Supplemental Appendix

Supplement to: Barratt J, Liew A, *et al.* Phase 2 Trial of Cemdisiran in Adult Patients with Immunoglobulin A Nephropathy: A Randomized Controlled Trial. *CJASN*. 2023.

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

Contents

Additional Methodological Details

Inclusion Criteria

Age and Sex

1. Male or female ≥18 years and ≤65 years of age at the time of informed consent.

Patient and Disease Characteristics

2. Clinical diagnosis of primary immunoglobulin A (IgA) nephropathy as demonstrated by historical biopsy collected within 60 months of screening.
3. Treated for IgA nephropathy with stable, optimal pharmacologic therapy. In general, stable and optimal treatment will include maximum-allowed or -tolerated angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker ≥3 months prior to start of run-in period.
4. Urine protein ≥1 g/24-hours at screening from a valid 24-hour urine collection, and mean urine protein ≥1 g/24-hours from two valid 24-hour urine collections at the end of the run-in period, prior to randomization.
5. Hematuria as defined by ≥10 red blood cells per high-powered field by microscopy or a positive urine dipstick (2+ [moderate] and above) measured by a central laboratory at screening.
6. Females of childbearing potential must have a negative pregnancy test, cannot be breastfeeding, and must be willing to use a highly effective method of contraception 14 days before first dose, throughout study participation, and for 90 days after last dose administration.

7. Previously vaccinated with meningococcal group ACWY conjugate vaccine and meningococcal group B vaccine, or willingness to receive these vaccinations as well as prophylactic antibiotic treatment, if required by local standard of care.
8. Previously vaccinated or willingness to receive vaccinations for *Haemophilus influenzae* type B and *Streptococcus pneumoniae* according to current national/local vaccination guidelines for vaccination use.

Informed Consent

9. Patient is willing and able to provide written informed consent and to comply with the study requirements.

Exclusion Criteria

Disease-Specific Conditions

1. Concomitant significant renal disease other than IgA nephropathy.
2. A diagnosis of rapidly progressive glomerulonephritis as measured by estimated glomerular filtration rate (eGFR) loss >30% over the duration of the run-in phase.
3. Secondary etiologies of IgA nephropathy (e.g., inflammatory bowel disease, celiac disease).
4. Diagnosis of IgA vasculitis (Henoch–Schonlein purpura).
5. eGFR <30 mL/min/1.73 m^2 2 weeks prior to randomization (local results may be used for assessment of eligibility).

Laboratory Assessments

6. Has any of the following laboratory parameter assessment: alanine transaminase >1.5 × upper limit of normal, international normalized ratio >2 (or >3.5 if on

anticoagulants), or total bilirubin >1.5 × upper limit of normal (unless bilirubin elevation is due to Gilbert's syndrome).

7. Confirmed positive IgG/IgM/IgA anti-drug antibodies to cemdisiran at screening.
8. Clinical laboratory test results considered clinically relevant and unacceptable in the opinion of the Investigator.
9. Positive hepatitis B virus (HBV) surface antigen, HBV core antibody, or hepatitis C virus (HCV) antibody (unless HCV viral load demonstrated negative).

Prior/concomitant Therapy

10. Treatment with systemic steroids for >7 days or other immunosuppressant agents in the 6 months prior to randomization.
11. Treatment with dual renin–angiotensin system blockade in the 3 months prior to entry into the run-in phase.
12. Received an investigational agent within the last 30 days or five half-lives, whichever is longer, prior to the first dose of study drug, or are in follow-up of another clinical study prior to study enrollment.

Medical Conditions

13. Known human immunodeficiency virus infection, HCV infection, or HBV infection.
14. Malignancy (except for non-melanoma skin cancers, cervical *in situ* carcinoma, breast ductal carcinoma *in situ*, or stage 1 prostate cancer) within the last 5 years.
15. Active psychiatric disorder, including but not limited to schizophrenia, bipolar disorder, or severe depression despite current pharmacologic intervention.
16. Known medical history or evidence of chronic liver disease or cirrhosis.

17. Has other medical conditions or comorbidities which, in the opinion of the Investigator, would interfere with study compliance or data interpretation.
18. History of multiple drug allergies or history of allergic reaction to an oligonucleotide or *N*-acetylgalactosamine.
19. History of intolerance to subcutaneous injection(s) or significant abdominal scarring that could potentially hinder study drug administration or evaluation of local tolerability.
20. Known contraindication to meningococcal vaccines (group ACWY conjugate and group B vaccines) required for this study. Refer to the most recent local product information for each vaccine for the current list of contraindications.
21. Unable to take antibiotics for meningococcal prophylaxis, if required by local standard of care.
22. Sustained blood pressure >140/90 mm Hg as defined by two or more readings during the run-in period, measured in supine position after 10 minutes of rest.
23. Receipt of an organ transplant (including hematologic transplant).
24. History of meningococcal infection within 12 months before screening.
25. Patients with systemic bacterial or fungal infections that require systemic treatment with antibiotics or antifungals.
26. Patients with functional or anatomic asplenia.

Alcohol Use

27. Patients who consume more than 14 units of alcohol per week (unit: one glass of wine [125 mL] = one measure of spirits [approximately one fluid ounce] = 0.5 pints of beer [approximately 284 mL]).

Randomization and Blinding

Randomization was performed using permuted block randomization and treatment assignments were maintained by the Interactive Response System. All site personnel and patients were blinded to study drug treatment during the efficacy period up to week 36, cemdisiran and placebo were packaged identically, and the syringe used for study drug administration was masked by a site pharmacist prior to administration by a healthcare professional.

The study drug was administered under the supervision of the Investigator or at a location other than the study site (e.g., at home) by a healthcare professional. If the patient was unable to come to the study site, and a visit by a healthcare professional for patients at a location other than the study site (e.g., at home) was not possible, study drug could be administered by the patient or the caregiver under the oversight of the Investigator and following consultation with the Medical Monitor, as allowed by applicable country and local regulations. In such cases, the patient or the caregiver was required to receive appropriate training on study drug administration prior to dosing. Syringe masking was not required if the study drug was administered at home by the patient or caregiver.

Baseline Definitions

Baseline value for 24-hour urine protein, urine protein-to-creatinine ratio (UPCR), and urine albumin-to-creatinine ratio was calculated as the average of two valid (as per section 6.4.1.1 of the protocol) collections at week –2 visit (i.e., the last measurement prior to the first dose of study drug).

Randomization Stratification Factors

The randomization was stratified based on the baseline 24-hour urine protein (≥ 1g/24-hours and <2 g/24-hours versus ≥2 g/24-hours). The mean of two valid 24-hour urine protein assessments at week –2 visit was used as the baseline. The stratification factor was recorded in both the Interactive Response System and the clinical database.

Missing Data

Missing values were not imputed, unless otherwise specified.

Patients who discontinued the study prior to week 36 visit were encouraged to remain on study and complete their remaining clinical visits (excluding pharmacokinetic assessments) through the visit at week 36. All data collected regardless of whether it was collected before or after treatment discontinuation were used for analysis. However, it is possible that data remained missing.

In case of missing date or partial date of adverse event onset, an adverse event was considered treatment-emergent unless it could be unequivocally determined (from the partial onset date and/or a partial or complete stop date) that the event occurred prior to the first dose of study drug. For medications with partial start or stop dates: the first day/month was imputed for start date, and the last day/month will be imputed for stop date. For medications with a completely missing start date, the medications were considered as started prior to the first dose of study drug in this study; medications were classified as prior or both prior and concomitant depending on the medication stop dates. If any medications had a completely missing stop date, then the medication was assumed to be ongoing.

Primary and Sensitivity Analyses

The primary endpoint of the study was the percent change from baseline in UPCR as measured in 24-hour urine at week 32. The analysis was conducted using the modified intent-to-treat (mITT) analysis set.

The primary analysis was performed using a restricted maximum likelihood-based mixed-effect model for repeated measures (MMRM) approach. The fixed effects used in the MMRM for the primary analysis included treatment (cemdisiran versus placebo), scheduled visits (week 16 and week 32), interaction term of treatment and scheduled visits, baseline 24-hour UPCR in log scale (continuous), and patient as a random effect.

Valid 24-hour urine protein values were included in the primary analysis (1 valid assessment at week 16 visit and the mean of two valid assessments at baseline and week 32 visit). All 24-hour urine samples needed to meet the validity criteria below. The 24-hour UPCR at baseline or week 32 was the average of two valid collections.

A 24-hour urine collection was considered valid if the following criteria were met:

- The collection was between 22 to 26 hours in duration between the initial discarded void and the last void or attempt to void.
- No voids were missed between the start and end time of the collection as indicated by the patient's urine collection diary.

The following criterion was also included at the start of the study, but was subsequently omitted because it was found to inappropriately exclude legitimately collected samples:

- At the start of the study, the 24-hour creatinine content was to be within 25% of the expected range as estimated by the following formula: [(140 – age) × weight]/5000, where weight is in kilograms. This result is multiplied by 0.85 in women. In case of a need for two valid samples, the maximum variation in total 24-hour urine creatinine between the two urine collections must be <25%.

Sensitivity Analyses

The first sensitivity analysis was conducted to evaluate the robustness of the primary model using normality of log-transformed UPCR data assumption. A stratified rank analysis of covariance (1) was conducted without using the normality assumption of log-transformed UPCR data. The following steps were performed:

1. Standardized ranks within each stratification stratum were derived across the two treatment groups for the baseline and the change from baseline at week 32 in 24-hour UPCR.
2. The linear regression model was fitted separately for each stratum where the standardized rank of the change from baseline at week 32 in 24-hour UPCR was the outcome variable; the standardized rank of the baseline was the only covariate.
3. The stratified mean score test was performed to compare the two treatment groups using the values of the residuals from the above model as scores and stratification factor as the stratum.
4. Cochran–Mantel–Haenszel *P*-value was obtained.

The second sensitivity analysis was to assess the impact of missing data and the robustness of the primary analysis. The analysis used the same MMRM on the imputed data, of which the missing 24-hour UPCR value was imputed with the spot UPCR (1,2) assessed closest to the date of missing value, when available. The need for this sensitivity analysis was judged depending on the extent of missing values.

Secondary Analyses

For secondary endpoints assessed in the double-blind period, the analysis compared randomized arms (cemdisiran versus placebo) using the mITT analysis set. The secondary endpoints, which were assessed beyond week 36, were summarized to describe the long-term efficacy of cemdisiran using the All Cemdisiran Treated Analysis Set by treatment sequence, i.e., cemdisiran/cemdisiran, placebo/cemdisiran, and All Cemdisiran.

Exploratory Analyses

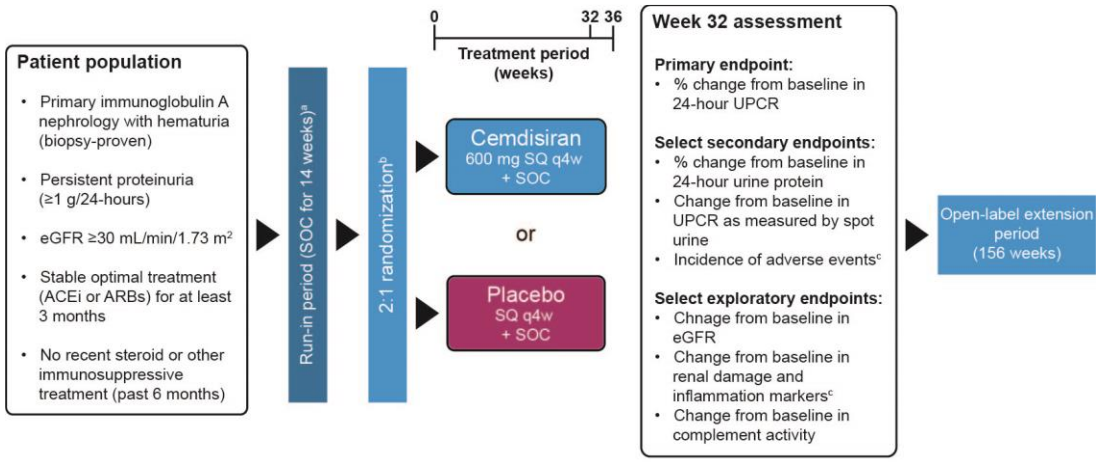
Exploratory endpoints were summarized descriptively by treatment arms during the double-blind period using the mITT analysis set. The slope of eGFR, computed for the entire study period, was also analyzed by treatment sequence cemdisiran/cemdisiran, placebo/cemdisiran using the All Cemdisiran Treated Analysis Set. However, the other exploratory endpoints were analyzed over the entire study if deemed appropriate.

eGFR Exploratory Analysis

The slope of eGFR was calculated for the first 36 weeks using all assessments, collected every 4 weeks, during the treatment period. A random coefficient model was used to analyze the slope of eGFR, including baseline eGFR, treatment sequence, time

(in years), and the interaction of treatment sequence and time as fixed effects, and intercept and time as random effects.

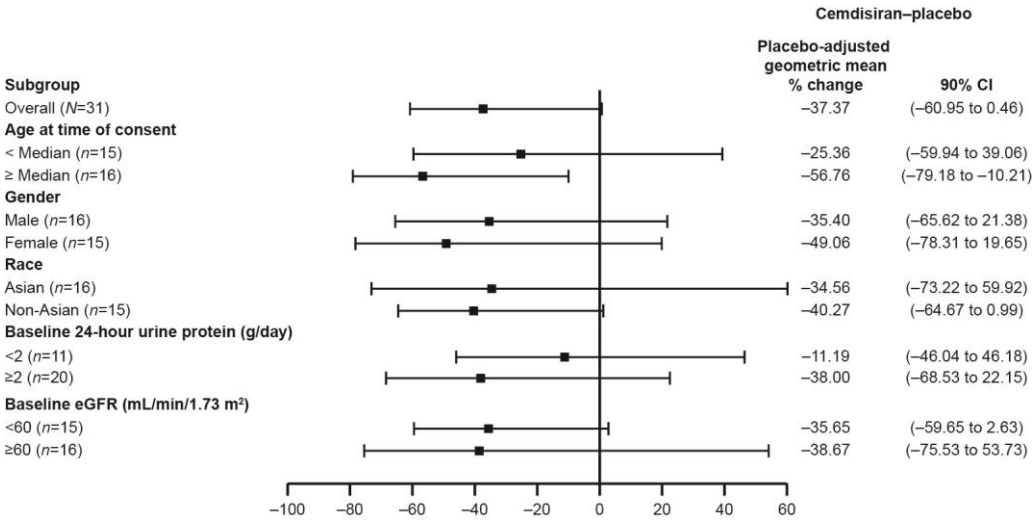
Supplemental Figures



Supplemental Figure 1. | Cemdisiran phase 2 IgA nephropathy study design.

[a]During the run-in period, patients' blood pressure, kidney function, hematuria, proteinuria, and treatment with SOC were documented by the Investigator. SOC was considered to be ACEi or ARB. Patients with proteinuria ≥1 g/24-hours within 2 weeks of the end of the run-in period, and who met blood pressure and eGFR criteria, were eligible to roll into the treatment period. [b]Stratified by baseline urine proteinuria levels (≥1 g/24-hours and

<2 g/24-hours versus ≥2 g/24-hours). [c]Monitored during the course of the study. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; eGFR, estimated glomerular filtration rate; IgA, immunoglobulin A; q4w, every 4 weeks; SOC, standard of care; SQ, single intravenous infusion; UPCR, urine protein-to-creatinine ratio.



Supplemental Figure 2. | Comparison of 24-hour UPCR at week 32 in predefined subgroups of patients treated with cemdisiran or placebo. CI, confidence interval; eGFR, estimated glomerular filtration rate; UPCR, urine protein-to-creatinine ratio.

Supplemental Tables

Supplemental Table 1. Representativeness of study participants

Category	Description
Disease, problem, or condition under investigation	IgA nephropathy
Special consideration related to:	
Sex and gender	• The male:female ratio is 2–3:1 in North America (4) and Europe, and about 1:1 in Asia (4)
Age	• Peak prevalence of IgA nephropathy occurs during the second and third decades of life (4)
Race or ethnic group[a]	• The prevalence of IgA nephropathy varies widely between racial/ethnic groups, being highest in persons of East-Asian descent, followed by Caucasians (4)

	• IgA nephropathy is relatively rare in individuals of sub-Saharan African ancestry (4)
Geography	• The prevalence of IgA nephropathy is markedly higher in East Asia compared with North America and Europe, although this may be partly due to a greater number of performance renal biopsies and national urine screening programs in East Asia (4)
Other considerations	• The true prevalence and incidence of IgA nephropathy may be higher than recognized because of likely undocumented subclinical cases (4) • Most cases of IgA nephropathy appear to be sporadic (90–95%) rather than in familial patterns (5–10%) (5)
Overall representativeness of this trial	• In line with the geographic variability of IgA nephropathy, in our study a total of 52% of patients were Asian, 39% were White, 3%

were Other, and 7% were of unreported race/ethnicity

- The ratio of males:females included in the current study was comparable (16/31, 52% of patients were male), with more male patients in the cemdisiran group compared with the placebo group (59% versus 33%, respectively)

- By design, the study excluded patients over the age of 65 years to minimize the burden of comorbid medical illness in this phase 2 study. Accordingly, the mean age of participants was 40.5 years in the cemdisiran group and 37.6 years in the placebo group

[a]Race and ethnic group information were self-reported by the patient

IgA, immunoglobulin A.

Supplemental Table 2. Change from baseline in 24-hour urine protein to week 32 in patients treated with cemdisiran compared with placebo (secondary endpoint)

Parameter	Placebo (*N*=9)	Cemdisiran (*N*=22)	Placebo-Adjusted Geometric Mean Change
Mean (standard deviation) 24-hour urine protein (g/day) at baseline	2.94 (1.34)	2.53 (1.46)	−
Mean (standard deviation) 24-hour urine protein (g/day) at week 32	3.14 (1.01)	2.15 (1.81)	−
Adjusted geometric mean ratio to baseline at week 32 (standard error to the mean)	1.05 (0.27)	0.67 (0.10)	-
Change from baseline, % in 24-hour urine protein at week 32	5.1	–32.9	–36.2

Supplemental Table 3. Change from baseline in week 32 in eGFR in patients treated with cemdisiran compared with placebo (exploratory endpoint)

	Placebo	Cemdisiran
Baseline		
N	9	22
eGFR (mL/min/1.73 m^2), mean (standard deviation)	61 (33)	72 (27)
Week 16		
n	9	21
Mean change from baseline (standard deviation) in eGFR (mL/min/1.73 m^2)	–2.78 (8.4)	–0.48 (10.9)
Week 32		
n	8	20
Mean change from baseline (standard deviation) in eGFR (mL/min/1.73 m^2)	–6.25 (4.8)	–2.90 (11.1)

Statistic			
Estimated eGFR slope[a,b] per year (standard deviation)	–11.90 (9.33)		–6.76 (10.92)
Difference in eGFR slopes (cemdisiran−placebo)		5.14	

[a]The random coefficient model for eGFR includes baseline eGFR, treatment, time from baseline assessment in years (baseline time denoted as zero), and the interaction of treatment and time as fixed effects, and intercept and time as random effects. The restricted maximum likelihood method was used. Asymptotic standard errors are used to model the within-patient errors and degrees of freedom are computed using the Kenward–Roger method.

[b]Estimated slope is based on week 36 data.

eGFR, estimated glomerular filtration rate.

Supplemental References

1. LaVange LM, Koch GG: Rank score tests. *Circulation* 114: 2528–2533, 2006
2. Ginsberg JM, Chang BS, Matarese RA, Garella S: Use of single voided urine samples to estimate quantitative proteinuria. *N Engl J Med* 309: 1543–1546, 1983
3. Viswanathan G, Upadhyay A: Assessment of proteinuria. *Adv Chronic Kidney Dis* 18: 243–248, 2011
4. Rajasekaran A, Julian BA, Rizk DV: IgA nephropathy: an interesting autoimmune kidney disease. *Am J Med Sci* 361: 176–194, 2021
5. Lai KN, Tang SC, Schena FP, et al.: IgA nephropathy. *Nat Rev Dis Primers* 11: 16001, 2016

Cemdisiran Phase 2 Study Investigators and Collaborators

The Medical University of South Carolina Health University Medical Center, SC, USA

Investigators: Anand Achanti, Primary Investigator; Milos Budisavljevic, Co-Investigator

Site staff: Linda Walker, Study Coordinator

AAA Clinical Research, Ontario, Canada

Investigators: Naresh Aggarwal, Primary Investigator

University of Santo Tomas Hospital, Manila, Philippines

Investigators: Stephanie Andres, Primary Investigator; Marie Stella Navarro, Sub-Investigator

Site staff: Haydee Gabuay, Study Coordinator

Karolinska University Hospital, Stockholm, Sweden

Investigators: Peter Barany, Primary Investigator; Olof Heimbürger, Co-Investigator

Site staff: Ulrika Jensen Durgé, Research Nurse

Providence Health Care Research Institute, University of British Columbia, Vancouver, British Columbia, Canada

Investigators: Sean Barbour, Primary Investigator

Site staff: Zainab Sheriff, Project Manager; Paula MacLeod, Clinical Research Coordinator

Leicester General Hospital, Leicester, UK

Investigators: Jonathan Barratt, Primary Investigator; Chee Kay Cheung, Sub-Investigator; Haresh Selvaskandan, Sub-Investigator

Site staff: Justyna Sklarzewicz, Lead Research Nurse

University Health Network, Toronto, Ontario, Canada

Investigators: Daniel Cattran, Primary Investigator

Site staff: Heather N. Reich, Investigator; Paul Ling, Coordinator; Arenn Jauhal, Investigator

GHR Mulhouse & Sud Alsace, Mulhouse, France

Investigators: Francois Chantrel, Primary Investigator; Jimmy Grellier, Co-Investigator

Site staff: Camille Alzina, Study Coordinator

Kaosiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan

Investigators: Jin Bor Chen, Primary Investigator

Chang Gung Memorial Hospital, Linkou Branch, Taoyuan City, Taiwan

Investigators: Kuan-Hsing Chen, Primary Investigator; Hsiang-Hao Hsu, Sub-Investigator; Huang-Yu Yang, Sub-Investigator; Kun-Hua Tu, Sub-Investigator

Fundació Puigvert, Barcelona, Spain

Investigators: Montserrat Diaz Encarnacion, Primary Investigator; Helena Marco Rusiñol, Sub-Investigator

Site staff: Luz San Miguel Amigo, Study Coordinator; Beatriz Bardaju de Quixano, Study Coordinator; Irene Silva Torres, Study Coordinator

Hospital Universitario Reina Sofía, Córdoba, Spain

Investigators: Mario Espinosa, Primary Investigator; Isabel López-López, Sub-Investigator

Site staff: Rocío Regalado, Study Coordinator

Medicine and Caring Sciences, Linköping University, Linköping, Sweden

Investigators: Anders Fernström, Primary Investigator

Site staff: Micael Gylling, Study Nurse; Fredrik Uhlin, Study Nurse

Mayo Clinic, Rochester, MN, USA

Investigators: Fernando Fervenza, Primary Investigator

Serdang Hospital, Selangor, Malaysia

Investigators: Bak Leong Goh, Primary Investigator; Fairol H. Ibrahim, Sub-Investigator; Aida Azlin Alias, Sub-Investigator; Tay Li Lian, Sub-Investigator

Amicis Research Center, CA, USA

Investigators: Billy Hour, Primary Investigator

Site staff: Tyrone Rosales, Coordinator; Veronica Macias, Coordinator

Northwest Louisiana Nephrology, LA, USA

Investigators: Marwan Kaskas, Primary Investigator

Normandie Univ, Centre Hospitalier Universitaire de Caen Normandie, Caen, France

Investigators: Antoine Lanot, Primary Investigator; Victor Gueutin, Co-Investigator

Site staff: Sylvie Brucato, Head of Clinical Research Center

Annonay Hospital, Annonay, France

Investigators: Eric Legrand, Primary Investigator

Site staff: Julie Cabantous, ARC

Hospital Universitari de Girona Doctor Josep Trueta, Girona, Spain

Investigators: Nadia Martin, Primary Investigator; Xoana Barros, Sub-Investigator

Site staff: Cristina Martínez, Study Coordinator; Montserrat Capdevila, Study Coordinator; Irene Rovira, Study Nurse

Hospital Tengku Ampuan Afzan, Pahang, Malaysia

Investigators: Fariz Safhan Mohamad Nor, Primary Investigator; Mohd Kamil Ahmad, Sub-Investigator; Wan Ahmad Syahril Rozli Wan Ali, Sub-Investigator; Tze Jian Ng, Sub-Investigator

Site staff: ‘Izzah ‘Atira Hisham, Study Coordinator; Nur Liyana Kamaronzaman, Study Coordinator; Nadia Shahirah Mohamed Asri, Pharmacist; Mohamad Haziq Abu Othman, Pharmacist; Mohd Shahril Mohd, Study Nurse

Centre Hospitalier Universitaire Caremeau, Nîmes, France

Investigators: Olivier Moranne, Primary Investigator

University of Malaysia, Kuala Lumpur, Malaysia

Investigators: Kok Peng Ng, Primary Investigator; Shok Hoon Ooi, Co-Investigator

Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Spain

Investigators: Joan Torras Ambros, Primary Investigator; Ana Coloma, Sub-Investigator; Juliana Draibe, Sub-Investigator

Site staff: Claudia Galofre, Study Coordinator

Hôpital du Sacré-Coeur de Montréal, Montreal, Quebec, Canada

Investigators: Stephan Troyanov, Primary Investigator

Site staff: Guylaine Marcotte, Nurse and Local Coordinator

National Kidney and Transplant Institute, Quezon City, Philippines

Investigators: Russell Villanueva, Primary Investigator; Donnah Franceska De Leon, Co-Investigator

Taichung Veterans General Hospital, Taichung, Taiwan

Investigators: Ming-Ju Wu, Primary Investigator

Site staff: Shan Lee, Study Nurse

Kuala Lumpur Hospital, Kuala Lumpur, Malaysia

Investigators: Rosnawati Yahya, Primary Investigator; Seow Yeing Yee, Sub-Investigator; Wan Hazlina Wan Mohamad, Sub-Investigator; Nurul Zaynah Nordin, Sub-Investigator; Muhamad Zaimi Abdul Wahab, Sub-Investigator

Site staff: Zurina Che Rohani, Study Coordinator; Mohd Alfaisal Mod Baharuddin, Study Nurse; Muhamad Nur Asswad Md Kassim, Study Nurse; Siti Nur Zuhafifah Mohd Zaki, Study Nurse

Tan Tock Seng Hospital, Singapore, Singapore

Investigators: See Cheng Yeo, Primary Investigator; Ru Sin Lim, Co-Investigator

Site staff: Siew Hwa Soh, Manager; Felicia Lee, Coordinator; Hongli Jiao, Coordinator/Nurse; Rosa Lim, Coordinator/Nurse; Kai Yan Lin, Coordinator

Centre Hospitalier Universitaire de Grenoble, La Tronche, France

Investigators: Philippe Zaoui, Primary Investigator; Pierre-Louis Carron, Sub-Investigator

Site staff: David Tartry, Study Coordinator; Mathilde Bugnazet, Study Coordinator; Farida Imerzoukene, Study Coordinator; Florence Theo, Study Nurse; Séverine Dhion, Study Nurse; Meryll Argoud, Study Nurse; Gaëlle Vial, Study Nurse; Audrey Lehman, Pharmacist; Thierry Romanet, Pharmacist