowledge of the events, unless, for certain SAEs, the protocol provides that no immediate reporting is required. Individual SAEs, except for SUSARs, will not be reported in CTIS or Eudravigilance. The sponsor includes an aggregated table with all SAEs in the Annual Safety Report (ASR) which will be submitted in CTIS.

SUSARs

A SUSAR is a serious adverse reaction (SAR) of which, the nature, severity or outcome is not consistent with the reference safety information (RSI). All SUSARs to investigational medicinal products or related to the same active substance occurring in a clinical trial from the same sponsor or another sponsor (part of the same parent company or joint development of medicinal product), should be reported electronically through Eudravigilance by the sponsor or another party on basis of a formal agreement with the sponsor of the trial.

13.3 Reporting of AEs and SAEs

13.3.1 Reporting of SAEs by the investigator to the sponsor

Since the IMP and AxMP's are marketed products with a known safety profile and used according to the conditions of the marketing authorization, a reduced safety data collection is thought to be appropriate.

Due to the severity (and subsequent withdrawal from the trial if occurring) events below are to be reported to the sponsor:

- Serious hepatotoxicity (an elevation in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) that is >3 times the upper limit of normal (ULN) and total bilirubin is >2 times the ULN) or a serious allergic reaction
- Serious allergic reaction

The investigator shall record and document all adverse events and report all SAEs to the sponsor, unless the protocol provides differently. The reporting of SAEs to the sponsor has to

be done without undue delay but not later than within 24 hours of obtaining knowledge of the events, unless, for certain SAEs, the protocol provides that no immediate reporting is required.

13.3.2 List of SAEs which do not require immediate reporting and procedure for reporting

- Hospitalizations for a pre-existing condition, unrelated to the study intervention
- Elective surgeries: surgeries or medical procedures planned prior or during the tria
- Disease progression: expected or unexpected progression of a pre-existing disease, or reoccurrences of a chronic disease, such as flare-ups or exacerbations, unrelated to the study intervention
- Death due to pre-existing terminal illness: in situations where participants have a pre-existing terminal condition and death is an expected outcome unrelated to the study intervention

13.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

13.5 Reporting of SUSARs by the sponsor to EudraVigilance

The sponsor will keep detailed records of all AEs which are reported to him/her by the investigator or investigators (CTR: Article 41(3)).

The sponsor will report electronically and without delay to EudraVigilance all relevant information about any SUSAR (CTR: Article 42).

The period for the reporting of SUSARs by the sponsor to EudraVigilance will take account of the seriousness of the reaction and will be as follows:

- In the case of fatal or life-threatening SUSARs, as soon as possible and in any event not later than **7 days** after the sponsor became aware of the reaction (CTR: Article 42(2(a))];
- In the case of non-fatal or non-life-threatening SUSARs, not later than **15 days** after the sponsor became aware of the reaction (CTR: Article 42(2(b))];
- In the case of a SUSARs which was initially considered to be non-fatal or nonlife threatening but which turns out to be fatal or life-threatening, as soon as possible and in any event not later than **7 days** after the sponsor became aware of the reaction being fatal or life-threatening (CTR: Article 42(2(c))).

Where necessary to ensure timely reporting, the sponsor may, in accordance with section 2.4 of Annex III, submit an initial incomplete report followed up by a complete report (CTR: Article 42(2)).

13.6 Annual safety report

Regarding investigational medicinal products other than placebo, the sponsor shall submit annually through CTIS to all Member States concerned a report on the safety of each investigational medicinal product used in a clinical trial (CTR: Article 43).

13.7 Unblinding procedures for safety reporting

Not applicable

13.8 Temporary halt for reasons of subject safety

The sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will submit the notification through CTIS without undue delay of a temporary halt but not later than in 15 days of the date of the temporary halt. It shall include the reasons for such action and specify follow-up measures. The study will be suspended pending a further positive decision by the concerned member state (CTR: Article 38). The investigator will take care that all subjects are kept informed.

13.9 Urgent safety measures and other relevant safety reporting

Where an unexpected event is likely to seriously affect the benefit-risk balance, the sponsor and the investigator will take appropriate urgent safety measures to protect the subjects. In addition, the sponsor will notify the Member States concerned, through CTIS, of the event and the measures taken. That notification will be made without undue delay but no later than **7 days** from the date the measures have been taken (CTR: Article 54).

13.10 Data Safety Monitoring Board (DSMB)/Data Monitoring Committee (DMC)

An independent data monitoring committee (IDMC/DSMB) will be established for this trial, if deemed necessary by the local EC. This committee will meet to ensure the safe and ethical treatment of trial participants, ensure the scientific integrity of the trial, and ensure the trial is conducted within the bounds of ethical medical practice. It may make recommendations to the sponsor and trial steering committee to amend or terminate the trial based on grounds of safety, futility, or greater than expected efficacy as defined by the accepted statistical practices and procedures to be detailed in their charter. The specific duties of the IDMC will be detailed in a separate IDMC charter document.

14. STATISTICAL ANALYSIS

14.1 Description of statistical methods

A detailed description of all statistical methods is described below in section 14.6.1 and section 14.6.2.

14.2 Analysis sets

Intention-to-Treat Population: this will encompass all included participants who received at least one dose of the study medication, irrespective of whether they received any further treatment or adhered to the protocol.

Per-Protocol Population: this includes only those participants who completed the trial and strictly adhered to the protocol, excluding any significant deviations or violations.

Control population: this includes all participants from both control groups (disease and healthy controls).

14.3 Participant demographics and other baseline characteristics

In addition to the above-mentioned parameters in section 11.3, we will collect patient data at baseline, including medical history, demographic data and current use of medication from the EPD. These findings will be summarized for each treatment group by presenting frequency distributions and descriptive statistics.

14.4 Randomisation and blinding

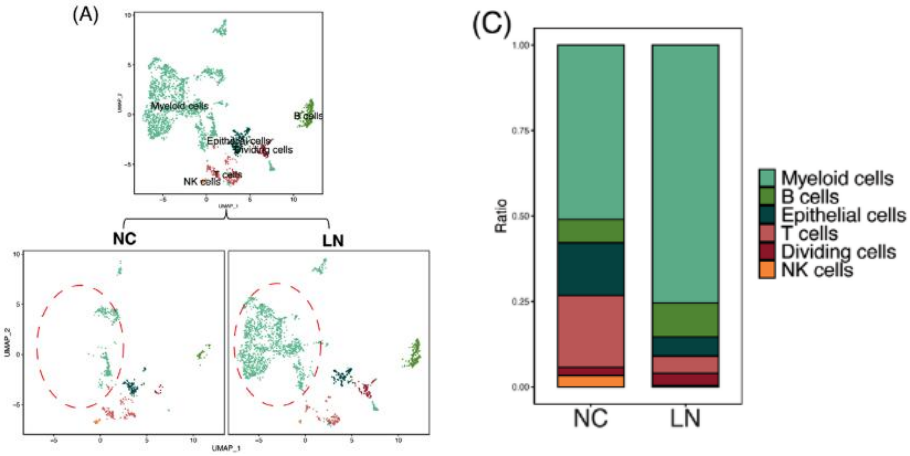
Patients participating in the study will be randomly assigned to receive either dual or triple therapy in an 1:1 allocation ratio. To ensure unbiased selection and maintain data integrity, Castor EDC will be used to assign subjects to their respective treatment groups. Castor EDC supports various randomization methods, and for this study, we will use variable block randomization without stratification with block sizes 2, 4, 6 based on treatment arm group size.

14.5 Sample size, trail power and level of significance used

Since scRNA-seq differences within the full macrophage spectrum (as stated in the primary endpoint) is a multifaceted goal, two power calculations were executed:

<u>Achievement of 'healthy' myeloid cell proportions at 3 months</u>

To date, no studies have directly compared immune cell populations in lupus nephritis (LN) patients before and after treatment. However, Tang *et al.* compared immune cell compositions in LN kidney tissue with healthy kidney tissue using single-cell RNA sequencing (21). In that study, the proportion of myeloid cells was expanded from approximately 50% of all immune cells in healthy kidney tissue (normal controls, NC) to roughly 75% in LN kidney tissue.



We hypothesize that, due to the additional effects of voclosporin on myeloid cell populations, 80% of patients receiving triple therapy will achieve a kidney myeloid cell proportion comparable to that observed in healthy kidney tissue at 3 months. In contrast, as this additive effect is not expected in the dual therapy group, we anticipate that only 30% of patients in this group will reach healthy myeloid cell proportions. Based on these assumptions, the estimated effect size (difference in proportions) is 0.5. With a power of 80% and a two-sided alpha level of 0.05, the required sample size is calculated to be approximately 14 patients per group.

Assessment of differences in immune cell proportions and gene expression levels at 3 months

There are no bulk transcriptomics data to draw from to perform adequate power calculations for gene expression levels. Concerning scRNA-seq, both Arazi et al. (24 kidney biopsies) (6) and Der et al. (21 kidney biopsies) (22) did not compare treatment differences. With the chosen number of samples we strike a balance between what in our experience is minimally needed for transcriptomics studies on treatment differences in patients and the number of patients that can realistically be included in this study. Note that we aim for explorative endpoints mainly, that may inform future clinical trials. Obtaining a detailed dataset may identify important differences between dual and triple treatment in specific cell types. For multi-sample single-cell transcriptomic experiments as proposed here a proper power analysis is challenging. Because of the high costs associated with this experiment, we nevertheless used a recently developed method (scPower;Schmid et al. (23)), which provides power estimates based on reference-derived priors for cell type–specific expression and for effect size distributions.

Power calculations were conducted using parameters that most closely reflect our single-cell experiment. Specifically, we selected “kidney” as the reference tissue and “macrophage” as the cell type of interest, and evaluated two scenarios representing different average effect sizes for differential gene expression, assuming 50 differentially expressed genes uniformly sampled from the top 5,000 genes ranked by expression (from high to low):

1. Neutral scenario: average fold change of 1.5, standard deviation 0.5, and macrophage proportion 0.1.
2. Optimistic scenario: average fold change of 2.0, standard deviation 0.5, and macrophage proportion 0.2.

In the neutral scenario, power estimates with 15 patients per arm were 69.1% when measuring 1,000 immune cells per sample and 78.4% when measuring 2,500 immune cells per sample. In the optimistic scenario, power increased to 93.8% and 95.9%, respectively.

Additional remarks: scPower controls the false positive rate in two complementary ways. First, it performs differential expression analysis on pseudobulk data, which approximates cell type–specific gene expression for each individual by summing counts across all cells of a given cell type. Second, it applies multiple testing correction across all genes (25,000 in our calculations) using the Benjamini–Hochberg false discovery rate. In addition to macrophages, differential gene expression analysis will also be performed for other major immune cell types. Power analyses for B cells, $CD4^+$ T cells, and $CD8^+$ T cells yielded estimates similar to those obtained for macrophages. It should be noted that in single-cell differential expression studies, multiple testing is generally controlled at the gene level rather than at the cell type level, since the number of genes tested is several orders of magnitude larger. Output of ScPower’s raw data for both scenarios is added as excel document in the RFI comment.

Resultantly, determining exact sample sizes for control groups is equally challenging due to limited prior data and the exploratory nature of this study. We selected 15 disease controls to closely match the total number of treated patients per treatment arm, enabling meaningful comparisons to identify treatment effects beyond disease-related changes. For the healthy controls, we propose 10 participants, which provides a sufficient baseline reference while balancing recruitment feasibility and expected lower variability.

14.6 Planned analysis

14.6.1 Analysis primary endpoint

The primary study endpoint is:

1. to determine there are scRNA-seq differences within the full macrophage spectrum between arm 1 vs arm 2 when comparing baseline kidney biopsy and kidney biopsy after 3 months of treatment

Endpoint 1

After processing of kidney biopsies, a 5um section will be analyzed for RNA quality and further processed for spatial single-cell RNA sequencing analysis using Nanostring technology or HD visium (10X genomics) technology.

After receiving mRNA counts, cells need to be annotated and the data need to be aligned. Demultiplexing of raw base call files and conversion to FASTQ files is performed with the Cell Ranger pipe-line (10XGenomics) using 'cellranger mkfastq'. Alignment to the human reference genome, read filtering, barcode correction, UMI counting and filtering of empty barcodes is performed using 'cellranger count' to generate feature counts for every single cell. Doublets are detected using DoubletFinder and excluded. Normalization and variance stabilization is performed using regularized negative binomial regression (Seurat, function SCTransform). Selected principal components are used in non-linear dimensionality reduction using UMAP and graph-based clustering at various resolutions. Cell type annotation is performed both 'manually', using appropriate clusters and cluster marker genes, and 'automat-ically', using a pre-trained CellTypist model for detailed annotation of immune cells (https://www.celltypist.org/models;Immune_All_Low). Data are annotated by mapping it to relevant reference datasets. Where appropriate biological replication is taken into account using pseudobulk-based DE methods.

First, myeloid cell subsets will be compared numerically and proportionally between treatment arms using the recently developed linear modelling approach *propeller (24)*. *Propeller* takes biological replicates into account, adjusts for additional covariates by including them in the linear model, and has shown good control of type I errors and power to detect differences in cell type proportions.

Second, gene expression levels per subset will be compared between treatment arms using a linear modelling approach. Recent studies showed that the standard approach of performing differential expression analysis at the cell level, used in single-cell analysis pipelines Seurat and

Scanpy, leads to an inflated false positive rate (25). Therefore, we will first aggregate the expression per replicate in each cell subset to construct so-called pseudobulk samples. Differential expression analysis of pseudobulk samples will be done using the linear modelling approach limma, one of the state-of-the-art methods for analyzing RNA-seq data (26), enabling adjustment for covariates. This approach will be used to investigate the association between the gene expression levels in macrophage/monocyte subsets and clinical/histological data and differences of these associations between treatment arms.

The following analyses will be performed:

- Primary analysis: intention-to-treat analysis of the primary endpoint (differences in cell type proportions and gene expression) in dual vs triple therapy
- Secondary analysis: per-protocol analysis of the primary endpoints (differences in cell type proportions and gene expression) in dual vs triple therapy

Missing data (less than 10%) will not be replaced by estimates. If a higher percentage of missing data is present, this will be handled using multiple (best and worst case) imputation methods to ensure that the statistical analysis remains unbiased. To assess the robustness of our primary results and the influence of various assumptions about the missing data, sensitivity analysis will be performed. This sensitivity analysis will involve comparing the results of a complete case analysis, which uses only cases with no missing data, with those obtained from the imputed dataset.

Given the two analytical approaches mentioned (intention-to-treat and per-protocol), it's essential to account for potential multiplicities. We will employ the FDR (false discovery rate, Benjamini-Hochberg) to account for multiple testing to adjust the significance level. By doing so, we will reduce the chances of type I errors.

14.6.2 Analysis secondary endpoint(s)

The secondary study endpoints are:

1. numerically distinguish residential macrophages and monocyte-derived macrophages in kidney tissue with spatial scRNAseq, comparing arm 1 vs arm 2;
2. to analyze rapid clinical remission of intensified treatment by proteinuria levels
3. to assess histological response of intensified treatment (arm 1) compared to SOC (arm 2);
4. to assess if early histological response is associated with clinical remission after two years and identify innovative early response determinants (including tissue monocyte/macrophage phenotyping)
5. to identify differences between LN, SLE and healthy subjects in peripheral monocyte phenotype by using bulk RNA sequencing and flowcytometric analyses, to be associated with scRNAseq data and clinical/biochemical parameters
6. To identify potential non-invasive urinary biomarkers of disease activity in the scRNA-seq data, to be tested in sequentially stored urine and/or serum from patients
7. attempt to render (repeat) kidney biopsy in LN obsolete by identifying reliable serum and/or urinary biomarkers that reflect tissue damage and treatment response;
8. confirm scRNA-seq identified macrophage markers immunohistochemically, identify spatial distribution (glomerular vs interstitial) and assess their predictive and prognostic use.

Secondary endpoint 1
Please refer back to the methods described under endpoint 1 in section 14.6.1

Secondary endpoint 2
For analyzing rapid clinical remission of intensified treatment based on proteinuria levels, time to complete response, defined as a reduction in proteinuria <0.5g/g (50mg/mmol) measured as the PCR from 24-hour urine collection, stabilization or improvement of kidney function (±10%–15% of baseline) within the follow-up duration. This is a time to event variable, and survival analysis will be used for assessing differences between groups. Kaplan-Meier curves will be used to visualize time-to-event data in each group and Cox's proportional hazards model will be used to assess the significance of the differences between treatment groups.

Additionally, when using complete response at end of follow up (24 months) as secondary endpoint, analysis can be done using a logistic regression model in order to model the odds of complete clinical response at end of follow-up.

Secondary endpoint 3
Histological response in the kidney tissue will be qualified using the most recent ISN/RPS (International Society of Nephrology/Renal Pathology Society) 2018 (27) classification. Baseline and repeat kidney biopsy will be assessed by a nephropathologist and total activity and chronicity indices (AI and CI) will be predicated in the pathology report. Additionally, biopsies will be scored base on percentage of tubulointerstitial, mesangial and capillary disease either represented by a percentage of affected tissue or absence/presence of lesions. This data is either numerical (discrete, non-normal distributed) or nominal (multi-category or binary). For assessing therapy effect specifically, the Δ AI and CI will be calculated. Comparing pathology reports, assessing differences per treatment arm and reporting significance will be done using medians and percentages using non-parametric tests: Spearman's for associations, Mann-Whitney U for comparing medians of two groups and Kruskal-Wallis for comparing medians of more than 2 groups.

Secondary endpoint 4
Early histological response will be defined by changes in the National Institutes of Health (NIH) Activity Index (AI), with response indicated by a decrease of ≥50 % and to ≤3 as proposed in a recent systematic literature review for determining definitions for histological treatment response in repeat biopsies in LN (28). Both early histological response and clinical remission after two years of follow-up are nominal (binary) and associations will be made using Spearman's correlation tests. If significant correlations are found, single-cell sequencing data can be consulted to assess if there are consequent associations between early histological response and specific monocyte/macrophage phenotype at baseline.

Secondary endpoint 5
Flow cytometric data (mean fluorescent intensity (MFI), continuous) of intended monocyte subpopulations will be calculated. Differences between groups will be assessed using either one-way ANOVA or Kruskal Wallis test based on distribution. Post hoc testing will be done using Tukey's or Dunn's post hoc test. For differences at different timepoints (e.g. at baseline

and 3 months after starting treatment) ΔMFI can be computed and compared with prior mentioned tests based on normality.

Associations between MFI in different groups and clinical parameters (e.g. eGFR, proteinuria, etc.) will be assessed using Spearman's test. Significantly increased MFI of specific monocyte subpopulations in specific groups can be cross-checked using the single-cell data by comparing differences in cluster proportions (percentages, continuous) between kidney tissue and peripheral blood monocytes using Mann-Whitney U test (assuming non-normal data distribution) with logit transformation if necessary. When testing multiple clusters, p-values will be adjusted for multiple testing.

Secondary endpoint 6 & 7
These endpoints are primarily explorative and highly dependent on previously collected data and tests. In the case a potential biomarker (e.g. peripheral circulating monocyte with specific surface protein expression) is extracted from single-cell sequencing and flowcytometric data, stored urine and peripheral blood samples of LN patients and control groups will be scanned for presence of the biomarker. Flowcytometric data (MFI, continuous) will be compared in both intervention and control groups and differences will be assessed using Kruskal-Wallis test (since we expect non-normally distributed data when assessing MFI between groups), and post-hoc testing using Dunn's.

Since the intended sample size of this study is small and the secondary endpoints are more focused on biomarker discovery instead of validation, validation of diagnostic and/or predictive performance using an ROC curve may be attempted however might be better suited to a separate study.

Secondary endpoint 8
When scRNA-seq identified macrophage markers (e.g. specific surface proteins) have been assessed during the first primary endpoint, these will be used as target antigen for color labelled antibodies. Biopsy staining will be semi-automatically assessed with Fiji (NIH) (29), an image processing package based on ImageJ2 software (NIH) (30). Glomeruli are either manually or automatically outlined around Bowman's capsule to indicate the glomerular compartment. Positively stained area will be quantified and expressed as the % of the total glomerular and tubulointerstitial area's (numerical, continuous).
Gathered data will be compared to the spatial single-cell data using Spearman's correlation test to check for a monotonic relationship between both methods, and if significant association is found, Bland-Altman analysis will be done to check agreement and/or bias.

If immunohistochemical analysis is deemed viable and equal to single-cell sequencing for the specific macrophage marker, further analysis may be done in order to assess the predictive and prognostic value (using ROC curve for evaluating the measurement as diagnostic test or Cohen's kappa for improving inter observer variability in pathologists). However, it is unknown if such results will be found, and the explorative value is deemed to be the more important endpoint than validation.

14.6.3 Analysis other study parameters/endpoints

Not applicable

14.7 Interim analysis

Not applicable

14.8 (Statistical) criteria for termination of the trial

If the rate of participant enrolment is significantly below the scheduled enrolment rate and is not anticipated to recover, this may lead to inadequate power to detect a clinically meaningful difference. If there are no strategies identified that can improve the rate to meet the required sample size within the proposed study duration, then the trial may be considered for termination.

14.9 Procedure for accounting for missing, unused and spurious data

We will review data to identify missing values, outliers, and spurious entries. This task will be facilitated by data validation checks and automated alert systems specifically designed to flag potential issues for an immediate review. Where data appears to be missing, efforts will be made to document the reasons, whether they stem from participant withdrawal, instances of being lost to follow-up, or any technical challenges. In scenarios where data is identified as unused or spurious, the initial approach will be to trace back to the source or identify the root cause of such discrepancies. For handling missing data, multiple imputations will be applied. Unused or spurious data will be cross-checked with the original source. Valid errors will either be corrected if the actual value is known or otherwise classified as missing. To ensure transparency, the final study report will detail the management of missing or erroneous data.

14.10 Procedure for reporting any deviation(s) from the original statistical plan

Any deviation(s) from the original statistical plan will be described and justified in the updated study protocol and/or the final report, as appropriate.

15. ETHICAL CONSIDERATIONS

15.1 Declaration of Helsinki

The study will be conducted according to the principles of the Declaration of Helsinki (version 2024) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

15.2 Recruitment and informed consent procedures

Informed consent will be obtained prior to any study related procedures being undertaken at screening. Informed consent will be written, dated and signed by the person performing the interview, and by the subject.

Possible eligible patients (LN patients and SLE without LN controls) will be asked by their treating physician if they would be interested in participating in the study. The treating phycisian is never the investigator. When interested, the investigator or his/her representative will be notified by the physician to explain the nature of the study to the subject, distribute the PIF and answer all questions regarding the study. In the interview it will be verified that the subject has understood the information. The subject will be provided with a copy of the document (or the record) by which informed consent has been given. The informed consent will be documented. Adequate time will be given for the subject to consider his or her decision to participate in the clinical trial (CTR: Article 29).

Possible eligible healthy volunteers will be recruited through the distribution of posters and flyers. In accordance with the Amsterdam UMC Research Code, direct colleagues will not be included as healthy participants. When interested to participate, volunteers can choose to contact the investigator through phone or email. The investigator or his/her representative will explain the nature of the study, distribute the PIF and answer all questions regarding the study. In the interview it will be verified that the subject has understood the information. The subject will be provided with a copy of the document (or the record) by which informed consent has been given. The informed consent will be documented. Adequate time will be given for the subject to consider his or her decision to participate in the clinical trial.

15.3 Benefits and risks assessment, group relatedness

Voclosporin in combination with MMF and prednisolone, and MMF/prednisolone without voclosporin are internationally recommended for treatment of proliferative (class III and IV) LN and are deemed safe when initiated and supervised by a qualified physician experienced in the diagnosis and treatment of lupus nephritis.

Most common adverse reactions of voclosporin are decreased eGFR (26.2 %) and hypertension (19.1 %). The most frequently reported serious adverse reactions with use of voclosporin are infections (10.1 %), acute kidney injury (3 %) and hypertension (1.9 %). In the first 4 weeks of treatment with voclosporin, haemodynamic reductions in eGFR are commonly experienced, which subsequently stabilize, even if treatment is continued.

Routine risk minimization measures are described in the SmPc and include advice to avoid direct UV light and regular monitoring for risk of infection, eGFR, risk of pure red cell aplasia (PRCA), serum potassium levels and blood pressure. Since the chance of potential risks is considered low and participants are closely monitored during regular follow-up visits (during which vital functions and lab results are consistently checked as is standard), adding voclosporin in the intervention group is deemed justified and poses no additional risks for patients participating in this study.

An additional biopsy will be performed 3 months after initiation of treatment to evaluate histological response and to conduct spatial single-cell RNA sequencing (scRNAseq) analysis. While renal biopsy is associated with a recognized risk of bleeding (estimated at approximately 1 in 1,000 procedures), this risk is influenced by factors such as concomitant anticoagulant use, anemia, and impaired renal function with elevated urea levels. Importantly, repeat biopsies are often a routine part of clinical management in lupus nephritis, particularly in cases of new active urinary sediment, rising serum creatinine, or worsening proteinuria during treatment, as no reliable biomarker currently exists to monitor therapeutic response or disease progression.

15.4 Compensation for injury

The sponsor has an insurance that is in accordance with the legal requirements in the Netherlands (Article 7 WMO, under 1). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

The sponsor/investigator has a liability insurance that is in accordance with article 7, under 9, of the WMO.

15.5 Compensation for subjects

Subjects will receive compensation for participating in the study based on estimated impact and

LN patients
Participating LN patients will receive €150 for their involvement in the trial. Since patients only attend their routine clinical care visits and no additional hospital visits are required, reimbursement for travel expenses is not applicable. The monetary payment specifically compensates participants for the additional renal biopsy procedure, blood vials, feces/urine samples and questionnaires mandated by the study. This amount is intended to acknowledge the time, effort, and inconvenience involved, while remaining modest enough to avoid serving as an undue external motivation that could influence voluntary participation.

SLE without LN controls
Participating SLE patients without LN will be asked for additional blood samples during their regular clinical follow-up and blood draw appointments. Since this involves no extra visits or procedures—only filling extra tubes during routine blood draws—no monetary compensation is proposed for this group.

Healthy controls
Since participating healthy controls are required to travel to and from the hospital on two separate occasions for blood draws and urine samples, reimbursement of travel expenses and/or parking costs was deemed sufficient compensation.

15.6 Compensation for investigators

The sponsor received funding for this trial (Category 3).

15.7 Other ethical considerations

Information regarding the race and ethnicity of patients with lupus nephritis (LN) will be obtained EPD. Race and ethnicity data are self-reported by patients and categorized such that each patient is assigned to a single category. The study team recognizes both the sensitivity of collecting race and ethnicity information and its critical importance for this study. Racial and ethnic disparities in LN are

well-documented; notably, non-white populations exhibit a higher incidence of LN and experience a more severe disease course and poorer prognosis compared to white populations(31, 32). Therefore, including race and ethnicity as variables is critical for understanding disease patterns and outcomes within the study population.

16. ADMINISTRATIVE ASPECTS, MONITORING AND CONFIDENTIALITY

The study will be conducted in strict compliance with the protocol, Clinical Trials Regulation No 536/2014, and the principles of good clinical practice.

16.1 Approval initial application and substantial modifications

The trial protocol, informed consent form, subject information leaflet, investigational medicinal product dossier, investigators brochure and any other documents required by the Regulation will be submitted for the regulatory approval before the clinical trial is started via CTIS.

The sponsor will also submit and obtain approval for substantial modifications to the original approved documents via CTIS.

A ‘substantial modification’ is defined in the CTR as any change to any aspect of the clinical trial which is made after notification of a decision referred to in Articles 8, 14, 19, 20 or 23 and which is likely to have a substantial impact on the safety or rights of the subjects or on the reliability and robustness of the data generated in the clinical trial.

16.2 Monitoring

The sponsor will assign an independent study monitor who will be responsible for overseeing various aspects of the clinical trial. This includes monitoring the informed consent process of participants, ensuring the accuracy of data in the raw data database, reporting adverse events, and verifying the recruitment procedure adheres to the specified inclusion and exclusion criteria.

Based on the NFU guidelines and our assessment of the low risk involved for participants, the study monitor will conduct monitoring visits the Amsterdam UMC. The informed consent forms will be verified for one to ten percent of the participants. Additionally, the inclusion and exclusion criteria for the first three participants will be examined, followed by verification for one to ten percent of the subsequent participants. Furthermore, the study monitor will assess the source data and the reporting of adverse events for one to ten percent of the participants. The extent and nature of this monitoring process will be determined by the sponsor, taking into consideration all characteristics of the clinical trial. A detailed monitoring plan, as outlined in CTR: Annex I D17ad, will be referred to for further specifics regarding the planned monitoring of the trial's conduct. This comprehensive approach will help ensure the trial is conducted with the highest standards of quality, safety, and adherence to the study protocol.

16.3 Recording, handling and storage of information

16.3.1 Handling of data and data protection

A Clinical Data Management System will be used that meets the General Data Protection Regulation (EU) 2016/679. Direct data entry will be done after all regular outpatient clinic visits. A validated database management system (CastorEDC) will be used to provide data management, data entry, participant survey and data validation functionalities. Data will be handled confidentially using a unique code. A subject identification code list will be used to link the data to the subject. The research team has access to the source data. The key to the code will be safeguarded by the PI. Data will be kept for at least 25 years.

In the event of a data breach, this will be reported to the competent authorities and data subjects within the time specified in accordance with GDPR. All measures will be taken to prevent further damage/spread.

16.3.2 Source documents and case report forms (CRF)

Source documents for this study will include hospital records (EPD), filled in questionnaires, procedure reports and data collection forms. These documents will be used to enter data on the CRFs. Data reported on the CRF that are derived from source documents must be consistent with the source documents or the discrepancies must be explained. All documents will be stored safely in confidential conditions. On all study-specific documents other than the signed consent, the subject will be referred to by the study subject identification code.

16.3.3 Clinical trial master file and data archiving

The sponsor and the investigator shall keep a clinical trial master file. The clinical trial master file shall at all times contain the essential documents relating to the clinical trial which allow verification of the conduct of a clinical trial and the quality of the data generated (CTR: Article 57).

The sponsor and the investigator shall archive the content of the clinical trial master file for at least 25 years after the end of the clinical trial, unless other EU law requires archiving for a longer period. The medical files of subjects shall be archived in accordance with national law (CTR: Article 58).

The content of the clinical trial master file shall be archived in a way that ensures that it is readily available and accessible, upon request (CTR: Article 57).

The clinical trial master file (CTMF) will be securely stored in a locked cabinet with a safe code to ensure restricted access. The cabinet will be located in a designated secure area at the sponsor's headquarters or a specified facility, complying with all relevant regulatory requirements and guidelines.

16.3.4 Collection and storage of biological samples

Samples will be stored for 5 years after the end of the study. If the participant signed the ICF for the biobank, samples will be stored for 50 years. Samples will be coded with a specific participant number, not traceable to the participant unless with the study subject identification code list.

16.4 Audits and inspections and direct access to source data/documents

This trial may be subject to internal or external monitoring, auditing or inspections procedure to ensure adherence to GCP. Access to all trial-related documents including direct access to source data will be given at that time.

16.5 Reporting of serious breaches

The sponsor will notify the Member States concerned about a serious breach of the Regulation or of the version of the protocol applicable at the time of the breach through CTIS without undue delay but not later than **seven days** of becoming aware of that breach (CTR: Article 52).

16.6 Notification of the start and the end of the recruitment

The sponsor will notify within 15 days each Member State concerned of the start of a clinical trial in relation to that Member State through CTIS (CTR: Article 36(1)).

The sponsor will notify within 15 days each Member State concerned of the first visit of the first subject in relation to that Member State through CTIS (CTR: Article 36(2)).

The sponsor will notify within 15 days each Member State concerned of the end of the recruitment of subjects for a clinical trial in that Member State through the EU (CTR: Article 36(3)).

16.7 Temporary halt/(early) termination

The sponsor will notify within 15 days each Member State concerned of the end of a clinical trial in relation to that Member State through CTIS (CTR: Article 37(1)).

The sponsor will notify within 15 days each Member State concerned of the end of a clinical trial in all Member States concerned and in all third countries in which the clinical trial has been conducted through CTIS (CTR: Article 37(3)).

16.7.1 Temporary halt/early termination for reasons not affecting the benefit-risk balance

The sponsor will notify with 15 days each Member State concerned of a temporary halt of a clinical trial in all Member States concerned for reasons not affecting the benefit-risk balance through CTIS (CTR: Article 37(5)).

When a temporarily halted clinical trial for reasons not affecting the benefit-risk balance is resumed the sponsor will notify each Member State concerned through CTIS (CTR: Article 37(6)).

The sponsor will notify to the EU portal CTIS of early termination of the clinical trial for reasons not affecting the benefit-risk balance through CTIS. The reasons for such action and, when appropriate, follow-up measures for the subjects will be provided as well (CTR: Article 37(7)).

16.7.2 Temporary halt/early termination for reasons of subject safety

In accordance to article 38 of the CTR, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The temporary halt or early termination of a clinical trial for reasons of a change of the benefit-risk balance will be notified to the Member States concerned through the EU portal CTIS without undue delay but not later than in 15 days of the date of the temporary halt or early termination. It shall include the reasons for such action and specify follow-up measures. The restart of the clinical trial following a temporary halt as referred to in paragraph 1 shall be deemed to be a substantial modification subject to the authorisation procedure laid down in Chapter III of the CTR (CTR: Article 38).

16.8 Summary of the results

Within one year from the end of a clinical trial in all Member States concerned, the sponsor will submit to the EU database CTIS a summary of the results of the clinical trial. The content of the summary of the results is set out in CTR Annex IV. It shall be accompanied by a summary written in a manner that is understandable to laypersons. The content of the summary is set out in CTR Annex V (CTR: Article 37(4)).

16.9 Public disclosure and publication policy

As per the CCMO publication Statement, we will share the study results after conducting statistical analyses. Our goal is to publish the findings in a peer-reviewed scientific journal within a reasonable timeframe, irrespective of the outcomes. Additionally, certain secondary outcomes will be published separately. Alongside the publication in scientific journals, we plan to present the data at international congresses, ensuring widespread dissemination of the study's outcomes.

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