Supplementary File

Efficacy and Safety of Iptacopan in Patients with C3 Glomerulopathy

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This file has been provided by the authors to give readers additional information about the work.

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Additional Methodology Details

Key Inclusion Criteria

1 Patients (aged ≥18 years, body mass index 18–38 kg/m^2 with systolic and diastolic blood pressure of 80–170 mm Hg and 50–105 mm Hg, respectively) with complement 3 glomerulopathy (C3G), confirmed by biopsy within 12 months prior to enrollment, with reduced C3 at screening (< 0.90 x lower limit of normal) in the case of the native cohort or those with C3G recurrence after transplant (confirmed by biopsy within 12 months prior to enrollment) in case of the recurrent KT cohort were eligible for the study

2 Patients with estimated glomerular filtration rate (eGFR, Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] formula) ≥ 30 mL/min/1.73 m^2 on a maximum recommended/tolerated dose of angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker (ACEi/ARB) and urinary protein-to-creatinine concentration ratio (UPCR) ≥ 100 mg/mmol sampled from first morning void at screening or baseline were included in cohort A

3 Recipients of kidney transplantation > 90 days before screening; recipients completing induction treatment (if applicable) > 30 days before the screening visit with eGFR (CKD-EPI formula) ≥ 30 mL/min/1.73 m^2 with normal or elevated urinary protein excretion at screening/baseline were included in the recurrent KT cohort

Key Exclusion Criteria

1 Patients using other investigational drugs within 5 half-lives of randomization or 30 days, or longer as per local regulations, or agents known to prolong the QT interval that cannot be discontinued for the duration of study

2 Known family history or known presence of long QT syndrome or Torsades de Pointes

3 Pregnant or nursing (lactating) women, where pregnancy was defined as the state of a female after conception and until the termination of gestation, confirmed by a positive human chorionic gonadotropin laboratory test

4 Donation or loss of 400 mL or more of blood within 8 weeks prior to initial dosing, or longer if required by local regulation

5 Plasma donation (> 200 mL) within 30 days prior to first dosing

6 Subjects who cannot receive vaccinations against *N. meningitidis*, *S. pneumoniae*, or *H. influenzae*

7 For recurrent KT cohort only:

- Patients who received any other transplant except a kidney allograft due to C3G end-stage kidney disease
- Clinical, histological, or laboratory signs of rejection
- Patients with known pro-thrombotic disorder
- Severe concurrent co-morbidities, e.g., advanced cardiac disease (NYHA class IV), severe pulmonary arterial hypertension (WHO class IV), or other conditions as judged by the investigator, both at screening and at baseline
- Kidney biopsy presenting with interstitial fibrosis/tubular atrophy or chronic allograft nephropathy of >50%

Trial Design

Screening/run-in period: The screening period was 60 days, including a screening visit and a run-in period. During the run-in period, a one-time first morning void urine collection (or a 24-hour urine collection) was collected and vaccinations or booster dose (in case of previous vaccinations) against *Neisseria meningitidis, Streptococcus pneumoniae,* and *Haemophilus influenzae* were administered before the first treatment, as per the local guidelines. If the most recent biopsy was older than 12 months in the case of the native cohort and 3 months in the case of the recurrent KT cohort, a renal biopsy was taken at any time during the screening period to confirm C3G diagnosis.

Baseline period: During the 30-day baseline period, baseline values for key complement biomarkers (as per the objectives) and efficacy-related proteinuria were established. The second of the 2 baseline visits was performed the day before dosing.

Treatment period: During the first 4 weeks of the 12-week treatment period, the iptacopan dose was escalated from 10 mg twice daily (bid) to 200 mg bid to allow collection of biomarkers and observe dose-response profiles. The 200 mg bid dose was continued for an additional 8 weeks (Week 5–12) for a total duration of 9 weeks. A kidney biopsy was collected at Week 12 for the recurrent KT cohort (optional for the native cohort). Patients were directly rolled over to the extension study if continuation of treatment with 200 mg bid was found to be beneficial by the investigator. If the extension clinical trial was not yet available at that site, patients continued a non-mandatory treatment extension for 12 weeks (Treatment Period 2). During the entire duration of the study, patients had to remain under stable doses of their supportive therapy, such as ACEis or ARBs, or immunomodulators, such as mycophenolate mofetil (MMF), systemic corticosteroids (CS) with or without diuretics and statins; no change in medication was allowed except for safety reasons. The stable dose was defined as a <25% dose change over at least 4 weeks prior to first baseline visit in the case of ACEi/ARBs, 30 days in the case of MMF, or 90 days in case of systemic CS (up to 7.5 mg daily prednisolone equivalent); the run-in period was extended to ensure stable medication at baseline.

Study Endpoints and Assessments

C3 Deposit Score

C3 deposits were scored on whole slide images of immunofluorescence semi quantitatively on a 0–3 scale for intensity values for C3 deposition with separate scores assigned for mesangial and capillary locations. The scores for each location (mesangial and capillary) were multiplied by a factor of 1 for segmental (defined as <50% of the tuft involved) and a factor of 2 for global ($\geq$ 50% of the tuft involved) extent of deposits. Deposits were diffuse in all cases in non-globally sclerosed glomeruli, and average overall intensity amongst sampled glomeruli was scored. The total score therefore ranged from 0–12. The score was evaluated independently by 3 independent pathologists. In case of discrepancies, the majority principle was applied to decide the score, if 2 out of 3 pathologists assigned the same score. In cases of initial non concordance, scores were adjudicated by assessment of images by the group of pathologists with agreement on a final score.

Other Endpoints

Secondary endpoints included:

Pharmacokinetics (PK): Plasma PK assessment, including non-compartmental parameter analysis, related to total drug, maximum observed plasma concentration (C_{max}), pre-dose observed plasma drug concentration (C_{trough}), time of maximum observed plasma concentration (T_{max}), area under the plasma concentration-time curve from time zero to time of last measurable concentration (AUC_{last}), and area under the plasma concentration-time curve over dosing interval (AUC_{tau}) after the first dose on Days 7, 14, 21, and 28, as well as C_{min} on Days 92 and 99 or 176 and 183.

Safety and tolerability: Electrocardiogram parameters, vital signs, safety laboratory assessments (including a panel of specific hormones), adverse events (AEs) and serious AEs.

Exploratory endpoints: Wieslab activity assay, urine sC5b9, urine lipocalin-2, urine immunoglobulin G, urine interleukin18, urine cystatin C, and histologic scores for disease activity and disease chronicity (based on light microscopy).

Study Assessments

Blood samples were collected for PK assessments at 0, 0.5, 1, 2,4,6, and 8 hours after 1 week of treatment at each dose level during the first 4 weeks, then pre-dose throughout the 200 mg treatment period. Blood collection for C3 and complement pathway biomarkers was done during baseline period, day 1 (pre-dose), during the first 4 weeks (after 1 week of treatment at each dose level, pre-dose, and 6 hours post-dose), day 36 (pre-dose), and on days 64 and 84. Additionally, C3 was also measured during screening
For proteinuria, urinary albumin, urinary protein, urinary albumin-to-creatinine ratio, and UPCR, 24-hour urine collection was done by domiciling between day −1 to 1, days 28–29, and days 84–85. The first morning void urine was collected once during run-in and baseline, and 5 times during the treatment period
The degree of complement system inhibition by LNP023 was assessed using the Wieslab assay. The Wieslab activity assay is based on the *in vitro* formation of the C5b9 complex, triggered by alternative pathway (AP) activation and is used to assess inhibition of the AP
Safety monitoring was done on every visit throughout the study

C3 Staining

Kidney biopsy material was placed in transport media and sent to a central laboratory, where the cores allocated for immunofluorescence were embedded in Optimal Cutting Temperature compound and frozen. Sections were cut and stained for C3 immunofluorescence using the Agilent antisera (anti-Human C3 Complement Rabbit polyclonal FITC-conjugated antibody Cat# F020102-2) and the slides were scanned
as whole slide images and reviewed as .svs files. In 3 cases, glomeruli were not available for frozen tissue immunofluorescence, and paraffin immunofluorescence was performed after pronase digestion on both baseline and day 84 biopsies. A panel made up by 3 independent renal pathologists reviewed the biopsies and scored the intensity of C3 immunofluorescence staining as described in the section above describing C3 deposit score.

Statistical Analysis

Missing values/censoring/discontinuations

Primary analysis included all available data up to the point of treatment discontinuation if applicable

UPCR concentrations below the lower limit of quantification (LLOQ) were imputed as LLOQ/2 for the purpose of graphical representation and statistical analyses

Missing UPCR values were assumed to be missing at random

All drug concentrations below the LLOQ were treated/reported as zero for calculation of PK parameters

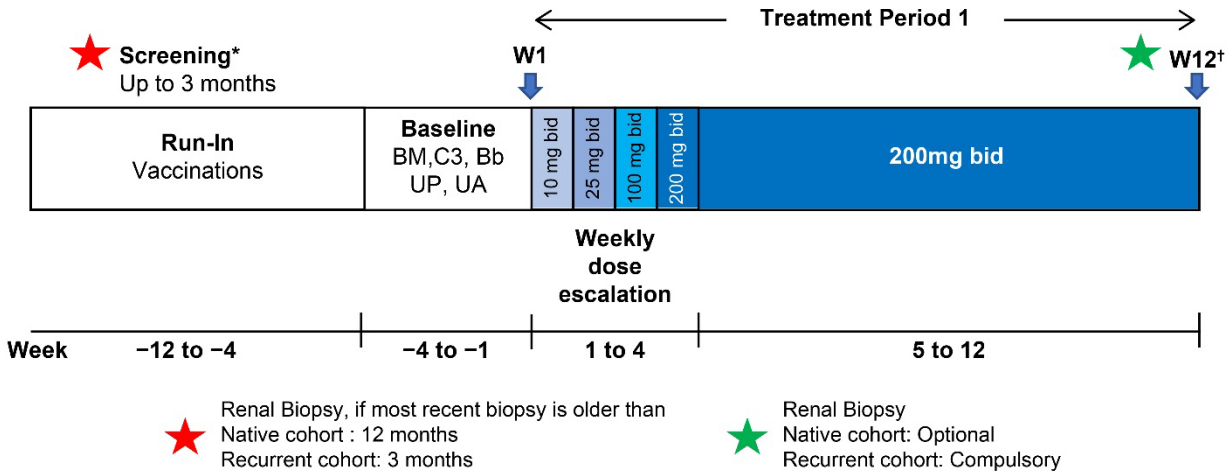
LLOQ values were treated as missing for the calculation of the geometric means and geometric coefficient of variation (CV%)

The change from baseline in UPCR (for the native cohort), as well as secondary endpoints related to proteinuria and renal function were analyzed using the mixed model repeated measures analysis of variance model. The model included the study day as a fixed effect and baseline log transformed endpoint (UPCR in case of primary endpoint) as a fixed covariate. An unstructured covariance matrix was used. Data were presented as the estimated mean value of endpoint with an 80% confidence interval. A generalized linear mixed model, with a common intercept, a pre-treatment slope, a change in the slope following iptacopan treatment, and cohort was used to predict the pre- and post-iptacopan change in eGFR over time. eGFR slope prior to iptacopan treatment and the change in eGFR slope after iptacopan treatment was explored and presented graphically

SAS version 9.4 was used for statistical analysis

PK analysis was conducted using the Phoenix WinNonlin (Version 8.0.0.3176)

Figure S1: Study design

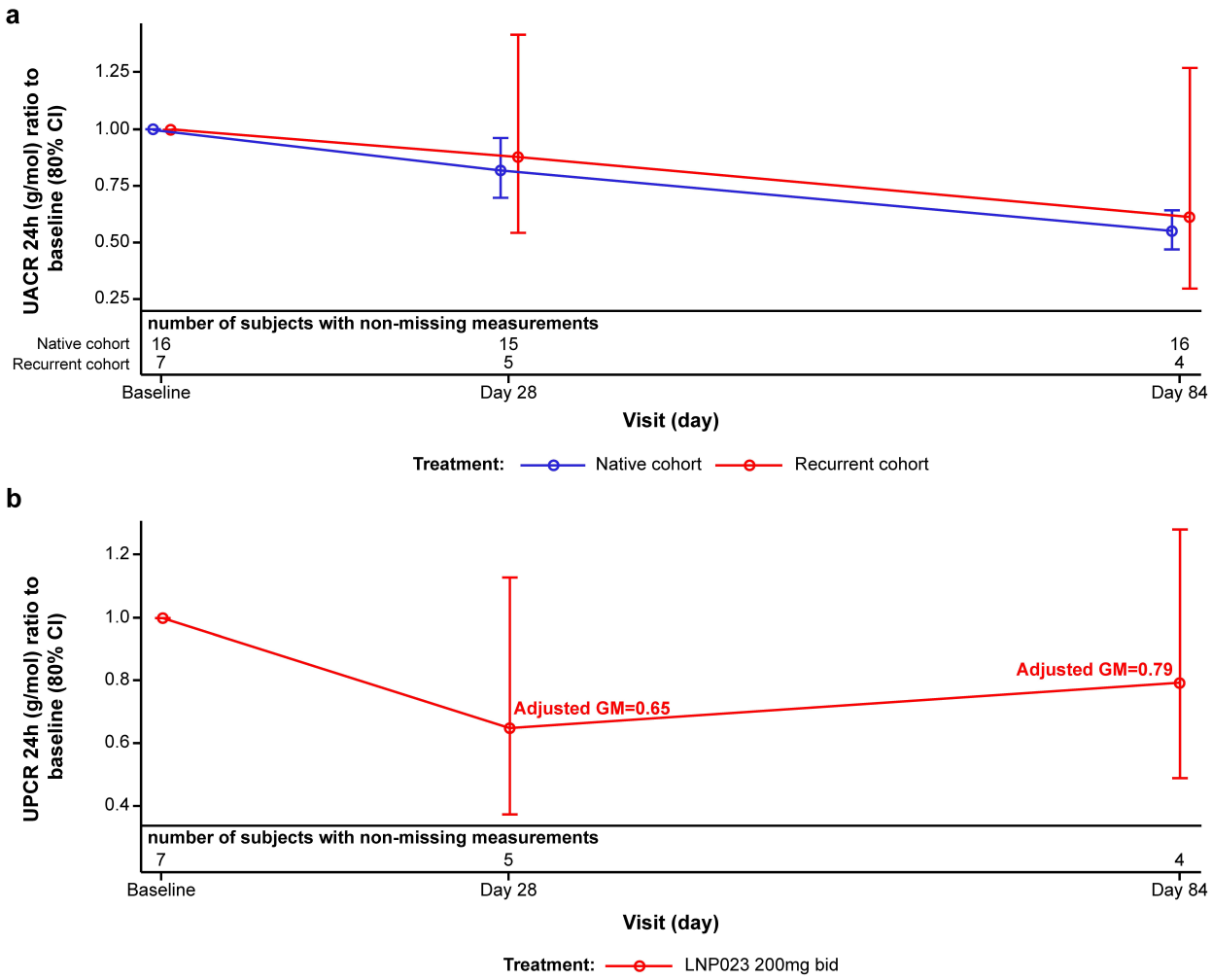


*Run-in period could be extended to ensure stable doses of ACEi/ARB, MMF, and/or systemic CS

†Patient may roll-over in a separate extension study (NCT03955445) or into a non-mandatory treatment extension of 12 weeks (Treatment Period 2) if the Investigator deems it clinically beneficial for the patient to continue treatment (200 mg bid dose) and if the planned extension study is not yet open at the site

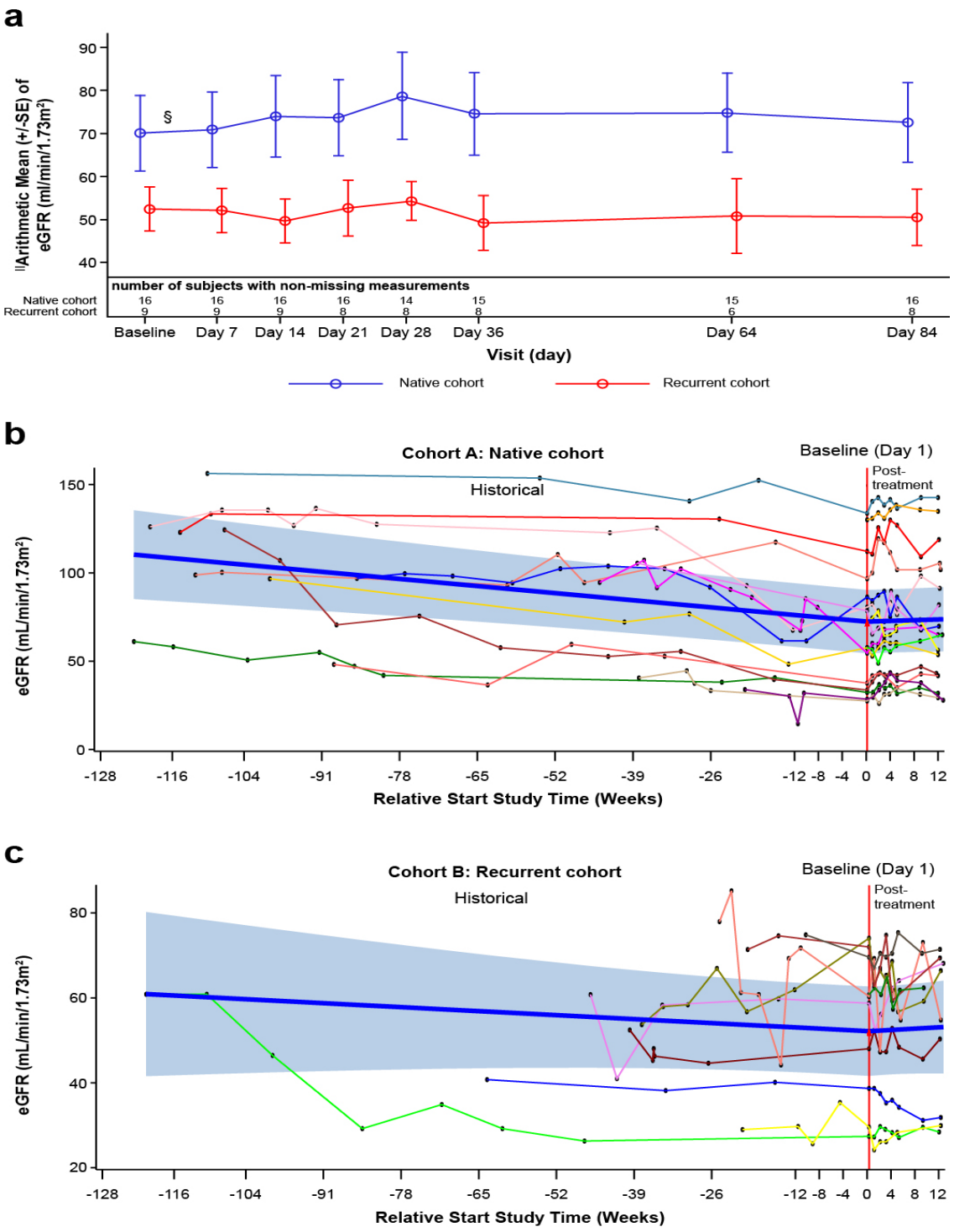
ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; Bb, cleaved factor B; bid, twice a day; BM, biomarkers; C3, component C3; CS, corticosteroids; MMF, mycophenolate mofetil; UA, urinary albumin; UP, urinary protein; W, week

Figure S2: Proteinuria after iptacopan treatment in the native cohort or recurrent KT cohort. UACR (adjusted GM [80% CI] of log ratio to baseline) in native and recurrent KT cohort (a), UPCR (adjusted GM [80% CI] of log ratio to baseline) in recurrent KT cohort (b) (PD analysis set 1)



Baseline is defined to be the 24-hour urine collection on day –1 to day 1
C3G, complement 3 glomerulopathy; CI, confidence interval; GM, geometric mean; PD, pharmacodynamic; UACR, urinary albumin-to-creatinine ratio; UPCR, urinary protein-to-creatinine ratio

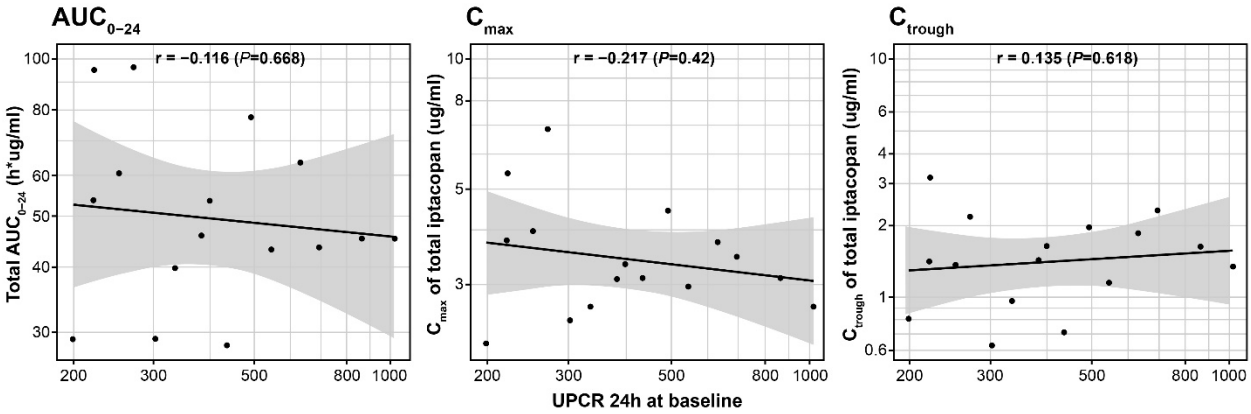
Figure S3: Kidney function as assessed by eGFR after iptacopan treatment in patients with native kidney or recurrent C3G after transplant. Kidney function as assessed by eGFR (AM [SE]) (a), individual patient eGFR slopes up to 2 years prior to and following commencement of iptacopan 12-weeks course in native cohort†† (b), individual patient eGFR slopes up to 2 years prior to and following commencement of iptacopan 12-week course in a recurrent KT cohort†† (c)



[§]A patient with recreational drug overdose was excluded from analysis; [‖]Baseline is defined to be the last available assessment prior to the first dose of study drug; [††]Mean eGFR slope and 95% CI indicated by blue line and surrounding shadowed area

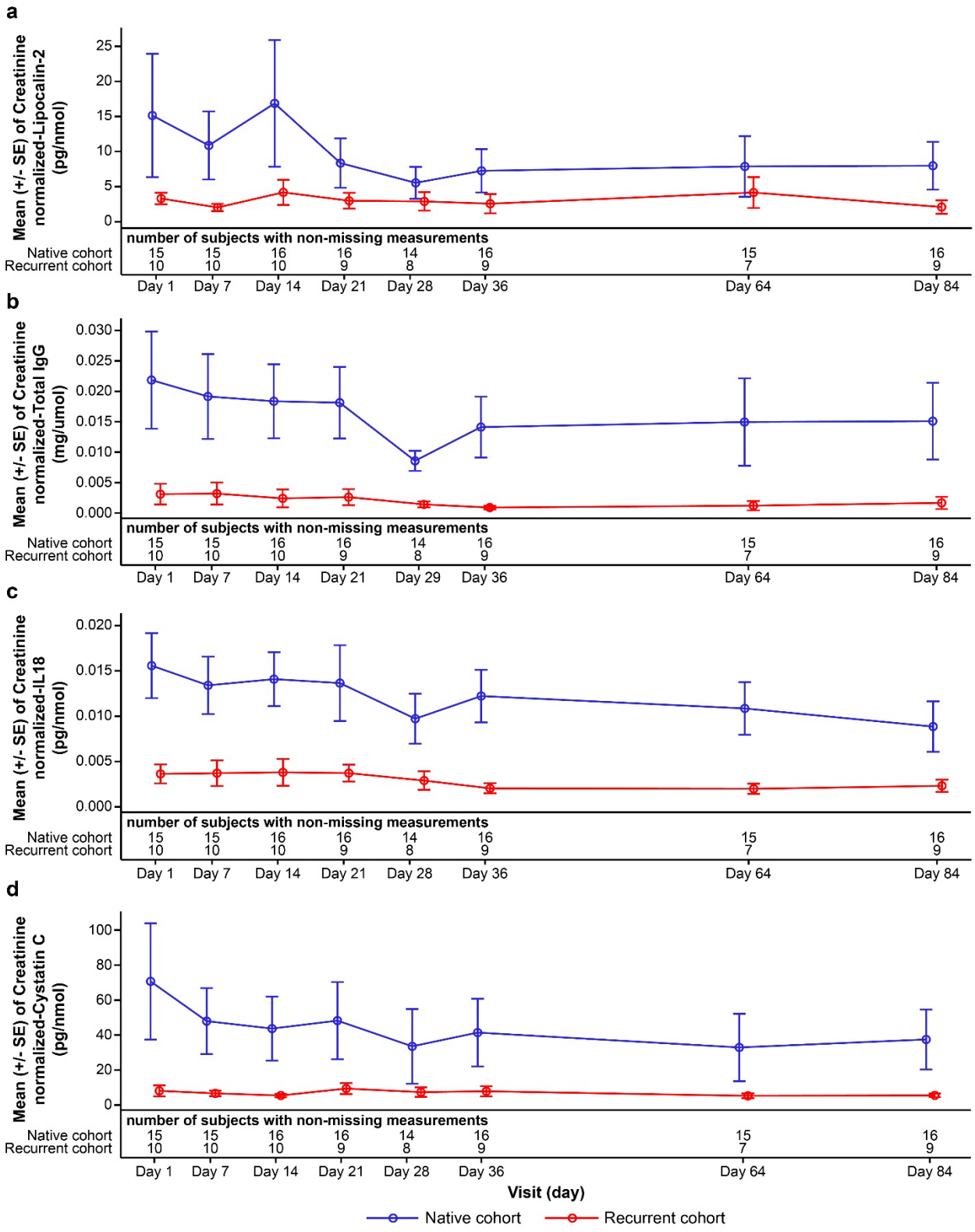
AM, arithmetic mean; C3G, complement 3 glomerulopathy; CI, confidence interval; eGFR, estimated glomerular filtration rate; SE, standard error

Figure S4: PK parameters and 24-hour UPCR in patients with native C3G**.** Total $AUC_{0–24}$ (a), C_{max} (b), C_{trough} (C)



AUC, area under the plasma concentration-time curve; C3G, complement 3 glomerulopathy; C_{max}, maximum observed plasma concentration; C_{trough}, the concentration just prior to the beginning of, or at the end, of a dosing interval; PK, pharmacokinetic; UPCR, urinary protein-to-creatinine ratio

Figure S5: Effect of iptacopan on urine biomarkers**.** Creatinine normalized-lipocalin-2 (a), IgG (b), IL18 (c), and cystatin C (d), PD analysis set 2



Values below LLOQ are imputed as LLOQ/2 and values above ULOQ are imputed as ULOQ for the analysis. Baseline is defined to be day 1 pre-dose assessment

Ig, immunoglobulin; IL, interleukin; LLOQ, lower limit of quantification; PD, pharmacodynamic; ULOQ, upper limit of quantification

Table S1: Prohibited medications

Medication	**Prohibited period**	**Action to be taken**
Live vaccines	Native and recurrent KT cohort: From baseline period until the end of treatment period	Discontinue study treatment
Cyclophosphamide and eculizumab	Native and recurrent KT cohort: A washout of 3 months prior to start of LNP023 treatment is required. It is prohibited until the end of treatment period	Discontinue study treatment
Mycophenolate mofetil or mycophenolate sodium	Native and recurrent KT cohort: If not on stable doses for at least 1 month before first treatment	Discontinue study treatment
Systemic corticosteroids	Native cohort: If not on stable dose (≤7.5 mg prednisolone equivalent) for at least 90 days before first treatment	Discontinue study treatment
Cyclosporine and standard immunotherapy	Native cohort: From baseline until the end of treatment period Recurrent KT cohort: If not on stable doses for at least 1 month before first treatment	Discontinue study treatment
Gemfibrozil (inhibition of multiple disposition pathways of LNP023) Strong CYP2C8 inhibitors (e.g., clopidogrel)	Native and recurrent KT cohort: 48 hours before first LNP023 dose until end of study	Discontinue study treatment

Table S2: Patient disposition and analysis sets

Treatment Period 1, n (%)	**Native cohort N=16**	**Recurrent KT cohort N=11**	**Overall N=27**
Patient disposition:			
Enrolled	16 (100)	11 (100)	27 (100)
Completed	16 (100)	11 (100)	27 (100)
Analysis sets			
Safety analysis set	16 (100)	11 (100)	27 (100)
PD analysis set 1	16 (100)	9* (81.8)	25 (92.6)
PD analysis set 2	16 (100)	10* (90.9)	26 (96.3)
PK analysis set	16 (100)	11 (100)	27 (100)
*A patient treated with cyclophosphamide and eculizumab while taking iptacopan was excluded from PD analysis sets 1 and 2; another patient took cocaine at day 71 of treatment with iptacopan and is excluded from PD analysis set 1 N, total number of patients; PD, pharmacodynamic; PK, pharmacokinetic			

Table S3: Summary statistics of LNP023 PK parameters after multiple dose administration of LNP023 10, 25, 100, or 200 mg to subjects in the native and recurrent KT cohorts (PK analysis set)

PK parameter (unit)				
Native Cohort	**LNP023 10 mg bid N = 15**	**LNP023 25 mg bid N = 1 6**	**LNP023 100 mg bid N = 16**	**LNP023 200 mg bid N = 15**
AUC_{last} (hr*ng/mL)	3690 ± 693 (18.8%)	5790 ± 1630 (28.1%)	13200 ± 4410 (33.4%)	20300 ± 8180 (40.2%)
AUC_{tau} (hr*ng/mL)	5020 ± 1110 (22.2%)	7970 ± 2250 (28.3%)	17800 ± 5800 (32.7%)	26900 ± 10900 (40.5%)
C_{max} (ng/mL)	637 ± 97.1 (15.2%)	941 ± 278 (29.5%)	2270 ± 805 (35.5%)	3600 ± 1230 (34.2%)
C_{trough} (ng/mL)	314 ± 133 (42.2%)	519 ± 133 (25.6%)	1090 ± 408 (37.4%)	1480 ± 653 (44.0%)
T_{max} (hr)	2.00 (0.800–6.00)	2.00 (0.900–4.00)	2.00 (0.500–4.00)	2.00 (1.00–4.00)
Recurrent KT Cohort	**LNP023 10 mg bid N = 11**	**LNP023 25 mg bid N = 11**	**LNP023 100 mg bid N = 11**	**LNP023 200 mg bid N = 9**
AUC_{last} (hr*ng/mL)	4560 ± 2060 (45.2%)	7880 ± 3830 (48.7%)	19600 ± 12100 (61.6%)	28100 ± 15900 (56.7%)
AUC_{tau} (hr*ng/mL)	6300 ± 3000 (47.7%)	10700 ± 5310 (49.8%)	26600 ± 16500 (62.0%)	37700 ± 22000 (58.5%)
C_{max} (ng/mL)	713 ± 292 (41.0%)	1280 ± 552 (43.0%)	3250 ± 1790 (55.1%)	4700 ± 2200 (46.8%)
C_{trough} (ng/mL)	417 ± 237 (56.8%)	644 ± 325 (50.5%)	1650 ± 1010 (61.3%)	2180 ± 1610 (73.9%)
T_{max} (hr)	2.00 (1.00–4.00)	2.00 (1.00–6.00)	2.00 (1.00–4.00)	2.00 (1.00–4.00)

Statistics are mean ± SD (CV%); CV%=SD/mean*100; for T_{max}, statistics are median (min-max)
AUC_{last}, area under the plasma concentration-time curve from time zero to time of last measurable concentration; AUC_{tau}, area under the plasma concentration-time curve over dosing interval; bid, twice a day; C_{max}, maximum observed plasma concentration; C_{min}, minimum observed plasma drug concentration; CV%, coefficient of variation (%); hr, hour; N, total number of patients; PK, pharmacokinetic; SD, standard deviation; T_{max}, time of maximum observed plasma concentration

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	NA
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	NA
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5-6
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	NA
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	NA
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	NA
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	NA
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	NA

			NA
		assessing outcomes) and how	NA
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	NA
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	7
	13b	For each group, losses and exclusions after randomisation, together with reasons	NA
Recruitment	14a	Dates defining the periods of recruitment and follow-up	NA
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	23
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	7
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	7-10
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	8-9
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	10
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	11
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.