Supplementary Material

Methods

Study Participants

Eligible patients must have a mean urine protein:creatinine ratio (UPCR) ≥1000 mg/g and a body mass index ≤40 kg/m^2. Patients treated with corticosteroids or receiving treatment with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, finerenone, aldosterone synthase inhibitors, or sodium-glucose cotransporter-2 inhibitors should be on a stable dose for ≥4 weeks prior to screening, with no plans to alter the dose during the study. In addition, concomitant use of calcineurin inhibitors, cytotoxic agents, or CD20 monoclonal antibodies (within five half-lives before screening); metformin, dofetilide, dabigatran, or digoxin (within five half-lives before screening); or strong inhibitors or inducers of CYP3A (within 1 week or five half-lives before screening) are not permitted in this study.

This trial will be conducted in compliance with the approved protocol and the ethical principles laid down in the Declaration of Helsinki, and in accordance with the International Conference on Harmonization Good Clinical Practice Guideline, relevant Boehringer Ingelheim (BI) standard operating procedures, EU regulation 536/2014, and local regulations. All patients will be required to provide signed, dated, freely written informed consent prior to admission to the study.

Treatment

Patients will enter a ≤30-day screening period prior to receiving the first dose of study drug. Following the 12-week treatment period, patients will enter a follow-up period with visits at 7 and 30 days after the end of treatment.

The doses in this trial were in part selected based on Phase I safety data demonstrating that treatment for 14 days was well tolerated in healthy volunteers (NCT04102462). Patients with primary focal segmental glomerulosclerosis are likely to have slightly higher exposure to BI 764198 than

healthy volunteers, due to their abnormal kidney function. Pharmacokinetics (PK) of Phase I data demonstrated an increase in area under the concentration-time curve (AUC) and maximum plasma concentration (C_{max}) in patients with moderate and severe abnormal kidney function after single doses of medium dose BI 764198 (NCT04176536). Based on data from these Phase I studies and preclinical assessments to demonstrate pharmacological effects in a mouse unilateral ureteral obstruction model (targeting >IC90), the therapeutic dose of BI 764198 is estimated to be between the low and medium dose. However, as target engagement is unable to be assessed in humans and due to the uncertainty of a demonstrable pharmacodynamics (PD) solely based on *in vitro* dose estimations, a high dose will also be assessed in this study. This high dose may be valuable to determine whether transient receptor potential cation channel, subfamily C, member 6 (TRPC6) inhibition induces a clinically meaningful UPCR response.

Decentralized Clinical Trial (DCT) Model

Informed consent and electronic trial data will be collected via a web-based platform (Science 37®), and visits will be conducted by mobile research nurses (MRNs). Remote visits and trial procedures, except eye examinations, will be conducted by MRNs, with oversight from the site investigator and staff. This option will be presented to patients prior to or at the time of informed consent, or patients can complete the screening visit at the study site and subsequently switch to the DCT model.

Endpoints and Assessments

PD endpoints are urinary biomarkers reflecting podocyte health (podocin [mRNA]: creatinine ratio [UPodCR], nephrin [mRNA]: creatinine ratio [UnephCR], and podocin [mRNA]:nephrin [mRNA] ratio [UPNR]) and drug target modulation (TRPC6 mRNA, nuclear factor of activated T-cells [NFAT] mRNA, and downstream markers of the calcineurin-NFAT pathway). The PK of BI 764198 are being assessed,

with parameters including C_{max}, time to maximum concentration, and AUC from t1 to t2 at steady state at Week 12.

Urine samples (24-hour) will be collected at screening, Day 4, and Weeks 12 and 13 to measure UPCR and urinary protein excretion. Spot urine samples will be collected at baseline (Visit 2), Day 4, and Weeks 4, 8, 12, and 13 for UPCR and urinary albumin:creatinine ratio calculations, and exploratory biomarker evaluation. At screening, first morning void urine samples will be collected to obtain the UPCR measurements required to determine trial eligibility. Serum samples will be collected at screening, baseline (Visit 2), Day 4, and Weeks 2, 4, 6, 8, 10, 12, and 13 to measure creatinine levels and determine estimated glomerular filtration rate (eGFR). Supplementary Table 1 summarizes the assessment of endpoints and timings for the study visits.

Randomization and Blinding

Patients, investigators, and all study personnel (except the trial pharmacometrician, pharmacokinetic programmer, and trial bioanalyst) will remain blinded to the randomized treatment assignments until study completion, according to the sponsor's standard operating procedures (SOPs). As needed, additional safety, efficacy, PK, and PD analyses may be performed during the trial by the clinical trial and project team, for which the database will be unblinded. Emergency unblinding will be available when the identity of the treatment must be known to the investigator to provide appropriate medical treatment, or otherwise ensure the safety of a trial participant.

Statistical Analysis

If ~40%, 30%, and 20% of patients in the high, medium, and low dose groups, respectively, reach a ≥25% reduction in UPCR after 12 weeks, compared with ~9% of patients receiving placebo, the proposed sample size provides a 73.4% probability for a difference of ≥25% in responder rates between at least one treatment group and placebo.

The dose–response relationship, based on the reduction in log of UPCR, may be investigated using a graphical approach. All secondary and other endpoints (except PK) will be assessed via descriptive statistics. An unblinded, exploratory analysis of PK, PD, and biomarker data will be performed during the trial.

Data Management and Trial Monitoring

A safety review committee, including a cardiologist, an ophthalmologist, a global pharmacovigilance member, and other trial team members, will be established by the sponsor to review individual and aggregated safety data at regular intervals. The primary objective of this committee will be to monitor the safety and tolerability of BI 764198. An independent data monitoring committee will not be established to monitor this trial; data management will be conducted in accordance with BI's SOPs and industry standards.

The investigator/institution will allow trial-related monitoring, audits, institutional review board/independent ethics committee review, and regulatory inspections. Direct access will be provided to all source documents/data, including progress notes and copies of laboratory and medical test results for review by the clinical research associate, auditor, and regulatory inspector.

Supplementary references

S1. Winn MP, Conlon PJ, Lynn KL, et al. A mutation in the TRPC6 cation channel causes familial focal segmental glomerulosclerosis. *Science*. 2005;308:1801–1804. https://doi.org/10.1126/science.1106215

S2. D'Agati VD, Kaskel FJ, Falk RJ. Focal segmental glomerulosclerosis. *N Engl J Med*. 2011;365:2398–2411. https://doi.org/10.1056/NEJMra1106556

S3. Miao J, Pinto EVF, Hogan MC, et al. Identification of genetic causes of focal segmental glomerulosclerosis increases with proper patient selection. *Mayo Clin Proc*. 2021;96:2342–2353. https://doi.org/10.1016/j.mayocp.2021.01.037

S4. Shalygin A, Shuyskiy LS, Bohovyk R, et al. Cytoskeleton rearrangements modulate TRPC6 channel activity in podocytes. *Int J Mol Sci*. 2021;22:4396. https://doi.org/10.3390/ijms22094396

S5. Heeringa SF, Moller CC, Du J, et al. A novel TRPC6 mutation that causes childhood FSGS. *PLoS One*. 2009;4:e7771. https://doi.org/10.1371/journal.pone.0007771

S6. Reiser J, Polu KR, Moller CC, et al. TRPC6 is a glomerular slit diaphragm-associated channel required for normal renal function. *Nat Genet*. 2005;37:739–744. https://doi.org/10.1038/ng1592

S7. 't Hart D, Li J, van der Vlag J, et al. Repurposing riociguat to target a novel paracrine nitric oxide-TRPC6 pathway to prevent podocyte injury. *Int J Mol Sci*. 2021;22:12485. https://doi.org/10.3390/ijms222212485

S8. Chen X, Sooch G, Demaree IS, et al. Transient receptor potential canonical (TRPC) channels: Then and now. *Cells*. 2020;9:1983. https://doi.org/10.3390/cells9091983

S9. Blaine J, Dylewski J. Regulation of the actin cytoskeleton in podocytes. *Cells*. 2020;9:1700. https://doi.org/10.3390/cells9071700

S10. Wang Q, Tian X, Wang Y, et al. Role of transient receptor potential canonical channel 6 (TRPC6) in diabetic kidney disease by regulating podocyte actin cytoskeleton rearrangement. *J Diabetes Res*. 2020;2020:6897390. https://doi.org/10.1155/2020/6897390

S11. Palygin O. The role of TRPC6 channel in chronic kidney disease. *Am J Physiol Renal Physiol*. 2022;322:F195–F196. https://doi.org/10.1152/ajprenal.00455.2021

S12. Goodson N, Wicks P, Morgan J, et al. Opportunities and counterintuitive challenges for decentralized clinical trials to broaden participant inclusion. *NPJ Digit Med*. 2022;5:58. https://doi.org/10.1038/s41746-022-00603-y

Supplementary Table 1. Study endpoints and assessments

Trial period	Screening period[a]	Randomized treatment					Follow-up	
Visit	**1**	**2**	**3**	**4**	**5**	**EoT**	**FU1**	**EoS**
Study day	–30	1	4	29	57	85	92	115
Study week		0	1	4	8	12	13	
Time window for visits (days)		0	+3	±3	±3	±3	±2	±3
Enrollment								
Informed consent	X							
Demographics	X							
Medical history	X	X						
Review of inclusion/exclusion criteria	X	X						
Intervention								
BI 764198								
Assessments								
Bodyweight	X			X	X	X	X	
Concomitant therapy	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	
Physical examination	X			X		X		
Resting 12-lead ECG	X	X	X	X	X	X	X	
Safety laboratory sampling	X	X	X	X	X	X	X	
Pregnancy testing	X	X		X	X	X	X	
Eye examinations	X					X		X
First morning void	X							
Spot urine		X	X	X	X	X	X	
24-hour urine collection	XX[b]		X			X	X	

Biomarker sampling (blood, urine)		X		X	X	X	X	
Pharmacogenomics		X						
PK sampling (blood)		X	X	X	X	X	X	
Randomization and dispense trial medication		X						
Dispense/review of medication diary		X	X	X	X	X		
Medication administration during study visit		X	X	X	X	X		
Adverse events	X	X	X	X	X	X	X	X
Return trial medication/ compliance check			X	X	X	X		

ECG, electrocardiogram; EoS, end of study; EoT, end of treatment; FU1, follow-up 1; PK, pharmacokinetics.

Phone calls will be carried out at Weeks 2, 6, and 10 (Days 15, 43, and 71, respectively) to assess adverse events and concomitant therapy use.

[a] Screening period may be shorter than 30 days.

[b] Two separate 24-hour urine samples will be collected on separate days prior to Visit 2.