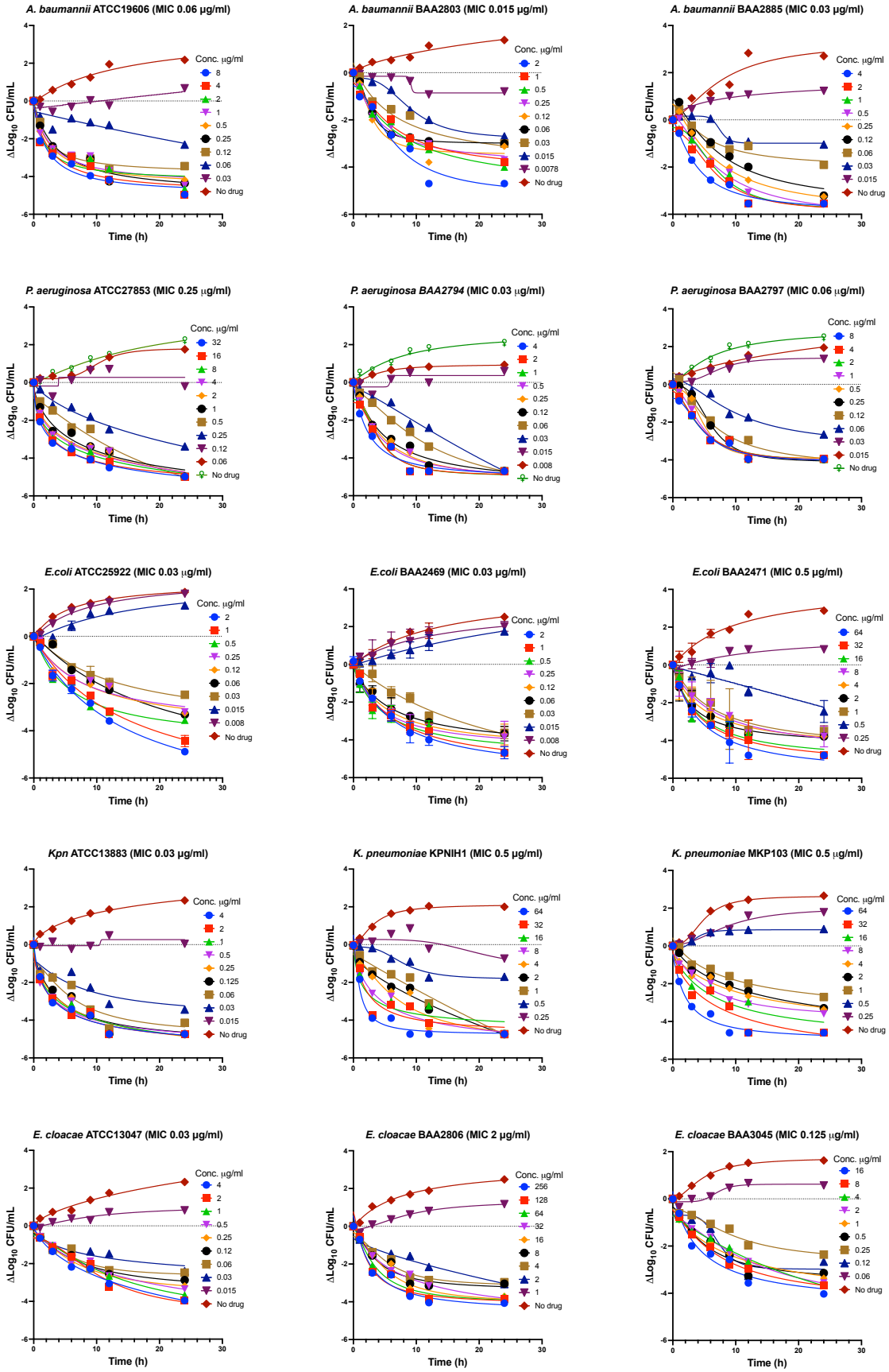
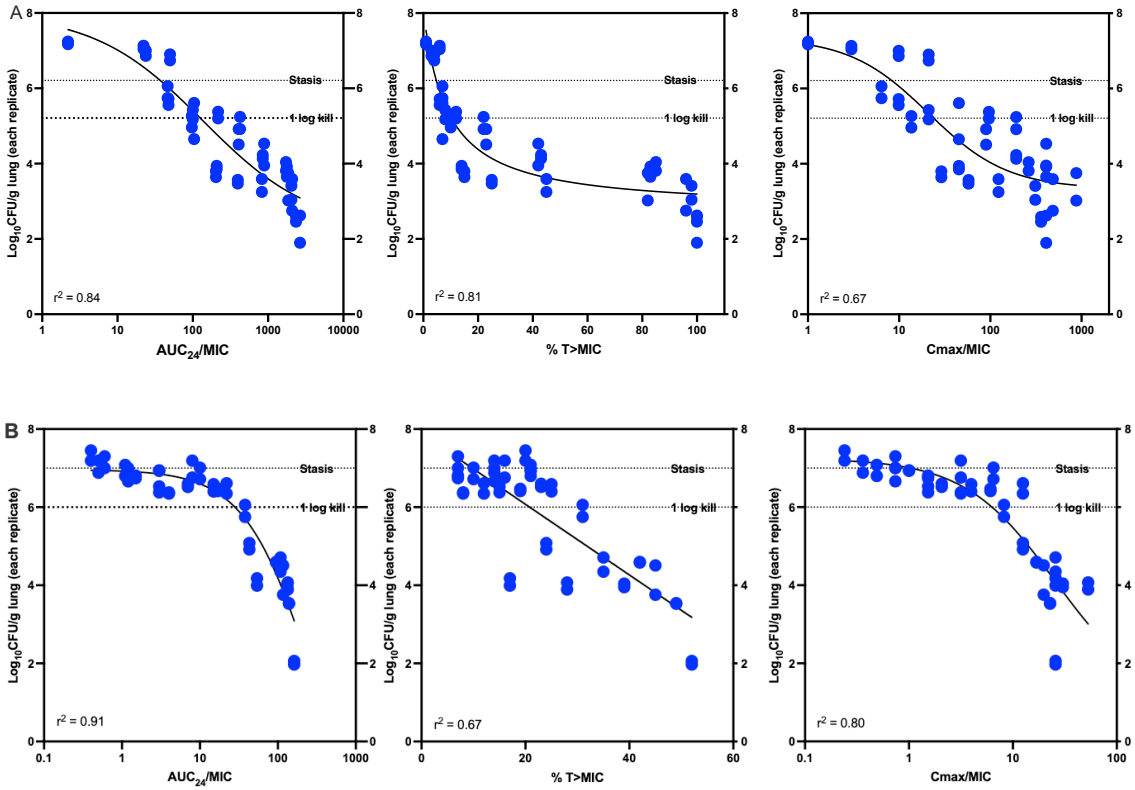
Supplementary Figure 1: *In vitro* killing kinetics against drug-susceptible and drug-resistant bacterial strains.

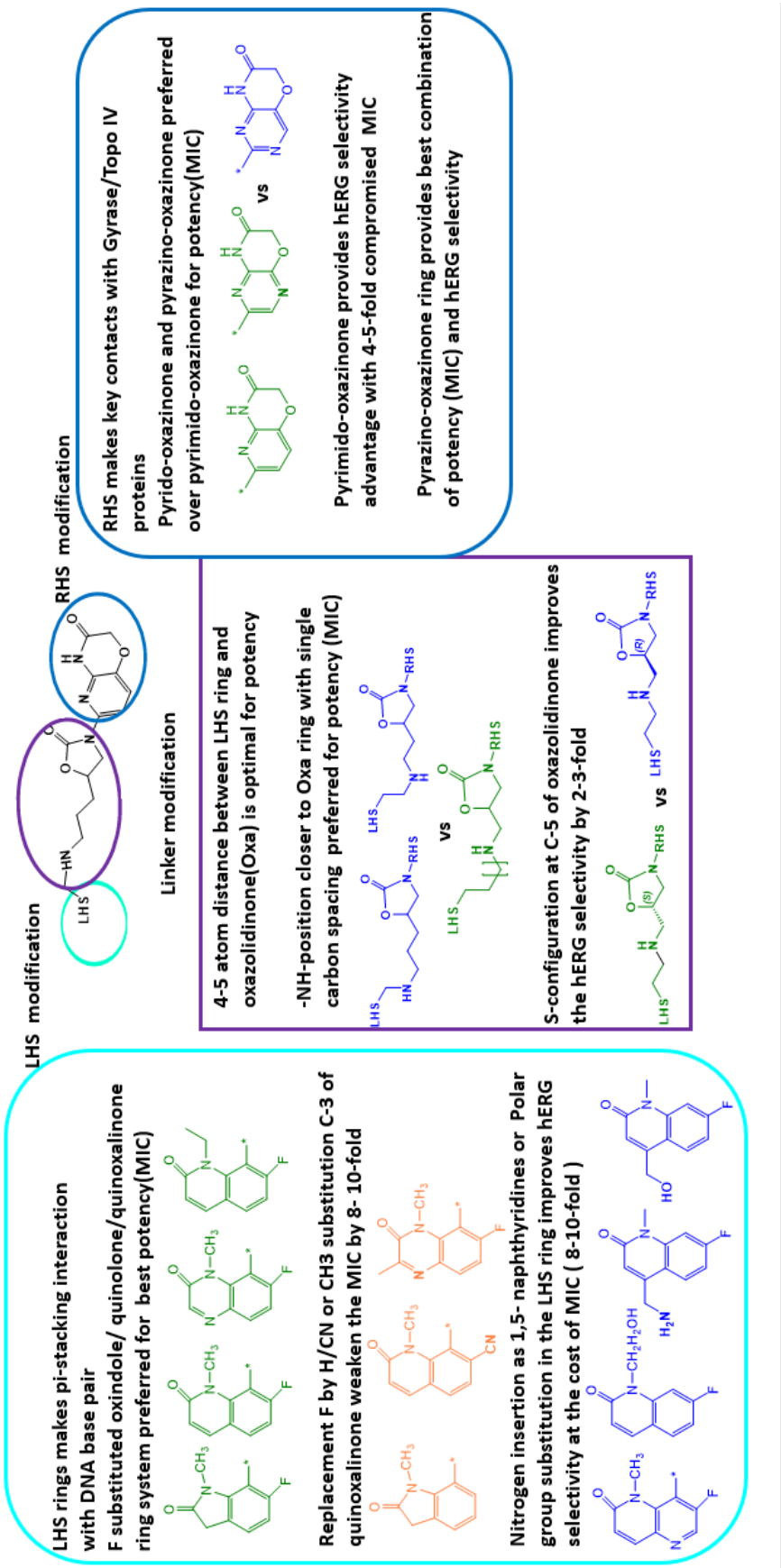


Following exposure to different concentrations (2-fold serial dilutions, nine concentrations, with start of either 256, 64, 32, 16, 8, 4, or 2 μg/ml) of BWC0977 for various time points, the bacterial kill was monitored *in vitro* against a panel of drug-susceptible and MDR strains of *E. coli*, *A. baumannii, K. pneumoniae*, *P. aeruginosa* and *E. cloacae*. The viable colony forming units (CFUs) were enumerated and the plots generated ($\Delta \log_{10}$CFU/ml Vs time) using Graph Pad Prism. The profiles demonstrated a limited increase in kill with increasing concentrations, but significant kill over time.

Supplementary Figure 2: PK/PD indices were obtained with *A. baumannii* ATCC19606 (Panel A; fAUC/MIC r^2=0.84, % t>MIC r^2= 0.81, fCmax/MIC r2=0.67); *K. pneumoniae* SKB067 (Panel B; fAUC/MIC r^2=0.91, fCmax/MIC r^2=0.80, %T>MIC r^2=0.67)

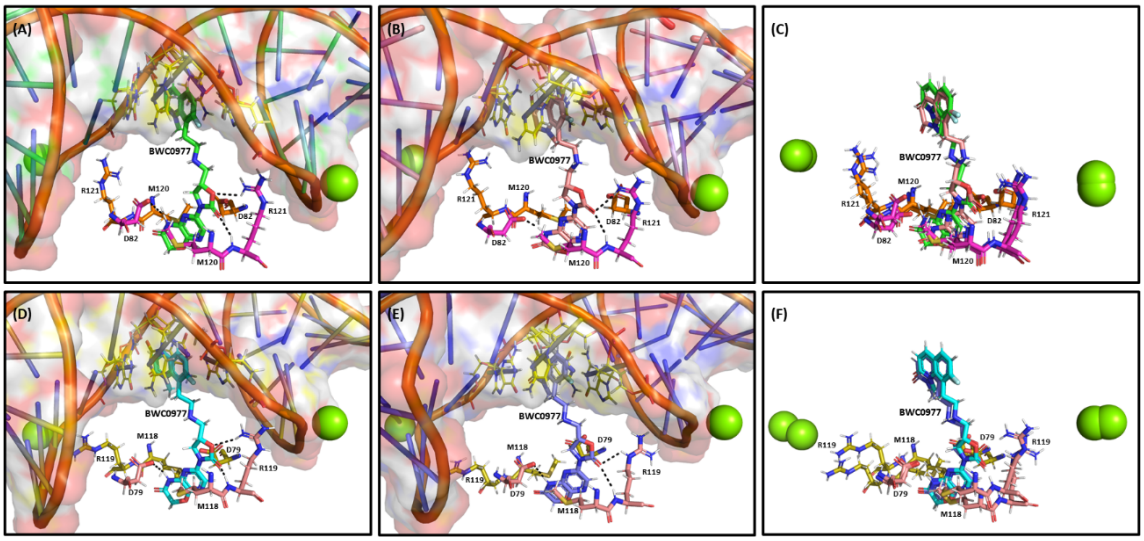


Supplementary Figure 3: Structure-Activity Relationships leading to the discovery of BWC0977.

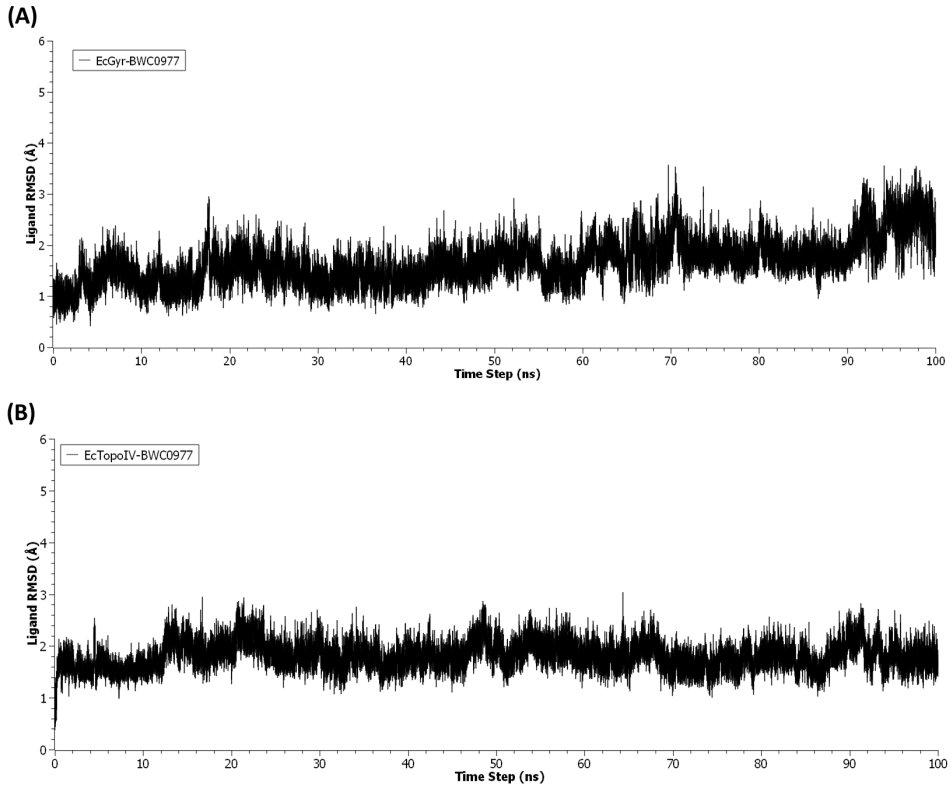


Supplementary Figure 4: Molecular Dynamics simulation poses before and after 100-nanoseconds (ns)

Binding orientation of BWC0977 with *E.coli* Gyrase and TopoIV in the docked pose (A & D), at the 100th ns of MD simulation (B & E), and their superimposition (C & F) are shown. Key interacting residues are depicted in sticks and colored based on the two protein chains. Ionic interactions are highlighted with broken lines, and Mg^{2+} is represented as a green sphere. The superimposed images of the docked pose and the 100th ns pose from the MD simulations clearly show no significant difference in the binding mode or interactions with key residues for both EcGyr and EcTopoIV.



Supplementary Figure 5: Ligand RMSD of BWC0977 with respect to the binding pocket of *E.coli* Gyrase (A) and TopoIV (B) is shown. The graph demonstrates that the RMSD of BWC0977 remains stable around 2Å throughout the 100ns molecular dynamics simulation, indicating a high affinity for binding.



Supplementary Table 1: Structure-activity relationship profile for compounds 1-9.

The progression of compounds C1-C9 synthesized and profiled with regards to gyrase and topoisomerase inhibition (IC_{50}), anti-bacterial spectrum, hERG inhibition liability and other properties. The systematic medicinal chemistry design modifications led to the convergence of improved antibacterial spectrum and absence of tox liability, while retaining the target inhibition and whole cell antibacterial activity.

Properties	C1	C2	C3	C4	C5	C6	C7	C8	C9
eLogD	-1.1	1.06	0.96	1.65	1.7	0.58	1.12	0.9	0.81
calculated pKa (Basic)	9.61	8.13	8.51	7.4	7.27	7.12	7.10	7.22	7.22
E. coli GyrA IC_{50} (μM)	13.2	0.054	0.1	0.036	0.034	0.041	0.011	0.013	0.0046
E. coli Topo IV IC_{50} (μM)	27	0.46	0.36	0.057	0.03	0.112	0.02	0.052	0.0096
Human Topo II decatenation IC_{50} (μM)	ND	ND	68.6	>120	>120	>120	>90	>120	>120
E. coli ATCC 25922 MIC (μg/ml)	80	0.25	0.06	0.03	0.015	0.06	0.06	0.06	0.03
S. aureus ATCC 29213 MIC (μg/ml)	160	0.13	0.06	0.03	0.008	0.06	0.008	0.008	0.008
K. pneumoniae ATCC 13883 MIC (μg/ml)	160	0.5	0.25	0.06	0.03	0.25	0.06	0.06	0.03
A. baumannii ATCC 19606 MIC (μg/ml)	160	0.25	0.06	0.03	0.015	0.25	0.25	0.25	0.06
P. aeruginosa ATCC 27853 MIC (μg/ml)	80	2	0.5	1	0.25	1	0.5	0.5	0.25
E. faecalis ATCC 29212 MIC (μg/ml)	160	0.25	0.25	0.125	0.03	0.25	0.125	0.06	0.06
hERG IC_{50} (μM)	ND	9	10	25	16.5	214	62	60	131
Aqueous solubility in PBS (μM)	ND	133	178	90	151	250	105	350	364
Human PPB %free	ND	9	8	2	<1	8.6	3.8	12	10
Hu_Heps_Clint (μl/min/million cells)	ND	ND	<8.2	9	ND	1.1	ND	4.1	2

ND: Not determined

Supplementary Table 2: MIC_{90} (µg/ml) of BWC0977 and known antibiotics determined at International Health Management Associates (IHMA) against a panel of multi-drug resistant Gram-positive isolates (independent study conducted by CARB-X).

Organism	*S. aureus*	*E. faecalis* / *E. faecium*	*S. pneumoniae*	*S. pyogenes*	Coagulase-negative Staphylococci
N	301	152	149	149	152
BWC0977	≤0.03	0.06	≤0.03	≤0.03	≤0.03
Azithromycin	>32	>32	>32	4	>32
Clindamycin	>32	>32	>32	0.125	>32
Daptomycin	0.5	2	0.125	0.06	0.5
Levofloxacin	32	>32	1	1	16
Linezolid	2	2	1	1	1
Vancomycin	1	32	0.5	0.5	2
Doxycycline	1	16	8	8	4
Penicillin	>32	>32	2	≤0.03	>32

Supplementary Table 3: Details of multiple laboratories across the world where the MIC_{90} studies were performed on a diverse range of bacterial pathogens. These pathogens were isolated from multiple infections, including complicated urinary tract infections (cUTI), complicated intra-abdominal infections (cIAI), wound infections, pneumonia, bloodstream infections, biothreat and cystic fibrosis infections.

MIC_{90} Study Site	Gram-Negative Isolates (N)	Gram-Positive Isolates (N)
St. John's Medical Hospital, Bangalore, India & Narayana Health, Bangalore, India	1392	194
JMI Laboratories, Iowa, USA	1952	34
International Health Management Associates (IHMA) Laboratories, Illinois, USA	3124	1119
Walter Reed Army Institute of Research (WRAIR), Maryland, USA	300	0
Colorado State University, USA	40	10
United States Army Medical Research Institute of Infectious Diseases, Maryland, USA	107	32
Eurofins, Taiwan	37	0
University of Texas Health Science Center at San Antonio, Texas, USA / National Institute of Allergy and Infectious Diseases (NIAID), Maryland, USA	138	0
University of Alabama, Birmingham, UK	45	0
University of Michigan, Ann Arbor, USA	36	4
Seattle Children's Hospital, Seattle, USA	16	11
TOTAL	7187	1404

Supplementary Table 4: MIC_{50} & MIC_{90} (µg/ml) of BWC0977 against a globally diverse panel of 7187 Gram-negative bacterial isolates**.**

Bacterial Pathogen	N	MIC Range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
Escherichia coli	1128	≤ 0.015 - 4	0.12	0.5
Pseudomonas aeruginosa	985	< 0.015 - 8	0.5	1
Klebsiella pneumoniae	945	0.03 – 32	0.5	2
Acinetobacter baumannii	860	0.03 – 4	0.25	0.5
Enterobacter cloacae	604	≤ 0.015 – 32	0.25	1
Citrobacter spp.	390	0.03 – 8	0.25	1
Proteus spp.	384	0.03 – 2	0.12	0.5
Serratia marcescens	367	0.03 – 32	0.25	1
Salmonella species	184	0.03 – 2	0.12	0.25
Morganella morganii	165	≤ 0.015 – 8	0.12	0.5
Klebsiella oxytoca, aerogenes	137	0.06 – 4	0.25	0.5
Neisseria gonorrhoeae	128	≤ 0.015 - 0.015	≤ 0.015	≤ 0.015
Providencia spp.	74	0.06 – 32	0.5	2
Campylobacter spp.	33	≤ 0.015 – 0.03	≤ 0.015	≤ 0.015
Shigella spp.	30	≤ 0.015 – 0.25	0.03	0.06
Helicobacter pylori	25	≤ 0.015 – 0.5	≤ 0.015	≤ 0.015
Enterobacter aerogenes	3	0.25	0.25	0.25
Hemophilus influenzae	2	≤ 0.015	≤ 0.015	≤ 0.015
Moraxella catarrhalis	1	≤ 0.015	≤ 0.015	≤ 0.015
Stenotrophomonas maltophilia	290	0.03 – 2	0.06	0.25
Burkholderia cepacia	75	0.03 – 2	0.06	0.5
Achromobacter spp.	40	≤ 0.015 – 0.5	0.06	0.12
Legionella pneumophila	35	≤ 0.015 – 0.015	≤ 0.015	≤ 0.015
Burkholderia gladioli	4	0.03 – 2	0.06	2
Burkholderia multivorans	4	0.5 – 2	1	2
Pandoraea apista	4	0.06 – 1	0.25	1
Ralstonia pickettii	4	0.03 – 0.06	0.03	0.06
Burkholderia pseudomallei	40	< 0.015 – 4	0.25	0.5
Burkholderia mallei	36	< 0.015 – 4	0.25	1
Francisella tularensis	36	≤ 0.015 – 0.015	≤ 0.015	≤ 0.015
Yersinia pestis	35	≤ 0.015 – 0.015	≤ 0.015	≤ 0.015
Mycoplasma spp.	35	≤ 0.015 – 0.015	≤ 0.015	≤ 0.015
Ureaplasma spp.	10	≤ 0.015 – 0.03	≤ 0.015	≤ 0.015
Fusobacterium spp.	33	≤ 0.015 – 0.5	≤ 0.015	≤ 0.015
Bacteroides fragilis	31	≤ 0.015 – 8	0.06	0.25
Prevotella sp.	30	≤ 0.015 – 1	≤ 0.015	≤ 0.015

Supplementary Table 5: MIC_{50} & MIC_{90} (µg/ml) of BWC0977 against a globally diverse panel of 1404 Gram-positive isolates.

Bacterial Pathogen	N	MIC Range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
Staphylococcus aureus	560	< 0.015 – 0.5	0.03	0.03
Coagulase negative Staphylococcus	208	≤ 0.015 – 0.03	≤ 0.015	≤ 0.015
Enterococcus faecalis	173	≤ 0.015 – 0.25	0.03	0.06
Streptococcus pyogenes	139	0.03 – 0.06	0.03	0.03
Enterococcus faecium	126	≤ 0.015 – 0.5	0.03	0.12
Streptococcus pneumoniae	122	0.03 – 0.06	0.03	0.03
Bacillus anthracis	42	≤ 0.015 - 0.015	≤ 0.015	≤ 0.015
Clostridium difficile	34	≤ 0.015 – 0.06	≤ 0.015	0.03

Supplementary Table 6: List of isolates used in the neutropenic mice thigh infection model dose response studies. All the strains were purchased from either the American Type Culture Collection (ATCC, USA) or the National Collection of Type Cultures (NCTC, UK).

Species	Strain	BWC0977 MIC (µg/ml)	Meropenem MIC (µg/ml)	Polymyxin MIC (µg/ml)
P. aeruginosa	ATCC 27853	0.5	0.5	0.5
P. aeruginosa	NCTC 13921	0.25	>64	1.0
P. aeruginosa	NCTC 13437	0.5	>64	1.0
A. baumannii	NCTC 13301	0.25	>64	0.5
A. baumannii	ATCC 17978	0.25	0.5	ND
A. baumannii	NCTC 13421	0.125	>16	ND
K. pneumoniae	NCTC 13465	0.25	0.06	0.5
K. pneumoniae	ATCC 43816	0.25	0.03	0.5
K. pneumoniae	ATCC 27736	0.5	0.06	1
E. coli	ATCC BAA-2523	0.06	0.25	0.25
E. coli	NCTC 13462	0.06	0.125	0.25

Supplementary Table 7: List of isolates and their MIC (µg/ml) used in the neutropenic rat lung infection model dose response studies.

Strain Name	BWC0977	Colistin	Ciprofloxacin	Meropenem
A. baumannii ATCC19606	0.06	0.5	0.4	0.25
A. baumannii SAC002 (Ciprofloxacin & carbapenem-resistant)	0.125	1	>16	>16
E. coli ATCC BAA-2469 (Ciprofloxacin & carbapenem-resistant)	0.06	1	>16	16
E. coli ATCC BAA-2471 (Ciprofloxacin & carbapenem-resistant)	0.5	0.5	>16	>32
E. coli SEC-015 (Ciprofloxacin & carbapenem-resistant))	0.25	1	>8	>8
K pneumoniae SKB067 (Colistin, Ciprofloxacin & carbapenem-resistant)	1	16	>16	>16
K. pneumoniae ATCC 13883	0.06	1	0.06	0.06
K. pneumoniae KPNIH1 (Ciprofloxacin & carbapenem-resistant)	1	2	>16	>32
K. pneumoniae MKP103 (Ciprofloxacin-resistant)	1	2	>16	<0.25
P. aeruginosa ATCC 27853	0.25	2	0.5	0.5
P. aeruginosa SPA041 (Ciprofloxacin-resistant)	1	2	>16	0.5

Origin of the strains used in the above studies: *A. baumannii* ATCC 19606, *E. coli* ATCC BAA-2469, *E. coli* ATCC BAA-2471, *K. pneumoniae* ATCC 13883 and *P. aeruginosa* ATCC 27853 were purchased from the American Type Culture Collection (ATCC, USA). *K. pneumoniae* KPNIH1 and MKP103 strains were purchased from Manoil Laboratory, University of Washington, USA. *E. coli* SEC-015, *A. baumannii* SAC002, *K. pneumoniae* SKB067, *P. aeruginosa* SPA041 were clinical isolates obtained from St. John's Hospital, Bangalore, India.

Supplementary Table 8: Dose fractionation design to determine the PK-PD index of BWC0977 in a neutropenic rat lung infection model following infection with *P. aeruginosa* ATCC 27853. Total doses of 300, 150, 75, 40, 20, 10 & 5 mg/kg were administered as intravenous infusion over 2 hours as once, twice or thrice in a day. Further, 150 mg/kg and 133 mg/kg were administered thrice in a day to yield a total dose of 450 and 400 mg/kg/day. Also, a total dose of 350 mg/kg was either fractionated as thrice or twice in a day. The calculated PK/PD indices are shown below:

Total dose [mg/kg]	Fractio-nated doses	Dose [mg/kg]	Regi-men	Log fC/MIC	fC/MIC	Log fAUC/MIC	fAUC/MIC per dose	Total fAUC/MIC	t>MIC [h]	Total t>MIC[h]	%t>MIC
450	3	150	q8	1.16	14.4	1.49	31.0	93	4.6	13.8	58
400	3	133	q8	1.10	12.6	1.44	27.4	82	4.3	13.0	54
350	2	175	q12	1.23	17.0	1.56	36.6	73	5.0	10.0	42
350	3	117	q8	1.04	10.9	1.38	23.8	71	4.1	12.3	51
300	1	300	q24	1.49	30.7	1.81	64.9	65	7.0	7.0	29
300	2	150	q12	1.16	14.4	1.49	31.0	62	4.6	9.2	38
300	3	100	q8	0.96	9.2	1.30	20.2	60	3.8	11.5	48
150	1	150	q24	1.16	14.4	1.49	31.0	31	4.6	4.6	19
150	2	75	q12	0.83	6.7	1.17	14.8	30	3.4	6.9	29
150	3	50	q8	0.63	4.3	0.98	9.6	29	3.0	9.1	38
75	1	75	q24	0.83	6.7	1.17	14.8	15	3.4	3.4	14
75	2	37.5	q12	0.50	3.1	0.85	7.1	14	2.8	5.7	24
75	3	25	q8	0.30	2.0	0.66	4.6	14	2.6	7.9	33
40	1	40	q24	0.53	3.4	0.88	7.6	8	2.9	2.9	12
40	2	20	q12	0.20	1.6	0.56	3.6	7	2.6	5.1	21
40	3	13	q8	0.01	1.0	0.37	2.4	7	2.5	7.4	31
20	1	20	q24	0.20	1.6	0.56	3.6	4	2.6	2.6	11
20	2	10	q12	-0.13	0.7	0.24	1.7	3	2.4	4.8	20
20	3	7	q8	-0.32	0.5	0.05	1.1	3	2.4	7.1	30
10	1	10	q24	-0.13	0.7	0.24	1.7	1.7	2.4	2.4	10
10	2	5	q12	-0.46	0.3	-0.08	0.8	1.7	2.3	4.7	19
10	3	3	q8	-0.65	0.2	-0.27	0.5	1.6	2.3	6.9	29
5	1	5	q24	-0.46	0.3	-0.08	0.8	0.8	2.3	2.3	10
5	2	2.5	q12	-0.79	0.2	-0.40	0.4	0.8	2.3	4.6	19
5	3	1.7	q8	-0.98	0.1	-0.59	0.3	0.8	2.3	6.8	29

Supplementary Table 9: **Mean (±SD) pharmacokinetic parameters of BWC0977 following single-dose intravenous bolus or intravenous-infusion (60 min) administration across various preclinical animal species.**

Species (gender)	Route	Dose (mg/kg)	C_0/ C_{max} (µg/mL)	T_{max} * (h)	AUC_{0-t} (h*µg/mL)	AUC_{0-inf} (h*µg/mL)	CLp (L/h/kg)	% GFR[b] %	V_d (L/kg)	V_{ss} (L/kg)	%TBW[c] %	$t_{1/2}$ (h)
BALB/c mouse (male)	IV bolus	3	2.98 ± 0.33[$]	NA	0.72 ± 0.12	0.72 ± 0.12	4.23 ± 0.73	504	3.40 ± 0.51	1.60 ± 0.22	221	0.56 ± 0.02
BALB/c mouse (male)	IV bolus	10	11.95 ± 1.66[$]	NA	2.68 ± 0.11	2.69 ± 0.11	3.72 ± 0.16	443	26.62 ± 38.31	1.89 ± 1.09	261	5.10 ± 7.41
Sprague Dawley rat (male)	IV Infusion	30	15.2 ± 2.2	1.0	14.7 ± 0.2	14.7 ± 0.2	2.04 ± 0.03	650	2.83 ± 0.09	0.84 ± 0.06	126	0.96 ± 0.02
Sprague Dawley rat (male)	IV Infusion	100	62.6 ± 6.7	1.0	73.7 ± 15.3	73.7 ± 15.3	1.40 ± 0.29	446	9.86 ± 1.68	0.90 ± 0.05	135	4.93 ± 0.5
Sprague Dawley rat (male)	IV Infusion	150	100.7 ± 14.5	1.0	103.9 ± 7.3	103.9 ± 7.3	1.45 ± 0.11	462	12.9 ± 1.0	0.86 ± 0.04	129	6.17 ± 0.42
Dunkin Hartley Guinea pigs (male)	IV Infusion	50	11.97 ± 3.51	0.50	16.5 ± 3.17	15.78 ± 3.01	3.26 ± 0.6	ND	NR	1.63 ± 1.3	ND	0.48 ± 0.29
Beagle dog (male)	IV infusion	3	3.28 ± 0.56	1.0	5.56 ± 0.78	5.25[a]	0.576[a]	157[a]	4.18[a]	1.08[a]	179[a]	5.05[a]
Beagle dog (male)	IV infusion	9	7.10 ± 0.15	1.0	15.70 ± 2.83	15.90 ± 3.12	0.55 ± 0.99	149	4.42 ± 1.56	1.23 ± 0.30	204	5.89 ± 3.16

*Median (Min-Max); [a](n=2); NA: Not applicable; ND: Not determined; NR: Not reported

[b] CL/GFR in corresponding species x 100; GFRs used for mice, rats and dogs were 0.840, 0.314 and 0.368L/h/kg, respectively (Davies 1993)

[c] V_{ss}/TBW in corresponding species x 100; TBW values used for mice, rats and dogs were 0.725, 0.668 and 0.604 L/kg, respectively (Davies 1993)

[$] C_0 form IV bolus administration

Supplementary Table 10: Comparison of BWC0977 concentrations in mice epithelial lining fluid and plasma (total and free fraction).

Dose (mg/kg)	Plasma (total) AUC	Plasma (free) AUC	ELF AUC
10	0.16 (AUC_{0-1})	0.02 ($fAUC_{0-1}$)	1.40 (AUC_{0-2})
40	2.84 (AUC_{0-6})	0.37 ($fAUC_{0-6}$)	5.04(AUC_{0-6})
80	10.40 (AUC_{0-24})	1.35 ($fAUC_{0-24}$)	16.33 (AUC_{0-8})
120	16.27 (AUC_{0-24})	2.12 ($fAUC_{0-24}$)	21.19 (AUC_{0-8})

For the PK studies, 10, 40, 80 and 120 mg/kg of BWC0977 was administered subcutaneously q24h in neutropenic CD-1 mice infected intramuscularly in the thigh with *Pseudomonas aeruginosa* NCTC 13921 and plasma samples taken at 0, 0.5, 1, 2, 4, 6, 8, and 24-hours post-dosing. The ELF was obtained by instilling 2ml of sterile saline into the lungs and removing saline from the lungs twice at 0, 0.5, 1, 2, 4, 6, 8 hours post-dosing. Plasma protein binding ~ 87%. The selected structural model was a 2-compartment model that had linear clearance. The absorption rate constant was 19 h^{-1}, total clearance 0.1 L/h, volume of distribution 0.05 L, the rate constant for BWC0977 distribution from the central to the peripheral compartment 0.7 h^{-1}, and rate constant for the BWC0977 distribution from the peripheral to the central compartment 0.3 h^{-1}. The goodness of fit of final population PK was r^2 of 0.98.

Supplementary Table 11: Comparison of BWC0977 concentrations in rat epithelial lining fluid and plasma (total and free fraction).

PK parameters (NCA)	ELF	Plasma (free)	Plasma (total)
C_{max} (μg/ml)	81 ± 11	2.7 ± 0.24	54 ± 4.8
AUC_{0-t} (hr.μg/ml)	113 ± 40	5.9 ± 2.2	117 ± 44
AUC_{0-inf} (hr.μg/ml)	114 ± 40	5.9 ± 2.2	117 ± 44
CL (mL/hr/Kg)	955 ± 321	19060 ± 8186	953 ± 409
V_{ss} (mL/Kg)	2875 ± 543	45732 ± 16729	2287 ± 836
$t_{1/2}$ (hr)	2.3 ± 0.93	2.8 ± 1.1	2.8 ± 1.1
AUC_{ELF}/AUC_{plasma}	-	19.4	0.97
$Cmax_{ELF}/Cmax_{plasma}$	-	26.3	1.3

BWC0977 was administered at 100 mg/kg as an intravenous infusion over 1-hour in neutropenic rats infected with *P. aeruginosa* ATCC27853. The ELF and plasma samples were taken at 0.5, 1 hour (during infusion), and post-infusion at 1.25, 1.5, 2, 3, 5, 9, 25 hours post-dosing. The ELF was obtained by instilling 2 ml of sterile saline into the lungs and removing saline from the lungs. The free plasma levels were calculated from the total plasma levels using the plasma protein binding value of 94%. The concentration of BWC0977 in the rat model was significantly higher in the ELF in comparison to free plasma levels. PK data analysis was done using the WinNonlin® software.
Abbreviations: NCA – non-compartmental analysis, AUC – Area Under the Curve, ELF – Epithelial lining fluid.

Supplementary Table 12: Pharmacokinetic parameters following intravenous infusion administration of BWC0977 in healthy human volunteers.

Cohort (Dose)	Summary statistics	C_{max} (µg/mL)	AUC_{0-t} (hr.µg/mL)	AUC_{0-inf} (hr.µg/mL)	CL (L/hr)	V_{ss} (L)	$t_{1/2}$ (hr)	$Ae_{(0-t)}$ (mg)	Fe %	CLr (L/hr)
C1 (120 mg)	Mean ± SD (%CV)	1.96 ± 0.49 (25)	5.75 ± 1.04 (18)	5.86 ± 1.04 (18)	21.08 ± 4.09 (19)	44.03 ± 25.18 (57)	3.31 ± 1.40 (42)	42.2 ± 7.35 (17)	35.2 ± 6.13 (17)	7.49 ± 1.54 (21)
C2 (240 mg)	Mean ± SD (%CV)	3.27 ± 0.69 (21)	10.08 ± 2.62 (26)	10.20 ± 2.61 (26)	25.17 ± 7.9 (31)	51.50 ± 12.30 (24)	4.70 ± 1.90 (40)	74.3 ± 23.13 (31)	31.0 ± 9.64 (31)	7.66 ± 2.34 (31)
C3 (480 mg)	Mean ± SD (%CV)	8.20 ± 2.50 (30)	24.40 ± 7.12 (29)	23.20 ± 7.11 (31)	22.56 ± 7.7 (34)	49.60 ± 15.43 (31)	6.64 ± 3.87 (58)	132 ± 10.11 (8)	27.5 ± 2.11 (8)	5.89 ± 2.03 (34)
C4 (720 mg)	Mean ± SD (%CV)	10.68 ± 1.56 (15)	30.46 ± 5.53 (18)	30.78 ± 5.62 (18)	24.32 ± 6.11 (25)	49.45 ± 10.18 (21)	6.35 ± 2.71 (43)	149 ± 48.38 (33)	20.6 ± 6.72 (33)	5.23 ± 2.77 (53)
C5 (1050 mg)	Mean ± SD (%CV)	17.93 ± 2.57 (14)	54.42 ± 8.44 (16)	54.67 ± 8.50 (16)	19.62 ± 3.26 (17)	44.5 ± 10.41 (23)	8.4 ± 2.54 (30)	211 ± 86.36 (41)	20.1 ± 8.23 (41)	3.89 ± 1.37 (35)

Median T_{max} was observed to be 2 hours; $Ae_{(0-t)}$ is the amount of drug excreted in urine from 0-last timepoint; CLr: Renal Clearance; Fe: Fraction of drug excreted unchanged in the urine.

Supplementary Table 13: Reference drugs and the solvents used for preparing the stock solutions.

Antimicrobial Agent	**Solvent**
Amikacin	Water
Ampicillin	Phosphate buffer pH 8.0, 0.1 M
Avibactam sodium	Water
Azithromycin	95% Ethanol
Aztreonam	Saturated sol sodium bicarbonate
Cefepime hydrochloride	Phosphate buffer pH 6.0, 0.1 M
Cefpodoxime free acid	0.1% (11.9mM) aqueous sodium bicarbonate
Ceftazidime pentahydrate	sodium carbonate 10% of the weight
Ceftriaxone sodium	Water
Ciprofloxacin hydrochloride	Water
Clindamycin hydrochloride	Water
Colistin sulfate	Water
Daptomycin	Water
Doxycycline hyclate	Water
Eravacycline dihydrochloride	Water
Gentamicin sulfate	Water
Levofloxacin	1/2 vol of water + 0.1 M NaOH dropwise to dissolve
Linezolid	Water
Meropenem	Water
Penicillin G potassium	Water

Supplementary Table 14: Bacterial strains used in the efficacy determination of BWC0977 in neutropenic mice dual thigh infection model.

Strain	Dose range (mg/kg) & doses (mg/kg) administered via SC q8h	Positive control q8h, SC
P. aeruginosa ATCC 27853	1-100 (1, 5, 10, 30, 60, and 100)	Meropenem 200mg/kg
P. aeruginosa NCTC 13437	5-120 (5, 10, 30, 60, 90, and 120)	Meropenem 200mg/kg
P. aeruginosa NCTC 13921	10-120 (10, 30, 60, 90, and 120)	Meropenem 200mg/kg and Polymyxin 25mg/kg*
A. baumannii NCTC 13301	10-120 (10, 30, 60, 90, and 120)	Meropenem 200mg/kg and Polymyxin 25mg/kg*
A. baumannii ATCC 17978	10-120 (10, 30, 60, 90, and 120)	Meropenem 200mg/kg and Polymyxin 25mg/kg*
A. baumannii NCTC 13421	2.5-120 (2.5,7.5,10,30,60, and 120)	Meropenem 200mg/kg and Polymyxin 25mg/kg*
K. pneumoniae NCTC 13465	10-120 (10, 30, 60, 90, and 120)	Meropenem 200mg/kg and Polymyxin 25mg/kg*
K. pneumoniae ATCC 43816	10-120 (10, 30, 60, 90, and 120)	Meropenem 200mg/kg and Polymyxin 25mg/kg*
K. pneumoniae NCTC 27736	2.5-120 (2.5,7.5,10,30,60,90 and 120)	Meropenem 200mg/kg and Polymyxin 25mg/kg*
E. coli ATCC - BAA-2523	10-120 (10, 30, 60, 90, and 120)	Meropenem 200mg/kg and Polymyxin 25mg/kg*
E. coli NCTC 13462	10-120 (10, 30, 60, 90, and 120)	Meropenem 200mg/kg and Polymyxin 25mg/kg*

*Meropenem and Polymyxin B represent 2 separate positive control groups within the same experiment

Supplementary Table 15: Details of the experimental conditions for evaluating BWC0977 as P-gp, BCRP and BSEP inhibitor.

Transporter	BSEP
Probe substrate	[^{3}H]-Taurocholic acid (0.4μM)
[Inhibitor (BWC0977)]	10 and 50μM
Positive control inhibitor	Cyclosporine (20μM)
Incubation time (min)	5
Incubation medium	HEPES-Tris (10mM), KNO_3 (100mM), $Mg(NO_3)_2$ (10mM) and sucrose (50mM)
Wash buffer	HEPES-Tris (10mM), KNO_3 (100mM) and sucrose (50mM)

Supplementary Table 16: Details of the experimental conditions for evaluating BWC0977 as P-gp, BCRP and BSEP substrate.

Transporter	P-gp	BCRP	BSEP
[Substrate (BWC0977)]	0.1, 1, 10 and 50μM	0.1, 1, 10 and 50μM	0.1, 1, 10 and 50μM
Inhibitors	Verapamil (100μM); Valspodar (10μM)	Ko143 (1μM); Sulfasalazine (30μM)	Cyclosporine (20μM); Glyburide (100μM)
Positive control substrate	N-Methylquinidine (0.5μM)	[^{3}H]-Estrone-3-sulfate (1μM)	[^{3}H]-Taurocholic acid (0.4μM)
BWC0977 incubation time (min)	1, 3 and 10	1, 3 and 10	1, 3 and 10
Positive control incubation time (min)	2	1	5
Incubation medium	Tris-HCl (10mM), $MgCl_2$ (10mM), sucrose (250mM)	MOPS-Tris (50mM), $MgCl_2$ (7.5mM), KCl (70mM)	HEPES-Tris (10mM), KNO_3 (100mM), $Mg(NO_3)_2$ (10mM) and sucrose (50mM)
Wash buffer	Tris-HCl (10mM), NaCl (100mM), sucrose (250mM)	MOPS-Tris (40mM), KCl (70mM)	HEPES-Tris (10mM), KNO_3 (100mM), sucrose (50mM), sodium taurocholate (0.1mM)

Supplementary Table 17: Experimental conditions for evaluating BWC0977 as OATP, OAT, OCT and MATE substrate and K_m / V_{max} determination.

Transporter	OATP1B1/ OATP1B3	OAT1	OAT3	OCT2	MATE1/ MATE2-K
[Substrate (BWC0977)]	0.5 and 5 µM	0.5 and 5 µM	0.5 and 5 µM	0.5 and 5 µM	0.5 and 5 µM
[Substrate (BWC0977)] K_m / V_{max} determinations	Not applicable	Not applicable	0.5, 1, 5, 10, 50, 100 and 500 µM	Not applicable	0.5, 1, 5, 10, 50, 100 and 500 µM (MATE2-K only)
Inhibitor	Rifampin (10 µM)	Probenecid (100 µM)	Probenecid (100 µM)	Quinidine (300 µM)	Cimetidine (20 µM[a]; 300 µM[b])
Positive control substrate	[^{3}H]-Estradiol-17β-glucuronide (50 nM)	[^{3}H]-p-Aminohippurate (1 µM)	[^{3}H]-Estrone-3-sulfate (50 nM)	[^{14}C]-Metformin (10 µM)	[^{14}C]-Metformin (10 µM)
Test article incubation time (min)	1 and 10	1 and 10	1 and 10	1 and 10	1 and 10
Positive control incubation time (min)	2	1	2	2	5

[a]For MATE1

[b]For MATE2 K

Supplementary Table 18: Pharmacokinetic Study design of BWC0977 in Mice, Rats, Guinea pigs and Dogs

Species, Strain, Gender	Route & feed condition	Dose (mg/kg)	Dose volume (mL/kg)	Formulation details
Mice, BALB/c, Male	IV bolus, Fed	3, 10	5	15% L-ascorbic acid in water, final pH adjusted to 4.0 with 3N NaOH
Rat, Sprague-Dawley, Male	IV infusion, Fed	30, 100, 150	20	10% L-ascorbic acid in water, pH adjusted to 4.0 with 1N NaOH
Rat, Sprague-Dawley, Male Bile duct cannulation study	IV infusion, Fed	100	10	15% L-ascorbic acid in water, final pH adjusted to 4.0 with 3N NaOH
Guinea pigs, Dunkin Hartley, Male	IV bolus, Fed	50	5	15% L-ascorbic acid in water, final pH adjusted to 4.0 with 3N NaOH
Dog, Beagle, Male	IV bolus / Fed	3, 10	5	10% L-ascorbic acid in water, pH adjusted to 4.0 with 1N NaOH

Supplementary Table 19: Single Ascending Dose (SAD) study design in healthy human volunteers.

Cohort	Dose (mg)	Number of doses	No. of days of dosing	Number of Subjects	
				BWC0977	Placebo
C1	120	1	1	6	2
C2	240	1	1	6	2
C3	480	1	1	6	2
C4	720	1	1	6	2
C5	1050	1	1	6	2
Total number of subjects				30	10

Supplementary Note 1: Treatment Emergent Adverse Events (TEAE)
Single Ascending Dose Phase
There were no deaths in Part A (SAD). Treatment-emergent AEs were reported for 26 of 30 subjects (87%) who received BWC0977, and for 4 of 10 subjects (40%) who received placebo with a total of 77 TEAEs. Most TEAEs were classified as mild in severity (67 of the 77 TEAEs [87%]), with 10 TEAEs (13%) classified as moderate in severity. The only moderate TEAE to occur in more than 1 subject was headache: 2 subjects in the 120 mg BWC0977 group, and 1 subject in the placebo group. No TEAEs were classified as severe and there was no clear BWC0977 dose-related trend in the severity of TEAEs. The most common TEAEs were dysgeusia and headache. Treatment-emergent AEs deemed to be related to study drug were reported in 21 of 30 subjects (70%) who received BWC0977 and in 2 of 10 subjects (20%) who received placebo, with a total of 36 treatment-related TEAEs. The most commonly occurring treatment-related TEAEs were dysgeusia and headache, which were only reported in subjects who received BWC0977. The incidence of dysgeusia and/or taste disorder appeared to increase with increasing BWC0977 dose. Most treatment-related TEAEs were classified as mild in severity, with 5 of 36 related TEAEs (14%) classified as moderate in severity. Moderate related TEAEs were only reported in subjects who received BWC0977 (4 of 30 subjects [13%]) and included: headache; diarrhoea; gastrooesophageal reflux disease, and nausea. Treatment-emergent AEs of infusion site reactions at the site of study drug administration were only reported in 2 of 30 subjects (7%) who received 720 mg BWC0977. Both TEAEs (infusion site pain and catheter site rash) were classified as mild in severity with one event considered related to study drug and one considered not related. Both these events resolved during the course of the study. There were no ECG, vital signs or clinical laboratory parameters that were assessed as clinically significant. There were no other clinically significant systemic findings observed.

Multiple Ascending Dose Phase
There were no deaths in Part B (MAD). A number of mitigatory measures were implemented to ensure least potential for infusion site reactions (ISRs): new intravenous (IV) injection infusion formulation was used, with increased pH of the BWC0977 and placebo formulation, to allow a better tolerability profile. The pH of both the active and placebo was modified from 4.3 to 4.7. Ibuprofen was allowed as premedication based on the Principal investigator's judgement to reduce the incidence of phlebitis. Doses of BWC0977 were administered over a shorter duration (30 (±5) minutes) via IV infusion, so that the flow rates were appropriate. Infusion site reaction assessment during and after infusion was adjusted to the new dose regimen. After implementation of the mitigatory measures the study was continued and two sentinel participants (n=2) received study BWC0977. Both participants received 20 mL saline flush pre-dose and post glucose flush at every IV infusion.

One participant reported infusion site pain on multiple occasions. Pain was generally mild, reported on most days and worsened after the third dose or 36 hours after each cannulation. Three re-cannulations were required (Day 3, Day 5 and Day 6). Ibuprofen was administered prior to dosing when the pain symptoms had developed. On Day 6 significant pain was reported which "ran up the arm" and thrombosis was confirmed on ultrasound. The participant withdrew consent for further dosing at conclusion of Day 6 (12/14 planned doses completed). The reason cited for withdrawal was the participant had to travel and hence will have difficulty in following up.

Second participant had fewer instances of pain at the infusion site, less cannula flushes and the lesser requirement for administration of ibuprofen (given only at the evening of Day 6). A single re-cannulation was performed 36 hours post cannula failure. Ultrasound was performed and showed thrombosis with phlebitis at the infusion site. The participant completed the planned 14/14 doses.

TITLE PAGE

Clinical Study Protocol: C001-2020-01

Study Title:	A randomized, double-blind, placebo-controlled, Phase 1 study of the safety, tolerability and pharmacokinetics of single and multiple ascending doses of BWC0977 in healthy adult volunteers
Protocol Number:	C001-2020-01
Study Phase:	I
Principal Investigator:	Dr Angela Molga
Sponsor:	Bugworks Research Inc. 2711, Centerville Road, Suite 400 Wilmington, Delaware 19808 USA
Name of Sponsor Signatory:	V.Balasubramanian, PhD
Protocol Version:	5.0
Date of Version:	06 April 2022

SPONSOR PROTOCOL APPROVAL SIGNATURE PAGE

The undersigned has reviewed and approved Protocol No. C001-2020-01 for issuance:

DocuSigned by:
Balasubramanian.V
Signer Name: Balasubramanian.V
Signing Reason: I approve this document
Signing Time: 05-Apr-2022 | 20:33:54 PDT
F5C1860FD1B5496E9BFA6E7083CB1D79

05-Apr-2022 | 20:34:00 PDT

Balasubramanian.V, PhD./ COO	Signature	Date

INVESTIGATOR SIGNATURE PAGE

I have read the attached protocol and agree that it contains all the necessary details for performing the study.

I will provide copies of the protocol and of the Investigator's Brochure (IB), which was furnished to me by the Sponsor, to all members of the study team responsible to me who participate in the study. I will discuss this material with them to assure that they are fully informed regarding the study treatment, including the potential risks and side effects, and the conduct of the study.

Once the protocol has been approved by the Institutional Review Board (IRB), I will not modify this protocol without obtaining the prior approval of the Sponsor and of the IRB. I will submit the protocol modifications and/or any informed consent form (ICF) modifications to the Sponsor and the IRB, and approval will be obtained before any modifications are implemented.

I understand the protocol and will work according to it, the principles of Good Clinical Practice [GCP; current International Council for Harmonization (ICH) guidelines], and the Declaration of Helsinki (1964) including all amendments up to and including the October 2013 revision.

Name of Site Principal Investigator	Signature	Date

Institution Name:
CMAX Clinical Research Pty Ltd
Level 5, 21-24 North Terrace
Adelaide, SA, 5000
AUSTRALIA

DOCUMENT HISTORY

Document	Version Date	Summary of Changes
Protocol V2.0	17 Mar 2021	N/A
Protocol V3.0	01 Sep 2021	Drug product presentation and respective details were updated in Sec 6 under Study Treatment
Protocol V4.0	21 Feb 2022	Addition of Holter monitoring for SAD Cohorts 4, 5 and 6 and all MAD cohorts. Addition of optional Cohort 6. Inclusion of details covered in Protocol Clarification Letters #1 and #2
Protocol V5.0	31 March 2022	Removal of QuantiFERON Gold (tuberculosis) testing at screening. Reduction of screening window to 28 days (previously 35 days). Reduction in the allowable window for concomitant medications to 14 days or 5 half-lives (previously 30 days or 5 half-lives). Incorporation of Protocol Clarification Letter #3 dated 10 Mar 2022 (relating to the requirement for Holter monitoring for SAD Cohort 3 onwards). Clarification that subjects are required to rest for at least 10 minutes prior to Holter ECG extraction timepoints, and for at least 5 minutes after.

This amendment incorporates all revisions to date including amendments made at the request of country health authorities, institutional review boards/ethics committees (IRBs/ECs), etc.

PROTOCOL SYNOPSIS

Title	A randomized, double-blind, placebo-controlled, Phase 1 study of the safety, tolerability and pharmacokinetics of single and multiple ascending doses of BWC0977 in healthy adult volunteers
Clinical Phase	Phase I
Investigational Product (IP)	**Name of the IP:** BWC0977 **Formulation:** Compounded Solution **Mode of administration:** Intravenous infusion
Comparator	**Placebo:** Compounded solution minus BWC0977
Primary Objective	To assess the safety and tolerability of BWC0977 following single and multiple ascending doses by intravenous (IV) infusion in healthy adult volunteers.
Secondary Objective	To assess the pharmacokinetic (PK) characteristics in plasma and urine of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult volunteers
Participant Number	This study will enroll approximately 72 healthy adult volunteers: Part A, Single Ascending Dose (SAD): Up to 48 healthy volunteers will be enrolled in a total of 6 cohorts (Cohort 6 optional). Each cohort will enroll 8 participants with 6 participants randomised to receive BWC0977 and 2 participants randomised to receive placebo. Part B, Multiple Ascending Dose (MAD): Up to 24 healthy volunteers are planned to be enrolled in up to 3 cohorts. Each cohort will enroll up to 8 participants, with 6 participants randomised to receive BWC0977 and 2 participants randomised to receive placebo. For each study part, efforts will be made to randomise approximately equal numbers of males and females to either active or placebo.
Sites	Single site (Australia)
Study Treatment	Part A: Single BWC0977 IV infusion (120, 240, 480, 720 mg plus doses of up to 1050 mg [actual dose to be determined based on safety, tolerability and PK data from Cohorts A1-A4]) Part B: Dose levels and dosing frequency to be determined based on safety, tolerability and PK data from Part A.
Study Duration	The total maximum duration for Part A (SAD) is **37 days**, inclusive of screening windows. The total maximum duration for Part B (MAD) is **45 days**, inclusive of screening windows.
Study Confinement Periods	For each participant enrolled in Part A, the confinement period will commence on Day -1, with dosing on Day 1 and discharge on Day 3.

<table>
<tr><td></td><td>For each participant enrolled in Part B, the confinement period will commence on Day -1, with dosing on each day 1-10 and discharge on Day 11.</td></tr>
<tr><td>Study Design</td><td>
This is a Phase 1, single-center, prospective, randomised, double-blind study of single-ascending doses and multiple-ascending doses of BWC0977 administered through IV infusion to healthy adult volunteers. This study is subdivided into parts, Part A (SAD) and Part B (MAD). The decision to escalate between dose levels and proceed to the next study part (Part B) will be based upon review of the available safety and PK data by the Safety Monitoring Group (SMG). In each part of the study, cohorts may be added or removed based upon emerging data.

In Part A healthy volunteers will be randomised to receive a single IV infusion doses of BWC0977 or placebo, to be infused over 120 (±10) minutes. The starting dose in Part A will be 120 mg, with 6 dose levels planned (Table S1). The dose level to be evaluated in Cohort A5 will be determined based on the safety, tolerability and PK data from the previous 4 cohorts. Cohort A6 is optional and may be included if a supra therapeutic dose is achieved by Cohort A3. This is to ensure that both sub therapeutic and supra-therapeutic doses are evaluated for QTc.

Table S1: Part A Single Ascending Dose Study Design
<table>
<tr><th rowspan="2">Cohort</th><th rowspan="2">Dose (mg)</th><th rowspan="2">No. of Doses Per Day</th><th colspan="2">Number of Participants</th></tr>
<tr><th>BWC0977</th><th>Placebo</th></tr>
<tr><td>A1</td><td>120</td><td>1</td><td>6</td><td>2</td></tr>
<tr><td>A2</td><td>240</td><td>1</td><td>6</td><td>2</td></tr>
<tr><td>A3</td><td>480</td><td>1</td><td>6</td><td>2</td></tr>
<tr><td>A4</td><td>720</td><td>1</td><td>6</td><td>2</td></tr>
<tr><td>A5</td><td>TBD*</td><td>1</td><td>6</td><td>2</td></tr>
<tr><td>A6</td><td>TBD**</td><td>1</td><td>6</td><td>2</td></tr>
<tr><td colspan="3" rowspan="2">Total Number of Participants</td><td>up to 36</td><td>up to 12</td></tr>
<tr><td colspan="2">up to 48</td></tr>
</table>
Abbreviations: TBD = to be determined.
*Dose not to exceed 1050 mg
**Cohort A6 is optional. Dose to be sub therapeutic. This cohort may be included if a supra therapeutic dose is achieved by Cohort A3.

Three dose levels are planned to be evaluated in Part B (Table S2), with the starting dose for Part B selected following completion of Part A, unless, based on emerging safety and PK data from Part A, the SMG deems it safe and appropriate to select a starting dose for Part B
</td></tr>
</table>

prior to completion of all dose level cohorts in Part A (a minimum of 3 cohorts from Part A must have been completed).

In Part B, participants will receive multiple doses of BWC0977 over 10 days. The doses and dosing frequencies of BWC0977 in Part B will be selected based on the safety, tolerability, and PK data of BWC0977 obtained in Part A and any preceding cohorts in Part B. A dose level will only be evaluated in Part B if determined to be safe and tolerable in Part A. In each part of this study, cohorts may be added or removed based upon emerging data.

Table S2: Part B Multiple Ascending Dose Study Design

Cohort	Dose (mg)*	Dosing frequency	No. of days of dosing	Number of Volunteers	
				BWC0977	Placebo
B1	TBD	TBD	10	6	2
B2	TBD	TBD	10	6	2
B3	TBD	TBD	10	6	2
Total Number of Volunteers				18	6
				24	

Abbreviations: TBD = to be determined.
*Dose not to exceed 1050 mg

Study Assessments

Safety and Tolerability

Clinical

- Medical history and concomitant medication use
- Evaluation of adverse events (AEs) and treatment-emergent AEs (TEAEs)
- Height and Weight
- Physical examination
- Vital signs
- 12-lead electrocardiograms (ECGs)
- Holter recording (SAD Cohorts 3, 4, 5 and 6 and all MAD Cohorts)

Laboratory Safety

- Haematology: Platelet Count, red blood cell (RBC) count, white blood cell (WBC) count (absolute) with differential, reticulocyte count, Hemoglobin, RBC indices.
- Coagulation: Prothrombin time (sec and INR), partial thromboplastin time
- Clinical chemistry: creatinine, glucose, sodium, urea, phosphate, creatine phosphokinase (CPK), potassium, chloride,

	bicarbonate, calcium, aspartate aminotransferase (AST) (SGOT), alanine aminotransferase (ALT) (SGPT), gamma glutamyltransferase (GGT), alkaline phosphatase, lactate dehydrogenase, total and direct bilirubin, uric acid, albumin, total Protein, lipid panel, creatinine (including calculated creatinine clearance [CrCl] using Cockcroft-Gault formula), albumin: creatinine ratio. • Urinalysis: pH, specific gravity, protein, blood, glucose, ketones, urobilinogen. Urine microscopic examination/sediment microscopy will be conducted in the instance of abnormal clinically significant urinalysis findings. **Infection Screening** • HIV (HIV1, HIV2) • Hepatitis B virus surface antigen (HBsAg) • Hepatitis C (anti HCV antibody) **Pregnancy and Follicle Stimulating Hormone testing** • Serum and urine human chorionic gonadotropin pregnancy testing will be performed. • Postmenopausal females will be tested for follicle stimulating hormone (FSH) levels. **Drugs of Abuse, Cotinine and Alcohol** • Drugs of abuse urine screen will include: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines. Urine cotinine and alcohol breath testing will also be performed. **Pharmacokinetics** Blood and urine samples for PK will be collected prior to dosing and at several timepoints post-dose.
Study Endpoints	<u>Primary Endpoints:</u> The safety and tolerability of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult participants will be evaluated based on the following endpoints: • Incidence of TEAEs and serious adverse events (SAEs) overall and by intensity • Changes from baseline in: o Safety laboratory test results o ECG parameters o Vital signs measurements

	○ Physical examination findings Secondary Endpoints: Following single dose: • Area under the curve $(AUC)_{(0\text{-}8)}$, $AUC_{(0\text{-}12)}$, $AUC_{(0\text{-}24)}$, $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}\infty)}$, maximum observed concentration (C_{max}), systemic clearance (CL), volume of distribution at steady state (Vdss), mean residence time (MRT), and terminal phase half-life ($t_{1/2}$) of BWC0977, as data permit. Following repeat dose: • $AUC_{(0\text{-}\tau)}$, C_{max}, concentration at the end of the dosing interval ($C\tau$), observed accumulation ratio (Ro) and CL of BWC0977, as data permit. Amount excreted in urine (Ae) of unchanged BWC0977, fraction of the dose excreted in urine (fe) and renal clearance (CLr), as data permit. Exploratory Endpoints: Cardiodynamic evaluation using 12-lead ECGs extracted from Holter recordings may be undertaken based on observed PK and other project considerations. In such case, the following endpoints will be used: • Change-from-baseline heart rate, PR, QRS and QTcF interval (ΔHR, ΔPR, ΔQRS and ΔQTcF). • Placebo-corrected ΔHR, ΔPR, ΔQTcF and ΔQRS • Categorical outliers for HR, PR, QRS and QTcF • Frequency of treatment emergent T- and U-wave abnormalities
Subject Selection Criteria	**Inclusion Criteria** Each subject must meet all of the following criteria to be eligible for study participation: 1. Healthy male or female 18 to 55 years of age, inclusive, at time of consent. 2. Body mass index (BMI) ≥ 19.0 and ≤ 30.0 (kg/m^2) and weight between 55.0 and 100.0 kg (inclusive). 3. Medically healthy without clinically significant abnormalities at the screening visit or Day -1, including: a) No findings in Physical examination or vital signs (including temperature, heart rate, respiratory rate, and blood pressure)

	that the Principal Investigator (PI) determines would interfere with interpretation of study results. b) Electrocardiograms (ECGs) without clinically significant abnormalities, including a QT duration corrected for heart rate by Fridericia's formula (QTcF) interval duration ≤450 msec (for males), and ≤470 msec (for females) obtained as an average from the triplicate screening ECGs after at least 5 minutes in a supine quiet-rest position. c) Clinically significant abnormalities in the screening clinical laboratory tests, as determined by the Investigator. Repeat testing could be performed at the Investigator's discretion. 4. Willing and able to provide written informed consent. 5. Agrees to be available for all study visits and cooperate fully with the requirements of the study protocol, including the schedule of assessments. 6. Willing to refrain from strenuous physical activity that could cause muscle aches or injury, including contact sports, at any time from 4 days prior to admission in the clinical research unit (CRU) until completion of the study (follow-up [FU] visit). 7. Willing to refrain from prescription medications from Screening visit until follow-up; and over-the-counter (OTC) medications, vitamin preparations and other food supplements, from Day -1 up to follow-up. 8. Have suitable venous access for drug administration and blood sampling. 9. If female of child-bearing potential, must agree to and comply with: a) Using 1 barrier method (e.g., female condom or male partner using a condom) plus 1 other highly effective method of birth control (e.g., oral contraceptive, implant, injectable, indwelling intrauterine device, vasectomized partner), or double-barrier method (use of a condom by the male partner with use of a diaphragm by the female partner), or b) Sexual abstinence, for the duration of the study (from signing of consent to FU visit) and for 30 days after last study drug administration, plus

c) Females of child-bearing potential must also agree not to donate ova or oocytes (ie, human eggs) during the study, and for one menstrual cycle after completion of the study.

To be considered of non-childbearing potential, a female must have either a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy (at least 6 weeks prior to screening), or menopause (last menstruation >12 months and follicle-stimulating hormone in menopausal range); provision of written documentation is not required for female sterilization and oral confirmation is adequate.

Female participants in same sex relationships do not need to utilize contraception.

10. Male volunteers, if sexually active with a female partner, must agree to and comply with using 1 barrier method of birth control (eg, male condom) plus 1 other highly effective method of birth control in their partner (eg, oral contraceptive; implant, injectable, indwelling intrauterine device), or double-barrier method (use of a condom by the male partner with use of a diaphragm by the female partner, or sexual abstinence, and must not donate sperm, for the duration of the study (from signing of consent to FU visit) and for 90 days after last study drug administration.

    To be considered surgically sterile, male participants must have had a vasectomy at least 3 months prior to screening with appropriate documentation of the absence of sperm in the ejaculate.

    Male participants in same sex relationships do not need to utilize contraception.

Exclusion Criteria

Volunteers who meet any of the following criteria will be excluded from the study:

1. Women who are pregnant and/or nursing.

2. History or presence of significant cardiovascular (including QT prolongation, clinically significant hypokalemia, or other proarrhythmic conditions), pulmonary, hepatic, renal, hematological, gastrointestinal, endocrine (including glucose intolerance, diabetes mellitus), immunologic (including asthma or seasonal allergies [that require intermittent use of steroids or other medication]), musculoskeletal (including tendinopathy), dermatologic, or neurological disease (including seizure disorders, psychiatric disorders), including any acute illness or surgery within the past 3 months determined by the PI to be clinically relevant.

	3. A serum creatinine value on Day -1 (check-in) that increased by more than 0.2 mg/dL (or 15.25 µmol/L) from the Screening value. Note: the serum creatinine test may be repeated prior to confirming exclusion. 4. History of photosensitivity to quinolones. 5. History of known or suspected *Clostridium difficile* infection. 6. Any condition that necessitated hospitalisation within the 3 months prior to Day -1 or is likely to require so during the study. 7. Positive test for hepatitis B virus surface antigen (HBsAg), hepatitis C virus antibody (anti-HCV antibodies), or human immunodeficiency virus antibody (antibodies to HIV-1, HIV-2) at screening. 8. Exposure to any prescription medications (small molecules, biologics and vaccines, including influenza and/or COVID-19 vaccines) or, systemically administered OTC drugs, dietary supplements or herbal remedies, within 14 days or 5 half-lives (if known), whichever is longer, prior to Day 1 (first dose). Participants should not receive any vaccinations (including influenza and/or COVID-19 vaccines) until after study completion. Discussion between the PI and the Sponsor Medical Monitor is encouraged regarding prior use of any medications during the pre-dose period. Note: An exception is made for hormonal contraceptives and a limited amount of paracetamol (a maximum of 4 doses per day of 500-mg paracetamol, and no more than 3 g per week) for the treatment of headache or any other pain. 9. Documented hypersensitivity reaction or anaphylaxis to any medication. 10. Smoker (including tobacco, e-cigarettes or marijuana) or nicotine user within 1 month prior to participation in the study and have a negative test for cotinine at the screening visit and at check in on Day -1 (*may be repeated once per timepoint, at the discretion of the PI, in the instance of a positive result*). 11. Positive urine drug/alcohol testing at screening or check-in (Day -1), or history of substance abuse or alcohol abuse (defined as greater than 2 standard drinks on average each and every day, where one standard drink is defined as containing 10 g of alcohol and is equivalent to 1 can or stubby of mid-strength beer, 30 ml nip spirits, or 100 ml wine) within the previous 5 years

	(may be repeated once per timepoint, at the discretion of the PI, in the instance of a positive result). 12. Donation of blood or plasma within 30 days prior to randomization, or loss of whole blood of more than 500 mL within 30 days prior to randomization, or receipt of a blood transfusion within 1 year of study enrollment. 13. Previous participation in this study or previous participation in another study within 5 half-lives (if known) of the agent, whichever is longer, of Day 1. Note: prior participation at any time in non-invasive methodology trials in which no drugs were given is acceptable. 14. Consumption of red wine, seville oranges, grapefruit or grapefruit juice, pummelos, other citrus fruits, grapefruit hybrids or fruit juices containing such products from 7 days prior to the first dose of study medication. 15. Employee or family member of an employee of the Sponsor, CRU, or clinical research organization at which the study will be conducted. 16. Unable to cooperate fully with the requirements of the study protocol, including the schedule of assessments, or likely to be non-compliant with any study requirements. 17. Any disease or condition (medical or surgical) that, by the determination of the PI, precludes the subject's participation in the study or would place the subject at risk as a result of participation in the study. Note: Volunteers should refrain from consumption of any foods containing poppy seeds within 48 hours (2 days) prior to screening and prior to Day -1 to avoid false positive drug screen results
Statistical Methods	Statistical analyses will be performed as detailed in the formal statistical analysis plan (SAP) for this clinical study. A Statistical Analysis Plan (SAP) will be prepared and finalized before database lock and analysis of data. Any deviations from the final SAP will be described and justified in the study report. All statistical analyses will be performed using SAS® (SAS Institute Inc. Cary NC USA). <u>Safety parameters:</u> All laboratory results, vital signs measurements, and safety ECG results will be summarized using appropriate descriptive statistics. The

	incidence of all AEs and treatment-emergent AEs will be described by MedDRA ® preferred term and system organ class and by other relevant AE description parameters (relationship to study drug, severity, change in study drug administration or discontinuation of study drug). PK parameters: Single and multiple-dose PK parameters will be derived from the plasma concentration time and urinary excretion data. A non-compartmental PK method will be used to analyze the plasma and urine concentrations of BWC0977. Descriptive statistics and graphs will be performed for plasma and urine concentrations and PK parameters. For the SAD and MAD parts, the dose-proportionality of PK plasma parameters C_{max}, AUC_{0-t} and AUC_{0-inf} will be evaluated using the Power model. For the MAD part, Accumulation Ratio, Time Invariance, the steady-state assessment of plasma concentration will be also studied using a one-way analysis of variance (ANOVA) after logarithmic transformation of plasma concentrations. Cardiodynamic Evaluation The primary analysis will be based on concentration-QTc modeling of the relationship between the plasma concentrations of BWC0977 and change-from-baseline QTcF (ΔQTcF) with the intent to exclude an effect of placebo-corrected ΔQTcF (ΔΔQTcF) > 10 ms at clinically relevant plasma concentrations. The effect of BWC0977 on the placebo-corrected ΔQTcF, ΔHR (heart rate), ΔPR, and ΔQRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) will also be evaluated at each post-dosing time point ('by-time point' analysis). In addition, an analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology and U-wave presence.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AChE	acetylcholine esterase
Ae	Urinary recovery of unchanged drug
Ae(0-x)	Urinary recovery of unchanged drug up to fixed nominal time-point x
Ae(0-t)	Complete urinary recovery of unchanged drug up to time of last measurable urinary concentration
Ae(0- τ)	Urinary recovery over a dosing interval
AE	Adverse Event
ALT	Alanine aminotransferase (SGPT)
AMR	Antimicrobial resistance
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase (SGOT)
AUC	Area under concentration-time curve
AUC(0-inf)	Area under the concentration-time curve from time zero (pre-dose) extrapolated to infinite time
AUC(0-x)	Area under the concentration-time curve from zero (pre-dose) to some fixed nominal time x
AUC(0-t)	Area under the concentration-time curve from time zero (pre-dose) to last time of quantifiable concentration within a subject across all treatments
AUC(0-τ)	Area under the concentration-time curve over the dosing interval
$AUMC_{inf}$	Area under the moment curve from time 0 extrapolated to infinity
β-HCG	Beta-human chorionic gonadotropin
BMI	Body mass index
BP	Blood pressure
CDC	Centre for Disease Control
CI	Confidence Interval
CL	Systemic clearance of parent drug
CLr	Renal clearance
Clr_{ss}	Renal clearance (at steady state)
C_{av}	Average concentration
C_{max}	Maximum observed concentration
C_{min}	Minimum observed concentration
CrCl	Creatinine clearance
Cτ	Pre-dose (trough) concentration at the end of the dosing interval
CPK	Creatine phosphokinase
CRF	Case Report Form
CRO	Contract research organizations
CRU	Clinical research unit
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
Δ	Change from baseline
ΔΔ	Placebo-corrected change from baseline
ECG	Electrocardiogram

eCRF	Case Report Form
EDC	Electronic data capture
EOS	End of Study
Fe	Fraction of the dose excreted in the urine
FDA	Food and Drug Administration
FIH	First in human
FSH	Follicle Stimulating Hormone
FU	Follow-up
GCP	Good Clinical Practice
GGT	Gamma glutamyltransferase
GLP	Good Laboratory Practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HR	Heart rate
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IND	Investigational New Drug
INR	International normalized ratio
IB	Investigator's Brochure
IEC	Independent Ethics Committee
IP	Investigational Product
IRB	Institutional Review Board
IV	Intravenous
IVRS/IWRS	Interactive Voice Response System and Interactive Web Response System
k_{el}	Terminal phase rate constant
LFTs	Liver function tests
MAD	Multiple ascending dose
MATE	Multidrug and toxin extrusion
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MRSA	methicillin-resistant *Staphylococcus aureus*
MRT	Mean residence time
NBTI	Novel Bacterial Type II Topoisomerase Inhibitors
NOAEL	No observed adverse effect level
OAT3	Organic anion transporter 3
OTC	Over-the-counter
PK	Pharmacokinetic
PI	Principal Investigator
QTcF	QT duration corrected for heart rate by Fridericia's formula
RBC	Red blood cells
Ro	Observed accumulation ratio
SAD	Single ascending dose

SAE	Serious adverse event(s)
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SMG	Safety Monitoring Group
SOP	Standard Operating Procedure
SBP	Systolic blood pressure
t	Time of last observed quantifiable concentration
t½	Terminal phase half-life
τ	Dosing interval
TEAE	Treatment-emergent adverse event
TGA	Australian Therapeutic Goods Administration
t_{max}	Time of occurrence of C_{max}
ULN	Upper limit of normal
Vdss	Volume of distribution at steady state of parent drug after intravascular (e.g., IV) administration
VRE	Vancomycin-resistant *Enterococcus*
WBC	White blood cells
WHO	World Health Organisation

1. INTRODUCTION

1.1 Background

Antimicrobial Resistance (AMR) is an increasingly serious threat to global public health that requires immediate action across all sectors and society. Without effective antibiotics, a key element in the armamentarium of modern clinical practice would be compromised. The cost of health care for patients with resistant infections is higher than care for patients with non-resistant infections due to longer duration of illness, additional tests, use of more expensive drugs and longer stay in hospitals. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases, resulting in prolonged illness, disability, and death. World Health Organization (WHO) published its first ever list of antibiotic-resistant "priority pathogens" in 2017, consisting of 12 families of bacteria that pose the greatest threat to human health. The most critical group includes multidrug resistant bacteria that pose serious threat in hospitals, nursing homes, and among critical care patients who are on devices such as ventilators and blood catheters. They include *Acinetobacter baumannii, Pseudomonas aeruginosa* and various Enterobacteriaceae (including *Klebsiella* sp., *E. coli*, *Serratia marcesens*, and *Proteus* spp.). They can cause severe and often deadly infections such as bloodstream infections and pneumonia. These bacteria have become resistant to a large number of antibiotics, including carbapenems and third generation cephalosporins – currently the best available antibiotics for treating multi-drug resistant bacteria (1).

In 2019, the Centre for Disease Control (CDC) published a comprehensive analysis outlining the top 18 antibiotic-resistant threats in the U.S. These 21 threats were further classified into urgent, serious, and concerning threats. The urgent threats include carbapenem-resistant *Enterobacteriaceae*, Carbapenem-resistant *Acinetobacter*, *Candida auris (C. auris)*, *Clostridioides difficile* & drug-resistant *Neisseria gonorrhoeae* (2). The serious threats include, multidrug-resistant *Acinetobacter* sp., drug-resistant *Campylobacter* sp., Extended-spectrum Beta-lactamase producing *Enterobacteriaceae*, Vancomycin-resistant *Enterococcus* (VRE), multidrug-resistant *Pseudomonas aeruginosa*, drug-resistant non-typhoidal *Salmonella* sp., drug-resistant *Salmonella typhi*, drug-resistant *Shigella* sp., methicillin-resistant *Staphylococcus aureus* (MRSA), & drug-resistant *Streptococcus pneumoniae*. Lastly the concerning threats include Erythromycin-Resistant Group A *Streptococcus* and Clindamycin-resistant Group B *Streptococcus*. In India, the most common pathogens encountered are *Enterobacter* spp, *Staphylococci*, *Klebsiella* sp., *Acinetobacter* spp, *Pseudomonas aeruginosa*, *Enterococcus* and *E. coli* (3).

Bugworks has discovered a novel bacterial topoisomerase inhibitor (NBTI), BWC0977, as a promising candidate that has activities against all the critical and high priority pathogens in WHO 2017, as well CDC published urgent & serous pathogen list. BWC0977, is a broad spectrum new chemical entity with dual target inhibition, potent activity against a very broad spectrum of Gram negative (including all the key members of Enterobacteriaceae and non-fermenters such as

Pseudomonas aeruginosa and *Acinetobacter baumannii*) and Gram-positive bacteria such as MRSA and VRE. Unlike the fluoroquinolone class of molecules, BWC0977 is equally active against deoxyribonucleic acid (DNA) gyrase and Topoisomerase IV. Extensive minimum inhibitory concentration studies with clinical isolates, demonstrated the lack of cross-resistance with fluoroquinolones. This is a novel lead that is differentiated from other reported NBTI series including gepotidacin, based on SciFinder and STN searches.

Both DNA gyrase and topoisomerase IV are clinically validated antibacterial targets inhibited by the quinolone family of antibiotics. NBTIs and quinolones bind to the same target proteins at a different binding pocket leading to a different mode of inhibition. They recognize distinctly different amino acids. Therefore, they inhibit different stages of the catalytic cycle of the target proteins.

BWC0977 has demonstrated in vitro and in vivo activity against Gram positive pathogens [including methicillin resistant Staphylococcus aureus (MRSA)] and gram-negative pathogens associated with respiratory tract, skin and soft tissue infections, including isolates resistant to existing classes of antimicrobials. BWC0977 has been shown to be efficacious in lung, thigh and urinary track bacterial infection model in rats caused by gram negative pathogens like *E.coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. BWC0977 selectively inhibits bacterial DNA replication by interacting in a unique way on the GyrA subunit of bacterial DNA gyrase and the ParC subunit of bacterial topoisomerase IV. This interaction appears to be highly specific to bacterial topoisomerases as evidenced by weak inhibition of human topoisomerase II, supporting the selective activity of BWC0977 against the bacterial target. As a consequence of its novel mode of action, BWC0977 is very potent against isolates carrying resistance determinants to established antibacterials and has shown no target-mediated cross-resistance with agents in current use, including fluoroquinolones.

1.2 Preclinical Safety Assesment of BWC0977

The dose limiting toxicity in the rat and dog studies (general toxicity, safety pharmacology and genetic toxicology) was clinical observations primarily consisting of decreased activity, semi-closed eyes, piloerection, involuntary twitching movements, fast or labored respiration, and mouth rubbing, the incidence and severity of which increased with the dose level. Due to the timing of the observations, they are considered attributable to maximum observed concentration (C_{max}).

Due to the inhibition of acetylcholine esterase (AChE) by BWC0977 it is plausible that peripheral inhibition of AChE could be the cause of the clinical observations noted in vivo. BWC0977 has been shown to not cross the blood brain barrier, therefore these clinical observations are unlikely to be centrally mediated. At the high dose levels the majority of these findings were reversible over the course of 2 hours postdose. The most sensitive species for these clinical observations was rat with a NOAEL determined to be at 75mg/kg/day with an observed C_{max} of 26 µg/ml and an observed area under concentration-time curve $(AUC)_{0\text{-}24}$ of 51.45 µg/ml*hr.

Due to the ability to monitor for these effects, the quick reversibility of the signs at the lower dose levels, and the clinical experience with AChE inhibitors, these effects are not considered to pose a significant risk to man. To mitigate any adverse effects observed related to C_{max}, a longer infusion time or fractionation of the total daily dose will be undertaken. A careful escalation of doses and close safety monitoring of volunteers is however required and will be performed in this study.

Repeat administration ≥80mg/kg/day administration through intravenous (IV) infusion for 120 min of BWC0977 in the 14-day Good Laboratory Practice (GLP) dog study led to increases in serum transaminases, alkaline phosphatase, gamma glutamyltransferase (GGT) and total bilirubin in all animals. This was in concordance with microscopic changes in the gall bladder, consisting of mucinous accumulation, in all animals administered 120mg/kg/day and in one male dog administered 80mg/kg/day.

At the end of the recovery phase, the clinical chemistry parameters had all decreased to near predose values and the microscopic findings in the gall bladder of animals previously administered 120 mg/kg/day were comparable with those of controls, which indicated complete reversibility.

The NOAEL for these changes in the dog was 40mg/kg/day with AUC_{0-24} of 91 h*µg/ml and C_{max} of 25 µg/ml.

The results from the genetic toxicity tests with BWC0977 are directly comparable with existing, marketed quinolone antibacterials.

BWC0977 has been assessed in a battery of nonclinical cardiovascular assays. In vivo, in one guinea pig study and the dog cardiovascular study, slight increases in QTc have been observed. BWC0977 also has a hERG IC50 of 56µM, however BWC0977 does not inhibit other cardiac ion channels up to 300µM. In the dog cardiovascular study the increase in QTc was observed at both dose levels assessed (non-statistically significant maximum increase of 4 msec at 30mg/kg; statistically significant maximum increase of 11 msec at 60mg/kg - both maximal increases were between 3-6 hours post start of 1 hour infusion), there was also a notable increase in heart rate at both dose levels. During the study there were clinical observations at both dose levels following the infusion, which were more significant at the higher dose level.

Due to the ability to monitor by cardiovascular changes by electrocardiogram (ECG), the transient nature of the changes in the parameters, and the ability to modulate the C_{max} with the length of infusion, or by fractionating the daily dose, these effects are not considered to pose a significant risk to man.

In vitro tests suggested that BWC0977 may have potential for phototoxicity. To mitigate this risk, participants in clinical studies will be requested to avoid sun exposure till end of follow-up.

A summary of the preclinical studies and the calculated safety margins for the minimum and maximum dose levels to be evaluated in this study is provided in Table 1.

Table 1. Safety Margins of BWC0977

Study	NOAEL/ NOEL			Proposed start dose 120mg		Maximum dose 1050 mg		
						Fold over C_{max}		Fold over AUC (either regimen)
	Dose level	C_{max} µg/ml	AUC_{0-24} h*µg/ml	Fold over C_{max}	Fold over AUC	(1050 mg UID)	(350 mg TID)	
Rat 14-day GLP study/ Rat Irwin / Rat Respiratory	75mg/kg/day	26	52	14	9	1.625	5	1
Rat In vivo MN and Comet	300mg/kg/day	>123.5*	>355*	54*	>60*	7*	19*	7*
Dog 14-day GLP study	40mg/kg/day	25	91	14	15	1.6	5	2
Dog cardiovascular Study	30mg/kg[#]	32	-	18	-	2	6	-

* Exposures based on exposure from 14-day rat study at 250mg/kg/day high dose;[#] 30mg/kg was not declared a no effect level due to the increases in heart rate and non-statistical change in QTc. Multiples of exposures achieved in the preclinical toxicology studies Vs the exposures predicted to be achieved from the proposed MRSD and the maximum dose. MRSD of 120mg is predicted to give a total C_{max} of 1.83 µg/ml and an AUC_{0-24} of 5.95 hr*µg/ml in a 70kg person when administered as a 2-hour intravenous infusion. Maximum dose of 1050mg is predicted to give a total C_{max} of 16.01 µg/ml and an AUC_{0-24} of 52.07 hr*µg/ml in a 70kg person when administered as 2-hour infusion.

1.3 Dose Selection Based on Preclinical Studies

The starting dose of BWC0977 for the first in human (FIH) study is selected based on Food and Drug Administration (FDA) (2005) and European Medicines Agency (2017) guidelines using nonclinical pharmacokinetic and toxicological data.

The no observed adverse effect level (NOAEL)-based maximum recommended starting dose (MRSD) according to the FDA was calculated using the NOAEL from the rat, which was the most sensitive species. The NOAEL, as derived from the 14-day toxicology study in rat, was 75 mg/kg/day. The human equivalent dose (HED) was calculated as 75 mg/kg / 6.2 = 12.1 mg/kg. Applying a standard safety factor of 10 (per FDA guidance), and a standard body weight of 70 kg, the NOAEL based maximum recommended starting dose (MRSD) would be (12.1 x 70)/10 = **85 mg**.

The NOAEL derived from the dog 14-day toxicology study was 40mg/kg/day. The HED is calculated as 40 mg/kg / 1.8 = 22.2 mg/kg. Applying the standard safety factor of 10 and a standard body weight of 70kg the safe starting dose would be (22.2 x 70)/10 = **155 mg**.

The NOAEL from the rat study was favoured as it was lower than the dog study NOAEL and was based on reversible observations.

Further, human pharmacokinetic (PK) values were predicted using PK model based allometric scaling of mice, rat, guinea pig and dog PK data fitting to an empirical PK model, and respective allometric exponents and coefficients of each of the PK parameters was derived after scaling based on simple allometry relationship. The allometric exponents and coefficients derived was used to calculate human PK parameters and predict human PK data after each dose. The models were used to determine a dose that will give an $AUC_{0\text{-}24}$ and C_{max} about 1/10 of the NOAEL dose.

Based on the animal NOAEL data and model-based predictions, a **starting dose of 120 mg is proposed for this FIH clinical study**. For a 70 kg person, this dose is predicted to elicit a C_{max} of 1.83 μg/mL (after a 2-hour intravenous infusion) and an $AUC_{0\text{-}24}$ of 5.95 μg*h/ml, that are approximately **14-fold below** the NOAEL-associated C_{max}, and **9-fold below** the NOAEL-associated $AUC_{0\text{-}24}$ value from the rat 14-day GLP study.

Likewise, a maximum dose of 1050 mg is proposed. This is predicted to give a total C_{max} of 16 μg/mL (after a 2-hour intravenous infusion) and an $AUC_{0\text{-}24}$ of 52 hr*μg/ml in a 70 kg person (i.e similar to the $AUC_{0\text{-}24}$ at the NOAEL dose).

1.4 Clinical Experience

There is no prior clinical experience with BWC0977. The proposed study is planned to be the first clinical study of BWC0977 in humans. The preclinical studies conducted to date do not contain information that precludes evaluation of BWC0977 in humans. Please refer to the Investigator's Brochure (IB) for BWC0977 for results from preclinical studies conducted as of date.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

1) To assess the safety and tolerability of BWC0977 following single and multiple ascending doses by intravenous (IV) infusion in healthy adult volunteers.

2.1.2 Secondary Objectives

1) To assess the pharmacokinetic (PK) characteristics in plasma and urine of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult volunteers

2.2 Endpoints

2.1.1 Primary Endpoints

Safety and tolerability of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult volunteers based on the following endpoints:

- Incidence of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) overall and by intensity
- Changes from baseline in:
    - Safety laboratory test results
    - ECG findings
    - Vital signs measurements
    - Physical examination findings

2.1.2 Secondary Endpoints

- Following single dose: $AUC_{(0-8)}$, $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, C_{max}, CL, volume of distribution at steady state (Vdss), mean residence time (MRT), and $t_{1/2}$ of BWC0977, as data permit.
- Following repeat dose: $AUC_{(0-\tau)}$, C_{max}, $C\tau$, Ro and CL of BWC0977, as data permit
- Amount excreted in urine (Ae) of unchanged BWC0977, fraction of the dose excreted in urine (fe) and renal clearance (CLr), as data permit

2.1.3 Exploratory Endpoints

Cardiodynamic evaluation using 12-lead ECGs extracted from Holter recordings may be undertaken based on observed PK and other project considerations. In such case, the following endpoints will be used:

- Change-from-baseline heart rate, PR, QRS and QTcF interval (ΔHR, ΔPR, ΔQRS and ΔQTcF).
- Placebo-corrected ΔHR, ΔPR, ΔQTcF and ΔQRS
- Categorical outliers for HR, PR, QRS and QTcF
- Frequency of treatment emergent T- and U-wave abnormalities

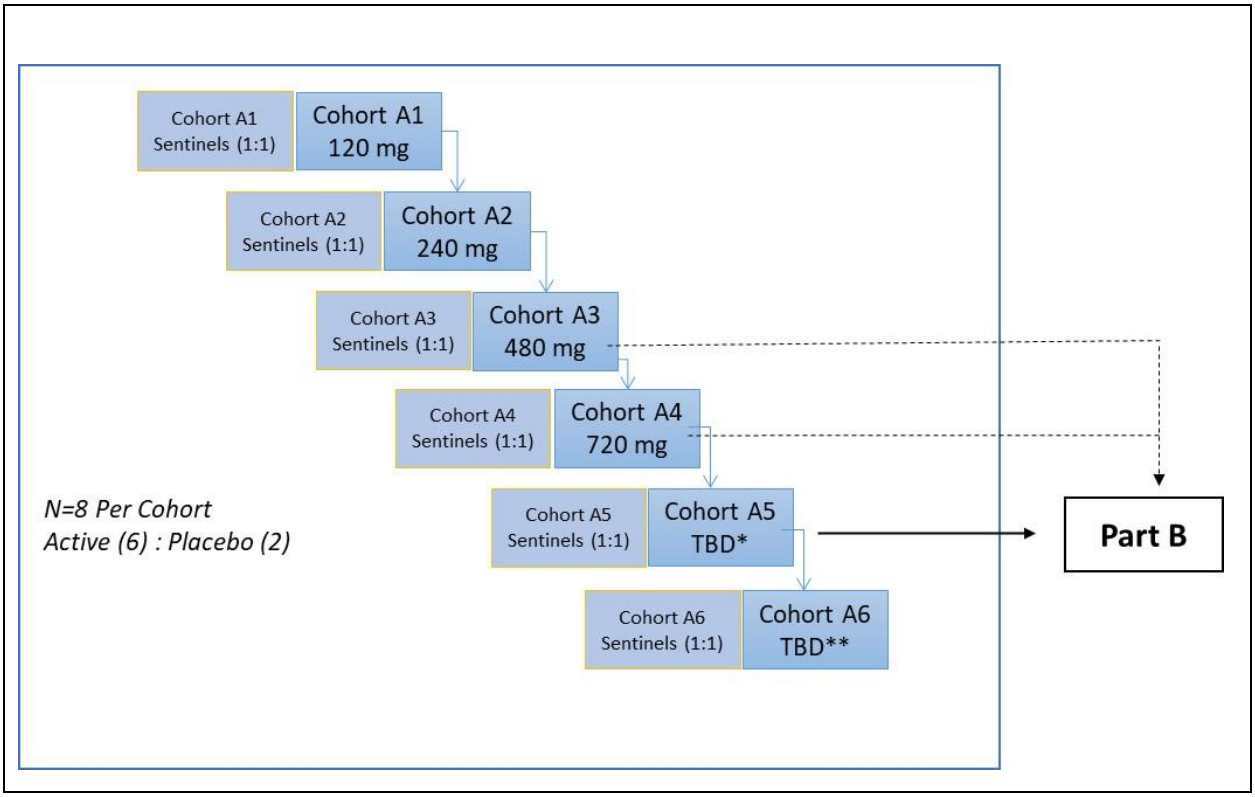
3. STUDY DESIGN

3.1 Overall Study Design and Plan

This will be a two-part, randomised, placebo-controlled, double-blind study to investigate the safety, tolerability and PK of BWC0977 in healthy adult volunteers. This study is subdivided into parts, Part A (SAD) and Part B (MAD). Part A will investigate escalating single IV doses of BWC0977. Part B will investigate multiple ascending doses of BWC0977. Volunteers may only be enrolled in one study part and randomised to one cohort per the randomisation schedule. The decision to escalate between dose levels and proceed to the next study part (Part B) will be based upon review of the available safety and PK data by the Safety Monitoring Group (SMG). In each part of the study, cohorts may be added or removed based upon emerging data.

The starting dose, and dose increments are based on available non-clinical data. In Part A, BWC0977 dose levels in the range of 120-1050 mg will be investigated (Figure 1). Sentinel dosing is planned for each cohort in Part A.

Figure 1. Diagrammatic Representation of Study Part A, SAD

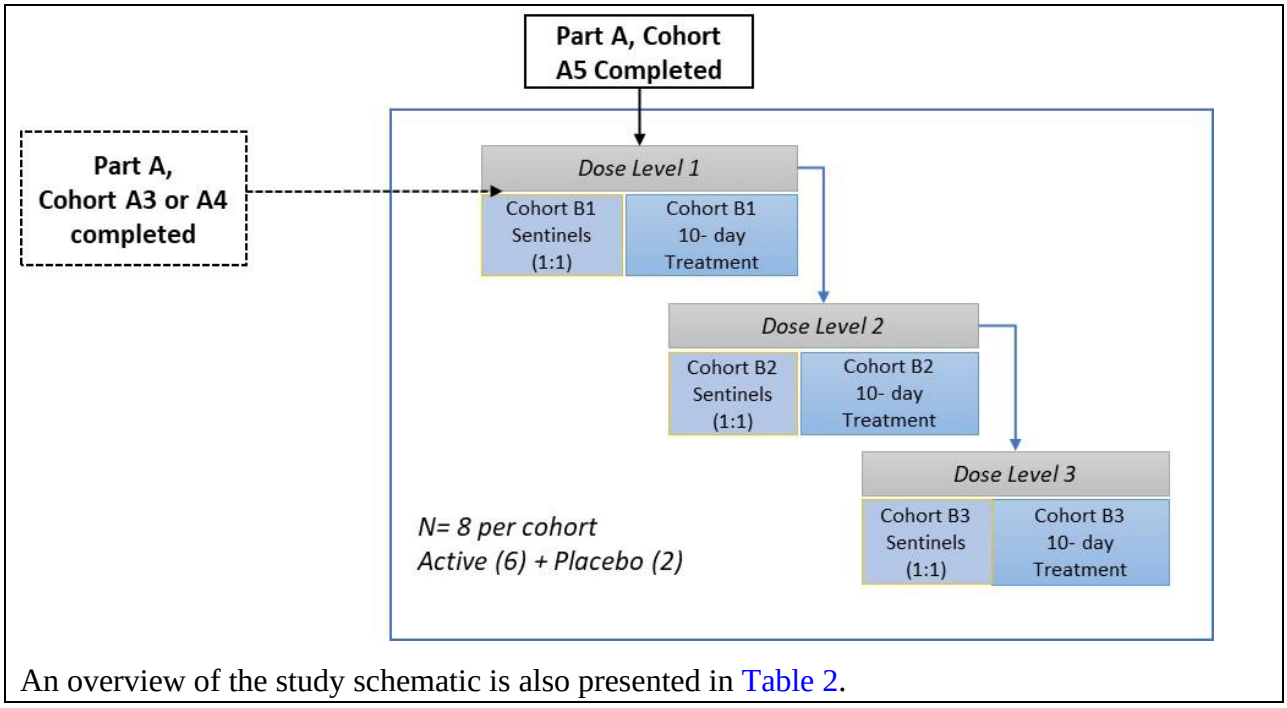


Abbreviations: TBD = to be determined.
* Dose level for Cohort A5 to be determined based on safety, tolerability and PK data from Cohorts A1-A4. Dose will not exceed 1050 mg.

**Cohort A6 is optional. Dose to be sub therapeutic, based on PK data from Cohorts A1-A5. This cohort may be included if a supra therapeutic dose is achieved by Cohort A3.

Up to three dose levels will be evaluated in Part B (Figure 2). The starting dose for Part B will be based on safety, tolerability and PK data from Part A and may be selected following completion of Part A, or, after at least 3 cohorts from Part A have completed if the SMG deems it safe and appropriate based on emerging safety and PK data from Part A. Sentinel dosing is planned for Part B, however, the requirement for sentinel dosing will be evaluated by the SMG following completion of the first MAD cohort. A dose level will only be evaluated in Part B, if it is determined to be safe and tolerable in Part A.

Figure 2. Diagrammatic Representation of Study Part B, MAD



An overview of the study schematic is also presented in Table 2.

Table 2. Dose Levels of BCW0977 Per Cohort

Cohort	Total Dose (mg)	Number of Doses Per Day	No. of days of dosing	Number of Participants	
				BWC0977	Placebo
A1	120	1	1	6	2
A2	240	1	1	6	2
A3	480	1	1	6	2
A4	720	1	1	6	2
A5	TBD*	1	1	6	2
A6	TBD#	1	1	6	2
Total Number of Participants (Part A)				up to 36	up to 12
				up to 48	
B1	TBD**	TBD**	10	6	2
B2	TBD**	TBD**	10	6	2
B3	TBD**	TBD**	10	6	2

Cohort	Total Dose (mg)	Number of Doses Per Day	No. of days of dosing	Number of Participants	
				BWC0977	Placebo
Total Number of Participants (Part B)				18	6
				24	
Total Number of Participants (Part A + Part B)				**up to 72**	

Abbreviations: TBD = to be determined.
* Dose level for Cohort A5 to be determined based on safety, tolerability and PK data from Cohorts A1-A4. Dose will not exceed 1050 mg.
# Cohort A6 is optional. Dose to be sub therapeutic. This cohort may be included if a supra therapeutic dose is achieved by Cohort A3.
** The doses and dosing frequencies of BWC0977 in Part B will be confirmed based on the safety, tolerability, and PK data of BWC0977 obtained in Part A, and any preceding cohorts in Part B.

3.1.1 Part A (Single Ascending Dose)

In Part A, healthy volunteers will be enrolled to receive a single dose of BWC0977 or placebo. The starting dose level in Part A will be 120 mg, and the dose range to be evaluated will be 120-1050 mg.

Dose escalation in Part A will be conducted in a total of 6 cohorts (Cohorts A1 to A6). Within each cohort, the 8 participants will be randomised to receive BWC0977 (6 participants) or placebo (2 participants). Cohort A6 is optional and may be included if a supra therapeutic dose is achieved by Cohort A3. This is to ensure that both sub therapeutic and supra-therapeutic doses are evaluated for QTc.

Healthy volunteers will be screened between Day -28 and Day -2. Participants will be admitted to the clinical facility on Day -1 with dosing to occur on Day 1. Participants will be confined at the clinical facility from Day -1 through to Day 3. Participants will be discharged following completion of all safety and PK assessments on Day 3.

Dosing in each cohort will start with two sentinel participants with one of the two sentinels randomised to receive BWC0977 and the other randomised to receive placebo. The safety and tolerability of each sentinel participant will be monitored in the clinic until Day 3 and will be reviewed prior to dosing the remainder of participants in each cohort. The study Principal Investigator (PI) will review safety/tolerability information available on the sentinel participants on Day 3 and in consultation with the SMG (if necessary), will make the decision to dose the remaining 6 participants in the cohort.

Dosing for each participant will be via IV infusion. The planned infusion duration time is 120 minutes, however the duration of infusion may be modified based upon review of safety, tolerability and available PK data from the preceding cohorts

Blood and urine samples for PK analysis will be collected pre-dose, and at several timepoints up to 48 hours post-dose. Continuous Holter monitoring will be performed from at least 1 hour prior to dosing and will continue to approximately 24 hours post-dose, with ECG extraction paired with

PK draws post-dose in SAD Cohorts 3, 4, 5 and 6. All on-study assessments to be performed in Part A are defined in Section 7 and the timing of each assessment is presented in Section 7.5 (Schedule of Events). Cohorts will be dosed in an escalating order. The dose of a cohort may be appropriately adjusted according to the safety, tolerability and available PK data from the previous dose cohort.

Participants will return to the clinical facility for final safety assessments at an End of Study (EOS) visit between Day 7-9 (inclusive). If any participants experience any clinically significant adverse events (AEs) during the confinement period, they may remain in the clinical facility for further observation at the discretion of the PI.

The decision to escalate between dose levels will be based upon review of blinded clinical and laboratory safety data from at least 5 out of 8 participants from the previous cohort (i.e., haematology, serum chemistry, coagulation, urinalysis, ECG, vital signs, physical examination findings and AE data) through to and including the final follow-up assessment on Day 7-9 and any available blinded PK data by the SMG.

Following completion of Day 7-9 for at least 5/8 participants, the SMG will review the data, discuss the findings, and recommend proceeding to the next cohort at the protocol-defined dose level, at another dose level (lower or higher) or to terminate enrolment in Part A. The SMG may also recommend commencing enrollment in Part B, providing that at least 3 cohorts from Part A have been completed.

Enrollment of participants in the previous cohort does not have to be completed before initiating enrollment in a new cohort provided the SMG has approved proceeding to the next dose level.

3.1.2 Part B (Multiple Ascending Dose)

In Part B, healthy volunteers will be enrolled to receive multiple doses of BWC0977 or placebo. The starting dose level in Part B will be determined based on the safety, tolerability and PK data from at least 3 completed cohorts from Part A.

Dose escalation in Part B will be conducted in a total of 3 cohorts (Cohorts B1 to B3). Within each cohort, the 8 participants will be randomised to receive BWC0977 (6 participants) or placebo (2 participants).

Healthy volunteers will be screened between Day -28 and Day -2. Participants will be admitted to the clinical facility on Day -1. Dosing will commence the following day (Day 1) and will continue until Day 10. The number of doses administered per day in Part B, as well as the total number of dosing days, will be determined based on the safety, tolerability and PK data from at least 3 completed cohorts from Part A. The dosing interval (τ) will be equal to 8 hours for thrice daily regimen or 12 hours for twice daily or 24 hours for once daily.

Participants will be confined at the clinical facility from Day -1 through to Day 11. Participants will be discharged following completion of all safety and PK assessments on Day 11.

Dosing in cohort B1 will start with two sentinel participants with one of the two sentinels randomised to receive BWC0977 and the other randomised to receive placebo. The safety and tolerability of each sentinel participant will be monitored in the clinic until Day 11 and will be reviewed prior to dosing the remainder of participants in each cohort. The study PI will review safety/tolerability information available on the sentinel participants on Day 11 and in consultation with the SMG (if necessary), will make the decision to dose the remaining 6 participants in the cohort.

Dosing for each participant will be via IV infusion. The planned infusion duration time for each dose 120 minutes, however the duration of infusion may be modified based upon review of safety, tolerability and available PK data from the preceding cohorts.

Blood and urine samples for PK analysis will be collected pre-dose, and at several timepoints up to post-dose. All on-study assessments to be performed in Part B are defined in Section 7 and the timing of each assessment is presented in Section 7.5 (Schedule of Events). Continuous Holter monitoring will be performed from at least 1 hour prior to dosing on Day 1 and 10 and will continue to approximately 24 hours post- Day 1 and Day 10 dose, with ECG extraction paired with PK sampling post-dose on both days in all MAD cohorts.

Participants will return to the clinical facility for final safety assessments at an End of Study (EOS) visit between Day 15-17 (inclusive). If any participants experience any clinically significant AEs during the confinement period, they may remain in the clinical facility for further observation at the discretion of the PI.

Cohorts will be dosed in an escalating order. The decision to escalate between dose levels will be based upon review of blinded clinical and laboratory safety data from at least 6 out of 8 participants from the previous cohort (i.e., haematology, serum chemistry, coagulation, urinalysis, ECG, vital signs, physical examination findings and AE data) through to and including the final follow-up visit on Day 15-17 and any available blinded PK data by the SMG.

Following completion of Day 15-17 for at least 6/8 participants, the SMG will review the data, discuss the findings, and recommend proceeding to the next cohort at the protocol-defined dose level, at another dose level (lower or higher) or to terminate enrolment in Part B. The SMG may also recommend use of an alternative dosing schedule for the next cohort in Part B, and/or determine whether use of sentinel dosing at the next dose level in Part B is warranted.

Enrollment of all participants in the previous cohort does not have to be completed before initiating enrollment in a new cohort provided the SMG has approved proceeding to the next dose level.

3.1.3 Duration of the Study

The total maximum study duration for participants in Part A is 37 days, inclusive of visit windows. This includes the screening period (Day -28 to Day -2), confinement to the clinical facility over 3 nights (Days -1 to Day 3) and one outpatient visit at the end of the study (Day 7-9). The total maximum study duration for participants in Part B is 45 days, inclusive of visit windows. This

includes the screening period (Day -28 to Day -2), confinement to the clinical facility over 11 nights (Days -1 to Day 11) and one outpatient visit at the end of the study (Day 15-17).

If any participants experience any clinically significant AEs during the confinement period in Part A or Part B, they may remain in the clinical facility for further observation at the discretion of the PI.

4. PARTICIPANT SELECTION

4.1 Planned Number of Participants

This study will enroll approximately 72 healthy adult participants (48 participants in Part A, 24 participants in Part B).

4.2 Inclusion Criteria

Volunteers must meet the following inclusion criteria for study participation:

1. Healthy male or female 18 to 55 years of age, inclusive, at time of consent

2. Body mass index (BMI) ≥ 19.0 and ≤ 30.0 (kg/m^2) and weight between 55.0 and 100.0 kg (inclusive)

3. Medically healthy without clinically significant abnormalities at the screening visit or Day -1, including:

    a) No findings in Physical examination or vital signs (including temperature, heart rate, respiratory rate, and blood pressure) that the Principal Investigator (PI) determines would interfere with interpretation of study results
    b) Triplicate ECGs without clinically significant abnormalities, including a QT duration corrected for heart rate by Fridericia's formula (QTcF) interval duration ≤450 msec (for males), and ≤470 msec (for females) obtained as an average from the triplicate screening ECGs after at least 5 minutes in a supine, quiet-rest position
    c) Hemoglobin/hematocrit, white blood cell (WBC) count, and platelet count within the normal range of the reference laboratory, unless deemed not clinically significant by the Investigator (eg, asymptomatic Gilbert's disease)
4. Willing and able to provide written informed consent

5. Agrees to be available for all study visits and cooperate fully with the requirements of the study protocol, including the schedule of assessments.

6. Willing to refrain from strenuous physical activity that could cause muscle aches or injury, including contact sports, at any time from 4 days prior to admission in the clinical research unit (CRU) until completion of the study (follow-up [FU] visit).

7. Willing to refrain from prescription medications from Screening visit until follow-up; and over-the-counter (OTC) medications, vitamin preparations and other food supplements, from Day -1 to follow-up

8. Have suitable venous access for drug administration and blood sampling

9. If female of child-bearing potential, must agree to and comply with:

    a) Using 1 barrier method (e.g., female condom or male partner using a condom) plus 1 other highly effective method of birth control (e.g., oral contraceptive, implant,

injectable, indwelling intrauterine device, vasectomized partner), or double-barrier method (use of a condom by the male partner with use of a diaphragm by the female partner), or

b) Sexual abstinence, for the duration of the study (from signing of consent to FU visit) and for 30 days after last study drug administration, plus

c) Females of child-bearing potential must also agree not to donate ova or oocytes (ie, human eggs) during the study, and for one menstrual cycle after completion of the study.

To be considered of non-childbearing potential, a female must have either a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy (at least 6 weeks prior to screening), or menopause (last menstruation >12 months and follicle-stimulating hormone in menopausal range); provision of written documentation is not required for female sterilization and oral confirmation is adequate.

Female participants in same sex relationships do not need to utilize contraception.

10. Male volunteers, if sexually active with a female partner, must agree to and comply with using 1 barrier method of birth control (eg, male condom) plus 1 other highly effective method of birth control in their partner (eg, oral contraceptive; implant, injectable, indwelling intrauterine device), or double-barrier method (use of a condom by the male partner with use of a diaphragm by the female partner), or sexual abstinence, and must not donate sperm, for the duration of the study (from signing of consent to FU visit) and for 90 days after last study drug administration.

    To be considered surgically sterile, male participants must have had a vasectomy at least 3 months prior to screening with appropriate documentation of the absence of sperm in the ejaculate.

    Male participants in same sex relationships do not need to utilize contraception.

4.3 Exclusion Criteria

Volunteers who meet any of the following criteria will be excluded from the study:

1. Women who are pregnant and/or nursing

2. History or presence of significant cardiovascular (including QT prolongation, clinically significant hypokalemia, or other proarrhythmic conditions), pulmonary, hepatic, renal, hematological, gastrointestinal, endocrine (including glucose intolerance, diabetes mellitus), immunologic (including asthma or seasonal allergies [that require intermittent use of steroids or other medication]), musculoskeletal (including tendinopathy), dermatologic, or neurological disease (including seizure disorders, psychiatric disorders), including any acute illness or surgery within the past 3 months determined by the PI to be clinically relevant

3. A serum creatinine value on Day -1 (check-in) that increased by more than 0.2 mg/dL (or 15.25 µmol/L) from the Screening value Note: the serum creatinine test may be repeated prior to confirming exclusion

4. History of photosensitivity to quinolones

5. History of known or suspected *Clostridium difficile* infection

6. Any condition that necessitated hospitalization within the 3 months prior to Day -1 or is likely to require so during the study

7. Positive test for hepatitis B virus surface antigen (HBsAg), hepatitis C virus antibody (anti-HCV antibodies), or human immunodeficiency virus antibody (antibodies to HIV-1, HIV-2) at screening.

8. Exposure to any prescription medications (small molecules, biologics and vaccines, including influenza and/or COVID-19 vaccines) or, systemically administered OTC drugs, dietary supplements or herbal remedies, within 14 days or 5 half-lives (if known), whichever is longer, prior to Day 1 (first dose). Participants should not receive any vaccinations (including influenza and/or COVID-19 vaccines) until after study completion. Discussion between the PI and the Sponsor Medical Monitor is encouraged regarding prior use of any medications during the pre-dose period.

   Note: An exception is made for hormonal contraceptives and a limited amount of paracetamol (a maximum of 4 doses per day of 500-mg paracetamol, and no more than 3 g per week) for the treatment of headache or any other pain.

9. Documented hypersensitivity reaction or anaphylaxis to any medication.

10. Smoker (including tobacco, e-cigarettes or marijuana) or nicotine user within 1 month prior to participation in the study and have a negative test for cotinine at the screening visit and at check in on Day -1 (*may be repeated once per timepoint, at the discretion of the PI, in the instance of a positive result*).

11. Positive urine drug/alcohol testing at screening or check-in (Day -1), or history of substance abuse or alcohol abuse (defined as greater than 2 standard drinks on average each and every day, where one standard drink is defined as containing 10 g of alcohol and is equivalent to 1 can or stubby of mid-strength beer, 30 ml nip spirits, or 100 ml wine) within the previous 5 years *(may be repeated once per timepoint, at the discretion of the PI, in the instance of a positive result).*

12. Donation of blood or plasma within 30 days prior to randomization, or loss of whole blood of more than 500 mL within 30 days prior to randomization, or receipt of a blood transfusion within 1 year of study enrollment.

13. Previous participation in this study or previous participation in another study within 5 half-lives (if known) of the agent, whichever is longer, of Day 1. Note: prior participation at any time in non-invasive methodology trials in which no drugs were given is acceptable.

14. Consumption of red wine, seville oranges, grapefruit or grapefruit juice, pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices containing such products from 7 days prior to the first dose of study medication.

15. Employee or family member of an employee of the Sponsor, CRU, or clinical research organization at which the study will be conducted.

16. Unable to cooperate fully with the requirements of the study protocol, including the schedule of assessments, or likely to be non-compliant with any study requirements.

17. Any disease or condition (medical or surgical) that, by the determination of the PI, precludes the subject's participation in the study or would place the subject at risk as a result of participation in the study.

Note: Volunteers should refrain from consumption of any foods containing poppy seeds within 48 hours (2 days) prior to screening and prior to Day -1 to avoid false positive drug screen results.

5. STUDY CONDUCT

For the exact timing and allowable windows for each procedure described in this section, please refer to Section 7.5, Schedule of Events.

5.1 Screening (Days -28 to -2 prior to Randomisation)

At the screening visit, potential volunteers will be given a detailed oral presentation describing the nature, purpose, risks, and requirements of the study and will receive detailed written information. Volunteers will be given ample time to consider participation and ask questions which will be adequately addressed by site personnel.

Once the volunteer is satisfied that he/she is willing to participate in the study, he/she will be asked to sign the study informed consent form (ICF, refer to Section 12.2.6). The investigational site personnel obtaining written consent from the volunteer will also sign the consent form.

Once signed, the Investigator will retain the original ICF for the participant's study records and provide the participant with a signed copy. The investigator will verify that informed consent has been obtained from each participant prior to enrollment into the study and prior to the participant undergoing any study-related procedures.

Screening activities after obtaining informed consent will be conducted and consist of the following:

- Completion of medical history
- Collection of demographic data (sex, age, race/ethnicity);
- Review of prior and current medications and supplements;
- Review inclusion and exclusion criteria.
- Physical examination (full).
- Measurement of height and weight.
- Measurement of vital signs.
- 12-lead ECG.
- Collection of blood and urine samples for:
    - Clinical safety labs, including hematology, serum chemistry (liver function tests (LFT's) and follicle stimulating hormone (FSH) [to confirm postmenopausal status in women]), and urinalysis (see Section 9.6.2 for list of tests);
    - Infectious serology (HIV, HBsAg, HCV);
    - Serum pregnancy test for women of childbearing potential
    - Drug substance abuse urine test and cotinine test
- Breath test for alcohol

For volunteers who meet eligibility criteria based on the Screening assessments, instruction will be provided on the following:

- Use of adequate contraceptive methods (see Section 4.2) for the duration of the study;
- Avoiding use of concomitant medications during the study, and mandatory prohibition of certain medications as defined in Section 9.6.6;
- Maintenance of usual dietary habits and avoidance of drastic changes

5.1.1 Screen Failure

A screen failure is defined as a participant who has signed the ICF, does not meet all the entry criteria outlined in Section 4 of this protocol (note that this includes assessments through Visit 1), and was not randomized to receive study treatment. The Investigator is responsible for keeping a record of all participants screened for entry into the study and subsequently excluded. The reason(s) for exclusion will be recorded in the source documents and on the Screening log. Screen failure participants will have only their consent, demographic and reason for screen failure (including, where applicable, the unmet inclusion or exclusion criteria) data entered into the electronic data capture (EDC) system. If an AE was responsible for the participant's screen failure, all data collected for that participant during the screening process will be entered into the EDC system.

5.2 Treatment Period

Clinical staff are required to perform assessments at the designated timepoints within the time windows indicated in this protocol (refer to Section 7.5, Schedule of Events). Actual times for each participant may vary depending on the scheduling and will be recorded in the electronic case report form (eCRF). When multiple procedures are scheduled at the same time point(s) relative to dosing, the following chronology of events should be adhered to, where possible: ECGs, vital signs, PK sampling and safety lab assessments.

5.2.1 Check-in (Day -1)

Participants will be admitted to the CRU on Day -1. Participants will be required to stay in the CRU for 3 nights (Part A) or 11 nights (Part B) and will be discharged on Day 3 and Day 11 for Part A (SAD) and Part B (MAD) respectively.

The following evaluations will be performed on Day -1:

- Completion of interim medical history review
- Review of prior and current medications and supplements;
- Physical examination (symptom-directed).
- Measurement of weight.
- Measurement of vital signs.
- 12-lead ECG.
- Collection of blood and urine samples for:

  - Clinical safety labs, including hematology, serum chemistry (LFT's and FSH [to confirm postmenopausal status in women]), and urinalysis (see Section 7.4.9.1 for list of tests);
  - Serum pregnancy Test for women of childbearing potential
- Urine for drug substance abuse and urine cotinine
- Breath test for alcohol
- Urine for pregnancy test for female of child-bearing potential.
- Estimated creatinine clearance
- Adverse events will be monitored and recorded.

All eligible participants will be randomized for each cohort and admitted into CRU.

5.2.2 Randomization and Dosing (Day 1)

For each cohort under SAD and MAD Part, participants who have passed the screening and were eligible as per inclusion and exclusion criterion will be randomised to the study treatment. The following procedures will be performed:

Prior to First Dose:

- Measurement of vital signs (supine systolic blood pressure (SBP)/diastolic blood pressure (DBP), temperature and heart rate) within 1 hour before study drug infusion;
- Review of prior and current medications and supplements;
- Physical examination within 4 hours before the start of study drug infusion.
- 12-lead ECG within 1 hour before the start of the first study drug infusion.
- Holter monitoring commences 60 minutes prior to dosing (SAD Cohorts 3, 4, 5 and 6 and all MAD Cohorts)
- Review inclusion and exclusion criteria.
- Randomisation
- Collection of blood and urine samples for:
  - Clinical safety labs, including hematology, serum chemistry
- For Part A: Blood for PK analysis will be collected immediately before (within 15 minutes before) the start of infusion of study drug;
- For Part B: Blood for PK analysis will be collected immediately before (within 15 minutes before) the start of infusion of study drug;
- For Part A: Obtain urine for PK analysis immediately before (within 2 hours before) the start of study drug;
- For Part B: Obtain urine for PK analysis immediately before (within 2 hours before) the start of the first study drug infusion

Following Dose Administration:

- For Part A: Measurement of vital signs (supine SBP/DBP, temperature and heart rate) 1, 2, 4, 6, 8, 10, and 12 hours (±10 minutes each) after the start of study drug infusion.

- For Part B: Measurement of vital signs (supine SBP/DBP, temperature and heart rate) 1, 2, and 4 hours (±10 minutes each) after the start of study drug infusion.
- 12-lead ECG at 1, 2 and 6 hours (±30 minutes each) after the start of study drug infusion.
- Holter monitoring continues to 24 hours post- Day 1 dose for SAD and MAD.
- For Part A: Blood for PK analysis will be collected at 5, 10,15, 20, 30, 45, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 10, and 12 hours (Day 1) (window: ± 2 minutes of the nominal timepoint up until the 20-minute timepoint and within ±10% of the nominal timepoint thereafter);
- For Part B: Blood for PK analysis will be collected at 30, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, and 8 hours (Day 1) window: within ±10% of the nominal timepoint);
- For Part A: Obtain urine for PK analysis at 0-2, >2-4, >4-8 and >8-12 hours (Day 1) (±10 minutes each) after the start of study drug infusion; collect and record urine volumes for each time point.
- For Part B: Obtain urine for PK analysis at 0-8h after the start of the first study drug infusion on Day 1.
- Adverse events will be monitored and recorded.
- Infusion reaction assessment during and immediately after infusion: 15 and 30 minutes, 1 and 2 hours post administration.
- Record all IV catheter site changes and the reason for change.

5.2.3 Post-treatment - SAD (Days 2-3):

Participants will continue to get safety evaluations done post treatment. The following procedures will be performed:

- Measurement of vital signs at 24 hours (Day 2) and 48 hours (Day 3) (±2 hours each) after the start of study drug infusion.
- Physical examination (symptom-directed) at 24 hours (Day 2) and 48 hours (Day 3) (±2 hours each) after the start of study drug infusion.
- 12-lead electrocardiogram at 24 hours (±30 minutes) after the start of study drug infusion (Day 2 only).
- Holter monitoring continues to approximately 24 hours post-dose on Day 2.
- Adverse events will be monitored and recorded.
- Record all IV catheter site changes and the reason for change.
- Blood and urine for safety laboratory assessments 24 hours (±10 minutes for blood samples, ±1 hour for urine samples) (Day 2 only)
- Blood for PK analysis at 24 hours (Day 2) and 48 hours (Day 3) window: within ±10% of the nominal timepoint) after the start of study drug infusion.
- Obtain urine at > 12-24, >24-36 and >36-48 hours (Days 2-3) (±10 minutes each) after the start of study drug infusion; collect and record urine volumes for each time point.

On day 3, participants will be discharged from the CRU after completing all safety and PK evaluations and post investigator reviews all clinical and safety related parameters of the participant.

5.2.4 Post-treatment - MAD (Day 2-11):

- Treatment administration as per randomization
- Measurement of vital signs at 24 hours (Day 2) and 48 hours (Day 3) (±2 hours each) after the start of each study drug infusion.
- Conduct symptom-directed physical examination
- Obtain 12-lead ECG measurements 24 hours after Day 1-10 dosing on Days 2, 4, 6, 8, and 10.
- Holter monitoring continues to approximately 24 hours post Day 1 dose (Day 2).
- Holter monitoring continues to approximately 24 hours post Day 10 dose (Day 11).
- Assess injection site for reactions if necessary (required if a reaction was observed on Day 1).
- Obtain blood (plasma) for PK analyses within 15 minutes prior to the start of the first infusion on days 5, 6, 7, 8, 9, and 10; and at 30, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 hours (window: within ±10% of the nominal timepoint) after the start of the last study drug infusion on Day 10. Exact schedule of blood sampling schedule will be fixed based on the dosage regimen to be followed post data review from Part A.
- Obtain urine for PK analysis at 0-8 hours after the start of the last study drug infusion on Day 10. Collect and record urine volumes for each time point. Exact schedule of urine sampling schedule will be fixed based on the dosage regimen to be followed post data review from Part A.
- Collect blood and urine for laboratory analyses on Days 2, 4, 6, 8, and 10 (at 4 hours after start of the study drug infusion on that day [(±10 minutes for blood samples, ±1 hour for urine samples]) and on Day 11 (at 24 hours after final Day 10 infusion [±10 minutes for blood samples, ±1 hour for urine samples]).
- Adverse events will be monitored and recorded.
- Prior and concomitant medications will be reviewed
- Infusion reaction assessment during and immediately after infusion: 15 and 30 minutes, 1 and 2 hours post administration on Days 2-10.
- Record all IV catheter site changes and the reason for change

On Day 11, participants will be discharged from the clinical unit after completing all safety and PK evaluations and post investigator reviews all clinical and safety related parameters of the participant.

Note: Assess patency of peripheral IV (PIV)/heplock, in accordance with site policies. If no longer patent remove and insert a new PIV/heplock in accordance with site policies.

5.3 Follow-up Visit

After discharge on Day 3 (SAD) or Day 11 (MAD), participants will return to the clinic for a single follow-up visit between days 7-9 (SAD) and between days 15-17 (MAD).

In SAD and MAD, the following procedures will be performed during follow-up:

- Adverse events since discharge will be monitored and recorded;
- Record all IV catheter site changes and the reason for change
- Physical examination
- Measurement of vital signs.
- 12-lead electrocardiogram (ECG).
- Blood and urine will be collected for clinical safety labs, including hematology, serum chemistry (LFT's).
- Pregnancy test (serum).
- Prior and concomitant medications will be reviewed.

5.4 Early Termination Visit

The following procedures will be performed if a participant terminates from the study early in Part A (SAD) or Part B (MAD):

- Adverse events will be monitored and recorded;
- Record all IV catheter site changes and the reason for change.
- Physical examination.
- Measurement of vital signs.
- 12-lead ECG.
- Blood and urine will be collected for clinical safety labs, including hematology, serum chemistry (LFT's).
- Pregnancy test (serum).
- Prior and concomitant medications will be reviewed.

5.5 Safety Review

The decision to enroll each study cohort will be made following evaluation of all available safety data and any available PK data by the SMG (details described in the study SMG Charter).

At a minimum, the SMG will be a comprised of the Investigator, an independent Medical Monitor and a clinical designate from the Sponsor (Sponsor's medical representative). Other individuals (e.g. medical experts) may be invited to participate at the discretion of the SMG to provide additional input into the review process if required.

Decisions to enroll the next cohort will be made by agreement between the PI(s), independent Medical Monitor and Sponsor's medical representative after completion of dosing in each study cohort.

The responsibilities and meeting schedule of the SMG will be outlined in a formal SMG Charter document.

Minutes following each SMG meeting will document the recommendation for dose escalation.

5.6 Early Withdrawal

If a randomized participant is withdrawn from the study prior to completing study treatment, the participant will be discharged from the study and the following procedures will be performed:

- Review of medications and supplements;
- Measurement of vital signs
- Physical examination;
- ECG assessment
- Collection of blood and urine samples for:
    - Clinical safety labs, including hematology, serum chemistry and urinalysis (see Section 9.6.2) for list of tests).
- Adverse events will be monitored and recorded;
- Discharge from the study.

The Investigator must continue to follow any participant with a possible study treatment related AE either until resolution or until the Investigator assesses them as chronic or stable.

5.6.1 Study Drug Discontinuation

Study drug must be discontinued if any of the following occur:

1. Adverse event/s as defined in the stopping criteria (refer Section 5.7)
2. Other findings that, at the discretion of the Investigator and/or Sponsor, indicate that study drug administration should be discontinued
3. Withdrawal of consent

The Investigator has full discretion to stop study drug infusion at any time, if clinically warranted or in the patient's best interest. All participants who have been dosed with any amount of study drug will continue to be followed for safety.

Participants who withdraw their consent will not receive any further study drug but will be offered all follow-up safety assessments. If a participant fails to attend scheduled study assessments, the Investigator must determine and document the reasons and the circumstances as completely and accurately as possible.

Participants who withdraw from the study prior to treatment (i.e. screened but nor randomized) may be replaced. Participants who withdraw after having received at least one dose of study drug will not be replaced.

Except in cases of emergency, the Investigator should consult with the Sponsor and the Medical Monitor before removing the participant from the study. In some circumstances it may be necessary to temporarily interrupt treatment as a result of AEs that may have an unclear relationship to study treatment. The Investigator should obtain approval from the Sponsor and Medical Monitor before restarting study treatments that were temporarily discontinued for an AE.

5.6.2 Study Withdrawal

Participants may choose to withdraw from this study at any time for any reason without penalty of jeopardizing their health care or loss of benefits to which the participant is otherwise entitled.

Within the provisions of informed consent and good clinical judgment with respect to safety, every attempt will be made to have participants complete the study. The following are reasons to terminate a participant's involvement in the study:

1. Significant protocol violation or noncompliance
2. Intercurrent illness that requires treatment that is not consistent with the protocol requirements, or intercurrent illness or the associated treatment that in the judgment of the Investigator poses a significant risk to the participant for continued participation in the study.
3. Participant wishes to withdraw for any reason.
4. Sponsor elects to end the study.
5. Any other reason that in the medical judgment of the Investigator poses unacceptable risk to the participant.

In the event that a participant discontinues the study prior to completion, the date the participant is withdrawn and the reason for discontinuation will be recorded in the source documents and eCRF. Although a participant will not be obliged to give his/her reason for withdrawing prematurely, the Investigator will make a reasonable effort to obtain the reason while fully respecting the participant's rights.

All participants who are randomized and treated (i.e., received any amount of study treatment) will be included in the safety analyses. Thus, every effort will be made to contact any participant who fails to attend any appointments/contacts, in order to ensure that he/she is in satisfactory health. If a participant withdraws from the study as a result of meeting discontinuation criteria after the start of study treatment administration, reasonable efforts should be made to have the participant return for the early withdrawal evaluations (Section 0).

Participants may choose to withdraw authorization to use and disclose their Personal health information (PHI) as defined by the HIPAA. Such withdrawal of authorization must be made to

the Investigator in writing. Any PHI collected by the Investigator prior to the date of such withdrawal will continue to be used and disclosed.

Randomized participants who are discontinued from this study for any reason will not be replaced.

If study termination criteria are met, enrollment of new participants and dosing of ongoing participants will be temporarily stopped. The Investigator, Sponsor, and the Medical Monitor will discuss whether a lower dose or any additional treatment guidelines should be implemented, or if the trial should be permanently stopped. Any proposed changes to the protocol to address such findings will be submitted for review and approval by the Institutional Review Board (IRB) prior to re-starting the trial.

5.7 Dose Escalation/Adjustment/Stopping Criteria

As described above, this protocol allows some alteration from the currently outlined dosing schedule. The maximum daily dose administered will not exceed the PK stopping criteria outlined in Section 5.7.4.

The decision to proceed to the next dose level of BWC0977 will be made by the SMG based on safety, tolerability and available PK data obtained from at least five participants at the prior dose level in Part A (SAD) and from at least 6/8 participants at the prior dose level in Part B (MAD). The actual doses to be administered may be adjusted based on safety, tolerability and preliminary PK data at previous dose levels.

The dosing schedule may also be adjusted to expand a dosing cohort to further evaluate safety and PK findings at a given dose level, or to add cohorts to evaluate additional dose levels. The study procedures for these additional participants(s) or cohort(s) will be the same as that described for other study participants.

Dosing in both Part A and Part B will proceed to the next dose level until evaluation of the nominal dose levels is completed, or the trial is stopped by the SMG. Temporary suspension of further dosing for a participant, dosing within a cohort or dose escalation between cohorts may be decided by the SMG is any of the criteria described in sections below are met.

For each of the stopping criteria described, the SMG will review all available safety data and will recommend if dosing should continue at that dose, or de-escalate to a lower dose level, or if the cohort, the dose level or the entire study should be terminated. No more healthy volunteers should be enrolled (or participants dosed) until this safety review is completed.

5.7.1 Liver Chemistry Stopping Criteria

Liver chemistry threshold stopping criteria have been designed to assure participant safety and to evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).

- Aspartate aminotransferase (AST)/ Alanine aminotransferase (ALT) > 3x upper limit of normal (ULN) is observed in two or more participants receiving BWC0977 within a cohort.
- One occurrence of Hy’s law criteria met, as defined by at least 3-fold elevations of ALT or AST above ULN, plus an elevation of serum total bilirubin to > 2 times ULN without elevated serum alkaline phosphatase, and no other disease or condition can be found to explain the liver test abnormalities

5.7.2 QTc Withdrawal Criteria

Decisions are to be based on an average QTc value of triplicate ECGs. If an ECG demonstrates a prolonged QT interval, obtain 2 more ECGs over a brief period, and then use the averaged QTc values of the 3 ECGs and other safety data to make a decision.

- Within a dose level, there are two or more occurrence of post-baseline QTcF prolongation, defined by average QTcF at least 501 ms and >60 ms change from baseline, that are determined to be clinically significant by the PI or Sponsor and are assessed as probably or possibly related to dosing with the investigational product.

5.7.3 Safety Stopping Criteria

The following events may result in temporary suspension or termination of administration of further dosing within a cohort or dose escalation between cohorts:

- Grade 3 gastrointestinal AE
- Grade 3 renal or urinalysis finding
- One occurrence of a SAE assessed to be probably or possibly related to dosing with the investigational product
- Participants with neutrophil counts below 1000 cells/μL will be discontinued from treatment and should be followed as appropriate until neutrophil counts normalize.
- One occurrence of elevation (>1.5 times ULN or a 2-fold increase as compared to baseline) in urea or serum creatinine
- Two or more severe AEs of the same character that are determined to be clinically significant by the PI or sponsor and are assessed as probably or possibly related to dosing with the investigational product
- If four or more participants experience the same or similar Grade 3 or higher AE which is possibly or probably related to the investigational product, the sponsor, the PI and Medical Monitor will review all available safety data and will recommend if dosing should continue at that dose, or de-escalate to a lower dose level, or if the study should be terminated. No more healthy volunteers should be enrolled (or participants dosed) until this safety review is completed.

Other findings such as a SAEs or severe AEs will be reviewed by the SMG. The SMG will determine how to proceed and if further dosing or dose escalation should be stopped

In case any safety or tolerability issues are experienced, a lower or intermediate dose may be administered in the next cohorts to gain more information on safety, tolerability and/or PK as recommended by the SMG.

The Investigator has full discretion to stop study drug infusion at any time, if clinically warranted (e.g. for an SAE or severe AE), or otherwise in the patient's best interest.

The grading for AEs is based on FDA's Guidance on Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

5.7.4 Pharmacokinetic Stopping Criteria

Dosing will be stopped if in any one individual participant, $AUC_{(0-24)}$> 52 µg.h/mL of BWC0977 has been observed or predicted at any given dose-level. However, at the discretion of the SMG, lower or intermediate doses may be explored in subsequent cohorts.

5.8 Study Termination

If the Sponsor or Investigator discovers conditions arising during the study that suggest the study should be halted, then this can happen only after appropriate consultation between the Sponsor and Investigator. Conditions that may warrant study termination include, but are not limited to:

- The discovery of any unexpected, significant, or unacceptable risk to the participants enrolled in the study.
- Insufficient adherence to the protocol requirements.
- A decision on the part of the Sponsor to discontinue the Study
- A decision on the part of the Sponsor to suspend or discontinue development of BWC0977.

The Investigator/Investigational Site has the right to close the site, at any time, although this should occur only after consultation between involved parties. The Investigator will notify the Independent Ethics Committee (IEC)/ IRB in writing of a premature termination of a study or closure of Investigational Site and will send a copy of the notification to the Sponsor.

6. STUDY TREATMENT

6.1 Description of Investigational Drug

6.1.1 Name of the Investigational Product

BWC0977.

6.1.2 Formulation

The drug product is presented as a compounded solution (BWC0977, 250 mg/vial). The solution will be diluted to a final concentration of 20 mg/mL using 5% dextrose for intravenous infusion and administered by IV infusion.

6.1.3 Preparation of Investigational Medicinal Product

A detailed Pharmacy manual will be provided which contains detailed instructions on preparation and dispensing of IMP as per randomization.

6.1.4 Method of Administration

BWC0977 will be infused intravenously via a drip system. The IV bag will be connected to an infusion line with integrated sterile filter (0.2 µm). The study drug will be infused continuously over 120 minutes, however the flow rate may be adjusted based on a review of available safety, tolerability and available PK data from previous cohorts by the SMG.

6.1.5 Packaging and Labeling

This study is a placebo-controlled study. All study medication will be compounded at the pharmacy and shipped to site. The Investigational Site pharmacist will be responsible for dispensing the appropriate treatment based on the randomization schedule. Study medication will be dispensed to the site with instructions for treatment administration.

BWC0977 will be compounded and supplied as solution vials with a content of 250 mg substance. The content of the required number of vials of BWC0977 will be diluted to a required volume using 5% dextrose. The solution of BWC0977 will be administered as continuous intravenous infusion over a period of 120 minutes.

Similarly, the content in the placebo vial will be diluted to a required volume using 5% dextrose. The solution will be administered as continuous intravenous infusion over a period of 120 minutes.

Bugworks Research Inc. will provide the required GMP like batch of drug substance and other excipients to Royal Adelaide Hospital (RAH) Pharmacy, who will compound the BWC0977 solution formulation as per the instructions in the Pharmacy manual. A Pharmacy manual will be provided to study site with instructions on how to prepare the specific solution for the respective treatment group.

The 20R vial (glass bottle) and the rubber closure for the container are appropriate for aqueous preparations for parenteral use according to international standards for packaging materials.

The treatment packages will be labeled with the following information:

- The notation- '*For Clinical Trial Use Only*';
- Study number
- Investigator/site identification
- Participant ID
- Kit No./Bottle ID/Batch number
- Retest date/expiry date
- Dosage Form/Content
- Directions for use, including route of administration
- Storage conditions
- Instructions to "keep out of reach of children"
- Caution: New Drug – Limited by TGA Australia law to investigational use.
- Name of Sponsor

6.1.6 Storage and Handling

All study treatment must be kept in an appropriate, secure area to prevent unauthorized access. The drug product (compounded solution) is to be stored at -10 to -30°C. The drug product can be stored at 2 to 8°C for up to 7 days. The diluted drug product (in the 5% Dextrose bag) is stable for up to 6 hours when stored at 15 to 25°C. Please refer to the Pharmacy manual for additional information. Storage conditions will be monitored, and appropriate monitoring logs maintained as source data. Deviations from the established temperature, should be documented, and the Sponsor should be notified.

6.2 Randomisation

This is a randomised, double blind controlled study.

In each cohort, the randomization will be blocked for 4 males and 4 females. Sentinels in each cohort will be 1 male and 1 female, randomised 1:1 for active:placebo, then the remaining 6 participants will be randomised 5:1 for active:placebo, with the placebo assigned to a participant of the opposite sex from the sentinel placebo. The requirement of equal numbers of males and females may be relaxed if there are fewer than 4 males or females available to be enrolled in a cohort.

The randomization schedule will be generated by Avance Clinical prior to the start of the study by an unblinded statistician. The Investigational Site pharmacist will follow this randomisation schedule to dispense the appropriate study treatment. Randomisation numbers will be assigned as participants qualify for the study and are assigned to treatment based on the randomisation schedule

6.3 Study Treatment Administration

Participants will be randomised 3:1 to either BWC0977 or Placebo.

6.4 Dose Modifications

Participants will be dosed the study treatment at the assigned (randomized) dose unless discontinuation criteria as defined in Section 5.6.1 are met. Dose modifications as recommended by the SMG may be considered in case any safety or tolerability issues are experienced.

6.5 Drug Accountability

In accordance with current Good Clinical Practice (GCP), the Investigational Site will account for all study treatment supplies. Details of receipt, storage, administration, and return or destruction will be recorded in the Investigational Drug accountability record according to the standard operating procedures (SOP) of the Investigational Site. Copies of the Investigational Drug accountability record will be provided to the Sponsor.

Study treatment will only be dispensed to participants enrolled in this protocol, and only as directed by this protocol. Administration of study treatment will be accurately recorded in each participant's source documents and eCRF.

6.6 Blinding

The following controls will be employed to maintain the double-blind status of the study:

- The infusion solution containing active drug and placebo will be indistinguishable in appearance
- The randomization list will be provided to the study center pharmacist for dispensing purposes and kept in the pharmacy, accessible to the pharmacist and authorized personnel
- PK results for the interim analyses between cohorts will be presented in a blinded fashion.

Individual code break envelopes will be provided for all participants by the contract research organization (CRO). Each sealed envelope containing the randomization code and treatment allocation information will be kept in a storage room at the clinical facility, which is locked with restricted access. To manage the participant's condition in case of a medical emergency, the Investigator (or delegate) is allowed to break the code to know whether a participant received BWC0977 or placebo. If opened, the name of the person who opened it, the date and time of opening and the reason for opening must be written on the envelope. The Sponsor will be informed in case of unblinding.

There are no specific antidotes for BWC0977. Knowledge of whether the participant received BWC0977 or placebo, may not necessarily help in the care of an individual participant. The need to break the code must therefore be carefully considered.

The laboratory where the PK samples will be analyzed will be provided a copy of the randomization code by the CRO since only samples of participants that have received the active drug BWC0977 will be analyzed.

6.7 Concomitant Medications and Supplements

All medications and supplements (other than study treatment) taken by the participant from screening/day-1 through day 3 (Part A) and day 11 (Part B) will be considered "concomitant" medications and supplements. Medications and supplements taken prior to screening /Day 1 that are no longer being taken at the time of screening /Day 1 will be considered "prior" medications and supplements.

All medications and supplements taken within 30 days prior to Day 1 (first dose) of study treatment and concomitant medications and supplements will be recorded in the participant's source documentation and in the eCRF.

The use of all prescribed medication is not allowed from within 14 days or 5 half-lives (if known), whichever is longer, prior to Day 1 (first dose). An exception is made for hormonal contraceptives, which are allowed throughout the study for women of child-bearing potential. The use of all over-the-counter medications, vitamin preparations and other food supplements, or herbal medications is also not allowed within 14 days or 5 half-lives (if known), whichever is longer, prior to Day 1 (first dose). An exception is made for paracetamol: a limited amount of paracetamol (a maximum of 4 doses per day of 500-mg paracetamol, and no more than 3 g per week) for the treatment of headache or any other pain is permitted.

Treatment with vaccines (including influenza and/or COVID-19 vaccines) is not permitted within 14 days prior to Day 1 (first dose), and throughout the duration of the study. Participants should not receive any vaccinations (including influenza and/or COVID-19 vaccines) until after study completion.

Medications to be avoided if there is a TEAE include succinylcholine or other depolarizing muscle relaxants and acetylcholinesterase inhibitors (including edrophonium, pyridostigmine, and neostigmine), drugs which are organic anion transporter 3 (OAT3) transporter inhibitors or substrates (e.g., probenecid, cefazolin, methotrexate, cephaloridine), and administration of multidrug and toxin extrusion (MATE)-2K transporter inhibitors or substrates (e.g., cimetidine, metformin, pyrimethamine, dolutegravir, cisplatin). If a participant requires the use of any medications and supplements during the study, the Investigator will contact the Sponsor and the Medical Monitor to discuss the participant's continued involvement in the study. Other medications to treat TEAEs may be prescribed if deemed necessary by the Investigator. If medications are used, the name of the drug, the dose and dosage regimen will be recorded in the eCRF.

6.8 Dietary and Other Restrictions

As described in the exclusion criteria, consumption of red wine, seville oranges, grapefruit or grapefruit juice, pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices containing such products are excluded from 7 days prior to the first dose of study medication. There are no other special requirements related to food and beverage intake during the study. Meals and snacks (such as decaffeinated coffee, herbal tea, fruit, crackers) will be provided according to study center's SOPs.

The use of alcohol and tobacco products is not allowed throughout the duration of the study.

Participants must be nonsmokers (including tobacco, e-cigarettes and marijuana) and not use nicotine within 1 month prior to participation in the study. Participants must have a negative cotinine test at screening and check-in (Day -1), and refrain from smoking for the duration of the study.

Strenuous exercise is not allowed within 4 days prior to Day -1 and during the entire study. Participants should avoid sun exposure till end of follow-up in view of the potential for phototoxicity of BWC0977.

Participants should not consume any foods containing poppy seeds within 48 hours (2 days) prior to Day -1 as this could cause a false positive drug screen result.

7. STUDY PROCEDURES AND ASSESSMENTS

7.1 Informed Consent

According to the ICH guideline for GCP (E6) and all institutional local, state, and federal laws, the Investigator will obtain and document informed consent for each volunteer screened for this study. All participants will be informed in writing of the nature of the protocol and Investigational Drug, its possible hazards, and their right to withdraw at any time, and will sign a form (ICF) indicating their consent to participate in the study prior to the initiation of study procedures. The participant's medical record should contain written documentation indicating that informed consent was obtained. The ICF must be reviewed and approved by the Investigator's designated IRB and by Bugworks Research Inc. designee prior to its use. Refer to Section 12.2.6 for further details regarding informed consent.

7.2 Pregnancy Testing

Serum β-human chorionic gonadotropin pregnancy (β-hCG) testing is to be performed for female participants of childbearing potential (ie, premenopausal or not surgically sterile) at screening and a urine pregnancy test before study drug infusion at Day -1. Any participant with a positive result is not eligible for study participation. All samples will be analyzed by the local laboratory. Any participant determined to be pregnant cannot participate in the study.

7.3 Medical History and Prior Medications

At Screening, a complete medical history will be collected by participant interview. Medications and supplements, recent blood donations, illnesses, and participation in other Investigational Drug trials or clinical trials will also be recorded.

7.4 Pharmacokinetic & Safety Assessments

7.4.1 Single Ascending Dose PK Assessment:

Blood:

The sampling schedule is planned to provide an adequate estimation of C_{max} and to cover the plasma concentration-time curve long enough to provide a reliable estimate of the extent of absorption. Blood (plasma) for PK analyses will be collected as per the Schedule of Events (refer to Table 6).

Urine:

Urine for PK analyses will be collected as per the Schedule of Events (refer to Table 6).

7.4.2 Multiple Ascending Dose PK Assessment:

Exact schedule of blood and urine sampling schedule will be fixed based on the dosage regimen to be followed post data review from Part A. However tentative schedule to be planned is as follows

Blood:

Blood (plasma) for PK analyses will be collected as per the Schedule of Events (refer to Table 7).

Urine:

Urine for PK analyses will be collected as per the Schedule of Events (refer to Table 7).

7.4.3 Pharmacokinetic Sample analysis of BWC0977

Blood samples (approximately 6 mL) to provide a minimum of 3 mL plasma for PK analysis will be collected at each nominated timepoint into appropriately labeled tubes.

The actual times may change but the number of samples will remain the same. All efforts will be made to obtain the PK samples at the exact nominal time relative to dosing. However, samples obtained within ± 2 minutes of the nominal timepoint up until 20-minutes post-dose and within 10% of the nominal time thereafter (eg, within 6 minutes of a 60 minute sample) from dosing will not be captured as a protocol deviation, as long as the exact time of the sample collection is noted on the source document and data collection tool (eg, eCRF).

Instructions for collecting and processing serum samples are provided in the study Laboratory Manual.

- Samples will be analyzed using a validated analytical method in compliance with standard operating procedures applicable at **Agilex BioLabs, Adelaide**.
- As part of understanding the pharmacokinetics of the study drug, samples may be used for metabolite identification and/or evaluation of the bioanalytical method. These data will be used for internal exploratory purposes and will not be included in the clinical study report. Samples collected for this purpose will be retained in accordance with local regulations and if not used within this timeframe, will be destroyed.

7.4.4 Safety Assessment

7.4.4.1 Weight and Height

Weight will be measured at visits as described in the schedule of events (Section 7.5). Height will be measured at Screening only with the participant wearing no shoes.

7.4.4.2 Vital Signs

Vital signs (respiratory rate, supine SBP/DBP, temperature and heart rate) will be recorded at visits as described in the schedule of events (Section 7.5). Blood pressure and heart rate will be measured after the participant has been supine for at least 5 minutes in a quiet environment and prior to any blood draw that occurs at the same time point.

7.4.4.3 Physical Examination

A physical examination will be performed at visits as described in schedule of events (Section 7.5), or in case of Early Withdrawal.

The physical examination will include the following: general appearance; skin; head, eyes, ears, nose, and throat; thyroid; lungs; cardiovascular; abdomen (liver, spleen); lymph nodes; and extremities, an abbreviated neurological examination and any other focused assessments suggested by the presence of specific symptoms.

7.4.4.4 12- Lead ECG

A triplicate 12-lead ECG will be performed at Screening, Day -1 and at the end of follow-up. A single standard 12-lead ECG will be obtained at other visits. The 12-lead ECG will be recorded after the participant has been resting at least 5 minutes in the supine position in a quiet environment. ECGs will be read for QT and QTcF (Federicia's) intervals and clinically significant abnormalities. At the Investigator's discretion, an additional ECG may be performed at any timepoint; however, it will be recorded in source documents as an unscheduled assessment.

7.4.4.5 Holter Monitoring

Continuous Holter monitoring will be performed for SAD Cohorts 3, 4, 5 and 6 and all MAD Cohorts. Holter monitoring will commence at least 1 hour pre-dose and will continue until approximately 24 hours post-dose on Day 1 for SAD and MAD. For MAD cohorts, a second Holter monitoring period will commence at least 1 hour pre-dose on Day 10, and continue until approximately 24 hours post-Day 10 dose (Day 11), as indicated in the Schedule of Events.

Subjects will rest for at least 10 minutes prior to Holter ECG extraction timepoints, and for at least 5 minutes after. Holter monitoring may be discontinued for 45 minutes daily for showering.

The 12-lead Holter and ECG equipment will be supplied and supported by Clario. All ECG data will be collected using a Global Instrumentation (Manlius, NY, USA) M12R ECG continuous 12 lead digital recorder. The continuous 12-lead digital ECG (Holter) data will be stored onto SD memory cards. ECGs to be used in the analyses will be selected by pre-determined time points as defined in the Table of Assessments and will be read centrally by Clario.

The following principals will be followed in ERT's core laboratory:

- ECG readers are blinded to the subject, visit and treatment allocation
- A limited number of readers will be employed for the study
- Baseline and on-treatment ECGs for a particular subject will be over-read on the same lead and will be analyzed by the same reader.
- The primary analysis lead is lead II. If lead II is not analyzable, then primary lead of analysis will be changed to another lead for the entire subject data set.

The following is a brief description of ECG analysis methods utilized by ERT's core laboratory.

TQT Plus ECG Extraction Technique

Ten 14-second digital 12-lead ECG tracings will be extracted from the continuous Holter recordings using the 'TQT Plus method', a computer-assisted and statistical process utilized by ERT. The method enables extraction of ECGs with the lowest HR variability and noise within the protocol-specified extraction time window (e.g., the HR and QT changes from beat-to-beat in the range of <10%). At each protocol-specified timepoint, 10 ECG replicates will be extracted from a 5-minute "ECG window" (typically, the last 5 minutes of the 10-minute period when the subject is maintained in a supine or semi-recumbent quiet position).

Expert-Precision QT Analysis

Expert-precision QT analysis will be performed on all analyzable (non-artifact) beats in the 10 ECG replicates. Statistical quality control procedures are used to review and assess all beats and identify "high" and "low" confidence beats using several criteria, including:

- QT or QTc values exceeding or below certain thresholds (biologically unlikely).
- RR values exceeding or below certain thresholds (biologically unlikely).
- Rapid changes in QT, QTc or RR from beat to beat.

Measurements of all primary ECG parameters (QT, QTc, RR) in all recorded beats of all replicates that are deemed "high confidence" is performed using COMPAS software. All low confidence beats are reviewed manually and adjudicated using pass-fail criteria. The final QC assessment is performed by a cardiologist. The beats found acceptable by manual review are included in the analysis. The median QT, QTc, and RR value from each extracted replicate is calculated, and then the mean of all available medians from a nominal timepoint is used as the subject's reportable value at that timepoint.

Morphological analyses will be performed with a focus on detecting changes in T-wave morphology and appearance of abnormal U waves. The analyses will evaluate change-from-baseline (i.e., treatment-emergent changes).

The analysis results for T-wave morphology and U-wave presence will be summarized in frequency tables with counts and percentages for both number of subjects and number of time points. The number and percentage of subjects in each treatment group having changes from baseline that represent the appearance of the morphological abnormality will be summarized. The total number of time points having a particular change event will be summarized in terms of number and percentage based on the number of observed time points across all subjects within a treatment group.

For T-wave morphology and U-wave presence, treatment-emergent changes will be assessed, i.e., changes not present at baseline. For each category of T-wave morphology and of U-waves, the category will be deemed as present if observed in any replicate at the time point. For baseline, the category will be deemed as present if observed in any replicate from all time points that constitute baseline.

7.4.4.6 Clinical Safety Laboratory tests

Blood and urine for clinical safety laboratory assessments will be collected and processed using standard procedures. A local laboratory will perform all clinical laboratory tests.

In the event of abnormal clinical laboratory values, the Investigator will make a judgment whether or not the abnormality is clinically significant. The Investigator may repeat any laboratory tests as deemed necessary to confirm out of range results. Cotinine testing may be repeated once per timepoint, at the discretion of the PI, in the instance of a positive result.

The clinical safety labs will include the following hematology, serum chemistry, urinalysis and other tests (Table 3):

Table 3. Clinical Laboratory Blood and Urinalysis Tests

Hematology			
Platelet Count	*RBC Indices*:	*Automated WBC Differential:*	
RBC Count	MCV	Neutrophils	
WBC Count (absolute)	MCH	Lymphocytes	
Reticulocyte Count	MCHC	Monocytes	
Hemoglobin		Eosinophils	
Hematocrit		Basophils	
Coagulation			
Prothrombin time (sec and INR)			
Partial thromboplastin time			
Clinical Chemistry			
Creatinine	Potassium	AST (SGOT)	Total and direct bilirubin
Glucose (random)	Chloride	ALT (SGPT)	Uric Acid
Sodium	Bicarbonate	GGT	Albumin
Urea	Calcium	Alkaline phosphatase	Total Protein
Phosphate		Lactate dehydrogenase	Lipid panel
CPK	Creatinine Clearance (calculated by Cockroft Gault formula)		Albumin:Creatinine ratio
Urinalysis			
urobilinogen	Specific gravity		
pH, glucose, protein, blood and ketones by dipstick			
Microscopic examination (if blood or protein is abnormal and assessed as clinically significant)			
Sedimentary microscopy will be performed if any of the above tests are abnormal and assessed as clinically significant. In such cases, microscopy will be performed for 1. White blood cells 2. Red blood cells 3. Hyaline casts 4. Granular casts 5. Cellular casts Any other cell types/casts found upon microscopic examination will be reviewed by the Pathology laboratory staff, and the Study Physician will be alerted if there are any concerns			
Infection and pregnancy screening			
HIV (HIV1 HIV2)			
Hepatitis B (HBsAg)			
Hepatitis C (anti HCV antibody)			
Serum (screening and follow-up) or urine pregnancy test (Day -1) (β–HCG)			
Other tests (Screening and Admission Only)			
Alcohol levels (measured by an alcohol breathalyzer/breath test performed at the study site) and urine for drug screen (drug screen to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines). Urine cotinine test.			
Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; β–HCG = Beta-human chorionic gonadotropin; CPK = creatine phosphokinase; GGT = gamma; glutamyl transferase; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human; immunodeficiency virus; INR = international normalized ratio; MCH = mean corpuscular hemoglobin; MCHC = mean cell haemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell; SGOT = serum glutamic-oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; WBC = white blood cell.			

Additional tests may be necessary to ensure participant safety and to ensure an adequate follow-up following an AE.

7.4.4.7 Follicle Stimulating Hormone Test

A serum FSH test will be performed in postmenopausal women.

7.4.4.8 Infusion Reaction Evaluation

Assessments of infusion reaction to monitor local tolerability to BWC0977 infusions will be performed up to at least 2 hours following study drug administration.

7.4.4.9 Infusion Site (IV Catheter Site) Reaction Evaluation

Assessments of infusion site (IV catheter) reaction to monitor local tolerability to BWC0977 infusions will be performed throughout the study. Particularly in MAD phase site tolerability assessments should continue after each dosing and will be followed up next day if infusion site pain or infusion site reaction characteristics continue to persist. The assessments should continue at regularly scheduled visits until the symptoms resolve.

7.4.4.10 Total Volume of Blood Collected

Blood samples will be collected from each participant in Part A throughout the study resulting in the total blood volumes per participant as shown in Table 4 and Table 5. Additional blood may be collected from each participant at any time if required for safety reasons.

Table 4. Blood Sample Volumes to be Collected per Participant (Part A – SAD)

Sample Type	***Volume of blood per sample (mL)***	***Number of Samples per Participant***					
		Screen	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 7-9**
Haematology	4	1	1	-	1	-	1
Serum chemistry [a]	9	1	1	-	1	-	1
Coagulation	3	1	1	-	1	-	1
Serology	9	1	-	-	-	-	-
PK samples	6 / 8[b]	-	-	18	1	1	-
Subtotal		**25 mL**	**16 mL**	**144 mL**	**22 mL**	**6 mL**	**16 mL**
Total blood volume collected per participant		**233 mL**					

Abbreviations: PK = pharmacokinetic; SAD = single ascending dose.
[a] *Includes serum pregnancy testing, where applicable*
[b] *8 mL per sample on Day 1 to account for volume in the blood collection line.*

Table 5. Blood Sample Volumes to be Collected per Participant (Part B – MAD)

Sample Type	***Volume of blood per sample (mL)***	***Number of Samples per Participant***								
		Screen	**Day -1**	**Day 1**	**Day 2**	**Day 4**	**Days 5, 7 and 9**	**Days 6, 8 and 11**	**Day 10**	**Day 15-17**
Haematology	4	1	1	-	1	1	-	1	1	1
Serum chemistry [a]	9	1	1	-	1	1	-	1	1	1
Coagulation	3	1	1	-	1	1	-	1	1	1
Serology	9	1	-	-	-	-	-	-	-	-
PK samples	6 / 8[a]	-	-	11		-	1	1	13	-
Subtotal		**25 mL**	**16 mL**	**88 mL**	**16 mL**	**16 mL**	**6 mL *(per day)***	**22 mL *(per day)***	**120 mL**	**16 mL**
Total blood volume collected per participant		**385 mL**								

Abbreviations: PK = pharmacokinetic; SAD = single ascending dose.
[a] *Includes serum pregnancy testing, where applicable*
[b] *8 mL per sample on Day 1 and Day 10 to account for volume in the blood collection line.*

7.5 Schedule of Events

Table 6 and Table 7 describe the daily schedule of events from Screening (28 days to 2 days before randomization) through Day 7-9 (Part A) and 15-17 (Part B) respectively.

Table 6. Part A (SAD) Daily Schedule of Events from Screening through Day 7-9

Protocol Activity	Day -28 to -2	Day -1	Day 1	Day 2	Day 3	Day 4-6	Day 7-9	Early Termin-ation
Admission to/Discharge from CRU	Screening[a]	Check-in[b]	→	→	Check out	→	X	X
Visit window (± days)	N/A	N/A	N/A	0	0		1	N/A
Informed Consent [c]	X							
Inclusion/Exclusion criteria	X	X	X					
Complete medical history	X							
Interim medical history		X						
Height, weight and BMI calculation	X	X[d]						
Vital signs [e]	X	X	X	X	X		X	X
Complete physical examination [f]	X						X	X
Symptom-directed physical examination [f]		X	X	X	X			
12-lead ECG [g]	X	X	X	X			X	X
Holter recording [h]			X	X				
Blood and urine for laboratory analyses [i]	X	X		X			X	X
Blood for HBsAg, HCV, HIV serology	X							
Breath test for alcohol	X	X						
Urine to test for drugs of abuse & cotinine	X	X						
Pregnancy test[j]	X	X					X	X
Estimated CrCl	X	X [d]						
Randomisation			X [k]					
Blood for PK analyses [l]			X	X	X			
Urine for PK analyses [m]			X	X	X			
Assess for adverse events [n]	X							
Assess for infusion reactions [o]			X					
Record all IV catheter site changes and the reason for change [p]			X					
Study Drug Administration			X					
Prior and concomitant medications [q]	X							

Abbreviations: BMI = Body mass index; CrCl = creatinine clearance; CRU = clinical research unit; ECG = electrocardiogram; FU = Follow-up; HBsAg = hepatitis B virus surface antigen; HCV = hepatitis C virus; HIV = Human immunodeficiency virus; IV = Intravenous; PK= Pharmacokinetic; SAD = single-ascending dose; UA = urinalysis.

Footnotes for Table 6 (Part A SAD)

a. Screening visit must occur within 28 days to 2 days before randomization.

b. Participants will be admitted to the CRU on Day -1 and confined to the CRU through 48 hours (Day 3) after the start of study drug infusion (Day 1); discharge may occur after all assessments and procedures are complete on Day 3.

c. Informed Consent must be obtained before initiating any study-related assessments or procedures.

d. Measure weight, calculate BMI, and estimate CrCl on Day -1 if >24 hours after Screening visit. Measure weight and serum creatinine on same day for estimation of CrCl.

e. Vital signs include supine blood pressure (systolic and diastolic, recorded after lying supine for 5 minutes), heart rate, respiratory rate and temperature. Vital signs will be obtained after at Screening visit, on admission to the CRU on Day -1, within 1 hour before and 1, 2, 4, 6, 8, 10, and 12 hours (±10 minutes each) after the start of study drug infusion, at 24 hours (Day 2) and 48 hours (Day 3) (±2 hours each) after the start of study drug infusion, and at the FU visit. In the event of overlap between safety and PK sampling timepoints, then safety will take preference over PK.

f. A complete physical examination, including general appearance, head, ears, eyes, nose, throat, dentition, thyroid, chest (heart, lungs), abdomen, skin, neurological, extremities, back, neck, musculoskeletal, lymph nodes will be performed at Screening visit and at the End of Study/FU visit. An abbreviated symptom directed physical examination will be performed on Day -1, within 4 hours before the start of study drug infusion on Day 1; and at 24 hours (Day 2) and 48 hours (Day 3) (±2 hours each) after the start of study drug infusion.

g. At the Screening visit and Day -1, triplicate ECGs will be obtained within a 15-minute period, separated by at least 1 minute. In addition, a standard single 12-lead safety ECG will be obtained within 1 hour before the start of the first study drug infusion on Day 1 and at 1, 2, 6, and at 24 hours (Day 2) (±30 minutes each) after the start of study drug infusion. At the FU visit, triplicate ECGs will be obtained within a 15-minute period, separated by at least 1 minute. All ECG recordings will be taken after at least 5 minutes in a supine, quiet-rest position and prior to obtaining any blood sample.

h. A continuous ECG recording (Holter) will be performed for approximately 25 hours, starting at least one hour pre-dose on Day 1 (SAD Cohorts 3, 4, 5 and 6) and continuing until approximately 24 hours post-dose. 12-lead ECGs will be extracted by the central ECG laboratory at 3 time points within one hour prior to dosing (e.g., -45, -30 and -15 minutes) and at the following time points, paired with PK sampling: 5, 10, 15, 20, 30, 45, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 10, and 12 and 24 hours post-dose. Subjects will be supinely resting for at least 10 minutes before and 5 minutes after each time point. When ECG extractions coincide with safety ECGs, vital signs assessment and blood draws, procedures will be carried out in said order.

i. Blood samples will be collected for serum chemistry (LFT's and FSH [to confirm postmenopausal status in women]), hematology, coagulation tests, and urine samples will be collected for UA (and urine microscopy if UA is positive for red blood cells, white blood cells, or protein and assessed as clinically significant) at Screening visit, upon admission to the CRU on Day -1, and at 24 hours (Day 2) (±10 minutes for blood samples, ±1 hour for urine samples) after the start of study drug infusion, and at the FU visit.

j. Females of child-bearing potential must have a negative serum pregnancy test (β–HCG) at Screening visit and a negative urine pregnancy test on Day –1 and agree to and comply with using a highly effective method of birth control from Day -1 through the FU visit. A serum pregnancy test will also be performed at the FU visit for females of child-bearing potential. To be considered not of childbearing potential, a female must have either a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy, or menopause (last menstruation >12 months and follicle-stimulating hormone test in menopausal range, unless previous follicle-stimulating hormone result documented in medical history and part of source documentation.

k. Randomize participant on Day 1.

l. Obtain blood (plasma) for PK analyses immediately before (within 15 minutes before) the start of infusion of study drug; at 5, 10, 15, 20, 30, 45, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 10, and 12 hours (Day 1) (window: window: ± 2 minutes of the nominal timepoint up until the 20-minute timepoint and within ±10% of the nominal timepoint thereafter); and at 24 hours (Day 2) and 48 hours (Day 3) (window: within ±10% of the nominal timepoint) after the start of study drug infusion.

m. Obtain urine for PK analysis immediately before (within 2 hours before) the start of study drug infusion and at 0-2, >2-4, >4-8, >8-12, >12-24 hours (Days 1-2), >24-36 and >36-48 hours (Days 2-3) (±10 minutes each) after the start of study drug infusion; collect and record urine volumes for each time point.

n. Adverse events are captured after the participant signs the Informed Consent Form up to the FU visit.
o. Infusion reactions to be assessed at during and immediately after infusion: 15 and 30 minutes, 1 and 2 hours post administration.
p. Following dosing, record all IV site (dosing catheter) changes and the reason(s) for change throughout study.
q. Prior and concomitant medication history includes all medications taken from Day -30 before the start of study drug infusion through the FU visit.

Table 7. Part B (MAD) Schedule of Assessments and Procedures

Protocol Activity	Screen -28 to -2	Day -1	Day 1-10	Day 11	Day 12-14	Day 15-17	Early Termin-ation
Admission to/Discharge from CRU	Screening[a]	Check-in[b]	→	Check-out	→	X	X
Visit window (±)	N/A	N/A	0	0		1	N/A
Informed consent [c]	X						
Inclusion/Exclusion criteria	X	X	X				
Complete medical history	X						
Interim medical history		X					
Height, weight and BMI calculation	X	X[d]					
Vital signs [e]	X	X	X	X		X	X
Complete physical examination [f]	X					X	X
Symptom-directed physical examination [f]		X	X	X			
12-lead ECG [g]	X	X	X			X	X
Holter recording [h]			X	X			
Blood and urine for laboratory analyses [i]	X	X	X	X		X	X
Blood for HBsAg, HCV, HIV serology	X						
Breath test for alcohol	X	X					
Urine to test for drugs of abuse	X	X					
Pregnancy test [j]	X	X				X	X
Estimated CrCl	X	X [d]					
Randomisation to study drug			X [k]				
Blood for PK analysis [l]			X	X			
Urine for PK analysis [m]			X	X			
Assess for adverse events [n]	X						
Assess for infusion reactions [o]			X				
Record all IV catheter site changes and the reason for change [p]			X				
Study Drug Administration			X [q]				
Prior and concomitant medications [r]	X						

Abbreviations: BMI = Body mass index; CrCl = creatinine clearance; CRU = clinical research unit; ECG = electrocardiogram; FU = Follow-up; HBsAg = hepatitis B virus surface antigen; HCV = hepatitis C virus; HIV = Human immunodeficiency virus; IV = Intravenous; MAD = multiple-ascending dose; PK= Pharmacokinetic; SAD = single-ascending dose; UA = urinalysis.

Footnotes for Table 7 (Part B MAD)

a. Screening visit must occur within 28 days before randomization.

b. Participants will be admitted to the CRU on Day -1 and confined to the CRU through 24 hours after the start of the Day 10 final study drug infusion (Day 11); discharge may occur after all assessments and procedures are complete on Day 11.

c. Informed Consent must be obtained prior to initiating any study-related assessments or procedures.

d. Measure weight, calculate BMI, and estimate CrCl on Day -1 if >24 hours after Screening visit. Measure weight and serum creatinine on same day for estimation of CrCl.

e. Vital signs include supine blood pressure (systolic and diastolic, recorded after lying supine for 5 minutes), heart rate, respiratory rate and temperature. Vital signs will be obtained at Screening visit, on admission to the CRU on Day -1, within 1 hour before and 1, 2, and 4 hours (±10 minutes each) after the start of the first study drug infusion on each dosing day, at 24 (±2) hours after the start of the final Day 10 study drug infusion (Day 11), and at the FU visit.

f. A complete physical examination (ie, general appearance, head, ears, eyes, nose, throat, dentition, thyroid, chest [heart, lungs], abdomen, skin, neurological, extremities, back, neck, musculoskeletal, lymph nodes) will be performed at Screening visit and at the End of Study/FU visit. An abbreviated symptom directed physical examination will be performed on Day -1, within 4 hours before the start of the first study drug infusion on each dosing day, at 24 (±2) hours after the start of the final Day 10 study drug infusion (Day 11).

g. At the Screening visit and Day -1, triplicate ECGs will be obtained within a 15-minute period, separated by at least 1 minute. In addition, a standard single 12-lead safety ECG will be obtained within 1 hour before the start of the first study drug infusion and at 1, 2, and 6 hours (each ±30 minutes) after the start of the first study drug infusion, and on Days 2, 4, 6, 8, and 10. At the FU visit, triplicate ECGs will be obtained within a 15-minute period, separated by at least 1 minute. All ECG recordings will be taken after at least 5 minutes in a supine, quiet-rest position and prior to obtaining any blood sample.

h. Two continuous ECG recordings (Holter) will be performed for approximately 25 hours each, the first starting at least one hour pre-dose on Day 1, and the second starting at least one hour pre-dose on Day 10 (all MAD Cohorts). Holter recordings will continue to approximately 24 hours post- Day 1 and Day 10 dose. A 12-lead safety ECG will be performed if abnormalities are observed during the continuous Holter monitoring. 12-lead ECGs will be extracted by the central ECG laboratory at 3 time points within one hour prior to dosing on Day 1 (e.g., -45, -30 and -15 minutes) and at the following time points, paired with PK sampling: 30, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8 hours post-dose, and on Day 10 at 15 minutes prior to dosing and then paired with PK at 30, 60, 75 and 90 minutes and 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 hours post-dose. Subjects will be supinely resting for at least 10 minutes before and 5 minutes after each time point. When ECG extractions coincide with safety ECGs, vital signs assessment and blood draws, procedures will be carried out in said order.

i. Blood samples will be collected for serum chemistry, hematology, coagulation tests,, and urine samples will be collected for UA (and urine microscopy if UA is positive for red blood cells, white blood cells, or protein and assessed as clinically significant) at Screening visit, upon admission to the CRU on Day -1, on Days 2, 4, 6, 8, and 10 (at 4 hours after start of the study drug infusion on that day [(±10 minutes for blood samples, ±1 hour for urine samples]), on Day 11 (at 24 hours after final Day 10 infusion [±10 minutes for blood samples, ±1 hour for urine samples]), and at the FU visit.

j. Females of child-bearing potential must have a negative serum pregnancy test (β–HCG) at Screening visit and a negative urine pregnancy test on Day –1, and agree to and comply with using a highly effective method of birth control from Day -1 through the FU visit. A serum pregnancy test will also be performed at the FU visit for females of child-bearing potential. To be considered not of childbearing potential, a female must have either a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy, or menopause (last menstruation >12 months and follicle-stimulating hormone test in menopausal range unless previous follicle-stimulating hormone result documented in medical history and part of source documentation.

k. Randomize participant on Day 1.

l. Obtain blood (plasma) for PK analyses immediately before (within 15 minutes before) the start of the first infusion of study drug on Day 1; at 30, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, and 8 hours (Day 1) (window:

within ±10% of the nominal timepoint); within 15 minutes prior to the start of the first infusion on days 5, 6, 7, 8, 9, and 10; and at 30, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 hours (window: within ±10% of the nominal timepoint) after the start of the last study drug infusion on Day 10. Exact schedule of blood sampling schedule will be fixed based on the dosage regimen to be followed post data review from Part A.

m. Obtain urine for PK analysis immediately before (within 2 hours before) the start of the first study drug infusion on Day 1; at 0-8h after the start of the first study drug infusion on Day 1; and at 0-8 hours after the start of the last study drug infusion on Day 10. Collect and record urine volumes for each time point. Exact schedule of urine sampling schedule will be fixed based on the dosage regimen to be followed post data review from Part A.

n. Adverse events are captured after the participant signs the Informed Consent Form up to the FU visit.

o. Infusion reactions to be assessed at during and immediately after infusion: 15 and 30 minutes, 1 and 2 hours post administration.

p. Following dosing, record all IV site (dosing catheter) changes and the reason(s) for change throughout study.

q. BWC0977 dose and dosing frequencies in the MAD cohorts will be determined based on safety and PK data from the SAD cohorts.

r. Prior and concomitant medication history includes all medications taken from Day -30 before the start of the first dose of study drug through the FU visit.

7.6 Review and Documentation of Medications and Supplements

All medications or supplements participants are taking or have taken within 30 days prior to Day 1 (first dose) through Day 9 of Part A (SAD) will be recorded in the participant's medical record and the medical history eCRF.

All medications and supplements (other than study treatment) taken by the participant after screening /Day 1 through Day 17 assessments of Part B (MAD) will be recorded in the participant's medical record and the medical history eCRF.

Medications and supplements taken prior to screening /Day 1 that are no longer being taken at screening /Day 1 will be considered "prior" medications and supplements.

Medications and supplements should be recorded according to the generic name when possible. Any medication or supplement used should have an indication recorded, and for concomitant medications and supplements, this indication must be represented as either for the treatment of an AE, for the management of a pre-existing condition, or for prophylaxis or other reasons.

Dosage increases for any concomitant medication or supplement should be noted and the reason for the dosage increase should be recorded. The side effects of concomitant medications will be recorded as AEs.

Any participant whose condition becomes disqualifying during the course of the study may be treated for that condition. If the condition is suspected during Screening, the participant should not be enrolled. Treatment of the condition should be instituted according to the Investigator's/attending physician's judgment.

Medications that have no treatment intent but rather maybe part of supportive routine should also be recorded in the participant's medical record and eCRF. This may include local anesthetics, intravenous solutions to maintain fluid balance and keep access open, medications used for prophylaxis.

8. ADVERSE EVENTS AND SAFETY REPORTING

8.1 Safety and Tolerability Assessments

Safety and tolerability will be assessed on an ongoing basis by review of reported AEs, physical examinations, vital signs (including weight, supine SBP/DBP, and heart rate), and clinical safety labs (hematology, serum chemistry, and urinalysis). Assessments will be performed in accordance with the schedule of events (Section 7.5, Table 6 and Table 7).

8.2 Definition of Adverse Event

An AE is defined in 21 CFR 312.32(a) as follows:

> Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug and does not imply any judgment about causality.

Worsening of a pre-existing medical condition, (ie, diabetes, migraine headaches, gout) is to be considered an AE if there is either an increase in severity, frequency, or duration of the condition or an association with significantly worse outcomes.

Adverse events will be recorded from the time of written informed consent through the last follow-up visit, or after the end of the study, if thought to be related to study drug. Any clinically significant observations in results of clinical laboratory, 12-lead ECGs, vital signs, or physical examinations will be recorded as AEs.

An AE which occurs prior to (the first) administration of the study drug will be considered a pre-treatment AE.

A treatment emergent AE (TEAE) is defined as any AE that starts or worsens (in frequency or severity) following exposure to study drug.

The Investigator is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual participant represents a significant change from baseline. In general, abnormal laboratory findings without clinical significance (based on the Investigator's judgment) should not be recorded as AEs; however, laboratory value changes requiring therapy or adjustment in prior therapy are considered AEs.

8.3 Definition of Serious Adverse Event

A SAE is defined in 21 CFR 312.32(a) as follows:

> An AE is considered "serious" if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death;
- Life-threatening AE;
- Inpatient hospitalization or prolongation of existing hospitalization;
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Congenital anomaly/birth defect.
- Is a medically important event or reaction

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An AE is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the participant at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

8.4 Eliciting and Reporting of Adverse Events

Participants will be asked in general terms about any AEs at regular intervals during the study. In addition, all AEs reported spontaneously during the course of the study will be recorded.

AE monitoring will start immediately following consent and will continue till end of follow-up. Any participant with a possible study treatment-related AE will be followed until resolution or stabilization of the event. Further, any SAE, whether or not related to study treatment, that occurs until the follow up visit following the last dose of study treatment, will be followed until resolution or stabilization of the event. This may require additional clinical assessments and laboratory tests. The follow-up results will be recorded in the participant 's source documentation and in the eCRF.

Participants will be instructed to report all AEs experienced during the study, and participants will be assessed for the occurrence of AEs throughout the study. At several time points before and after drug administration participants will be asked general, non-leading questions to determine the occurrence of AEs.

Medical conditions existing at Screening should be recorded as medical history. New or worsening pre-existing medical conditions or diseases are considered AEs if they arise or worsen after the Screening visit and should be recorded as AEs.

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an AE if it occurs or is detected following the first dose of study treatment through follow-up visit. Conditions leading to planned

surgical procedures are not AEs if the condition(s) was (were) known before study treatment. In the latter case, the condition should be reported as medical history.

8.4.1 Routine Reporting of Adverse Events

All AEs, whether or not associated with the study treatment, that are observed by the Investigator, other Investigational Site personnel, or those reported by the participant will be recorded in the participant's source documentation and on the AE page of the eCRF. Copies of the SAE case report form (CRF) pages or an SAE listing generated based on the eCRF pages will be submitted to the Sponsor at regularly scheduled intervals to allow the Sponsor to meet expedited regulatory reporting requirements under 21 CFR 312.32 (see Section 8.4.3 for further detail) and regular regulatory reporting requirements under 21 CFR 312.33.

For each AE, the following information will be entered in the eCRF:

- Medical diagnosis of the event in standard medical terminology (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event);
- Date of onset of any new AE or worsening of a previously observed AE;
- Date of resolution of the event (or confirmation ongoing);
- Whether the event is serious (per definition in Section 8.3), and if so, the reason it is considered serious;
- Severity of AE (per definition in Section 8.6);
- Assessment of the attributability of the AE to the study treatment (per definition in Section 8.5);
- Whether the event is expected (per definition in Section 8.7);
- Action taken on account of the AE: No action; concomitant medications or therapies required; tests required; hospitalization required (or prolonged); treatment unblinded; and/or change in the study treatment administration or dose (i.e. whether the study treatment was temporarily interrupted or discontinued);
- Outcome of AE (per definition in Section 8.8).

Due to the coronavirus SARS-COV-2 (COVID-19) pandemic, Investigators must also ensure compliance with local governing legislation and reporting requirements associated with COVID-19 infections.

8.4.2 Suspected Adverse Reaction

A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of Investigational New Drug Application (IND) safety reporting, "reasonable possibility" and/or at least possibly related means there is evidence to suggest a causal

relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

8.4.3 Reporting of Serious Adverse Events, Including Death

The Sponsor will adhere to all expedited regulatory reporting requirements as per 21 CFR 312.32.

SAEs, including death due to any cause, which occur during this study or within 30 days following the last dose of the study treatment, whether or not related to the administration of study treatment, must be reported by the Investigator or other Investigational Site personnel to the Medical Monitor by telephone or fax **within 24 hours of learning of the event**. The contact information for the Medical Monitor is provided below.

Medical Monitor:
Dr. Abhijeeth Chandrasekaran
Clinical Scientist
RxMD
320/1, Lloyds Road, Royapettah,
Chennai 600 014, India
Tel +91.44.2466 2270, Mobile: +91 99417 35679
Email: abhijeeth.chandra@rxmd.com

Sponsor designated safety officer details for SAE reporting:
Pilar Garzon / Elena Bercu / Neha Madan
Phone: +61 478 034 138
Email: safety@avancecro.com

SAE Forms will be provided by the Sponsor or Sponsor designated CRO as described in the study safety reporting plan. If all information is not known at the time of initial reporting, an initial report should still be made. In the event there is a question as to whether the experience is serious, the information should be forwarded to the Medical Monitor for review. The Investigator is responsible for following up on completion of the SAE Form. The Investigator will submit substantiating data in hard copy form, such as diagnostic test reports and progress notes, to the Medical Monitor. In the case of fatality, autopsy reports will be furnished to the Medical Monitor as soon as available. If the Medical Monitor is informed of a SAE via a telephone call, preliminary information will be obtained, and the study site will be instructed to fax an SAE Form.

The initial SAE Form and any subsequent follow-up SAE Forms submitted to provide more accurate, corrected, or new information must be signed by the Investigator. The Investigator and Investigational Site Personnel must make every reasonable effort to obtain, from other institutions if necessary, all supporting medical case records as needed to comply with expedited IND safety reporting requirements.

If the SAE involves expedited IND safety reporting (as determined by the Sponsor or designee), all supporting medical records must be submitted to the Sponsor or designee within 4 calendar days for death or life-threatening events, and 10 calendar days for all other events. In cases where medical records and supporting documentation are unobtainable, the Investigator must generate a narrative of the event, utilizing when necessary, interviews with the participant, their family members and care givers as appropriate.

The Investigator must also promptly inform the governing IRB of the SAE in accordance with the governing IRB's requirements. If an SAE is determined by the Sponsor to be reportable to the FDA as an IND Safety Report (as defined in 21 CFR 312.32), it will be reported to FDA by the Sponsor or designee within the specified time frame. All IND Safety Reports will also be promptly provided to the Investigator for submission to his/her IRB. Similarly, any SAE that is determined by the Sponsor to require expedited reporting to other regulatory authorities will be reported to the appropriate authorities by the Sponsor or designee within the specified time frames, and will be provided to the Investigator for submission to his/her IRB.

The Investigator, Medical Monitor, and Sponsor will review each SAE report and evaluate the relationship of the adverse experience to study treatment and to underlying disease. Based on this assessment, a decision will be made concerning the need for further action. The primary consideration governing further action is whether new findings affect the safety of participants participating in the clinical trial. If the discovery of a new adverse experience related to the study treatment raises concern over the safety of continued administration of study treatment, the Sponsor will take immediate steps to notify the regulatory authorities.

Further action that may be required includes the following:

1. Alteration of existing research by modification of the protocol;
2. Discontinuation or suspension of the study;
3. Alteration of the informed consent process by modification of the existing consent form and informing current study participants of new findings;

8.4.4 Exposure *In Utero* Management and Reporting

In instances of pregnancies or suspected pregnancies identified or reported for any female participant (or male participant's female partner), including a positive pregnancy test regardless of age, following administration of study treatment, the pregnant female participant (or the male participant's female partner) will be advised to notify her healthcare provider. Study drug administration will be discontinued immediately in the event of a reported (or suspected) pregnancy in a female participant.

The Investigator will notify the Sponsor and designated study safety officer of this event and document the pregnancy on the EIU form as described in the study Safety Reporting Plan.

Informed consent will be sought from the pregnant female participant (or male participant's female partner) in order to allow for the Investigator to conduct follow-up access and review of relevant medical records throughout the gestational period and on the infant following delivery. The Investigator shall follow-up newborn infants that have been exposed to investigational product (IP) in utero for a minimum of 12 months. Upon discovery of any congenital anomalies (or neonatal deaths) the Investigator shall submit a follow-up report to the Sponsor (and study safety officer) using an SAE Form (as per study Safety Reporting Plan) including information regarding the status of the newborn. A miscarriage or abortion or any congenital anomaly diagnosed in the infant exposed in utero shall also be reported by the Investigator to the study Safety Officer (and the Sponsor) using an SAE Form.

8.4.5 Protocol Deviations Due to an Emergency or Adverse Event

Departures from the protocol will be determined as allowable on a case-by-case basis and only in the event of an emergency. The Investigator or other physician in attendance in such an emergency must contact the Medical Monitor as soon as possible to discuss the circumstances of the emergency.

The Medical Monitor, in conjunction with the Investigator, will decide whether the participant should continue to participate in the study. All protocol deviations and reasons for such deviations must be noted in the Clinical Trial Management System.

8.5 Causality Assessment of Adverse Events

For all AEs, the Principal Investigator will provide an assessment of causal relationship to the study treatment (active or placebo). The causality assessment must be recorded in the participant's source documents and on the AE eCRF. Causal relationship will be classified according to the following criteria:

Relationship between Study Drug and AE:					
	Category				
AE (is):	None	Unlikely	Possibly	Likely	Definitely
Clearly the result of an external factor	Yes	No	No	No	No
Probable/possibly the result of another factor	No	Yes	Yes	No	No
Has a chronological relationship with the time of administration and /or represents a known reaction to Study Drug	No	No	Yes	Yes	Yes
Disappears or decreases after discontinuation of the Study Drug	NA	NA	NA	Yes	Yes
Recurs on renewed administration (re-challenge)	No	No	NA	NA	Yes or NA**
** A rechallenge is not required; if done, rechallenge would be expected to be positive, NA: Not Applicable					

8.6 Adverse Event Severity Assessment

The severity of each AE will be graded according to the FDA Guidance - Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (FDA Adult adolescent volunteer vaccine guidance). The severity of AEs that are not specifically listed in the FDA Guidance will be categorized according to the general guidelines for systemic illness (i.e. illness or clinical AE) provided in the FDA Guidance, as summarized in the table below.

General Guidelines for Severity Assessment of Clinical Adverse Event

(Mild) Grade 1: No interference with activity
(Moderate) Grade 2: Some interference with activity not requiring medical intervention.
(Severe) Grade 3: Prevents daily activity and requires medical intervention
(Potentially life threatening) Grade 4: ER visit or hospitalization

Note the distinction between the severity and the seriousness of an AE. A severe AE is not necessarily a SAE. For example, a headache may be severe (prevents daily activity or requires use of narcotic pain reliever) but would not be classified as serious unless it met one of the criteria for SAEs, listed above in Section 8.3.

8.7 Expectedness of Adverse Event

An unexpected AE is defined in 21 CFR 312.32(a) as follows:

An AE is considered "unexpected" if it is not listed in the IB or is not listed at the specificity or severity that has been observed; or, if an IB is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

8.7.1 Serious and Unexpected Suspected Adverse Reaction

A suspected unexpected serious adverse reaction (SUSAR) is any event that meets all 3 of the following definitions:

1) suspected adverse reaction (Section 8.4.2);

2) serious (Section 8.3); and

3) unexpected (as described in text above, Section 8.7).

8.8 Assessment of Adverse Event Outcome

Outcome of AEs will be defined according to ICH Topic E2B, ICH Guideline.

- **Recovered/Resolved:** The participant has recovered fully from the AE without any remaining effects or impairment.
- **Recovering/Resolving:** The participant is recovering, but with an after effect possibly due to disease or treatment.
- **Recovered/Resolved with Sequelae**: The participant has recovered, but with an after effect possibly due to disease or treatment.
- **Not Recovered/Not Resolved:** The condition is still present.
- **Fatal:** Fatal should only be used when death is possibly related to the AE.
- **Unknown**: The primary outcome is not known at the time of the final assessment. If an outcome for an AE is not available at the time of the initial report, follow-up will proceed until an outcome is known or followed up to the Final Study Visit. The Investigator must continue to follow all SAEs and non-serious AEs considered to be at least possibly related to study drug either until resolution or the Investigator assesses them as chronic or stable. This follow-up may extend after the end of the study.

8.9 Clinical Findings

Any significant clinical findings will be followed until the condition returns to pre-study status, stabilizes, or can be explained as not being study treatment related. If the clinical finding is reported as an AE (per the criteria outlined in Section 8.2), the follow-up procedures for AEs defined above will apply.

9. STATISTICAL METHODS

This section describes the statistical methods to be used for the analysis and reporting of data collected under Protocol No. C001-2020-01. Additional details will be provided in the statistical analysis plan (SAP). Any major modifications of the primary endpoint definition and/or its analysis will be reflected in a protocol amendment.

9.1 Healthy Volunteer Disposition

All participants screened and randomized will be accounted for. All post-randomization discontinuations will be summarized by reason for discontinuation. The number of participants screened and not randomized will be presented.

9.2 Analysis Populations

The study analysis populations will consist of:

- Randomized population: All participants will be analyzed who are assigned a randomization number in the Treatment phase.
- Safety population: All randomized participants will be analyzed who receive any study treatment in the Treatment phase.
- Pharmacokinetic Population: All randomized participants who have taken at least one dose of BWC0977 without protocol deviation affecting PK evaluation, and with available PK data to determine plasma concentrations of BWC0977 will be included in the PK data analysis

9.3 Protocol Deviations

The criteria for protocol deviations considered major with the implication of data exclusions from the Per Protocol (PP) analysis will be determined prior to database lock and unblinding.

9.4 Trial Population

9.4.1 Demographics and other Baseline Characteristics

Baseline and demographic characteristics will be summarized for all participants in the safety population by treatment group and overall. Continuous variables will be displayed via summary

statistics (mean, median, sample size, standard deviation, minimum, and maximum). Categorical variables will be summarized via counts and percentages.

9.4.2 Medical History, Concomitant Medication and Other Safety Evaluations

Medical history will be coded by system organ class (SOC) and preferred term (PT) using the currently available version of the Medical Dictionary for Regulatory Activities (MedDRA) and summarized for all participants in the population analysis sets by treatment group.

The number and percentage of participants with medical history conditions will be summarized by SOC and PT.

9.5 Statistical and Analysis methods

9.5.1 Sample Size Determination

This study is exploratory in nature; no pre-planned hypothesis testing is to be performed. The sample size determination is not based on statistical power considerations. The sample size has been selected to provide information on safety, tolerability and PK following single doses of BWC0977. Any p-values to be calculated according to the SAP will be interpreted in the perspective of the explorative character of this study. Dose cohort size selected for this study is 8 participants with a 6:2 ratio of active drug to placebo in a randomized manner in both Part A and Part B of the study. Additional participants may be added at a dose level to further evaluate safety and/or tolerability after discussions between the Bugworks study team and the investigator/s.

9.5.2 Pharmacokinetic Analysis

9.5.2.1 Derivation of Pharmacokinetic Parameters

PK parameters following single and multiple-dose administration will be derived from the concentration-time data, as data permits. Table 8 and Table 9 provide the details of derived plasma PK parameters. Table 10 and Table 11 provide the details of derived urine PK parameters.

The total amount of BWC0977 excreted in urine and dose amount recovered (as a percentage of total dose) will be determined from urine samples collected and amounts listed for each subject and summarised for each dose group.

Table 8. Single Dose Plasma PK Parameters

Parameter	Definition	Method of Determination
AUC_{last}	Area under the plasma concentration-time profile from time zero to the time of the last quantifiable concentration (C_{last})	Linear/Log trapezoidal method
AUC_{inf}	Area under the plasma concentration-time profile from time zero extrapolated to infinite time	AUC_{last} + (C_{last}*/kel), where C_{last}* is the predicted plasma concentration at the last quantifiable time point estimated from the log-linear regression analysis.
C_{max}	Maximum plasma concentration	Observed directly from data
T_{max}	Time for C_{max}	Observed directly from data as time of first occurrence
t½	Terminal elimination half-life	Ln(2)/kel, where kel is the terminal phase rate constant calculated by a linear regression of the log-linear concentration-time curve. Only those data points judged to describe the terminal log-linear decline will be used in the regression.
Vz	Apparent volume of distribution	Dose / (AUC_{inf} * kel)
Vdss	Volume of distribution at Steady state	CL • MRT, where MRT is the mean residence time calculated as ($AUMC_{inf}$/AUC_{inf} - Infusion duration/2); $AUMC_{inf}$ is area under the moment curve from time 0 extrapolated to infinity
CL	Clearance	Dose / AUC_{inf}
AUC_{last}(dn)	Dose normalized AUC_{last}	AUC_{last} / Dose
AUC_{inf}(dn)	Dose normalized AUC_{inf}	AUC_{inf} / Dose
C_{max}(dn)	Dose normalized C_{max}	C_{max} / Dose

Table 9. Multiple Dose Plasma PK Parameters at Steady State

Parameter	Definition	Method of Determination
AUC_{τ}	Area under the concentration-time profile from time zero to time tau (τ), the dosing interval	Linear/Log trapezoidal method
C_{max}	Maximum serum concentration	Observed directly from data
C_{τ}	Concentration at the end of the dosing interval	As observed from concentration data
C_{av}	Average concentration	AUC_{τ}/τ
T_{max}	Time for C_{max}	Observed directly from data as time of first occurrence
CL	Clearance	Dose / AUC_{τ}
C_{min}	Lowest concentration observed during the dosing interval	Observed directly from data
$t_{½}$	Terminal elimination half-life	Ln(2)/kel, where k_{el} is the terminal phase rate constant calculated by a linear regression of the log-linear concentration-time curve. Only those data points judged to describe the terminal log-linear decline will be used in the regression.
Vdss	Steady-state volume of distribution	CL • MRT, where MRT is the mean residence time calculated as ($AUMC_{\tau}/AUC_{\tau}$ - Infusion duration/2); $AUMC_{\tau}$ is area under the moment curve over the dosing interval
R_o	Observed accumulation ratio based on AUC	$AUC_{\tau,ss}$ / AUC_{τ} Day 1
$R_{o,Cmax}$	Observed accumulation ratio based on Cmax	$C_{max,ss}$ / C_{max} Day 1
AUC_{τ}(dn)	Dose normalized AUC_{τ}	AUC_{τ} / Dose
C_{max}(dn)	Dose normalized C_{max}	C_{max} / Dose
C_{min}(dn)	Dose normalized C_{max}	C_{min} / Dose

Abbreviations: ss = steady state.

Note: Actual PK sampling times will be used in the derivation of PK parameters.

Table 10. Single Dose Urine PK Parameters

Parameter	Definition	Method of Determination
$Ae_{(0\text{-last})}$	Cumulative amount of unchanged drug excreted in urine	∑(Concentration*volume)
Fe	Cumulative fraction of dose excreted unchanged in urine over the entire collection interval	100* ($Ae_{(0\text{-last})}$/dose)
CLr	Renal clearance	$Ae_{(0\text{-last})}/AUC_{(0\text{-last})}$

Table 11. Multiple Dose Urine Parameters

Parameter	Definition	Method of Determination
$Ae_{0\text{-}\tau}$	Cumulative amount of unchanged drug excreted in urine over the entire collection interval	∑(Concentration*volume) Calculated for Days 1 and 10
$Fe_{0\text{-tss}}$	Cumulative fraction of the dose excreted as unchanged in urine over the entire collection interval (at steady state);	100* ($Ae_{0\text{-}\tau}$ dose) Calculated for Days 1 and 10
CLr_{ss}	Renal clearance (at steady state)	$Ae_{0\text{-}\tau}$/ $AUC\tau$ Calculated for Days 1 and 10

9.5.2.2 Statistical Methods of PK analysis

The Plasma PK parameters for BWC0977 and its metabolites will be summarized descriptively by dose level. Plasma concentrations will be listed and summarized descriptively by dose and nominal PK sampling time. Individual participant, summary profiles (mean and median plots) of the Plasma concentration-time data will be plotted by treatment and PK sampling time. For summary statistics and summary plots, the nominal PK sampling time will be used. For individual participant plots, the actual PK sampling time will be used, whilst the pre-dose time will be set to zero. Summary plots will be presented on both linear-linear and log-linear scales.

Dose normalized parameters for BWC0977 and its metabolites will be plotted against dose and will include individual participant values and the geometric means for each dose. For SAD, AUC_{inf}, AUC_{last}, and C_{max} will be plotted; for MAD, AUC_{τ} and C_{max} (Day 1 and at steady state) and C_{min} (at steady state) will be plotted. These plots will be used to help understand the relationship between the PK parameters and dose.

9.5.2.3 Estimate of Variability

Following log-transformation, AUC_{inf} and C_{max} of BWC0977 and its metabolites for single dosing groups will be analyzed separately by mixed effect models fitting dose as fixed effect term. The repeat dose groups will be analyzed separately by mixed effect models fitting dose, day, dose-by-day interaction as fixed effect terms and participant as a random effect term. Point estimate and 90% confidence interval (CI) will be constructed using the appropriate error term and then be exponentially back-transformed to provide point estimate and 90% CI of PK

parameters at each dose level on Day 1. Model-based within- participant and between-participant coefficients of variations will be calculated based on appropriate error term.

Following log-transformation, AUC(0-τ) of BWC0977 and its metabolites on Day 10 will be analyzed separately by mixed effect models fitting dose as a fixed effect. Point estimate and 90% CI will be constructed using the appropriate error term and then be exponentially back-transformed to provide point estimate and 90% CI of the PK parameters at each dose level. Model-based between- participant coefficients of variations will be calculated based on appropriate error terms.

9.5.2.4 Dose Proportionality

Dose proportionality of AUC_{inf} and C_{max} on Day 1 and for repeat dose groups AUC(0-τ) and C_{max} of BWC0977 on Day 10 will be assessed separately using the power model as described below:

$y = \alpha * dose^{\beta}$

where y denotes the PK parameter being analyzed and α depends on the random error in the repeat dose phase where participants take the study drug in a parallel-group fashion. Dose proportionality implies that β=1 and will be assessed by estimating β along with its 90% CI. The exponent, β, in the power model will be estimated by regressing the loge-transformed PK parameter on loge-transformed dose. The power model will be fitted by restricted maximum likelihood (REML) using Statistical Analysis Software (SAS) Proc Mixed, with a fixed effect term for dose. The PK parameter endpoint and the factor dose will be loge-transformed prior to the analysis. An estimate of slope (with corresponding 90% CI) will be provided as a measure of potential dose proportionality (i.e. a slope ≈ 1 implies dose proportionality).

In the case where dose proportionality is not established over the entire dosing range, secondary analysis of dose proportionality will be assessed for select doses over the higher dosing range with the power model or by pair-wise analysis of variance (ANOVA) using the SAS Mixed models procedure. If the secondary analysis using the pair-wise ANOVA approach is performed, a reference dose would be chosen based on the lowest clinically relevant dose over which the pharmacokinetics can be adequately described and the other doses would be treated as test doses.

9.5.2.5 Accumulation Ratio

For the repeat dose groups of Part B, the accumulation ratio (Ro) will be calculated as the ratio of AUC(0-τ) on Day 10 to AUC(0-τ) on Day 1 for each participant.

The dosing interval (τ) will be equal to 8 hours for thrice daily regimen or 12 hours for twice daily or 24 hours for once daily. The accumulation ratio will be listed and summarized along with other PK parameters.

Following log-transformation, AUC(0-τ) of BWC0977 on Days 1 and 10 will be analyzed by a mixed effect model, fitting dose, day and dose-by-day interaction as fixed effects and subject as a random effect. For each dose, point estimate and 90% CI for the difference

"AUC(0-τ) on Day 10 - AUC(0-τ) on Day 1" will be constructed using the appropriate error term. The point estimate and associated 90% CI will then be exponentially back-transformed to provide point and 90% CI estimates for the ratios "AUC(0-τ) on Day 10: AUC(0-τ) on Day 1". If dose-by-day interaction is not significant, then a single point estimate and 90% CI pooled across all doses for the ratio AUC(0-τ) on Day 10 : AUC(0-τ) on Day 1 may be constructed with all estimates for each dose.

9.5.2.6 Steady State Assessment

To evaluate whether steady state was achieved, statistical analysis of steady-state trough concentrations (Cτ) will be performed after loge-transformation of Cτ on Days 7, 8 and 9, Days 10. A mixed effect model will be fitted by dose and day (as a continuous covariate) as a fixed effect term and participant as a random effect term. The coefficients for the slope of the day effect on the loge-scale will be used to evaluate steady-state for each dose group. Using the pooled estimate of variance, the 90% CIs for the slope will be calculated. Distributional assumptions underlying the statistical analyses will be assessed by visual inspection of residual plots. Normality will be examined by normal probability plots, while homogeneity of variance will be assessed by plotting the residuals against the predicted values for the model. Alternative analyses of the data will be performed if any of the model assumptions appear to be violated. Time of occurrence of C_{max} (T_{max}) of BWC0977 will be separately analyzed using non-parametric Wilcoxon rank test to compute point estimates and associated 90% CIs for the median differences.

9.5.3 Cardiodynamic Evaluation

The cardiodynamic ECG endpoints include change-from-baseline in heart rate (HR), QTcF, PR and QRS (ΔHR, ΔQTcF, ΔPR and ΔQRS); placebo-corrected ΔHR, ΔQTcF, ΔPR and ΔQRS (ΔΔHR, ΔΔQTcF, ΔΔPR and ΔΔQRS); categorical outliers for HR, QTcF, PR, QRS; and frequency of treatment-emergent changes for T-wave morphology and U-wave presence.

The primary analysis will be based on concentration-QTc modeling of the relationship between the plasma concentrations of BWC0977 and change-from-baseline QTcF (ΔQTcF) with the intent to exclude an effect of placebo-corrected ΔQTcF (ΔΔQTcF) > 10 ms at clinically relevant plasma concentrations. The effect of BWC0977 on the placebo-corrected ΔQTcF, ΔHR (heart rate), ΔPR, and ΔQRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) will also be evaluated at each post-dosing time point ('by-time point' analysis). In addition, an analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology and U-wave presence.

More details on cardiodynamic ECG evaluation will be described in a separate SAP.

9.6 Safety Evaluation

All safety data analysis will be performed on the safety analysis set, which includes all enrolled participants who receive at least one dose of study medication. Adverse events, ECGs, blood

pressure, heart rate, continuous cardiac monitoring (SAD only), ambulatory blood pressure monitoring (MAD cohorts only), and safety laboratory data will be reviewed and summarized on an ongoing basis during the study to evaluate the safety of participants. Any clinical laboratory, ECG, blood pressure (BP), and PR abnormalities of potential clinical concern will be described. Safety data will be presented in tabular and/or graphical format and summarized descriptively, where appropriate.

Medical history, ECGs and physical exam information collected during the course of the study will be captured for inclusion into the study database, unless otherwise noted. Data collected at Screening that is used solely for inclusion/exclusion criteria, such as laboratory data, ECGs and vital signs will be considered source data, and will not be captured for inclusion into the study database, unless otherwise noted (note: screening physical exam is a comprehensive exam and will be captured in the study database as the baseline exam). Demographic data collected at Screening will be included in the study database.

Summary statistics and data listings will be provided for the following endpoints:

- Incidence of dose limiting or intolerable treatment related AEs.
- Incidence, severity and causal relationship of treatment emergent AEs (TEAEs).
- Incidence of abnormal laboratory findings (clinical chemistry, hematology and urinalysis).
- Changes from baseline in safety laboratory assessments.
- Abnormal and clinically relevant changes in vital signs, BP, and ECG parameters.
- Incidence of anti-drug-antibodies (ADA).

9.6.1 Adverse Events

AEs will be coded using the most current version of MedDRA. The severity of AEs will be graded according to the schema presented in Section 8.6. TEAEs will be collected starting with Day 1 through Day 7-9 (Part A) and Day 15-17 (Part B). AEs occurring between screening and Day 1 will be regarded as "pretreatment" if they occur before IP administration at Day 1. TEAEs are defined as any AE that starts or worsens (increases in frequency or severity) after the first randomized dose of study IP on Day 1.

The incidence of TEAEs will be tabulated by MedDRA preferred term, system organ class, treatment group, severity, and assigned relationship to study treatment. The incidence for each TEAE will be provided as the total number of participants that experienced the TEAE, as well as the percentage of the population that this represents. If a TEAE is reported more than once for a given participant, the greatest severity and the worst-case attribution will be presented in the summary tables.

TEAEs will be listed for individual participants, along with information regarding onset dates and end dates, onset time where available, severity, seriousness, relationship to study treatment, action taken, and outcome. A similar listing will be prepared for the pretreatment AEs.

Pretreatment AEs and TEAEs that lead to withdrawal from the study will be separately listed and summarized. Similarly, separate tabulations and listings will be prepared for pretreatment and treatment-emergent SAEs.

Descriptive statistics will be generated as appropriate (i.e., frequency for categorical data). Inferential statistical analysis comparing the AE data between active and placebo is not planned. However, crude incidence rates will be provided

9.6.2 Laboratory Evaluations

Individual clinical safety lab (hematology, serum chemistry, and urinalysis) values will be listed by treatment and summarized using descriptive statistics as appropriate (i.e., mean, median, range, and standard deviation for continuous data and frequency for categorical data). Individual change from baseline (Screening) in laboratory values will be calculated and summarized descriptively. A clinically significant change from baseline (Screening) will be recorded as an AE if deemed appropriate by the Investigator.

9.6.3 Vital Signs

Individual vital sign measurements (respiratory rate, supine SBP/DBP, temperature and heart rate) will be listed by measurement time and summarized using descriptive statistics as appropriate (i.e., mean, median, range, and standard deviation). Individual change from baseline (Screening) in vital sign measurements will be calculated and summarized descriptively. A clinically significant change from baseline (Screening) will be recorded as a TEAE if deemed appropriate by the Investigator.

9.6.4 12-lead ECG

Individual 12-lead ECG assessments for each visit will be listed.

9.6.5 Physical Examination

Individual physical examination findings will be listed for each visit in which a physical examination occurred. A clinically significant change from baseline (Screening) will be recorded as an AE if deemed appropriate by the Investigator.

9.6.6 Prior and Concomitant Medications and Supplements

Medications and supplements will be coded using the most current version of the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system.

All medications and supplements (other than study treatment) taken by the participant from Day 1 through Day 7-9 (Part A) and Day 15-17 (Part B) will be considered “concomitant” medications and supplements. Medications and supplements taken prior to the first dose of BWC0977 that are no longer being taken at the time of the first dose of BWC0977 will be considered “prior” medications and supplements.

Concomitant medications and supplements will be listed for individual participants. A similar listing will be prepared for prior medications and supplements taken within 30 days prior to the first dose of study treatment. The frequency of use of these prior and concomitant medications and supplements will be summarized.

9.6.7 Handling of Missing, Unused, or Spurious Data

No substitution of missing data will be used in any calculations. Data points that appear to be spurious will be investigated and will not be excluded from the listings. An explanation will be given for all missing, unused and spurious data in the relevant sections of the CSR.

10. DATA MANAGEMENT

10.1 Data Collection

All data required by the study protocol will be collected in a validated database according to the CRO's SOPs.

10.2 Electronic Data Capture

Data from the source documents will be entered into the EDC system by authorized Investigational Site personnel. Data Management staff, using both electronic and manual checks, will systematically check the data. Errors or omissions will result in queries (which can be issued by the Study Monitor or Data Management staff), which will be presented to the Investigational Site within the EDC system. The Investigational Site will resolve the queries within the EDC system. The Study Monitor and Data Management staff will review the responses as part of the query resolution process. The EDC system will track the queries with the corresponding responses.

Medications and supplements entered into the database will be coded in the EDC system using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. AEs and Medical History will be coded in the EDC system using MedDRA terminology.

Clinical safety laboratory blood and urinalysis samples will be processed by Australian Clinical Labs. All lab results will be sent electronically to the clinical site. The clinical laboratory results will be imported into the database.

10.3 Quality Assurance and Database Lock

A 100% critical variable review of all key safety and secondary endpoint data in the database will be performed. Following this review, a data quality control audit or a random sample equal to the square root plus 1 of the total population will be performed.

When the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement between the Sponsor, the Investigator, the lead data manager, and the study biostatistician.

11. AMENDMENTS/MODIFICATIONS OF THIS PROTOCOL

The Investigator will ensure that the study is conducted in accordance with the procedures and evaluations described in this protocol. As the study progresses it may become necessary to change or modify parts of the protocol. The Sponsor or designee is responsible for submitting protocol amendments to the appropriate government regulatory authorities. The Investigator is responsible for submitting protocol amendments to the appropriate IRB. Approval by the IRB must be obtained before changes are implemented.

When an emergency occurs that requires a departure from the protocol for an individual, a departure will be only for that participant. The Investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact the Medical Monitor immediately by telephone. Such contacts will be made as soon as possible to permit a decision as to whether or not the participant (for whom the departure from protocol was affected) is to continue in the study. The eCRF and source documents will completely describe the departure from the protocol and state the reasons for such departure. In addition, the IRB will be notified in writing of such departure from protocol.

12. ETHICAL, LEGAL AND ADMINISTRATIVE CONSIDERATIONS

12.1 Regulatory Documentation

Before the trial starts, Essential Documents as defined in ICH E6 will be generated and placed in both the Investigator's and Sponsor's files. Additional Essential Documents will be added to both files as new information becomes available and at the completion or termination of the trial as defined in ICH E6.

12.2 Protection of Human Participants

12.2.1 Declaration of Helsinki

The Investigator will conduct this study in accordance with the Declaration of Helsinki (1964) including all amendments up to and including the October 2013 revision.

12.2.2 Good Clinical Practice and Regulatory Compliance

The Investigator will conduct this study in accordance with the principles of GCP (current ICH guidelines) and the requirements of all local regulatory authorities regarding the conduct of clinical trials and the protection of human participants.

The study will be conducted as described in the approved protocol, with amendments and in accordance with the obligations of clinical Investigators set forth in the Form FDA 1572 and in 21 CFR 50, 54, 56 and 312.

12.2.3 Independent Ethics Committee / Institutional Review Board

The Investigator is responsible for the submission of the protocol, ICF, and other written materials (such as advertisements and diaries), along with relevant supporting data (e.g., IB), to the appropriate IEC/IRB for review and approval before the study can be initiated. The Investigator is also responsible for submitting amendments to the protocol and ICF to the IEC/IRB for review and approval prior to implementation of the change. The Investigator is responsible for providing the Sponsor with a letter documenting the IEC/IRB approval prior to initiation of the study or implementation of the changes, respectively.

The Investigator will not have authority to implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB of an amendment, except where necessary to eliminate an immediate hazard to study participants. Any significant deviation from the approved protocol will be documented in the source documents and eCRF.

Any deviation or change to the protocol required to eliminate an immediate hazard prior to obtaining IEC/IRB approval/favorable opinion, will be submitted as soon as possible to:

- IEC/IRB for review and approval/favorable opinion.
- The Sponsor via appropriate designees.
- Regulatory Authorities, if required by local regulations.

Documentation of IEC/IRB approval signed by the chairperson or designee of the IRB will be provided to the Sponsor via appropriate designees.

If an Amendment substantially alters the study design or increases the potential risk to the participant: (1) the ICF will be revised and submitted to the IEC/IRB for review and approval/favorable opinion; (2) the revised ICF will be used to obtain consent from participants currently enrolled in the study if they are affected by the Amendment; and (3) the new ICF will be used to obtain consent from any new participants prior to enrollment.

The Investigator is responsible for informing the IRB of all reportable AEs. IND Safety Reports provided by the Sponsor to the Investigator will be promptly forwarded to the IEC/IRB by the Investigator. Updates to the IB provided by the Sponsor to the Investigator will be submitted to the IEC/IRB by the Investigator.

The Investigator is also responsible for informing the IEC/IRB of the progress of the study and for obtaining annual IRB renewal. The Investigator must inform the IEC/IRB when the study is completed or terminated. After completion or termination of the study, the Investigator will submit the final clinical study report to the IEC/IRB, prepared by the Sponsor (or Sponsor's delegate). The structure and content of the report will meet that described in Structure and Content of Clinical Study Reports E3 (ICH Harmonized Tripartite Guideline, dated November 30, 1995).

12.2.4 Regulatory Notification

The requirements for the conduct of clinical trials in accordance with the applicable regulations of the Australian Therapeutic Goods Administration (TGA) under the Clinical Trial Notification scheme will be met before commencement of this study.

At the end of the study, the HREC and relevant regulatory authorities (TGA) will be notified by the sponsor (or delegate) according to applicable regulatory and HREC requirements.

12.2.5 Clinical Laboratory Certification and Reference Ranges

Before the initiation of this study, the PI, or nominee, will obtain a copy of the certification form, with certification number and expiration date for all clinical laboratories used in the study. Reference ranges for each clinical laboratory test used in this study will be obtained from the appropriate laboratory that will perform the test for the study

12.2.6 Participant Informed Consent

The Investigator must comply with informed consent regulations (21 CFR Part 50) and relevant state regulations.

The informed consent form (ICF) will be prepared by the Sponsor (or Sponsor delegate). The ICF will clearly describe the nature, scope, and potential risks and benefits of the study, in a language that the participant understands. The ICF will conform to all the requirements for informed consent according to ICH GCP and US FDA guidelines (21 CFR 50) and will include any additional elements required by the Investigator's institution or local regulatory authorities. The ICF will adhere to the ethical principles that have their origin in the Declaration of Helsinki.

Prior to the beginning of the study, the Investigator will obtain the IRB's written approval/favorable opinion of the written ICF. The IRB approved ICF will be given to each prospective participant. The participants will be given adequate time to discuss the study with the Investigator or site staff and to decide whether or not to participate. Each participant who agrees to participate in the trial and who signs the ICF will be given a copy of the signed, dated, and witnessed document. The original signed ICF will be retained by the Investigator in the study files.

The ICF and any other information provided to participants will be revised whenever important new information becomes available that is relevant to the participant 's consent, and the Investigator will obtain the IRB's written approval/favorable opinion prior to the use of the revised documents. The Investigator, or a person designated by the Investigator, will fully inform the participant of all pertinent aspects of the study and of any new information relevant to the participant 's willingness to continue participation in the study. Participants will read and sign any and all revised ICFs.

12.3 Pandemic Preparedness

Clinical sites should have in place procedures and strategies to accommodate the current COVID-19 pandemic (or other pandemics/epidemics/outbreaks as appropriate). Such procedures should include requirements in relation to criteria such as:

- Attendance (e.g. who is permitted to be on site during a pandemic, limitations, records of attendance, plans for suppliers and deliveries);
- Physical layout (e.g. physical distancing requirements and signage);
- Flexibility (e.g. procedures for scaling, ability to respond to outbreak);
- Support for remote interactions with Sponsors and study teams (e.g. communication including infrastructure);
- The local environment (e.g. contact with local health authorities, ability to access up-to-date pandemic information);
- Any other site-specific relevant criteria.

COVID-19 testing will be done for any participants who are clinically indicated as per the Investigators discretion and/or as directed by Local Health Authorities.

12.4 Participant Confidentiality

All information obtained during the conduct of the study with respect to the volunteers' state of health will be regarded as confidential. This is detailed in the ICF provided to the participant. An agreement for the use or disclosure of any such Personal health information (PHI) will be obtained from the participant in writing (e.g. HIPAA authorization or country-specific guidelines as applicable) prior to performing any study-related procedures. Disclosure of participant medical information obtained as a result of this study to third parties other than those noted below is prohibited.

Medical information resulting from a participant's involvement in this study may be given to the participant's personal physician or to the appropriate medical personnel responsible for the participant's welfare. Data generated as a result of this study are to be available for inspection on request by FDA or other government regulatory agency auditors, the Sponsor (or designee), and the IRB.

The information developed in this clinical study will be used by the Sponsor in the clinical development of the Study treatment and therefore may be disclosed by the Sponsor as required for disclosure as a public company to other clinical investigators, to other pharmaceutical companies, to the FDA, and to other government agencies. All reports and communications relating to volunteers in this study will identify each participant only by their initials and participant number.

12.5 Entering Data into EDC

All data required by the study protocol will be recorded in the electronic database provided by the EDC vendor. Data from the source documents will be entered into the EDC system by authorized Investigational Site personnel. The data will be updated at the time of each participant visit. Results of tests performed outside the Investigational Site will be entered as soon as available to the Investigational Site. The Principal Investigator must verify that all data entries are accurate and correct by electronically signing the participant's investigator signature screen.

12.6 Source Documentation

All data entered in the eCRF must be verifiable against source documentation. Source documents may include, but are not limited to, a participant's medical record, hospital charts, clinic charts, the Principal Investigator's study files, as well as the results of diagnostic tests.

12.7 Retention of Records

The Investigator has the responsibility of maintaining a comprehensive and centralized filing system containing all study-related documentation. These files must be available for inspection by the Sponsor or designee, the IRB, and regulatory authorities (i.e., FDA or international regulatory authorities) at any time and should consist of the Essential Documents as defined in ICH E6, which include, but are not limited to, the following elements:

- Participant files, containing the completed eCRFs, supporting source documentation from the medical record, including laboratory data, and the signed ICF;
- Regulatory files, containing the protocol with all amendments and Sponsor and Investigator signature pages, copies of all other regulatory documentation, and all correspondence between the site and the IRB and Sponsor; and
- Drug accountability files, including a complete account of the receipt and disposition of the Study treatment (active and placebo).

The Investigator will retain all study records for at least 2 years after the last approval of a marketing application in an ICH region (i.e., United States, Europe, or Japan), and until there are no pending or contemplated marketing applications in an ICH region. If no application is filed or if the application is not approved for such indication, the Investigator will retain all study records for at least 2 years after the investigation is discontinued and regulatory authorities have been notified. The Sponsor will provide written notification when it is appropriate for the Investigator to discard the study-specific documents referenced above.

The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, or the study Sponsor, standards/procedures; otherwise, the retention period will default to 15 years.

The Investigator will notify the Sponsor prior to destroying any study records. Should the Investigator wish to assign the study records to another party or move them to another location, the Sponsor will be notified in writing in advance.

If the Investigator cannot guarantee this archiving requirement at the Investigational Site for any or all of the documents, special arrangements will be made between the Investigator and the Sponsor for storage. If source documents are required for continued care of the participant, appropriate copies for storage off site will be made.

12.8 Clinical Study Report

After completion or termination of the study, a clinical study report will be prepared. The structure and content of the report will meet that described in Structure and Content of Clinical Study Reports E3 (ICH Harmonized Tripartite Guideline, dated November 30, 1995).

The Principal Investigator and the Sponsor's representative must verify that all information and data in the clinical study report is accurate and correct by signing the clinical study report.

13. STUDY ADMINISTRATION

13.1 Study Monitoring

This study will be monitored by the Sponsor or designee to evaluate the progress of the study, to verify the accuracy and completeness of the eCRFs, to assure that all protocol requirements, applicable laws and/or regulations, and Investigator's obligations are being fulfilled, and to resolve any inconsistencies in the study records.

The Investigator will allow the Study Monitor to periodically review, at mutually convenient times during the study and after the study has been completed, all eCRFs and office, hospital, and laboratory records supporting the participation of each participant in the study.

The Study Monitor will compare the eCRF data against source documentation in order to verify its accuracy and completeness. The Investigator and Investigational Site staff will collaborate with the Study Monitor to resolve any identified data discrepancies in a timely manner.

The Study Monitor will record any protocol deviations identified, including, but not limited to, volunteers that were enrolled even though they did not meet all eligibility criteria, volunteers who took concomitant medications specifically prohibited by the protocol, participants who received the wrong study treatment or incorrect dose, and participants who failed to comply with the protocol-defined dietary restrictions. The Investigator and Investigational Site staff will collaborate with the Study Monitor to identify the reason for each protocol deviation.

The Study Monitor will compare the Investigational Site study treatment accountability record against the study treatment inventory (unused and used) at the site. The Investigator and

Investigational Site staff will collaborate with the Study Monitor to resolve any identified discrepancies in a timely manner.

Each issue identified during study monitoring visits will be documented and reported to both the Sponsor and the Investigator.

13.2 On-Site Audits

The TGA, or other regulatory authorities, may request access to all study records for inspection and copying. The Principal Investigator and Investigational Site staff will cooperate with the inspectors and allow access to all source documents supporting the eCRFs and other study-related documents. The Investigator will immediately notify the Sponsor when contacted by any regulatory authority for the purpose of conducting an inspection.

The Sponsor or designee may also request to visit the Investigator's site to conduct an audit of the study. Prior to initiating this audit, the Investigator will be contacted by the Sponsor to arrange a convenient time for this visit. The Principal Investigator and Investigational Site staff will cooperate with the auditors and allow access to all source documents supporting the eCRFs and other study-related documents.

13.3 Data Quality Assurance

All eCRFs must be completed by authorized Investigational Site personnel who have undergone eCRF training. Data will be entered into the eCRF as information becomes available on a visit-by-visit basis. All data recorded on the eCRFs must be supported by source documentation. The Principal Investigator must verify that all data entries in the eCRF are accurate and correct by electronically signing and dating the eCRF.

All eCRF corrections must be made by the Principal Investigator or authorized Investigational Site personnel. The Principal Investigator must authorize changes to the recorded data, and this authorization must be documented in the source documents.

Refer to Section 10 for further details regarding Data Management quality assurance, including query generation and resolution, final data review, and database lock.

13.4 Publication Policy

All information and data obtained in the course of the study are the property of the Sponsor and are considered confidential. To avoid disclosures that could jeopardize proprietary rights, the institution and/or the Investigator agree to certain restrictions on publications (e.g., abstracts, speeches, posters, manuscripts, and electronic communications), as detailed in the clinical trial agreement.

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, HIPAA or equivalent country-specific laws.

This trial will be registered in a publicly accessible database (e.g. clinicaltrials.gov or country-specific registries) not later than 21 days after enrollment of the first participant. Results of this trial, including negative and inconclusive, as well as positive results, will be made publicly available.

13.5 Disclosure and Confidentiality

The information in this document is confidential and is not to be disclosed without the written consent of the Sponsor except to the extent that disclosure would be required by law and for the purpose of evaluating and/or conducting a clinical study for the Sponsor. You are allowed to disclose the contents of this document only to your IEC/IRB and study personnel directly involved with conducting this protocol. Persons to whom the information is disclosed must be informed that the information is confidential and proprietary to the Sponsor and that it may not be further disclosed to third parties

14. REFERENCES

1. https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (Accessed on 11 June 2020)
2. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf (Accessed on 11 June 2020)
3. Walia K, Madhumathi J, Veeraraghavan B, Chakrabarti A, Kapil A, Ray P, Singh H, Sistla S, Ohri V C. Establishing Antimicrobial Resistance Surveillance & Research Network in India: Journey so far. Indian J Med Res 2019;149:164-79
4. BWC0977 Investigator's Brochure *(ver2.0 dt 22 Feb 2021)*
5. FDA Guidance for Industry. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. September 2007

TITLE PAGE

Clinical Study Protocol: C002-2023-01

Study Title:	A randomized, double-blind, placebo-controlled, Phase 1 study of the safety, tolerability, and pharmacokinetics of single and multiple ascending doses of BWC0977 in healthy adult volunteers
Protocol Number:	C002-2023-01
Study Phase:	I
Principal Investigator:	Dr Nicholas Farinola
Sponsor:	Bugworks™ Research Inc. 2711, Centerville Road, Suite 400 Wilmington, Delaware 19808 USA Bugworks Research Australia Pty. Ltd (Subsidiary of Bugworks Research Inc., USA) GPO Box 939, Adelaide, SA 5001 Australia
Name of Sponsor Signatory:	Dr. V. Balasubramanian, PhD
Protocol Version:	5.0
Date of Version:	15 Nov 2023

SPONSOR PROTOCOL APPROVAL SIGNATURE PAGE

The undersigned has reviewed and approved Protocol No. C002-2023-01 for issuance:

Name	Designation	Signature & Date
Dr. V. Balasubramanian, PhD	Chief Operations Officer	

INVESTIGATOR SIGNATURE PAGE

I have read the attached protocol and agree that it contains all the necessary details for performing the study.

I will provide copies of the protocol and of the Investigator's Brochure (IB), which was furnished to me by the Sponsor, to all members of the study team responsible to me who participate in the study. I will discuss this material with them to assure that they are fully informed regarding the study treatment, including the potential risks and side effects, and the conduct of the study.

Once the protocol has been approved by the Institutional Review Board (IRB)/ Independent Ethics Committee (IEC), I will not modify this protocol without obtaining the prior approval of the Sponsor and of the IRB/IEC. I will submit the protocol modifications and/or any informed consent form (ICF) modifications to the Sponsor and the IRB/IEC, and approval will be obtained before any modifications are implemented.

I understand the protocol and will work according to it, the principles of Good Clinical Practice [GCP; current International Council for Harmonization (ICH) guidelines], and the Declaration of Helsinki (1964) including all amendments up to and including the October 2013 revision.

Name of Site Principal Investigator	Signature	Date

Institution Name:
CMAX Clinical Research Pty Ltd
Level 5, 21-24 North Terrace
Adelaide, SA, 5000
Australia

DOCUMENT HISTORY

Document	Version Date	Summary of Changes	Rationale
Protocol V1.0	05Jul2023	N/A	N/A
Protocol V2.0	26Jul2023	Showering time during Holter monitoring on Day 1 and last day of infusion.	Clarity is provided further as to when Holter monitoring could be paused
		Addition of infusion site reaction evaluation criteria under section 8.4.6. and 15.2	Included as part of safety monitoring to evaluate the infusion site reaction
		Added sequence of cohort recruitment in MAD and SAD for better understanding.	Provided the sequence of cohort recruitment for clarity
		Holder readings for pre-dose evaluation are revised from -45, -30 and -15 to pre-dose 1, 2 and 3.	To enable baseline evaluation during Holter monitoring
		Revised the study rationale to describe the sequence of cohort enrolment.	Provided further clarity in the study rationale
		Revised the Table 1 footnotes to include objective of initiating the MAD with 240 mg.	Provided further clarity in the starting dose rationale
		Replaced the term "infusion reaction assessment" to "infusion site reaction assessment."	For better clarity
		Minor administrative changes including version number, date, and addition of document history	Administrative changes
Protocol V3.0	09Aug2023	Updated safety officer(s) details i.e., removed Neha Madan and added Alexander Lenov.	Administrative change
		Updated concomitant medications information in section 6.7.	To provide better understanding of concomitants medications during the trial
		Removed showering during Holter monitoring on Day 1 and last day of infusion.	To avoid the interruption during the Holter Monitoring

Protocol V4.0	19Sep2023	Updated Partial Thromboplastin Time (PTT) to Activated Partial Thromboplastin Time (aPTT)	For better understanding of safety profile
		Updated VIP Scoring Assessment Timepoints from 15 mins, 30 mins, 1 hour and 2 hours to 15 minutes, 2-hours (i.e., at end of infusion) and 4 hours post start of administration.	Clarity is provided for the assessment of Infusion Site Reactions (ISR) timepoints
		Appendix II: SAD PK sampling points added	For better understanding of timepoints
		Review and documentation of medications and supplements	Administrative Changes
		-	Administrative Changes
Protocol V5.0	15 Nov2023	Updated concomitant medications in exclusion criteria and section 6.7 with addition of Ibuprofen as premedication.	To provide better understanding of concomitants medications during the trial
		Updated randomization criteria	Administrative Changes
		Updated Urine PK & A4 blood PK collection timepoints	Administrative Changes
		Addition of a window to the ISR assessments	Administrative Changes
		Updated Cohort A1 study duration from over 120 minutes to 30 minutes	Administrative Changes
		Modified the PK, ECGs Holter evaluation timepoints to accommodate the infusion duration of Cohort A1	Administrative Changes
		-	Administrative Changes

PROTOCOL SYNOPSIS

Title	A randomized, double-blind, placebo-controlled, Phase 1 study of the safety, tolerability, and pharmacokinetics of single and multiple ascending doses of BWC0977 in healthy adult volunteers
Clinical Phase	Phase I
Investigational Product (IP)	**Name of the IP:** BWC0977 **Formulation:** Compounded Solution **Mode of administration:** Intravenous Infusion
Comparator	**Placebo:** Compounded solution minus BWC0977
Primary Objective	To assess the safety and tolerability of BWC0977 following single and multiple ascending doses by intravenous (IV) infusion in healthy adult volunteers.
Secondary Objective	To assess the pharmacokinetic (PK) characteristics in plasma and urine of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult volunteers.
Exploratory Objective	To assess the QT/ corrected QT (QTc) prolongation potential of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult volunteers.
Participant Number	This study will enroll approximately 56 healthy adult volunteers: Part A, Multiple Ascending Dose (MAD): Up to 40 healthy volunteers are planned to be enrolled in up to 5 cohorts including an optional cohort. Each cohort will enroll up to 8 participants, with 6 participants randomized to receive BWC0977 and 2 participants randomized to receive placebo. Part B, Single Ascending Dose (SAD): Up to 16 healthy volunteers will be enrolled in a total of 2 cohorts. Each cohort will enroll 8 participants with 6 participants randomized to receive BWC0977 and 2 participants randomized to receive placebo. For each study part, efforts will be made to randomize approximately equal numbers of males and females to either active or placebo.
Sites	Single site (Australia)
Study Treatment	Part A: BWC0977 IV infusion. The dose levels and frequencies are as below: a) A1: 240 mg, BID for 7 days b) A2: 750 mg, BID for 10 days c) A3: 1250 mg, BID for 10 days d) A4: 1000 mg, TID for 10 days e) A5: Optional Cohort, if required Part B: Single BWC0977 IV infusion B1:1500 mg B2: dose to be determined based on safety, tolerability, and PK data from

<table>
<tr><td></td><td>Cohort B1.</td></tr>
<tr><td>Study Duration</td><td>The total maximum duration in Part A (MAD) for Cohort A1 is 42 days inclusive of screening period and 7-day dosing and 45 days for Cohorts A2, A3 and A4 inclusive of screening period and 10-day dosing. Cohort A5 is an optional cohort with duration dependent on dosing regimen selected.

The total maximum duration for Part B (SAD) is 37 days, inclusive of screening windows.</td></tr>
<tr><td>Study Confinement Periods</td><td>For each participant enrolled in Part A, the confinement period will commence on Day -1, with dosing and discharge as below:

A1: Dosing Day 1-7 and discharge on Day 8.
A2, A3 & A4: Dosing Day 1-10 and discharge on Day 11
A5: Optional Cohort, if required.

For each participant enrolled in Part B, the confinement period will commence on Day -1, with dosing on Day 1 and discharge on Day 3.</td></tr>
<tr><td>Study Design</td><td>This is a Phase 1, single-center, randomized, double-blind, placebo-controlled study of single-ascending doses and multiple-ascending doses of BWC0977 administered through IV infusion to healthy adult volunteers. This study is subdivided into 2 parts, Part A (MAD) and Part B (SAD). In each part of the study, cohorts may be added or removed based upon emerging data.

Up to 5 dose levels are planned to be evaluated in Part A Table S1 including an optional cohort, with the starting dose for Part A selected based on nonclinical data and the previous study (C001-2020-01).

In Part A, participants will receive multiple doses of BWC0977 or placebo over 7 and 10 days with cohorts, doses and dosing frequencies presented in the table below (see Table S1).
Table S1: Part A Multiple Ascending Dose Study Design
<table>
<tr><th colspan="7">Part A – MAD (Double-Blinded)</th></tr>
<tr><th rowspan="2">Cohort</th><th rowspan="2">Dose (mg)</th><th rowspan="2">No. of Doses</th><th>Duration</th><th rowspan="2">Dosing Frequency</th><th colspan="2">Number of Subjects</th></tr>
<tr><th>Days</th><th>BWC0977</th><th>Placebo</th></tr>
<tr><td>A1*</td><td>240</td><td>14</td><td>7</td><td>BID</td><td>6</td><td>2</td></tr>
<tr><td>A2</td><td>750</td><td>20</td><td>10</td><td>BID</td><td>6</td><td>2</td></tr>
<tr><td>A3*</td><td>1250</td><td>20</td><td>10</td><td>BID</td><td>6</td><td>2</td></tr>
<tr><td>A4*</td><td>1000</td><td>30</td><td>10</td><td>TID</td><td>6</td><td>2</td></tr>
<tr><td>A5</td><td>TBD</td><td>TBD</td><td>TBD</td><td>TBD</td><td>6</td><td>2</td></tr>
<tr><td colspan="5">Total (Active + Placebo)</td><td>30</td><td>10</td></tr>
<tr><td colspan="5">Total Number of Subjects</td><td colspan="2">40</td></tr>
<tr><td colspan="7">Note: *Holter monitoring only for these cohorts.
Every effort will be made to ensure that at a minimum 2 male and 2 female participants will be enrolled into each cohort.</td></tr>
</table></td></tr>
</table>

Doses will be administered via IV infusion over 30 (±5) minutes (A1) and120 (±10) minutes (A2-A4). Dosing in each cohort is planned to start with two sentinel participants with one of the two sentinels randomized to receive BWC0977 and the other randomized to receive placebo.

The decision to escalate between dose levels will be based upon review of blinded clinical and laboratory safety data from at least 6 out of 8 participants from the previous cohort through to and including the final follow-up visit and any available blinded PK data by the Safety Monitoring Group (SMG). The SMG will review the data, discuss the findings, and recommend proceeding to the next cohort at the protocol-defined dose level, at another dose level (lower or higher) or to terminate enrolment in Part A. The SMG may also recommend use of an alternative dosing schedule for the next cohort in Part A, and/or determine whether use of sentinel dosing at the next dose level in Part A is warranted.

In Part B healthy volunteers will be randomized to receive a single IV infusion dose of BWC0977 or placebo, to be infused over 120 (±10) minutes.

In the first cohort of this SAD, the dose level to be evaluated in Cohort B1 will be dose at 1500 mg. Cohort B1 will be initiated after the completion of Cohort A2 of MAD. Cohort B2 dose will be based on PK, safety, and tolerability of B1 (Table S2).

Cohort B2 is included as a potentially supra-therapeutic dose. This is to ensure that both sub-therapeutic and supra-therapeutic doses are evaluated for QTc and as an optional cohort.

Table S2: Part B Single Ascending Dose Study Design

Part B - SAD (Double-Blinded)						
Cohort	**Dose (mg)**	**No. of Doses**	**Duration**	**Dosing Frequency**	**Number of Subjects**	
			Days		**BWC0977**	**Placebo**
B1	1500	1	1	1	6	2
B2*	TBD (Optional Cohort)				6	2
Total (Active + Placebo)					12	4
Total Number of Subjects					**16**	

Note: **Optional cohort to be utilized only if needed.*
Every effort will be made to ensure that at a minimum 2 male and 2 female participants will be enrolled into each cohort.

Dosing in each cohort will start with two sentinel participants with one of the two sentinels randomized to receive BWC0977 and the other randomized to receive placebo.

The decision to escalate between dose levels will be based upon review of blinded clinical and laboratory safety data from at least 5 out of 8 participants from the previous cohort through to and including the final follow-up assessment on Day 8±1 and any available blinded PK data by the SMG. The

	SMG will review the data, discuss the findings, and recommend proceeding to the next cohort at another dose level (lower or higher) or to terminate enrolment in Part B. The sequence of cohort enrollment in MAD & SAD is A1/A2/B1/A3/A4/A5 (Optional)/B2 (Optional).
Study Assessments	**Safety and Tolerability:** **Clinical:** • Medical history and concomitant medication use • Evaluation of adverse events (AEs) and treatment-emergent AEs (TEAEs) • Height and Weight • Physical examination • Vital signs • 12-lead electrocardiograms (ECGs) • Holter recording (All SAD cohorts and MAD cohorts 1, 3, 4 and 5). **Laboratory Safety:** • Haematology: Platelet Count, red blood cell (RBC) count, white blood cell (WBC) count (absolute) with differential, reticulocyte count, Hemoglobin, Hematocrit, RBC indices. • Coagulation: Prothrombin time (sec and INR), activated partial thromboplastin time (aPTT). • Clinical chemistry: creatinine, glucose, sodium, urea, phosphate, creatine phosphokinase (CPK), potassium, chloride, bicarbonate, calcium, aspartate aminotransferase (AST) (SGOT), alanine aminotransferase (ALT) (SGPT), gamma glutamyltransferase (GGT), alkaline phosphatase, lactate dehydrogenase, total and direct bilirubin, uric acid, albumin, total protein, lipid panel, calculated creatinine clearance using Cockcroft-Gault formula), albumin:creatinine ratio. • Urinalysis: pH, specific gravity, protein, blood, glucose, ketones, urobilinogen. Urine microscopic examination/sediment microscopy will be conducted in the instance of abnormal clinically significant urinalysis findings. **Infection Screening:** • HIV (HIV1, HIV2) • Hepatitis B virus surface antigen (HbsAg) • Hepatitis C (anti HCV antibody) **Pregnancy and Follicle Stimulating Hormone testing:** • Serum and urine human chorionic gonadotropin pregnancy testing will be performed. • Postmenopausal females will be tested for follicle stimulating hormone

	(FSH) levels. **Drugs of Abuse, Cotinine and Alcohol:** • Drugs of abuse urine screen will include: methamphetamine, amphetamines, barbiturates, cocaine, opiates, cannabinoids, phencyclindine, methadone, tricyclic antidepressants, and benzodiazepines. • Urine cotinine and alcohol breath testing will also be performed. **Pharmacokinetics:** Blood and urine samples for PK will be collected prior to dosing and at several timepoints post-dose.
Study Endpoints	**Primary Endpoints:** The safety and tolerability of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult participants will be evaluated based on the following endpoints: • Incidence of TEAEs and serious adverse events (SAEs) overall and by intensity. • Changes from baseline in: o Safety laboratory test results o ECG parameters o Vital signs measurements o Physical examination findings **Secondary Endpoints:** **Following repeat dose:** • Area under the concentration-time profile from time zero to time tau (τ), the dosing interval ($AUC_{(0-\tau)}$), maximum observed concentration (C_{max}), time to maximum concentration (T_{max}), concentration at the end of the dosing interval ($C\tau$), observed accumulation ratio (Ro), volume of distribution at steady state (Vdss) and systemic clearance (CL) of BWC0977, as data permit. • Amount excreted in urine (Ae) of unchanged BWC0977, fraction of the dose excreted in urine (fe) and renal clearance (CLr), as data permit. **Following single dose:** • Area under the curve $(AUC)_{(0-8)}$, $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, C_{max}, T_{max}, CL, Vdss, mean residence time (MRT), and terminal phase half-life ($t_{1/2}$) of BWC0977, as data permit. • Amount excreted in urine (Ae) of unchanged BWC0977, fraction of the dose excreted in urine (fe) and renal clearance (CLr), as data permit.

	Exploratory Endpoints: Cardiodynamic evaluation using 12-lead ECGs extracted from Holter recordings may be undertaken based on observed PK and other project considerations. In such case, the following endpoints will be used: • Change-from-baseline heart rate (HR), PR, QRS and QT duration corrected for heart rate by Fridericia's formula (QTcF interval) (ΔHR, ΔPR, ΔQRS and ΔQTcF). • Placebo-corrected ΔHR, ΔPR, ΔQTcF and ΔQRS (ΔΔHR, ΔΔQTcF, ΔΔPR and ΔΔQRS). • Categorical outliers for HR, PR, QRS and QTcF. • Frequency of treatment emergent T- and U-wave abnormalities.
Subject Selection Criteria	**Inclusion Criteria:** Each subject must meet all the following criteria to be eligible for study participation: 1. Healthy male or female 18 to 55 years of age, inclusive, at time of consent. 2. Body mass index (BMI) ≥ 19.0 and ≤ 30.0 (kg/m^2) and weight between 55.0 and 100.0 kg (inclusive). 3. Medically healthy without clinically significant abnormalities at the screening visit, Day -1 or Day 1, including: a) No findings in Physical examination or vital signs (including temperature, HR, respiratory rate, and blood pressure) that the Investigator determines would interfere with interpretation of study results. b) Electrocardiograms (ECGs) without clinically significant abnormalities, including a QTcF interval duration ≤450 msec (for males), and ≤470 msec (for females) obtained as an average from the triplicate screening ECGs after at least 5 minutes in a supine quiet-rest position. c) Clinically significant abnormalities in the screening clinical laboratory tests, as determined by the Investigator. Repeat testing could be performed at the Investigator's discretion. 4. Willing and able to provide written informed consent. 5. Agrees to be available for all study visits and cooperate fully with the requirements of the study protocol, including the schedule of events. 6. Willing to refrain from strenuous physical activity that could cause muscle aches or injury, including contact sports, at any time from 4 days prior to admission in the clinical research unit (CRU) until completion of the study (follow-up [FU] visit).

	7. Willing to refrain from prescription medications from Screening visit until follow-up; and over-the-counter (OTC) medications, vitamin preparations and other food supplements, from Day -1 up to follow-up. 8. Have suitable venous access for drug administration and blood sampling. 9. If female of child-bearing potential, must agree to and comply with: a) Using 1 barrier method (e.g., female condom or male partner using a condom) plus 1 other highly effective method of birth control (e.g., oral contraceptive, implant, injectable, indwelling intrauterine device, vasectomized partner), or double-barrier method (use of a condom by the male partner with use of a diaphragm by the female partner), from signing the consent form until 30 days after last study drug administration, or b) Sexual abstinence, for the duration of the study (from signing of consent to FU visit) and for 30 days after last study drug administration, plus c) Females of child-bearing potential must also agree not to donate ova or oocytes (i.e., human eggs) during the study, and for one menstrual cycle after completion of the study. d) To be considered of non-childbearing potential, a female must have either a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy (at least 6 weeks prior to screening), or menopause (last menstruation >12 months and FSH in menopausal range); provision of written documentation is not required for female sterilization and oral confirmation is adequate. e) Female participants in same sex relationships do not need to utilize contraception. 10. Male volunteers, if sexually active with a female partner, must agree to and comply with using 1 barrier method of birth control (e.g., male condom) plus 1 other highly effective method of birth control in their partner (e.g., oral contraceptive; implant, injectable, indwelling intrauterine device), or double-barrier method (use of a condom by the male partner with use of a diaphragm by the female partner, or sexual abstinence, and must not donate sperm, for the duration of the study (from signing of consent) and for 90 days after last study drug administration. To be considered surgically sterile, male participants must have had a vasectomy at least 3 months prior to screening with appropriate documentation of the absence of sperm in the ejaculate. Male participants in same sex relationships do not need to utilize contraception.

Exclusion Criteria:

Volunteers who meet any of the following criteria will be excluded from the study:

1. Women who are pregnant and/or lactating.
2. History or presence of significant cardiovascular (including QT prolongation, clinically significant hypokalemia, or other proarrhythmic conditions), pulmonary, hepatic, renal, hematological, gastrointestinal, endocrine (including glucose intolerance, diabetes mellitus), immunologic (including asthma or seasonal allergies [that require intermittent use of steroids or other medication]), musculoskeletal (including tendinopathy), dermatologic, or neurological disease (including seizure disorders, psychiatric disorders), including any acute illness or surgery within the past 3 months, as determined by the Investigator to be clinically relevant.
3. A serum creatinine value on Day -1 (check-in) that increased by more than 0.2 mg/Dl (or 15.25 µmol/L) from the Screening value. *Note:* the serum creatinine test may be repeated prior to confirming exclusion.
4. History of photosensitivity to quinolones.
5. History of known or suspected *Clostridium difficile* infection.
6. Any condition that necessitated hospitalization within the 3 months prior to Day -1 or is likely to require so during the study.
7. Positive test for HbsAg, anti-HCV antibodies, or antibodies to HIV-1, HIV-2 at screening.
8. Exposure to any prescription medications (small molecules, biologics, and vaccines, including influenza and/or COVID-19 vaccines) or, systemically administered OTC drugs, dietary supplements, or herbal remedies, within 14 days or 5 half-lives (if known), whichever is longer, prior to Day 1 (first dose). Participants should not receive any vaccinations (including influenza and/or COVID-19 vaccines) until after study completion. Discussion between the PI and the Sponsor Medical Monitor is encouraged regarding prior use of any medications during the pre-dose period.

Note: An exception is made for hormonal contraceptives, paracetamol (a maximum of 4 doses per day of 500-mg, and no more than 3 g per week) for the treatment of headache or any other pain, and ibuprofen as premedication for thrombophlebitis as per PI's judgement.

9. Documented hypersensitivity reaction or anaphylaxis to any medication.
10. Smoker (including tobacco, e-cigarettes, or marijuana) or nicotine user within 1 month prior to dosing and have a negative test for cotinine at

	check in on Day -1 (*may be repeated once, at the discretion of the Investigator, in the instance of a positive result*). 11. Positive urine drug/alcohol testing at screening or check-in (Day -1), or history of substance abuse or alcohol abuse (defined as greater than 2 standard drinks on average each and every day, where one standard drink is defined as containing 10 g of alcohol and is equivalent to 1 can or stubby of mid-strength beer, 30 ml nip spirits, or 100 ml wine) within the previous 5 years *(may be repeated once per timepoint, at the discretion of the Investigator, in the instance of a positive result).* 12. Donation of blood or plasma within 30 days prior to randomization, or loss of whole blood of more than 500mL within 30 days prior to randomization, or receipt of a blood transfusion within 1 year of study enrollment. 13. Previous participation in this study or previous participation in another study within 5 half-lives (if known) of the agent, or 30 days, whichever is longer, of Day 1. *Note:* prior participation at any time in non-invasive methodology trials in which no drugs were given is acceptable. 14. Consumption of red wine, Seville oranges, grapefruit, or grapefruit juice, pummelos, other citrus fruits, grapefruit hybrids or fruit juices containing such products from 7 days prior to the first dose of study medication. 15. Employee or family member of an employee of the Sponsor, clinical research unit, or clinical research organization at which the study will be conducted. 16. Unable to cooperate fully with the requirements of the study protocol, including the schedule of events, or likely to be non-compliant with any study requirements. 17. Any disease or condition (medical or surgical) that, by the determination of the Investigator, precludes the subject's participation in the study or would place the subject at risk as a result of participation in the study. *Note:* Volunteers should refrain from consumption of any foods containing poppy seeds within 48 hours (2 days) prior to screening and prior to Day -1 to avoid false positive drug screen results
Statistical Methods	Statistical analyses will be performed as detailed in the formal statistical analysis plan (SAP) for this clinical study. The SAP will override the analysis methodology provided here. A SAP will be prepared and finalized before database lock and analysis of data. Any deviations from the final SAP will be described and justified in the clinial

	study report. All statistical analyses will be performed using SAS® (SAS Institute Inc. Cary NC USA). **Safety Parameters:** All laboratory results, vital signs measurements, and safety ECG results will be summarized using appropriate descriptive statistics. The incidence of all AEs and TEAEs will be described by MedDRA ® preferred term and system organ class and by other relevant AE description parameters (relationship to study drug, severity, led to withdrawal from the study, was an SAE). **PK Parameters:** Single and multiple-dose PK parameters will be derived from the plasma concentration time and urinary excretion data. A non-compartmental PK method will be used to analyze the plasma and urine concentrations of BWC0977. Descriptive statistics and graphs will be performed for plasma and urine concentrations and PK parameters. For the MAD parts, the dose-proportionality of PK plasma parameters C_{max}, $AUC_{0-\tau}$ and AUC_{0-inf} will be evaluated using the Power model. For the MAD part, accumulation ratio, time invariance, and the steady-state assessment of plasma concentration will be also studied using a one-way analysis of variance (ANOVA) after logarithmic transformation of plasma concentrations. **Cardiodynamic Evaluation:** The primary analysis will be based on concentration-QTc modeling of the relationship between the plasma concentrations of BWC0977 and ΔQTcF with the intent to exclude an effect of ΔΔQTcF > 10 ms at clinically relevant plasma concentrations. The effect of BWC0977 on the ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS will also be evaluated at each post-dosing time point ('by-time point' analysis). In addition, an analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology and U-wave presence.

TABLE OF CONTENTS

List of In-Text Tables:

List of In-Text Figures:

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AchE	Acetylcholine esterase
Ae	Urinary recovery of unchanged drug
Ae(0-x)	Urinary recovery of unchanged drug up to fixed nominal time-point x
Ae(0-t)	Complete urinary recovery of unchanged drug up to time of last measurable urinary concentration
Ae(0- τ)	Amount excreted in urine over a dosing interval
AE	Adverse Event
ALT	Alanine aminotransferase (SGPT)
AMR	Antimicrobial resistance
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase (SGOT)
AUC	Area under concentration-time curve
AUC(0-inf)	Area under the concentration-time curve from time zero (pre-dose) extrapolated to infinite time
AUC(0-x)	Area under the concentration-time curve from zero (pre-dose) to some fixed nominal time x
AUC(0-t)	Area under the concentration-time curve from time zero (pre-dose) to last time of quantifiable concentration within a subject across all treatments
AUC(0-τ)	Area under the concentration-time curve over the dosing interval
$AUMC_{inf}$	Area under the moment curve from time 0 extrapolated to infinity
β-HCG	Beta-human chorionic gonadotropin
BMI	Body mass index
BP	Blood pressure
CDC	Center for Disease Control
CI	Confidence Interval
CL	Systemic clearance of parent drug
CLr	Renal clearance
Clr_{ss}	Renal clearance (at steady state)
C_{av}	Average concentration
C_{max}	Maximum observed concentration
C_{min}	Minimum observed concentration
CrCl	Creatinine clearance
Cτ	Pre-dose (trough) concentration at the end of the dosing interval
CPK	Creatine phosphokinase
CRF	Case Report Form
CRO	Contract research organizations
CRU	Clinical research unit
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
Δ	Change from baseline
ΔΔ	Placebo-corrected change from baseline
ECG	Electrocardiogram

eCRF	Electronic Case Record Form/Case Report Form
EDC	Electronic data capture
EOS	End of Study
Fe	Fraction of the dose excreted in the urine
FDA	Food and Drug Administration
FIH	First in human
FSH	Follicle Stimulating Hormone
FU	Follow-up
GCP	Good Clinical Practice
GGT	Gamma glutamyltransferase
GLP	Good Laboratory Practice
HbsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HR	Heart rate
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IND	Investigational New Drug
INR	International normalized ratio
IB	Investigator's Brochure
IEC	Independent Ethics Committee
IP	Investigational Product
IRB	Institutional Review Board
IV	Intravenous
IVRS/IWRS	Interactive Voice Response System and Interactive Web Response System
k_{el}	Terminal phase rate constant
LFTs	Liver function tests
MAD	Multiple ascending dose
MATE	Multidrug and toxin extrusion
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MRSA	methicillin-resistant *Staphylococcus aureus*
MRT	Mean residence time
NBTI	Novel Bacterial Type II Topoisomerase Inhibitors
NOAEL	No observed adverse effect level
OAT3	Organic anion transporter 3
OTC	Over-the-counter
PK	Pharmacokinetic
PI	Principal Investigator
QTcF	QT duration corrected for heart rate by Fridericia's formula
RBC	Red blood cells
Ro	Observed accumulation ratio
SAD	Single ascending dose

SAE	Serious adverse event(s)
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SGOT	Serum glutamic-oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SMG	Safety Monitoring Group
SoA	Schedule of Activities
SOP	Standard Operating Procedure
SBP	Systolic blood pressure
t	Time of last observed quantifiable concentration
t½	Terminal phase half-life
τ	Dosing interval
TEAE	Treatment-emergent adverse event
TGA	Australian Therapeutic Goods Administration
t_{max}	Time of occurrence of C_{max}
ULN	Upper limit of normal
Vdss	Volume of distribution at steady state of parent drug after intravascular (e.g., IV) administration
VIP	Visual Infusion Phlebitis score
VRE	Vancomycin-resistant *Enterococcus*
WBC	White blood cells
WHO	World Health Organisation

1. INTRODUCTION

1.1 Background

Antimicrobial Resistance (AMR) is an increasingly serious threat to global public health that requires immediate action across all sectors and society. Without effective antibiotics, a key element in the armamentarium of modern clinical practice would be compromised. The cost of health care for patients with resistant infections is higher than care for patients with non-resistant infections due to longer duration of illness, additional tests, use of more expensive drugs and longer stay in hospitals. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases, resulting in prolonged illness, disability, and death. World Health Organization (WHO) published its first ever list of antibiotic-resistant "priority pathogens" in 2017, consisting of 12 families of bacteria that pose the greatest threat to human health. The most critical group includes multidrug resistant bacteria that pose serious threat in hospitals, nursing homes, and among critical care patients who are on devices such as ventilators and blood catheters. They include *Acinetobacter baumannii, Pseudomonas aeruginosa* and various Enterobacteriaceae (including *Klebsiella* sp., *E. coli*, *Serratia marcesens*, and *Proteus* spp.). They can cause severe and often deadly infections such as bloodstream infections and pneumonia. These bacteria have become resistant to a large number of antibiotics, including carbapenems and third generation cephalosporins – currently the best available antibiotics for treating multi-drug resistant bacteria (1).

In 2019, the Center for Disease Control (CDC) published a comprehensive analysis outlining the top 18 antibiotic-resistant threats in the U.S. These 21 threats were further classified into urgent, serious, and concerning threats. The urgent threats include carbapenem-resistant *Enterobacteriaceae*, Carbapenem-resistant *Acinetobacter*, *Candida auris (C. auris)*, *Clostridioides difficile* and drug-resistant *Neisseria gonorrhoeae* (2). The serious threats include, multidrug-resistant *Acinetobacter* sp., drug-resistant *Campylobacter* sp., Extended-spectrum Beta-lactamase producing *Enterobacteriaceae*, Vancomycin-resistant *Enterococcus* (VRE), multidrug-resistant *Pseudomonas aeruginosa*, drug-resistant non-typhoidal *Salmonella* sp., drug-resistant *Salmonella typhi*, drug-resistant *Shigella* sp., methicillin-resistant *Staphylococcus aureus* (MRSA), & drug-resistant *Streptococcus pneumoniae*. Lastly the concerning threats include Erythromycin-Resistant Group A *Streptococcus* and Clindamycin-resistant Group B *Streptococcus*. In India, the most common pathogens encountered are *Enterobacter* spp, *Staphylococci*, *Klebsiella* sp., *Acinetobacter* spp, *Pseudomonas aeruginosa*, *Enterococcus* and *E. coli* (3).

Bugworks has discovered a novel bacterial Type IV topoisomerase inhibitor (NBTI), BWC0977, as a promising candidate that has activities against all the critical and high priority pathogens in WHO 2017, as well CDC published urgent and serous pathogen list. BWC0977, is a broad spectrum new chemical entity with dual target inhibition, potent activity against a very broad spectrum of Gram negative (including all the key members of Enterobacteriaceae and non-

fermenters such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) and Gram-positive bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA) and VRE. Unlike the fluoroquinolone class of molecules, BWC0977 is equally active against deoxyribonucleic acid (DNA) gyrase and Topoisomerase IV. Extensive minimum inhibitory concentration studies with clinical isolates, demonstrated the lack of cross-resistance with fluoroquinolones. This is a novel lead that is differentiated from other reported NBTI series including gepotidacin, based on SciFinder and STN searches.

Both DNA gyrase and topoisomerase IV are clinically validated antibacterial targets inhibited by the quinolone family of antibiotics. NBTIs and quinolones bind to the same target proteins at a different binding pocket leading to a different mode of inhibition. They recognize distinctly different amino acids. Therefore, they inhibit different stages of the catalytic cycle of the target proteins.

BWC0977 has demonstrated in vitro and in vivo activity against Gram positive pathogens (including MRSA) and Gram-negative pathogens associated with respiratory tract, skin, and soft tissue infections, including isolates resistant to existing classes of antimicrobials. BWC0977 has been shown to be efficacious in lung, thigh and urinary track bacterial infection model in rats caused by gram negative pathogens like *E.coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. BWC0977 selectively inhibits bacterial DNA replication by interacting in a unique way on the GyrA subunit of bacterial DNA gyrase and the ParC subunit of bacterial topoisomerase IV. This interaction appears to be highly specific to bacterial topoisomerases as evidenced by weak inhibition of human topoisomerase II, supporting the selective activity of BWC0977 against the bacterial target. As a consequence of its novel mode of action, BWC0977 is very potent against isolates carrying resistance determinants to established antibacterials and has shown no target-mediated cross-resistance with agents in current use, including fluoroquinolones.

1.2 Preclinical Safety Assesment of BWC0977

The dose limiting toxicity in the rat and dog studies (general toxicity, safety pharmacology and genetic toxicology) was clinical observations primarily consisting of decreased activity, semi-closed eyes, piloerection, involuntary twitching movements, fast or labored respiration, and mouth rubbing, the incidence and severity of which increased with the dose level. Due to the timing of the observations, they are considered attributable to maximum observed concentration (C_{max}).

Due to the inhibition of acetylcholine esterase (AchE) by BWC0977 it is plausible that peripheral inhibition of AchE could be the cause of the clinical observations noted in vivo. BWC0977 has been shown to not cross the blood brain barrier, therefore these clinical observations are unlikely to be centrally mediated. At the high dose levels the majority of these findings were reversible over the course of 2 hours post-dose. The most sensitive species for these clinical observations was rat with a no observed adverse effect level (NOAEL) determined to be at 75mg/kg/day with

an observed C_{max} of 26 µg/ml and an observed area under concentration-time curve $(AUC)_{0-24}$ of 51.45 µg/ml*hr.

Due to the ability to monitor for these effects, the quick reversibility of the signs at the lower dose levels, and the clinical experience with AchE inhibitors, these effects are not considered to pose a significant risk to man. To mitigate any adverse effects observed related to C_{max}, a longer infusion time or fractionation of the total daily dose will be undertaken. A careful escalation of doses and close safety monitoring of volunteers is however required and will be performed in this study.

Repeat administration ≥80mg/kg/day administration through intravenous (IV) infusion for 120 min of BWC0977 in the 14-day Good Laboratory Practice (GLP) dog study led to increases in serum transaminases, alkaline phosphatase, gamma glutamyltransferase (GGT) and total bilirubin in all animals. This was in concordance with microscopic changes in the gall bladder, consisting of mucinous accumulation, in all animals administered 120mg/kg/day and in one male dog administered 80mg/kg/day.

At the end of the recovery phase, the clinical chemistry parameters had all decreased to near predose values and the microscopic findings in the gall bladder of animals previously administered 120 mg/kg/day were comparable with those of controls, which indicated complete reversibility.

The NOAEL for these changes in the dog was 40mg/kg/day with AUC_{0-24} of 91 h*µg/ml and C_{max} of 25 µg/ml.

The results from the genetic toxicity tests with BWC0977 are directly comparable with existing, marketed quinolone antibacterials.

BWC0977 has been assessed in a battery of nonclinical cardiovascular assays. In vivo, in one guinea pig study and the dog cardiovascular study, slight increases in Corrected QT Interval (QTc) have been observed. BWC0977 also has a hERG IC_{50} of 56µM, however BWC0977 does not inhibit other cardiac ion channels up to 300µM. In the dog cardiovascular study, the increase in QTc was observed at both dose levels assessed (non-statistically significant maximum increase of 4 msec at 30mg/kg; statistically significant maximum increase of 11 msec at 60mg/kg – both maximal increases were between 3-6 hours post start of 1 hour infusion), there was also a notable increase in heart rate at both dose levels. During the study there were clinical observations at both dose levels following the infusion, which were more significant at the higher dose level.

Due to the ability to monitor by cardiovascular changes by electrocardiogram (ECG), the transient nature of the changes in the parameters, and the ability to modulate the C_{max} with the length of infusion, or by fractionating the daily dose, these effects are not considered to pose a significant risk to man.

In vitro tests suggested that BWC0977 may have potential for phototoxicity. To mitigate this risk, participants in clinical studies will be requested to avoid sun exposure till end of follow-up.

The formulation used in the first-in-human (FIH) clinical study C001-2020-01 had been assessed in dog for local vascular irritancy. Within the dog local tolerance study there were BWC0977-related microscopic findings at the infusion sites, the nature and severity of which were predominately related to the number of infusions that occurred at each dose site. At the lower concentration, the changes consisted predominately of relatively minor perivascular and vascular changes after up to 10 dosing occasions. After a single dosing occasion there was perivascular hemorrhage without any accompanying vascular changes. In the previous FIH study (C001-2020-01), infusion site findings were observed in all four healthy volunteers (one receiving placebo, three receiving active) dosed at 240 mg three times a day (TID). These reactions began on day two of administration in all subjects, with one serious treatment-emergent adverse event (TEAE) (infusion site thrombosis) reported. Therefore, further work was conducted in the rat and dog to select a formulation which had less potential for vascular irritation than the previous formulation administered to humans. A rat tail vein tolerability study was conducted, followed by a dog study assessing, the prototype termed PT2 (consisting of BWC0977) which was ultimately selected as a suitable formulation for progression into clinical studies. A clear comparison between the two local tolerance studies in dogs, comparing the previously used clinical formulation and PT2, is challenging due to variations in the number of dosing occasions for each individual dosing site, differing dose levels assessed in the two local tolerance studies and daily vs alternate days of dosing at individual dosing sites. However, based on histopathological assessment the effects at the infusion site caused by PT2 in rats and dogs were significantly less than the previous clinical formulation dosed to humans and appeared similar to control formulation containing only excipients suggesting they were mainly due to the venipuncture procedure (further details are provided in the Investigator's Brochure [IB] (4).

1.3 Following understanding on formulation tolerance and identifying better prototype PT2, a pharmacokinetic (PK) study in dogs at 10mpk following 1 hour infusion which compared relative bioavailability of previous formulation studied in C001-202-01 to the new prototype (PT2) demonstrated the PK of both formulations to be similar and no change in the PK (further details are provided in the IB).Clinical Safety Data

BWC0977 is currently in Phase I clinical development. The FIH study (C001-2020-01) of this compound was "A randomized, double-blind, placebo-controlled, Phase 1 study of the safety, tolerability, and pharmacokinetics of single and multiple ascending doses of BWC0977 in healthy adult volunteers" in 44 healthy adult subjects. Data from this study demonstrated that single IV infusion doses infused over 120 min ranging from 120 mg to 1050 mg were generally well tolerated. The most commonly occurring treatment-related TEAEs were dysgeusia and headache.

The incidence of dysgeusia and/or taste disorder appeared to increase with increasing BWC0977 dose.

When administered as an IV infusion TID, both BWC0977 and the placebo were not tolerated with local infusion site reactions leading to study drug discontinuation in all 4 subjects dosed. One TEAE (infusion site thrombosis) following administration of BWC0977 was considered serious and related to study drug. No systemic findings pertaining to BWC0977 were observed.

All adverse events (AEs)/serious AEs (SAEs) resolved prior to study end.

The PK results from the FIH study (C001-2020-01) showed that for a single dose of BWC0977 administered as an intravenous infusion over 120 minutes, plasma C_{max} and AUC increased proportionally with dose. For most subjects, the time of occurrence of C_{max} (T_{max}) occurred at the end of the infusion. The average apparent terminal phase half-life ($t_{1/2}$) increased gradually with dose, from 3.3 hours for 120 mg BWC0977 to 8.4 hours for 1050 mg BWC0977. The amount of drug excreted in urine in the 48-hour interval following dose administration was less than dose proportional to increasing dose, with the fraction of dose excreted in urine gradually decreasing from 35% for 120 mg BWC0977 to 20% for 1050 mg BWC0977.

Based on animal NOAEL, model-based predictions, and similarity of PK between the clinical formulation used in the FIH study and the current study. A **starting dose of 240 mg is proposed for this Phase I clinical study**. For a 70 kg person, this dose is predicted to elicit a C_{max} of 3.27 μg/mL (after a 2-hour IV infusion) and an AUC_{0-24} of 10.2 μg*h/mL, that are approximately **8-fold below** the NOAEL-associated C_{max}, and **5-fold below** the NOAEL-associated AUC_{0-24} value from the rat 14-day GLP study.

Once the multiple ascending dose (MAD) component of the study commences, we will evaluate single dose of 1500 mg (B1) after completion of A2. Cohort B2 is to facilitate evaluation of ECG on supra-therapeutic doses of BWC0977 after completion of B1.

1.4 Rationale

1.4.1 Study Rationale

The BWC0977 FIH trial (C001-2020-01) was conducted using an IV clinical formulation (CF). Since BWC0977 administered in the form of CF was not tolerated during the multiple dose administration with all 4 healthy volunteers (100% dosed) experiencing TEAEs of infusion site reactions (mild or moderate in severity) at the site of study drug administration the study was terminated. There were no ECG, vital signs or clinical laboratory parameters that were assessed as clinically significant (CS). There were no other CS or persistent systemic findings observed. Hence, the events that led to study drug withdrawal of both BWC0977 and placebo: infusion site thrombosis; infusion site pain; infusion site erythema, and infusion site warmth would have to be overcome with a new formulation to complete the multiple dose cohorts.

Different new IV injection formulations were developed and evaluated to understand the risk of vascular toxicity in preclinical species (rat and dog). Additional non-clinical studies *(in-vitro cell culture)* were conducted to explore candidate formulations with lower risk for injection site reactions (ISRs).. Of the three candidate formulations tested namely PT1, PT2, and PT3 in the dog local tolerance study, each formulation demonstrated histologic improvements over the initial clinical formulation given in FIH study. The decision to progress PT2 was made by placing priority on incremental differences observed in the histology (over PT1 and PT3), given that it is an objective finding in vivo. However, given recurrence of ISRs in the current study, we re-evaluated PT1 and PT3 to identify if, taken holistically (pH, osmolality, buffering capacity, etc.), they have less potential for tolerability issues than PT2. PT3 did not show a better profile than PT2. PT1 provided the most tolerable profile, and is expected to be a substantial improvement over PT2 after multiple doses. In brief, increased pH, decreased osmolarity, improved buffering capacity and decreased precipitation potential underpin the superiority of PT1 formulation in comparison to PT2. The strength (mg/mL) and flow rate (mL/min) will be optimized to ensure least potential for ISRs. Therefore, the duration of infusion may vary with cohorts.

Please refer to the IB for BWC0977 for results from preclinical studies conducted as of date (4).

1.4.2 Dose Rationale

Single and repeat IV dose levels are planned in this study. New IV injection infusion formulation will be evaluated at single IV infusion dose of 240 mg in the Cohort-1 of Part-A of the study, based on the experience on the safety and PK of BWC0977 from the old formulation evaluated in FIH study (C001-2020-01). From the Day 1, 240 mg will be administered twice a day (BID) for 7 days to assess TEAEs of infusion site reactions which became apparent in C001-2020-01 in all 4 subjects after 3-4 doses administration.

In the earlier study C001-2020-01 it was decided to explore 1500 mg dose since no safety and tolerability issues were observed at 1050 mg single dose which resulted in C_{max} and AUC_{0-24} of 17.93 µg/mL and 54.42 µg*h/mL, respectively. However, due to TEAEs observed in the MAD B1 cohort in C001-2020-01, the 1500 mg single dose cohort was not undertaken. In the current study, we plan to dose subjects at 1500 mg single dose level with the new formulation.

In the dog cardiovascular study, the increase in QTc was observed at both dose levels assessed (a non-statistically significant maximum increase of 4 msec at 30mg/kg; a statistically significant maximum increase of 11 msec at 60mg/kg both maximal increases were between 3-6 hours post start of 1-hour infusion), there was also a notable increase in heart rate at both dose levels. The mean C_{max} achieved in the dog cardio dynamic study equates to approximately 32 µg/mL (4µM free) concentrations at 30 mg/kg. Though 30mg/kg was not declared a no-effect level there were variable changes in the heart rate and non-statistical changes in QTc.

In the C001-2020-01 FIH study at 1050mg single dose in humans the Cmax achieved was 17.93 ± 2.57µg/mL, this concentration was 1.7x fold lower than the Cmax achieved at 30mpk in dog QTc study.

At 1500mg single dose in humans, it was predicted to achieve a C_{max} of 26 µg/mL, approximately similar to the C_{max} achieved in dog QTc study at 30mg/kg. From the in vivo efficacy studies in non-clinical species, it was observed the PK/PD index for the efficacy of BWC0977 is fAUC/MIC which could be achieved by fractionating the total dose by multiple dose administration hence, it is proposed to explore 1250mg BID (2500mg total daily dose) and 1000mg TID (3000mg total daily dose). Hence, to not pose a significant QTc risk to humans in the study it is proposed to not explore a dose >1500mg as a single dose in the current study.

Considering the intended use of BWC0977 will include treatment of urinary tract infections, renal excretion data following IV dosing in humans will continue to be collected in this study.

The safety and PK data emerging from each cohort will be assessed by the Safety Monitoring Group (SMG) post each dose level, prior to enrolling the next higher dose level.

For each study part, if the SMG deems that the dose level was well tolerated, SMG will recommend escalating to the next higher dose. It is noted that:

- Cohort B1 (SAD) may commence following SMG review of data from Cohort A2. Cohorts A3-A5 may only be enrolled following SMG review of Cohort B1.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

To assess the safety and tolerability of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult volunteers.

2.1.2 Secondary Objective

To assess the PK characteristics in plasma and urine of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult volunteers.

2.1.3 Exploratory Objective

To assess the QT/QTc prolongation potential of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult volunteers.

2.2 Endpoints

2.1.1 Primary Endpoints

Safety and tolerability of BWC0977 following single and multiple ascending doses by IV infusion in healthy adult volunteers based on the following endpoints:

- Incidence of TEAEs and SAEs overall and by intensity
- Changes from baseline in:
  - Safety laboratory test results
  - ECG findings
  - Vital signs measurements
  - Physical examination findings

2.1.2 Secondary Endpoints

Following repeat dose: $AUC_{(0-\tau)}$, C_{max}, T_{max}, $C\tau$, Ro, Vdss and CL of BWC0977, as data permit. Amount excreted in urine (Ae) of unchanged BWC0977, fraction of the dose excreted in urine (fe) and renal clearance (CLr), as data permit.

Following single dose: $AUC_{(0-8)}$, $AUC_{(0-12)}$, $AUC_{(0-24)}$, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, C_{max}, T_{max}, CL, Vdss, mean residence time (MRT), and $t_{1/2}$ of BWC0977, as data permit. Amount excreted in urine (Ae) of unchanged BWC0977, fraction of the dose excreted in urine (fe) and renal clearance (CLr), as data permit.

2.1.3 Exploratory Endpoints

Cardiodynamic evaluation using 12-lead ECGs extracted from Holter recordings may be undertaken based on observed PK and other project considerations. In such case, the following endpoints will be used:

- Change-from-baseline heart rate (HR), PR, QRS and QT duration corrected for heart rate by Fridericia's formula (QTcF) interval (ΔHR, ΔPR, ΔQRS and ΔQTcF).
- Placebo-corrected ΔHR, ΔPR, ΔQTcF and ΔQRS (ΔΔHR, ΔΔQTcF, ΔΔPR and ΔΔQRS).
- Categorical outliers for HR, PR, QRS and QTcF.
- Frequency of treatment emergent T- and U-wave abnormalities.

3. STUDY DESIGN

3.1 Overall Study Design and Plan

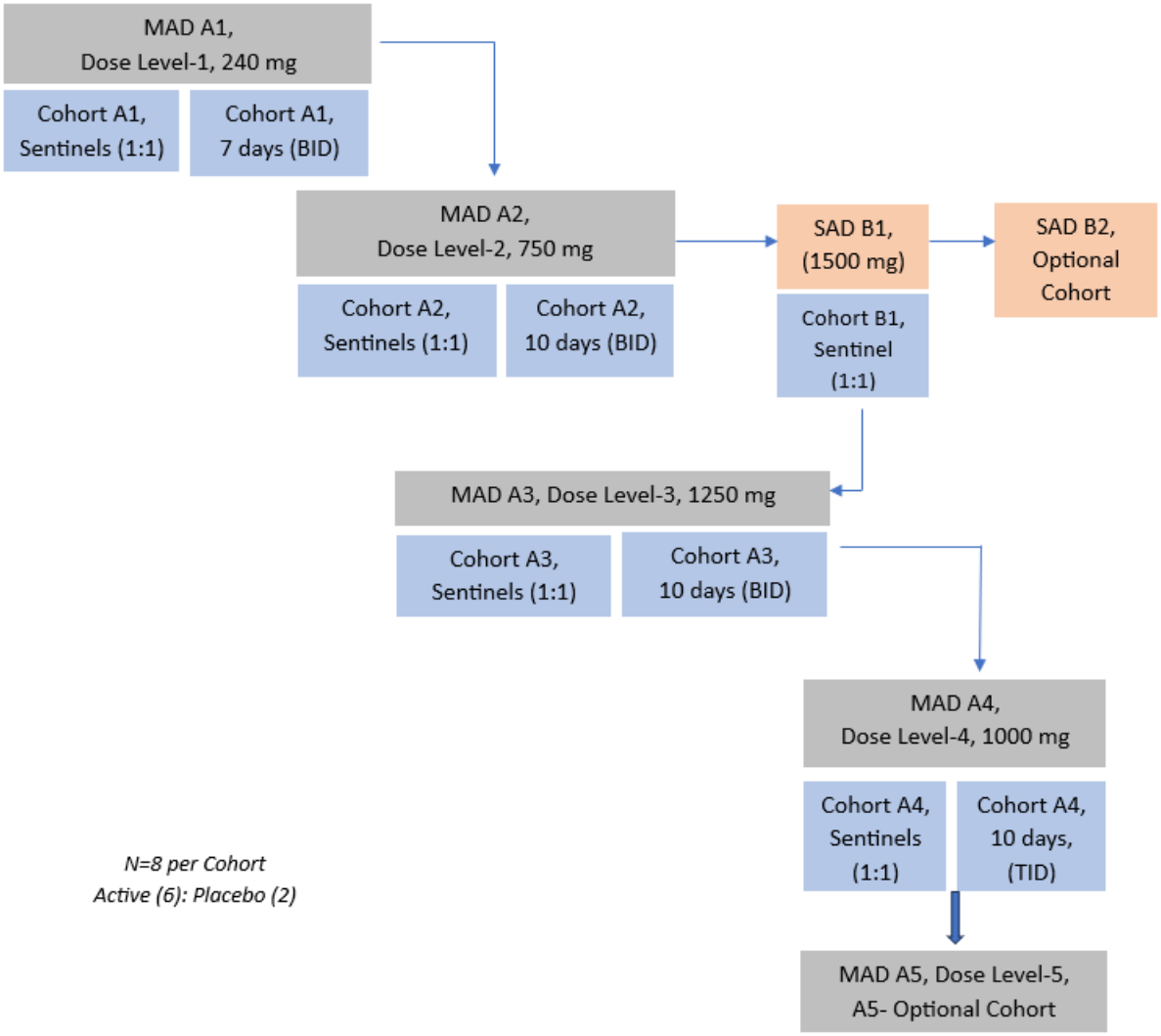
This will be a two-part (Part A and B), randomized, placebo-controlled, double-blind study to investigate the safety, tolerability, and PK of BWC0977 in healthy adult volunteers. This study is subdivided into parts, Part A (MAD) and Part B single ascending dose (SAD). Part A will investigate multiple ascending doses of BWC0977. Part B will investigate escalating single IV doses of BWC0977. Volunteers may only be enrolled in one study part and randomized to one cohort per the randomization schedule. The decision to escalate between dose levels and proceed to the next study part (Part A) will be based upon review of the available safety and PK data by the SMG. In each part of the study, cohorts and doses may be decided based upon emerging data and cohorts may be added or removed.

The starting dose, and dose increments are based on available non-clinical data and information received from the previous study (C001-2020-01). In the first cohort of this study, the dose level

to be evaluated in Cohort A1 will be dose at 240 mg (BID). Cohort A2 (750 mg, BID), A3 (1250 mg, BID), A4 (1000 mg, TID) and A5 is an optional cohort (

Figure 1). Sentinel dosing is planned for each cohort in Part A however, the requirement for sentinel dosing will be evaluated by the SMG following completion of the first MAD cohort. The dosing of Cohort B1 will be initiated after MAD A2. Cohorts A3-A5 may only be enrolled following completion of Cohort B1. The sequence of cohort enrollment in MAD & SAD is A1/A2/B1/A3/A4/A5 (Optional)/B2 (Optional).

Figure 1. Diagrammatic Representation of Study Part A, MAD and Part B, SAD



Abbreviations: TBD = to be determined.
**Cohort B2 is an optional cohort and dose level will be decided based on safety, tolerability, and pharmacokinetics data from the SAD-B1 cohort.*

An overview of the study schematic is also presented in **Table 1**.

Table 1. Dose Levels of BCW0977 Per Cohort

Cohort	Dose (mg)	No. of Doses	Duration	Dosing Frequency	Number of Subjects	
			Days		BWC0977	Placebo
Part A - MAD (Double-Blinded)						
A1*	240	14	7	BID	6	2
A2	750	20	10	BID	6	2
A3*	1250	20	10	BID	6	2
A4*	1000	30	10	TID	6	2
A5**	TBD	TBD	TBD	TBD	6	2
Total (Active + Placebo)					30	10
Total Number of Subjects (Part A)					40	
Part B - SAD (Double-Blinded)						
B1*	1500	1	1	1	6	2
B2**	TBD (Optional Cohort)				6	2
Total (Active + Placebo)					12	4
Total Number of Subjects (Part B)					16	
Total Number of Subjects (Part A + B) = Up to 56						

Note: *IV infusion duration specified in section 6.1.*
**Holter monitoring for these cohorts.*
***Optional Cohort to be utilized only if needed.*
Abbreviations: TBD = to be determined.

Hence, in C002-2023-01 (current study) it is intended to start 240mg BID for 7 days in MAD A1 cohort of Part-A to evaluate the safety, and tolerability of BWC0977 post-new formulation administration to humans.
Post the completion of this MAD A1 cohort, SMG would meet and evaluate the safety and tolerability data and potentially decide to escalate to the next higher cohorts in Part A and Part B respectively.

Based on POPPK modelling and simulations using the human PK data from C1-C5 cohorts in C001-2020-01 study, dosing SAD B1 at 1500mg is predicted to yield a C_{max} 26 µg/mL & AUC 73 µg.hr/mL. Similarly following 1000mg TID (3000mg total daily dose) is predicted to achieve at steady state Cmax of 19.8 µg/mL and AUC0-24,ss of 153.9 µg.hr/mL.

PK/PD data from the preclinical studies indicate that the target efficacious exposure (AUC) is ~120 µg.hr/mL, to cover all the key pathogens including K. pneumoniae, P. aeruginosa, A. baumannii, and E. coli.

3.1.1 Part A (Multiple Ascending Dose)

In Part A, healthy volunteers will be enrolled to receive multiple doses of BWC0977 or placebo. The starting dose level in Part B (240 mg) is determined from the previous study (C001-2020-01).

Dose escalation in Part A will be conducted in a total of 5 cohorts. Within each cohort, the 8 participants will be randomized to receive BWC0977 (6 participants) or placebo (2 participants).

Healthy volunteers will be screened between Day -28 and Day -2. Participants will be admitted to the clinical facility on Day -1. Dosing will commence the following day (Day 1) and will continue as per the schedule of events (SoA). The dosing interval (τ) will be equal to 8 hours for thrice daily regimen or 12 hours for twice daily or 24 hours for once daily.

Dosing in Cohorts A1 will be for 7 days (BID), A2 and A3 will be for 10 days (BID), A4 will be for 10 days (TID), and A5 is an optional cohort, with the dose level and dosing frequency to be determined prior to enrolment of participants into these cohorts. Cohorts A3-A5 may only be enrolled following completion cohort B1.

Participants will be confined at the clinical facility from Day -1 through to the end of dosing. Participants will be discharged following completion of all safety and PK assessments as per the SoA.

Dosing in Cohort A1 will start with two sentinel participants with one of the two sentinels randomized to receive BWC0977 and the other randomized to receive placebo. The study PI will review safety/tolerability information available on the sentinel participants for a minimum of 48 hours post-dose and in consultation with the SMG (if necessary), will make the decision to dose the remaining 6 participants in the cohort. During the trial enrolment, 48-hour sentinel observation will be performed before proceeding with non-sentinel dosing.

Dosing for each participants will be via IV infusion.

Blood and urine samples for PK analysis will be collected pre-dose, and at several timepoints up to post-dose. All on-study assessments to be performed in Part A are defined in Section 7 and the timing of each assessment is presented in Section 7.5 (Schedule of Events). Continuous Holter monitoring will be performed from at least 1 hour prior to dosing on Day 1 and end of the dosing day and will continue to approximately 24 hours post- Day 1 and end of the day dose, with ECG extraction paired with PK sampling post-dose on both days in MAD cohorts (1, 3 and 4).

Participants will return to the clinical facility for final safety assessments at an End of Study (EOS) visit between Day 13±1 (A1), and 16±1 (A2, A3 & A4, 10-day dosing) (inclusive, as per SoA). If any participants experience any CS AEs during the confinement period, they may remain in the clinical facility for further observation at the discretion of the PI.

Cohorts will be dosed in an escalating order as per the emerging data. The decision to escalate between dose levels will be based upon review of blinded clinical and laboratory safety data from at least 6 out of 8 participants from the previous cohort (i.e., haematology, serum chemistry,

coagulation, urinalysis, ECG, vital signs, physical examination findings and AE data) through to and including the final follow-up visit and any available blinded PK data by the SMG.

Following completion of at least 6/8 participants, the SMG will review the data, discuss the findings, and recommend proceeding to the next cohort at the protocol-defined dose level, at another dose level (lower or higher) or to terminate enrolment in Part A. The SMG may also recommend use of an alternative dosing schedule for the next cohort in Part A, and/or determine whether use of sentinel dosing at the next dose level in Part A is warranted. Following completion of Cohort A2, the SMG may recommend enrolment proceeds for Cohort B1. Enrolment of Cohorts A3-A5 may commence following completion of Cohort B1.

Enrollment of all participants in the previous cohort does not have to be completed before initiating enrollment in a new cohort provided the SMG has approved proceeding to the next dose level.

3.1.2 Part B (Single Ascending Dose)

Part B may commence following completion of Cohort A2. In the first cohort of this SAD study, the dose level to be evaluated in Cohort B1 will be dosed at 1500 mg. Dosing in Cohort B2 will be based on PK, safety, and tolerability of B1.

Dose escalation in Part B will be conducted in a total of 2 cohorts (Cohorts B1 & B2). Within each cohort, the 8 participants will be randomized to receive BWC0977 (6 participants) or placebo (2 participants). Cohort B2 is included as a potentially supra therapeutic dose. This is to ensure that both sub therapeutic and supra-therapeutic doses are evaluated for QTc.

Healthy volunteers will be screened between Day -28 and Day -2. Participants will be admitted to the clinical facility on Day -1 with dosing to occur on Day 1. Participants will be confined at the clinical facility from Day -1 through to Day 3. Participants will be discharged following completion of all safety and PK assessments on Day 3.

Dosing in each cohort will start with two sentinel participants with one of the two sentinels randomized to receive BWC0977 and the other randomized to receive placebo. The safety and tolerability of each sentinel participant will be monitored in the clinic until Day 3 and will be reviewed prior to dosing the remainder of participants in each cohort. The study Principal Investigator (PI) will review safety/tolerability information available on the sentinel participants on Day 3 and in consultation with the SMG (if necessary), will make the decision to dose the remaining 6 participants in the cohort, if required.

Dosing for each participant will be via IV infusion. The planned infusion duration time is 120 minutes (±10 minutes); however, the duration of infusion may be modified based upon review of safety, tolerability, and available PK data from the preceding cohorts.

Blood and urine samples for PK analysis will be collected pre-dose, and at several timepoints up to 48 hours post-dose. Continuous Holter monitoring will be performed from at least 1 hour prior to dosing and will continue to approximately 24 hours post-dose, with ECG extraction paired with PK draws post-dose in SAD Cohorts 1 and 2. All on-study assessments to be performed in Part B

are defined in Section 7 and the timing of each assessment is presented in Section 7.5 (Schedule of Events). Cohort will be dosed in an escalating order. The dose of a cohort may be appropriately adjusted according to the safety, tolerability, and available PK data from the previous dose cohort.

Participants will return to the clinical facility for final safety assessments at an EOS visit between Day 8±1 (inclusive). If any participants experience any CS AEs during the confinement period, they may remain in the clinical facility for further observation at the discretion of the PI.

The decision to escalate between dose levels will be based upon review of blinded clinical and laboratory safety data from at least 5 out of 8 participants from the previous cohort (i.e., haematology, serum chemistry, coagulation, urinalysis, ECG, vital signs, physical examination findings and AE data) through to and including the final follow-up assessment on Day 8±1 and any available blinded PK data by the SMG.

Following completion of Day 8±1 for at least 5/8 participants, the SMG will review the data, discuss the findings, and recommend proceeding to the next cohort at another dose level (lower or higher) or to terminate enrolment in Part B.

Enrollment of participants in the previous cohort does not have to be completed before initiating enrollment in a new cohort provided the SMG has approved proceeding to the next dose level.

3.1.3 Duration of the Study

The total duration for Part A Cohort A1 is **42 days** inclusive of 7-day dosing and screening and **45 days** including 10-day dosing and screening schedule for cohorts A2, A3 and A4. Cohort A5 is an optional cohort with duration dependent on dosing regimen selected.

The total duration for Part B (SAD) is **37 days**, inclusive of screening windows.

If any participants experience any CS AEs during the confinement period in Part A or Part B, they may remain in the clinical facility for further observation at the discretion of the PI.

4. PARTICIPANT SELECTION

4.1 Planned Number of Participants

This study will enroll approximately 56 healthy adult participants (40 participants in Part A, 16 participants in Part B).

4.2 Inclusion Criteria

Volunteers must meet the following inclusion criteria for study participation:

1. Healthy male or female 18 to 55 years of age, inclusive, at time of consent

2. Body mass index (BMI) ≥ 19.0 and ≤ 30.0 (kg/m^2) and weight between 55.0 and 100.0 kg (inclusive)

3. Medically healthy without clinically significant abnormalities at the screening visit, Day -1 or pre-dose Day 1, including:

    a) No findings in Physical examination or vital signs (including temperature, HR, respiratory rate, and blood pressure) that the Investigator determines would interfere with interpretation of study results
    b) Triplicate ECGs without CS abnormalities, including a QTcF interval duration ≤450 msec (for males), and ≤470 msec (for females) obtained as an average from the triplicate screening ECGs after at least 5 minutes in a supine, quiet-rest position
    c) Clinically significant abnormalities in the screening clinical laboratory tests, as determined by the Investigator. Repeat testing could be performed at the Investigator's discretion.

4. Willing and able to provide written informed consent

5. Agrees to be available for all study visits and cooperate fully with the requirements of the study protocol, including the schedule of events.

6. Willing to refrain from strenuous physical activity that could cause muscle aches or injury, including contact sports, at any time from 4 days prior to admission in the clinical research unit (CRU) until completion of the study (follow-up [FU] visit).

7. Willing to refrain from prescription medications from Screening visit until follow-up; and over-the-counter (OTC) medications, vitamin preparations and other food supplements, from Day -1 to follow-up

8. Have suitable venous access for drug administration and blood sampling

9. If female of child-bearing potential, must agree to and comply with:

    a) Using 1 barrier method (e.g., female condom or male partner using a condom) plus 1 other highly effective method of birth control (e.g., oral contraceptive, implant, injectable, indwelling intrauterine device, vasectomized partner), or double-barrier

method (use of a condom by the male partner with use of a diaphragm by the female partner), from signing the consent form until 30 days after last study drug administration, or

b) Sexual abstinence, for the duration of the study (from signing of consent) and for 30 days after last study drug administration, plus

c) Females of child-bearing potential must also agree not to donate ova or oocytes (ie, human eggs) during the study, and for one menstrual cycle after completion of the study.

d) To be considered of non-childbearing potential, a female must have either a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy (at least 6 weeks prior to screening), or menopause (last menstruation >12 months and follicle-stimulating hormone levels in menopausal range); provision of written documentation is not required for female sterilization and oral confirmation is adequate.

e) Female participants in same sex relationships do not need to utilize contraception.

10. Male volunteers, if sexually active with a female partner, must agree to and comply with using 1 barrier method of birth control (e.g., male condom) plus 1 other highly effective method of birth control in their partner (e.g., oral contraceptive; implant, injectable, indwelling intrauterine device), or double-barrier method (use of a condom by the male partner with use of a diaphragm by the female partner), or sexual abstinence, and must not donate sperm, for the duration of the study (from signing of consent) and for 90 days after last study drug administration.

    To be considered surgically sterile, male participants must have had a vasectomy at least 3 months prior to screening with appropriate documentation of the absence of sperm in the ejaculate.

    Male participants in same sex relationships do not need to utilize contraception.

4.3 Exclusion Criteria

Volunteers who meet any of the following criteria will be excluded from the study:

1. Women who are pregnant and/or lactating.

2. History or presence of significant cardiovascular (including QT prolongation, clinically significant hypokalemia, or other proarrhythmic conditions), pulmonary, hepatic, renal, hematological, gastrointestinal, endocrine (including glucose intolerance, diabetes mellitus), immunologic (including asthma or seasonal allergies [that require intermittent use of steroids or other medication]), musculoskeletal (including tendinopathy), dermatologic, or neurological disease (including seizure disorders, psychiatric disorders), including any acute illness or surgery within the past 3 months, as determined by the Investigator to be clinically relevant.

3. A serum creatinine value on Day -1 (check-in) that increased by more than 0.2 mg/dL (or 15.25 µmol/L) from the Screening value Note: the serum creatinine test may be repeated prior to confirming exclusion

4. History of photosensitivity to quinolones

5. History of known or suspected *Clostridium difficile* infection

6. Any condition that necessitated hospitalization within the 3 months prior to Day -1 or is likely to require so during the study

7. Positive test for hepatitis B virus surface antigen (HBsAg), hepatitis C virus antibody (anti-HCV antibodies), or human immunodeficiency virus antibody (antibodies to HIV-1, HIV-2) at screening.

8. Exposure to any prescription medications (small molecules, biologics, and vaccines, including influenza and/or COVID-19 vaccines) or, systemically administered OTC drugs, dietary supplements, or herbal remedies, within 14 days or 5 half-lives (if known), whichever is longer, prior to Day 1 (first dose). Participants should not receive any vaccinations (including influenza and/or COVID-19 vaccines) until after study completion. Discussion between the PI and the Sponsor Medical Monitor is encouraged regarding prior use of any medications during the pre-dose period.

Note: An exception is made for hormonal contraceptives, paracetamol (a maximum of 4 doses per day of 500-mg, and no more than 3 g per week) for the treatment of headache or any other pain, and ibuprofen as premedication for thrombophlebitis as per PI's judgement.

9. Documented hypersensitivity reaction or anaphylaxis to any medication.

10. Smoker (including tobacco, e-cigarettes, or marijuana) or nicotine user within 1 month prior to dosing and have a negative test for cotinine at check in on Day -1 (*may be repeated once, at the discretion of the Investigator, in the instance of a positive result*).

11. Positive urine drug/alcohol breath testing at screening or check-in (Day -1), or history of substance abuse or alcohol abuse (defined as greater than 2 standard drinks on average each and every day, where one standard drink is defined as containing 10 g of alcohol and is equivalent to 1 can or stubby of mid-strength beer, 30 ml nip spirits, or 100 ml wine) within the previous 5 years *(may be repeated once per timepoint, at the discretion of the Investigator, in the instance of a positive result).*

12. Donation of blood or plasma within 30 days prior to randomization, or loss of whole blood of more than 500 mL within 30 days prior to randomization, or receipt of a blood transfusion within 1 year of study enrollment.

13. Previous participation in this study or previous participation in another study within 5 half-lives (if known) of the agent, or 30 days, whichever is longer, of Day 1.

    Note: prior participation at any time in non-invasive methodology trials in which no drugs were given is acceptable.

14. Consumption of red wine, Seville oranges, grapefruit, or grapefruit juice, pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices containing such products from 7 days prior to the first dose of study medication.

15. Employee or family member of an employee of the Sponsor, CRU, or clinical research organization at which the study will be conducted.

16. Unable to cooperate fully with the requirements of the study protocol, including the schedule of events, or likely to be non-compliant with any study requirements.

17. Any disease or condition (medical or surgical) that, by the determination of the Investigator, precludes the subject's participation in the study or would place the subject at risk as a result of participation in the study.

Note: Volunteers should refrain from consumption of any foods containing poppy seeds within 48 hours (2 days) prior to screening and prior to Day -1 to avoid false positive drug screen results.

5. STUDY CONDUCT

For the exact timing and allowable windows for each procedure described in this section, please refer to Section 7.5, SoA.

5.1 Screening (Days -28 to -2 prior to Randomization)

At the screening visit, potential volunteers will be given a detailed oral presentation describing the nature, purpose, risks, and requirements of the study and will receive detailed written information. Volunteers will be given ample time to consider participation and ask questions which will be adequately addressed by site personnel.

Once the volunteer is satisfied that he/she is willing to participate in the study, he/she will be asked to sign the study informed consent form (ICF, refer to Section 12.2.6). The investigational site personnel obtaining written consent from the volunteer will also sign the consent form.

Once signed, the Investigator will retain the original ICF for the participant's study records and provide the participant with a signed copy. The investigator will verify that informed consent has been obtained from each participant prior to enrollment into the study and prior to the participant undergoing any study-related procedures.

Screening activities after obtaining informed consent will be conducted and consist of the following:

- Completion of medical history
- Collection of demographic data (sex, age, race/ethnicity);
- Review of prior and current medications (including alternate or herbal medications) and supplements;
- Review inclusion and exclusion criteria.
- Physical examination (full).
- Measurement of height and weight.
- Measurement of vital signs.
- 12-lead ECG.
- Collection of blood and urine samples for:
    - Clinical safety labs, including hematology, serum chemistry (liver function tests (LFT's) and follicle stimulating hormone (FSH) [to confirm postmenopausal status in women]), and urinalysis (see Section 7.4.4.6 for list of tests);
    - Infectious serology (HIV, HBsAg, HCV);
    - Serum pregnancy test for women of childbearing potential
    - Drug substance abuse and urine test
- Breath test for alcohol
- Estimated creatinine clearance
- All assessments may be repeated once at investigator discretion

For volunteers who meet eligibility criteria based on the Screening assessments, instruction will be provided on the following:

- Use of adequate contraceptive methods (see Section 4.2) for the duration of the study;
- Avoiding use of concomitant medications during the study, and mandatory prohibition of certain medications as defined in Section 9.6.6;
- Maintenance of usual dietary habits and avoidance of drastic changes alongside exposure to the sun.

5.1.1 Screen Failure

A screen failure is defined as a participant who has signed the ICF, does not meet all the entry criteria outlined in Section 4 of this protocol (note that this includes assessments through Visit 1), and was not randomized to receive study treatment. The Investigator is responsible for keeping a record of all participants screened for entry into the study and subsequently excluded. The reason(s) for exclusion will be recorded in the source documents and on the Screening log. Screen failure participants will have only their consent, demographic, and reason for screen failure (including, where applicable, the unmet inclusion or exclusion criteria) data entered the electronic data capture (EDC) system. If an AE was responsible for the participant's screen failure, all data collected for that participant during the screening process will be entered into the EDC system.

5.2 Treatment Period

Clinical staff are required to perform assessments at the designated timepoints within the time windows indicated in this protocol (refer to Section 7.5, SoA). Actual times for each participant may vary depending on the scheduling and will be recorded in the electronic case report form (eCRF). When multiple procedures are scheduled at the same time point(s) relative to dosing, the following chronology of events should be adhered to, where possible: ECGs, vital signs, PK sampling and safety lab assessments.

5.2.1 Check-in (Day -1)

Participants will be admitted to the CRU on Day -1. Participants will be required to stay in the CRU for the maximum 11 nights (Part A, as per SoA) or 3 nights (Part B) and will be discharged as per the SoA.

The following evaluations will be performed on Day -1:

- Completion of interim medical history review
- Review of prior and current medications (including alternative and herbal medications) and supplements;
- Physical examination (symptom-directed).
- Measurement of weight.
- Measurement of vital signs.
- 12-lead ECG.

- Collection of blood and urine samples for:
    - Clinical safety labs, including hematology, coagulation, serum chemistry (LFT's), and urinalysis (see Section 7.4.4.6 for list of tests);
    - Urine pregnancy test for women of childbearing potential
- Urine for drugs of abuse and urine cotinine
- Breath test for alcohol
- Urine for pregnancy test for females of child-bearing potential.
- Estimated creatinine clearance
- Adverse events will be monitored and recorded.

All eligible participants will be randomized for each cohort and admitted into CRU on Day -1.

5.2.2 Randomization and Dosing (Day 1)

For each cohort under SAD and MAD Parts, participants who have passed the screening and are eligible as per inclusion and exclusion criterion will be randomized to the study treatment. The following procedures will be performed:

Prior to First Dose:

- Measurement of vital signs (supine systolic blood pressure (SBP)/diastolic blood pressure (DBP), temperature, respiratory rate, and heart rate) within 1 hour before study drug infusion;
- Review of prior and current medications (including alternative and herbal medications) and supplements;
- Symptom directed physical examination within 4 hours before the start of study drug infusion.
- 12-lead ECG within 1 hour before the start of the first study drug infusion.
- Holter monitoring commences 60 minutes prior to dosing (SAD Cohorts 1, 2 and MAD Cohorts 1, 3 & 4).
- Review inclusion and exclusion criteria.
- Randomization.
- For Part A: Blood for PK analysis will be collected immediately before (within 60 minutes before) the start of infusion of study drug;
- For Part B: Blood for PK analysis will be collected immediately before (within 60 minutes before) the start of infusion of study drug;
- For Part A: Obtain urine for PK analysis immediately before (within 2 hours before) the start of study drug;
- For Part B: Obtain urine for PK analysis immediately before (within 2 hours before) the start of the first study drug infusion.
- Ibuprofen as thrombophlebitic premedicationas per PI's judgement .

Following Dose Administration:

- For Part A: Measurement of vital signs (supine SBP/DBP, temperature, respiratory rate, and heart rate) 1, 2, 4, and 12 hours (±10 minutes each) after the start of 1st drug infusion each dosing day as per SoA.
- For Part B: Measurement of vital signs (supine SBP/DBP, temperature and heart rate) 1, 2, 4, 6, 8, 10, and 12 hours (±10 minutes each) after the start of study drug infusion as per SoA.
- 12-lead ECG at for cohort A1 0.5 (±10 minutes), 2, and 6 hours (±30 minutes each) after the start of study drug infusion. For cohort A2-4 at 1, 2, 4 and 6 hours (±30 minutes each) after the start of study drug infusion.
- Holter monitoring continues to 24 hours post- Day 1 dose from 1st infusion on Day 1 for MAD (except A2) and SAD.
- For Part A:

Cohort A1: Blood for PK analysis will be collected at 5, 15, 30, 60, 75, and 90 minutes and 2, 4, 6, 8, and 12 hours post first Day 1 infusion. Refer to Appendix I for PK sampling windows.

Cohort A2 & A3: Blood for PK analysis will be collected at 30, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, and 12 hours post first Day 1 infusion. Refer to Appendix I for PK sampling windows.

Cohort A4: Blood for PK analysis will be collected at 30, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, and 8 hours post first Day 1 infusion. Refer to Appendix I for PK sampling windows.

- For Part B: Blood for PK analysis will be collected at 5, 10, 15, 20, 30, 45, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 10, and 12 hours (Day 1) (window: ± 2 minutes of the nominal timepoint up until the 20-minute timepoint and within ±10% of the nominal timepoint thereafter) (Appendix II);
- For Part A: Obtain urine for PK analysis at 0-3, >3-6,>6-8h and 8-12 hours after the start of the first study drug infusion on Day 1 (8-12 h is applicable for A1-A3). Refer to Section 7.5 for PK sampling windows.
- For Part B: Obtain urine for PK analysis at 0-3, >3-6, >6-8 and >8-12 hours (Day 1) (±10 minutes each) after the start of study drug infusion on Day 1; collect and record urine volumes for each time point.
- Adverse events will be monitored and recorded.
- Infusion site reaction assessment during and after each infusion:
  Cohort A1: 15 minutes (±5 minutes), 30 minutes (±5 minutes) (i.e., at end of infusion), and 2 hours (±5 minutes) post start of administration.

Cohorts A2-A4 & all SAD cohort(s): 15 minutes (±5 minutes), 2-hours (±5 minutes) (i.e., at end of infusion) and 4 hours (±5 minutes) post start of administration.

- Record all IV catheter site changes and the reason for change.

- For Part A: The participants will be administered with additional doses of IMP from Day-2 as per SoA.

5.2.3 During Treatment – MAD (Days 2-10)

- Treatment administration as per randomization schedule.
- Measurement of vital signs are to be performed at 1, 2, 4, and 12 hours (±10 minutes each) after the start of the first study drug infusion on each dosing day and at 24 hours (±2 hours) after the start of the first Day 7 (A1), and 10 (A2, A3 & A4) study drug infusion.
- Conduct symptom-directed physical examination within 4 hours before the start of the first study drug infusion on each dosing day and at 24 (±2) hours after the start of the final Day of study drug infusion.
- Obtain 12-lead ECG measurements for cohort A1 0.5 (±10 minutes), 2, and 6 hours (±30 minutes each) after the start of study drug infusion. For cohort A2-4 at 1, 2, and 6 hours (±30 minutes each) after the start of study drug infusion.
- Holter monitoring continues to approximately 24 hours post Day 1 dose (Day 2).
- Holter monitoring continues to approximately 24 hours post Day 7 (A1) and Day 10 (A3 to A4) dose.
- Collect blood and urine for laboratory analyses Days 2, 4, 6, 7) (A1, 7-day dosing), and 2, 4, 6, 8, 10) (A2, A3 & A4, 10-day dosing)
- Obtain blood (plasma) for PK analyses. Refer to Appendix I for PK sampling times and windows.
- Collect urine for PK Adverse events will be monitored and recorded.
- Concomitant medications will be reviewed.
- Infusion site reaction assessment during and after infusion:
  Cohort A1: 15 minutes (±5 minutes), 30 minutes (±5 minutes) (i.e., at end of infusion), and 2 hours (±5 minutes) post start of administration.
  Cohort A2-A4: 15 minutes (±5 minutes), 2-hours (±5 minutes) (i.e., at end of infusion) and 4 hours (±5 minutes) post start of administration on Days 2-10, as per the SoA.
- Record all IV catheter site changes and the reason for change.
- Ibuprofen as thrombophlebitic premedicationas per PI's judgement .

On Day 8 (A1, 7-day dosing), and 11 (A2, A3 & A4, 10-day dosing) participants will be discharged from the clinical unit after completing all safety and PK evaluations and post investigator reviews of all clinical and safety related parameters of the participant.

Note: Assess patency of peripheral IV (PIV)/heplock, in accordance with site policies. If no longer removes and insert a new PIV/heplock in accordance with site policies.

5.2.4 Post-treatment - SAD (Days 2-3):

Participants will continue to get safety evaluations done post treatment. The following procedures will be performed:

- Measurement of vital signs at 24 hours (Day 2) and 48 hours (Day 3) (±2 hours each) after

the start of study drug infusion.

- Physical examination (symptom-directed) at 24 hours (Day 2) and 48 hours (Day 3) (±2 hours each) after the start of study drug infusion.
- 12-lead electrocardiogram at 24 hours (±30 minutes) after the start of study drug infusion (Day 2 only).
- Holter monitoring continues to approximately 24 hours post-dose on Day 2.
- Adverse events will be monitored and recorded.
- Concomitant medications will be reviewed.
- Record all IV catheter site changes and the reason for change.
- Blood and urine for safety laboratory assessments 24 hours (±10 minutes for blood samples, ±1 hour for urine samples) (Day 2 only).
- Blood for PK analysis at 24 hours (Day 2) and 48 hours (Day 3) window: within ±10% of the nominal timepoint) after the start of study drug infusion.
- Obtain urine at > 12-24, >24-36 and >36-48 hours (Days 2-3) (±10 minutes each) after the start of study drug infusion; collect and record urine volumes for each time point.

On Day 3, participants will be discharged from the CRU after completing all safety and PK evaluations and post investigator reviews all clinical and safety related parameters of the participant.

5.3 Follow-up Visit

After discharge on as per the SoAs, participants will return to the clinic for a single follow-up visit between Days 13±1 (A1, 7-day dosing), 16±1 (A2, A3&A4, 10-day dosing) (MAD) and Days 8±1 (SAD).

In MAD and SAD, the following procedures will be performed during follow-up:

- Adverse events since discharge will be monitored and recorded;
- Complete physical examination
- Measurement of vital signs.
- 12-lead electrocardiogram (ECG).
- Blood and urine will be collected for clinical safety labs, including hematology, coagulation, serum chemistry (LFT’s) and urinalysis.
- Estimated CrCl (MAD cohorts only)
- Pregnancy test urine.
- Prior and concomitant medications will be reviewed.

5.4 Early Termination Visit

The following procedures will be performed if a participant terminates from the study early in Part A (MAD) or Part B (SAD):

- Adverse events will be monitored and recorded;

- Record all IV catheter site changes and the reason for change.
- Complete physical examination.
- Measurement of vital signs.
- 12-lead ECG.
- Blood and urine will be collected for clinical safety labs, including hematology, serum chemistry (LFT's).
- Pregnancy test (serum).
- Prior and concomitant medications will be reviewed.

5.5 Safety Review

The decision to enroll each study cohort will be made following evaluation of all available safety data and any available PK data by the SMG (details described in the study SMG Charter).

At a minimum, the SMG will be a comprised of the Investigator, an independent Medical Monitor and a clinical designate from the Sponsor (Sponsor's medical representative). Other individuals (e.g., medical experts) may be invited to participate at the discretion of the SMG to provide additional input into the review process if required.

Decisions to enroll the next cohort will be made by agreement between the PI(s), independent Medical Monitor and Sponsor's medical representative after completion of dosing for 6/8 participants in each study cohort in Part A and 5/ 8 participants in each cohort in Part B.

The responsibilities and meeting schedule of the SMG will be outlined in a formal SMG Charter document.

Minutes following each SMG meeting will document the recommendation for dose escalation.

5.6 Early Withdrawal

If a randomized participant is withdrawn from the study prior to completing study treatment, the participant will be discharged from the study and the following procedures will be performed:

- Review of medications and supplements;
- Measurement of vital signs
- Physical examination;
- ECG assessment
- Collection of blood and urine samples for:
    - Clinical safety labs, including hematology, serum chemistry, coagulation, and urinalysis (see Section 7.4.4.6) for list of tests).
    - Serum pregnancy test
- Adverse events will be monitored and recorded;
- Discharge from the study.

The Investigator must continue to follow any participant with a possible study treatment related AE either until resolution or until the Investigator assesses them as chronic or stable.

5.6.1 Study Drug Discontinuation

Study drug must be discontinued if any of the following occur:

1. Adverse event/s as defined in the stopping criteria (refer Section 5.7).
2. Other findings that, at the discretion of the Investigator and/or Sponsor, indicate that study drug administration should be discontinued.
3. Withdrawal of consent.

The Investigator has full discretion to stop study drug infusion at any time, if clinically warranted or in the patient's best interest. All participants who have been dosed with any amount of study drug will continue to be followed for safety.

Participants who withdraw their consent will not receive any further study drug but will be offered all follow-up safety assessments. If a participant fails to attend scheduled study assessments, the Investigator must determine and document the reasons and the circumstances as completely and accurately as possible.

Participants who withdraw from the study prior to treatment (i.e., screened but not randomized) may be replaced. Participants who withdraw after having received at least one dose of study drug will not be replaced.

Participants will continue to be monitored for safety till end of study follow up

Except in cases of emergency, the Investigator should consult with the Sponsor and the Medical Monitor before removing the participant from the study. In some circumstances it may be necessary to temporarily interrupt treatment as a result of AEs that may have an unclear relationship to study treatment. The Investigator should obtain approval from the Sponsor and Medical Monitor before restarting study treatments that were temporarily discontinued for an AE.

5.6.2 Study Withdrawal

Participants may choose to withdraw from this study at any time for any reason without penalty of jeopardizing their health care or loss of benefits to which the participant is otherwise entitled.

Within the provisions of informed consent and good clinical judgment with respect to safety, every attempt will be made to have participants complete the study.

The following are reasons to terminate a participant's involvement in the study:

1. Significant protocol violation or noncompliance
2. Intercurrent illness that requires treatment that is not consistent with the protocol requirements, or intercurrent illness or the associated treatment that in the judgment of the

Investigator poses a significant risk to the participant for continued participation in the study.

3. Participant wishes to withdraw for any reason.
4. Sponsor elects to end the study.
5. Any other reason that in the medical judgment of the Investigator poses unacceptable risk to the participant.

In the event that, a participant discontinues the study prior to completion, the date the participant is withdrawn and the reason for discontinuation will be recorded in the source documents and eCRF. Although a participant will not be obliged to give their reason for withdrawing prematurely, the Investigator will make a reasonable effort to obtain the reason while fully respecting the participant's rights.

All participants who are randomized and treated (i.e., received any amount of study treatment) will be included in the safety analyses. Thus, every effort will be made to contact any participant who fails to attend any appointments/contacts, in order to ensure that he/she is in satisfactory health. If a participant withdraws from the study as a result of meeting discontinuation criteria after the start of study treatment administration, reasonable efforts should be made to have the participant return for the early withdrawal evaluations (Section 5.4).

Randomized and treated participants who are discontinued from this study for any reason will not be replaced.

If study termination criteria are met, enrollment of new participants and dosing of ongoing participants will be temporarily stopped. The Investigator, Sponsor, and the Medical Monitor will discuss whether a lower dose or any additional treatment guidelines should be implemented, or if the trial should be permanently stopped. Any proposed changes to the protocol to address such findings will be submitted for review and approval by the Institutional Review Board (IRB) /Independent Ethics Committee (IEC) prior to re-starting the trial.

5.7 Dose Escalation/Adjustment/Stopping Criteria

As described above, this protocol allows some alteration from the currently outlined dosing schedule, as amended.

The decision to proceed to the next dose level of BWC0977 will be made by the SMG based on safety, tolerability and available PK data obtained from at least 6/8 participants at the prior dose level in Part A (MAD) and from at least 5/8 participants at the prior dose level in Part B (SAD). The actual doses to be administered may be adjusted based on safety, tolerability, and preliminary PK data at previous dose levels.

The dosing schedule may also be adjusted to expand a dosing cohort to further evaluate safety and PK findings at a given dose level, or to add cohorts to evaluate additional dose levels. The study procedures for these additional participants(s) or cohort(s) will be the same as that described for other study participants.

Dosing in both Part A and Part B will proceed to the next dose level until evaluation of the nominal dose levels is completed, or the trial is stopped by the SMG. Temporary suspension of further dosing for a participant, dosing within a cohort or dose escalation between cohorts may be decided by the SMG if any of the criteria described in sections below are met.

For each of the stopping criteria described, the SMG will review all available safety data and will recommend if dosing should continue at that dose, or de-escalate to a lower dose level, or if the cohort, the dose level, or the entire study should be terminated. No more healthy volunteers should be enrolled (or participants dosed) until this safety review is completed.

5.7.1 Liver Chemistry Stopping Criteria

Liver chemistry threshold stopping criteria have been designed to assure participant safety and to evaluate liver event etiology (in alignment with the FDA premarketing clinical liver safety guidance).

- Aspartate aminotransferase (AST)/ Alanine aminotransferase (ALT) > 3x upper limit of normal (ULN) is observed in two or more participants receiving BWC0977 within a cohort.
- One occurrence of Hy's law criteria met, as defined by at least 3-fold elevations of ALT or AST above ULN, plus an elevation of serum total bilirubin to > 2 times ULN without elevated serum alkaline phosphatase, and no other disease or condition can be found to explain the liver test abnormalities.

5.7.2 QTc Withdrawal Criteria

Decisions are to be based on an average QTc value of triplicate ECGs. If an ECG demonstrates a prolonged QT interval, obtain 2 more ECGs over a brief period, and then use the averaged QTc values of the 3 ECGs and other safety data to make a decision.

- Within a dose level, there are two or more occurrence of post-baseline QTcF prolongation, defined by average QTcF at least 501 ms and >60 ms change from baseline, that are determined to be clinically significant by the PI or Sponsor and are assessed as probably or possibly related to dosing with the investigational product.

5.7.3 Safety Stopping Criteria

The following events may result in temporary suspension or termination of administration of further dosing within a cohort or dose escalation between cohorts:

- Grade 3 gastrointestinal AE.
- Grade 3 renal or urinalysis finding.
- One occurrence of a SAE assessed to be probably or possibly related to dosing with the investigational product.
- Participants with neutrophil counts below 1000 cells/μL will be discontinued from treatment and should be followed as appropriate until neutrophil counts normalize.
- One occurrence of elevation (>1.5 times ULN or a 2-fold increase as compared to baseline) in urea or serum creatinine.

- Two or more severe AEs of the same character that are determined to be clinically significant by the PI or sponsor and are assessed as probably or possibly related to dosing with the investigational product.
- If four or more participants experience the same or similar Grade 3 or higher AE which is possibly or probably related to the investigational product, the sponsor, the PI, and Medical Monitor will review all available safety data and will recommend if dosing should continue at that dose, or de-escalate to a lower dose level, or if the study should be terminated. No more healthy volunteers should be enrolled (or participants dosed) until this safety review is completed.

Other findings such as a SAEs or severe AEs will be reviewed by the SMG. The SMG will determine how to proceed and if further dosing or dose escalation should be stopped.

In case any safety or tolerability issues are experienced, a lower or intermediate dose may be administered in the next cohorts to gain more information on safety, tolerability and/or PK as recommended by the SMG.

The Investigator has full discretion to stop study drug infusion at any time, if clinically warranted (e.g., for an SAE or severe AE), or otherwise in the patient's best interest.

The grading for AEs is based on FDA's Guidance on Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

5.8 Study Termination

If the Sponsor or Investigator discovers conditions arising during the study that suggest the study should be halted, then this can happen only after appropriate consultation between the Sponsor and Investigator. Conditions that may warrant study termination include, but are not limited to:

- The discovery of any unexpected, significant, or unacceptable risk to the participants enrolled in the study.
- Insufficient adherence to the protocol requirements.
- A decision on the part of the Sponsor to discontinue the Study
- A decision on the part of the Sponsor to suspend or discontinue development of BWC0977.

The Investigator/Investigational Site has the right to close the site, at any time, although this should occur only after consultation between involved parties. The Investigator will notify the IRB/IEC in writing of a premature termination of a study or closure of Investigational Site and will send a copy of the notification to the Sponsor.

6. STUDY TREATMENT

6.1 Description of Investigational Drug

6.1.1 Name of the Investigational Product

BWC0977.

6.1.2 Formulation

The drug product is presented as a compounded solution (BWC0977, 250 mg/vial). The solution will be diluted to a final concentration of 10 mg/mL using 5% dextrose for IV infusion and administered by IV infusion.

The placebo is the compounded solution using same excipients and process, without BWC0977.

During the course of this study, the pH of the BWC0977 and placebo formulation was increased to allow a better tolerability profile based on sentinel volunteer findings in the A1 cohort. The pH of both the active and placebo was modified from 4.3 to 4.7.

6.1.3 Preparation of Investigational Medicinal Product

A detailed Pharmacy manual will be provided which contains detailed instructions on preparation and dispensing of IMP as per randomization.

6.1.4 Method of Administration

BWC0977 will be infused intravenously via a drip system. The IV bag will be connected to an infusion line with integrated sterile filter (0.2 µm).

The study drug will be infused continuously as follows

- Cohort-A1:30 minutes (±5 minutes) and
- Cohort A2-A4: 120 minutes (±10 minutes) (all remaining cohorts);

However, the flow rate may be adjusted based on a review of available safety, tolerability, and available PK data from previous cohorts by the SMG.

6.1.5 Packaging and Labeling

This study is a placebo-controlled study. All study medication will be compounded at the pharmacy and shipped to site. The Investigational Site pharmacist will be responsible for dispensing the appropriate treatment based on the randomization schedule. Study medication will be dispensed to the site with instructions for treatment administration.

BWC0977 will be compounded and supplied as a solution in vials with a content of 250 mg substance. The content of the required number of vials of BWC0977 will be diluted to a required volume using 5% dextrose. The solution of BWC0977 will be administered as continuous intravenous infusion.

Similarly, the content in the placebo vial will be diluted to a required volume using 5% dextrose. The solution will be administered as continuous IV infusion.

Bugworks Research Inc. will provide the required GMP batch of drug substance and other excipients to Royal Adelaide Hospital (RAH) Pharmacy, who will compound the BWC0977 solution formulation as per the instructions in the Pharmacy manual. A Pharmacy manual will be provided to study site with instructions on how to prepare the specific solution for the respective treatment group.

The 20R vial (glass bottle) and the rubber closure for the container are appropriate for aqueous preparations for parenteral use according to international standards for packaging materials.

The treatment package labels will include, but not be limited to, the following information:

- The notation- '*For Clinical Trial Use Only*;'
- Study number
- Study drug name
- Investigator/site identification
- Participant ID
- Kit No./Bottle ID/Batch number
- Retest date/expiry date
- Dosage Form/Strength
- Directions for use, including route of administration
- Storage conditions
- Name of Sponsor

6.1.6 Storage and Handling

All study treatment must be kept in an appropriate, secure area to prevent unauthorized access. The drug product (compounded solution) can be stored at 2-8°C. for10 days. The diluted drug product [in the Sterile Water for Injection (SWFI) or 5% Dextrose bag)] is stable for up to 24 hours when stored at 1825°C. Please refer to the Pharmacy manual for additional information. Storage conditions will be monitored, and appropriate monitoring logs maintained as source data. Deviations from the established temperature, should be documented, and the Sponsor should be notified.

6.2 Randomization

This is a randomized, double blind controlled study.

In each cohort, the randomization will be blocked for 4 males and 4 females. Every effort will be made to ensure that in sentinels of each cohort will be 1 male and 1 female, randomized 1:1 for active:placebo, then the remaining 6 participants will be randomized 5:1 for active:placebo, with the placebo assigned to a participant of the opposite sex from the sentinel placebo. The

requirement of equal numbers of males and females may be relaxed if there are fewer than 4 males or females available to be enrolled in a cohort.

The randomization schedule will be generated by Avance Clinical prior to the start of the study by an unblinded statistician. The Investigational Site pharmacist will follow this randomization schedule to dispense the appropriate study treatment. Randomization numbers will be assigned as participants qualify for the study and are assigned to treatment based on the randomization schedule.

Overall, in each cohort, participants will be randomized 3:1 to either BWC0977 or placebo.

6.3 Study Treatment Administration

The participants in MAD Cohort A1 will receive the 7-day dosing, and participants in cohort A2, A3 and A4 will receive 10-day dosing. Participants in part B (SAD) will receive a single dose on Day 1.

6.4 Dose Modifications

Participants will be dosed the study treatment at the assigned (randomized) dose unless discontinuation criteria as defined in Section 5.6.1 are met. Dose modifications as recommended by the SMG may be considered in case any safety or tolerability issues are experienced.

6.5 Drug Accountability

In accordance with current Good Clinical Practice (GCP), the Investigational Site will account for all study treatment supplies. Details of receipt, storage, administration, and return or destruction will be recorded in the Investigational Drug accountability record according to the standard operating procedures (SOP) of the Investigational Site. Copies of the Investigational Drug accountability record will be provided to the Sponsor.

Study treatment will only be dispensed to participants enrolled in this protocol, and only as directed by this protocol. Administration of study treatment will be accurately recorded in each participant's source documents and eCRF.

6.6 Blinding

The following controls will be employed to maintain the double-blind status of the study (PI and Participant):

- The infusion solution containing active drug and placebo will be indistinguishable in appearance.
- The randomization list will be provided to the study center pharmacist for dispensing purposes and kept in the pharmacy, accessible to the pharmacist and authorized personnel
- PK results for the interim analyses between cohorts will be presented in a blinded fashion.

Individual code break envelopes will be provided for all participants by the contract research organization (CRO). Each sealed envelope containing the randomization code and treatment allocation information will be kept in a storage room at the clinical facility, which is locked with restricted access. To manage the participant's condition in case of a medical emergency, the Investigator (or delegate) is allowed to break the code to know whether a participant received BWC0977 or placebo. If opened, the name of the person who opened it, the date and time of opening and the reason for opening must be written on the envelope. The Sponsor will be informed in case of unblinding.

There are no specific antidotes for BWC0977. Knowledge of whether the participant received BWC0977 or placebo, may not necessarily help in the care of an individual participant. The need to break the code must therefore be carefully considered.

The laboratory where the PK samples will be analyzed will be provided a copy of the randomization code by the CRO since only samples of participants that have received the active drug BWC0977 will be analyzed.

6.7 Concomitant Medications and Supplements

All medications and supplements (other than study treatment) taken by the participant from day 1 through Day 13/16 (Part A, MAD and as applicable) and Day 8 (Part B) will be considered "concomitant" medications and supplements. Medications and supplements taken prior to Day 1 that are no longer being taken at the time of Day 1 will be considered "prior" medications and supplements.

All medications and supplements taken within 28 days prior to Day 1 (first dose) through the FU visit will be recorded in the participant's source documentation and in the eCRF.

The use of all prescribed medication is not allowed from within 14 days or 5 half-lives (if known), whichever is longer, prior to Day 1 (first dose). An exception is made for hormonal contraceptives, which are allowed throughout the study for women of child-bearing potential. The use of all over-the-counter medications, vitamin preparations and other dietary supplements, or herbal medications is also not allowed within 14 days or 5 half-lives (if known), whichever is longer, prior to Day 1 (first dose). An exception is made for paracetamol and ibuprofen. A limited amount of paracetamol (a maximum of 4 doses per day of 500-mg, and no more than 3 g per week) for the treatment of headache or any other pain is permitted. Likewise, Ibuprofen is allowed as premedication for thrombophlebitis based on the PI's judgement.

Treatment with vaccines (including influenza and/or COVID-19 vaccines) is not permitted within 14 days or 5 half-lives (if known), whichever is longer, prior to Day 1 (first dose), and throughout the duration of the study. Participants should not receive any vaccinations (including influenza and/or COVID-19 vaccines) until after study completion.

Medications to be avoided if there is a TEAE include succinylcholine or other depolarizing muscle relaxants and acetylcholinesterase inhibitors (including edrophonium, pyridostigmine, and neostigmine), drugs which are organic anion transporter 3 (OAT3) transporter inhibitors or substrates (e.g., probenecid, cefazolin, methotrexate, cephaloridine), multidrug and toxin extrusion (MATE)-2K transporter inhibitors or substrates (e.g., cimetidine, metformin, pyrimethamine, dolutegravir, cisplatin), selective MAO-B inhibitors (rasagiline, selegiline, safinamide), 5-HT1A inhibitors (e.g. pindolol), and adrenergic α1A inhibitors (e.g. silodosin, tamsulosin). If a participant requires the use of any medications and supplements during the study, the Investigator will contact the Sponsor and the Medical Monitor to discuss the participant's continued involvement in the study. Other medications to treat TEAEs may be prescribed if deemed necessary by the Investigator. If medications are used, the name of the drug, the dose and dosage regimen, route of administration, indication and start/stop dates will be recorded in the eCRF.

6.8 Dietary and Other Restrictions

As described in the exclusion criteria, consumption of red wine, Seville oranges, grapefruit, or grapefruit juice, pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices containing such products are excluded from 7 days prior to the first dose of study medication. There are no other special requirements related to food and beverage intake during the study. Meals and snacks (such as decaffeinated coffee, herbal tea, fruit, crackers) will be provided according to study center's SOPs.

The use of alcohol and tobacco products is not allowed throughout the duration of the study.

Participants must be nonsmokers (including tobacco, e-cigarettes, and marijuana) and not use nicotine within 1 month prior to dosing in the study. Participants must have a negative cotinine test at check-in (Day -1), and refrain from smoking for the duration of the study.

Strenuous exercise is not allowed within 4 days prior to Day -1 and during the entire study.

Participants should avoid sun exposure from Day 1 of dosing till end of follow-up i.e., A1 (14 days, as maximum), A2, A3 & A4 (17 days, as applicable) and B1 & B2 (9 days) in view of the potential for phototoxicity of BWC0977.

Participants should not consume any foods containing poppy seeds within 48 hours (2 days) prior to screening and prior to Day -1 as this could cause a false positive drug screen result.

7. STUDY PROCEDURES AND ASSESSMENTS

7.1 Informed Consent

According to the ICH guideline for GCP (E6) and all institutional local, state, and federal laws, the Investigator will obtain and document informed consent for each volunteer screened for this study. All participants will be informed in writing of the nature of the protocol and Investigational Drug, its possible hazards, and their right to withdraw at any time, and will sign a form (ICF) indicating their consent to participate in the study prior to the initiation of study procedures. The participant's medical record should contain written documentation indicating that informed consent was obtained. The ICF must be reviewed and approved by the Investigator's designated IRB/IEC and by Bugworks Research Inc. designee prior to its use. Refer to Section 12.2.6 for further details regarding informed consent.

7.2 Pregnancy Testing

Serum β-human chorionic gonadotropin pregnancy (β-hCG) testing is to be performed for female participants of childbearing potential (i.e., Premenopausal, or not surgically sterile) at screening and a urine pregnancy test before study drug infusion at Day -1. Any female participant with a positive result is not eligible for study participation. All samples will be analyzed by the local laboratory. Any participant determined to be pregnant cannot participate in the study.

7.3 Medical History and Prior Medications

At Screening, a complete medical history will be collected by participant interview. Medications and supplements, recent blood donations, illnesses, and participation in other Investigational Drug trials or clinical trials will also be recorded.

7.4 Pharmacokinetic & Safety Assessments

7.4.1 Multiple Ascending Dose PK Assessment:

The exact schedule of blood and urine sampling schedule will be fixed based on the dosage regimen to be followed post data review from Part A. However tentative schedule to be planned is as follows:

Blood:

Blood (plasma) for PK analyses will be collected as per Appendix I.

Urine:

Urine for PK analyses will be collected as per the SoA (refer to Section 7.5).

7.4.2 Single Ascending Dose PK Assessment:

Blood:

The sampling schedule is planned to provide an adequate estimation of C_{max} and to cover the plasma concentration-time curve long enough to provide a reliable estimate of the extent of absorption. Blood (plasma) for PK analyses will be collected as per the Table 7.

Urine:

Urine samples will be collected as per the Table 7 (Section 7.5).

7.4.3 Pharmacokinetic Blood Sample Analysis of BWC0977

Blood samples (approximately 6 mL) to provide a minimum of 3 mL plasma for PK analysis will be collected at each nominated timepoint into appropriately labeled tubes.

The actual times may change but the number of samples will remain the same. All efforts will be made to obtain the PK samples at the exact nominal time relative to dosing. However, for Part B, samples obtained within ± 2 minutes of the nominal timepoint up until 20-minutes post-dose and within 10% of the nominal time thereafter (e.g., within 6 minutes of a 60-minute sample) from dosing will not be captured as a protocol deviation, as long as the exact time of the sample collection is noted on the source document and data collection tool (e.g., eCRF). For sampling windows for Part A, refer to Appendix 1 and for Part B, refer to Appendix II

Instructions for collecting and processing serum samples are provided in the study Laboratory Manual.

Samples will be analyzed using a validated analytical method in compliance with standard operating procedures applicable at **Agilex BioLabs, Adelaide**.

7.4.4 Safety Assessment

7.4.4.1 Weight and Height

Weight will be measured at visits as described in the schedule of events (Section 7.5). Height will be measured at Screening only with the participant wearing no shoes.

7.4.4.2 Vital Signs

Vital signs (respiratory rate, supine SBP/DBP, temperature and HR) will be recorded at visits as described in the schedule of events (Section 7.5). Blood pressure and heart rate will be measured after the participant has been supine for at least 5 minutes in a quiet environment and prior to any blood draw that occurs at the same time point.

7.4.4.3 Physical Examination

A physical examination will be performed at visits as described in schedule of events (Section 7.5), or in case of Early Withdrawal.

A complete physical examination will include the following: General Appearance, HEENT, Neck (incl Thyroid & Nodes), Cardiovascular, Respiratory, Gastrointestinal, Renal, Neurological, Musculoskeletal, Skin and any other focused assessments suggested by the presence of specific symptoms.

Otherwise, a symptom-directed physical examination will be performed at visits as described in schedule of events (Section 7.5).

7.4.4.4 12- Lead ECG

A triplicate 12-lead ECG will be performed at Screening, Day -1 and at the end of follow-up. A single standard 12-lead ECG will be obtained at other visits. The 12-lead ECG will be recorded after the participant has been resting at least 5 minutes in the supine position in a quiet environment. ECGs will be read for ventricular HR, PR interval, QRS, QT and QTcF (Federicia's) intervals and CS abnormalities. At the Investigator's discretion, an additional ECG may be performed at any timepoint; however, it will be recorded in source documents as an unscheduled assessment.

7.4.4.5 Holter Monitoring

Continuous Holter monitoring will be performed for all SAD Cohorts and MAD Cohorts 1, 3 and 4. Holter monitoring will commence at least 1 hour pre-dose and will continue until approximately 24 hours post-dose on Day 1 for SAD and MAD. For MAD cohorts, a second Holter monitoring period will commence at least 1 hour pre-dose of 1st infusion on the last day of dose as per SoA, and continue until approximately 24 hours post-first dose of the day, as indicated in the SoA. Subjects will rest for at least 10 minutes prior to Holter ECG extraction timepoints, and for at least 5 minutes after.

The 12-lead Holter and ECG equipment will be supplied and supported by Clario. All ECG data will be collected using a Global Instrumentation (Manlius, NY, USA) M12R ECG continuous 12 lead digital recorder. The continuous 12-lead digital ECG (Holter) data will be stored onto SD memory cards. ECGs to be used in the analyses will be selected by pre-determined time points as defined in the SoA and will be read centrally by Clario.

The following principals will be followed in ERT's core laboratory:

- ECG readers are blinded to the subject, visit and treatment allocation
- A limited number of readers will be employed for the study
- Baseline and on-treatment ECGs for a particular subject will be over-read on the same lead and will be analyzed by the same reader.
- The primary analysis lead is lead II. If lead II is not analyzable, then primary lead of analysis will be changed to another lead for the entire subject data set.

The following is a brief description of ECG analysis methods utilized by ERT's core laboratory.

TQT Plus ECG Extraction Technique:
Ten 14-second digital 12-lead ECG tracings will be extracted from the continuous Holter recordings using the 'TQT Plus method', a computer-assisted and statistical process utilized by ERT. The method enables extraction of ECGs with the lowest HR variability and noise within the protocol-specified extraction time window (e.g., the HR and QT changes from beat-to-beat in the range of <10%). At each protocol-specified timepoint, 10 ECG replicates will be extracted from a 5-minute "ECG window" (typically, the last 5 minutes of the 10-minute period when the subject is maintained in a supine position).

Expert-Precision QT Analysis:
Expert-precision QT analysis will be performed on all analyzable (non-artifact) beats in the 10 ECG replicates. Statistical quality control procedures are used to review and assess all beats and identify "high" and "low" confidence beats using several criteria, including:

- QT or QTc values exceeding or below certain thresholds (biologically unlikely).
- RR values exceeding or below certain thresholds (biologically unlikely).
- Rapid changes in QT, QTc or RR from beat to beat.

Measurements of all primary ECG parameters (QT, QTc, RR) in all recorded beats of all replicates that are deemed "high confidence" is performed using COMPAS software. All low confidence beats are reviewed manually and adjudicated using pass-fail criteria. The final QC assessment is performed by a cardiologist. The beats found acceptable by manual review are included in the analysis. The median QT, QTc, and RR value from each extracted replicate is calculated, and then the mean of all available medians from a nominal timepoint is used as the subject's reportable value at that timepoint.

Morphological analyses will be performed with a focus on detecting changes in T-wave morphology and appearance of abnormal U waves. The analyses will evaluate change-from-baseline (i.e., treatment-emergent changes).

The analysis results for T-wave morphology and U-wave presence will be summarized in frequency tables with counts and percentages for both number of subjects and number of time points. The number and percentage of subjects in each treatment group having changes from baseline that represent the appearance of the morphological abnormality will be summarized. The total number of time points having a particular change event will be summarized in terms of number and percentage based on the number of observed time points across all subjects within a treatment group.

For T-wave morphology and U-wave presence, treatment-emergent changes will be assessed, i.e., changes not present at baseline. For each category of T-wave morphology and of U-waves, the category will be deemed as present if observed in any replicate at the time point. For baseline, the

category will be deemed as present if observed in any replicate from all time points that constitute baseline.

7.4.4.6 Clinical Safety Laboratory tests

Blood and urine for clinical safety laboratory assessments will be collected and processed using standard procedures. A local laboratory will perform all clinical laboratory tests.

In the event of abnormal clinical laboratory values, the Investigator will make a judgment whether or not the abnormality is CS. The Investigator may repeat any laboratory tests as deemed necessary to confirm out of range results. Cotinine testing may be repeated once per timepoint, at the discretion of the PI, in the instance of a positive result.

The clinical safety labs will include the following hematology, serum chemistry, urinalysis, and other tests (Table 2):

Table 2. Clinical Laboratory Blood and Urinalysis Tests

Hematology			
Platelet Count	*RBC Indices*:	*Automated WBC Differential:*	
RBC Count	MCV	Neutrophils	
WBC Count (absolute)	MCH	Lymphocytes	
Reticulocyte Count	MCHC	Monocytes	
Hemoglobin		Eosinophils	
Hematocrit		Basophils	
Coagulation			
Prothrombin time (sec and INR)			
Activated partial thromboplastin time (aPTT)			
Clinical Chemistry			
Creatinine	Potassium	AST (SGOT)	Total and direct bilirubin
Glucose (random)	Chloride	ALT (SGPT)	Uric Acid
Sodium	Bicarbonate	GGT	Albumin
Urea	Calcium	Alkaline phosphatase	Total Protein
Phosphate		Lactate dehydrogenase	Lipid panel
CPK	Creatinine Clearance (calculated by Cockroft Gault formula)		Albumin:Creatinine ratio
FSH (at screening)			
Urinalysis			
urobilinogen	Specific gravity		
pH, glucose, protein, blood, and ketones by dipstick			
Microscopic examination (if blood or protein is abnormal and assessed as clinically significant)			
Sedimentary microscopy will be performed if blood or protein tests are abnormal and assessed as clinically significant. In such cases, microscopy will be performed for 1. White blood cells 2. Red blood cells 3. Hyaline casts 4. Granular casts 5. Cellular casts Any other cell types/casts found upon microscopic examination will be reviewed by the Pathology laboratory staff, and the Study Physician will be alerted if there are any concerns.			
Infection and pregnancy screening			
HIV (HIV1 HIV2)			
Hepatitis B (hBsAg)			
Hepatitis C (anti HCV antibody)			
Serum (screening and follow-up) or urine pregnancy test (Day -1) (β–HCG)			
Other tests (Screening and Admission Only)			
Alcohol levels (measured by an alcohol breathalyzer/breath test performed at the study site) and urine for drug screen (drug screen to include at minimum: Methamphetamine, Opiates, Cocaine, THC, Phencyclidine, Benzodiazepines, Barbiturates, Methadone, Tricyclic Antidepressants, Amphetamine; Urine cotinine test.			
Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; β–HCG = Beta-human chorionic gonadotropin; CPK = creatine phosphokinase; GGT = gamma; glutamyl transferase; hBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human; immunodeficiency virus; INR = international normalized ratio; MCH = mean corpuscular hemoglobin; MCHC = mean cell haemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell; SGOT = serum glutamic-oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; WBC = white blood cell.			

Additional tests may be necessary to ensure participant safety and to ensure an adequate follow-up following an AE.

7.4.4.7 Follicle Stimulating Hormone Test (FSH)

A serum FSH test will be performed to confirm postmenopausal status in women.

7.4.4.8 Infusion Site Reaction Evaluation

Assessments of infusion site reactions to monitor local tolerability to BWC0977 infusions will be performed at each study drug administration as indicated in the Schedule of Events (to be followed up the next day if infusion site reactions are present). If infusion site pain or infusion site reactions persist, they should be followed up at regularly scheduled visits until the symptoms resolve.

7.4.4.9 Total Volume of Blood Collected

Blood samples will be collected from each participant in Part A and Part B throughout the study resulting in the total blood volumes per participant as shown in **Table 3** and **Table 4**, respectively.

Additional blood may be collected from each participant at any time if required for safety reasons.

Table 3. Blood Sample Volumes to be Collected per Participant (Part A – MAD)

Cohort A1:

Sample Type	***Volume of blood per sample (mL)***	***Number of Samples per Participant***										
		Screen	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 13±1 (FU)**
Haematology	4	1	1	-	1	-	1	-	1	1	1	1
Serum chemistry [a]	9	1	1	-	1	-	1	-	1	1	1	1
Coagulation	3	1	1	-	1	-	1	-	1	1	1	1
Serology	9	1	-	-		-	-	-	-	-	-	-
PK samples	6 / 8[a]	-	-	11	2	1	1	1	1	11	2	-
Subtotal		**25 mL**	**16 mL**	**88 mL**	**32 mL**	**6 mL**	**22 mL**	**6 mL**	**22 mL**	**104 mL**	**32 mL**	**16 mL**
Total blood volume collected per participant		**369 mL**										

Abbreviations: FU = follow-up; PK = pharmacokinetic; MAD = multiple ascending dose.
[a] Includes serum pregnancy testing, where applicable
8 mL per sample on Day 1/Day 2 and Day 7/Day 8 to account for volume in the blood collection line.

Cohorts A2, A3 & A4 (10-day dosing):

Sample Type	***Volume of blood per sample (mL)***	***Number of Samples per Participant***												
		Screen	**Day -1**	**Day* 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day* 10**	**Day 11**	**Day 16±1 (FU)**
Haematology	4	1	1	-	1	-	1	-	1	-	1	-	1	1	1
Serum chemistry [a]	9	1	1	-	1	-	1	-	1	-	1	-	1	1	1
Coagulation	3	1	1	-	1	-	1	-	1	-	1	-	1	1	1
Serology	9	1	-	-	-	-	-	-	-	-	-	-	-	-	-
PK samples	6 / 8[a]	-	-	11	2	1	1	1	1	1	1	1	11	2	-
Subtotal		**25 mL**	**16 mL**	**88 mL**	**32 mL**	**6 mL**	**22 mL**	**6 mL**	**22 mL**	**6 mL**	**22 mL**	**6 mL**	**104 mL**	**32 mL**	**16 mL**
Total blood volume collected per participant		**393 mL**													
Abbreviations: FU = follow-up; PK = pharmacokinetic; MAD = multiple ascending dose.															
[a] Includes serum pregnancy testing, where applicable															
8 mL per sample on Day 1/Day 2 and Day 10/Day 11 to account for volume in the blood collection line.															
** for Cohort A4, on Day 1 and Day 10, the number of PK samples are 10. Hence, the total volume of blood loss is 377 ml.*															

Table 4. Blood Sample Volumes to be Collected per Participant (Part B – SAD)

Sample Type	***Volume of blood per sample (mL)***	***Number of Samples per Participant***					
		Screening	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 8±1**
Haematology	4	1	1	-	1	-	1
Serum chemistry [a]	9	1	1	-	1	-	1
Coagulation	3	1	1	-	1	-	1
Serology	9	1	-	-	-	-	-
PK samples	6 / 8[b]	-	-	18	1	1	-
Subtotal		**25 mL**	**16 mL**	**144 mL**	**22 mL**	**6 mL**	**16 mL**
Total blood volume collected per participant		**229 mL**					
Abbreviations: PK = pharmacokinetic; SAD = single ascending dose. *[a] Includes serum pregnancy testing, where applicable.* *[b] 8 mL per sample on Day 1 to account for volume in the blood collection line.*							

7.5 Schedule of Events

Table 5, **Table 6** and **Table 7** describe the daily schedule of events from Screening (-28 days to -2 days before randomization) through Day 13±1 (A1, 7 day dosing), and 16±1 (A2, A3 and A4, 10 day dosing) as per SoA (Part A) and 8±1 (Part B), respectively.

Table 5. Cohort A1 (MAD) Daily Schedule of Events from Screening through Day 13±1

Protocol Activity	Screen -28 to -2	Day -1	Day 1-7	Day 8	Day 13 ±1 day (FU)	Early Termination
Admission to/Discharge from CRU	Screening[a]	Check-in[b]	→	Check-out	→	X
Visit window (±)	N/A	N/A	0	0	N/A	N/A
Informed consent [c]	X					
Demographics	X					
Inclusion/Exclusion criteria	X	X	X[d]			
Complete medical history	X					
Interim medical history		X				
Height, weight, and BMI calculation	X	X[e]				
Vital signs [f]	X	X	X	X	X	X
Complete physical examination [g]	X				X	X
Symptom-directed physical examination[g]		X	X	X		
12-lead ECG [h]	X	X	X		X	X
Blood and urine for laboratory analyses[i]	X	X	X	X	X	X
Blood for HBsAg, HCV, HIV serology	X					
Breath test for alcohol	X	X				
Urine to test for drugs of abuse	X	X				
Cotinine test		X				
Pregnancy test [j]	X	X			X	X
Estimated CrCl	X	X [e]			X	
Randomization to study drug			X [k]			
Blood for PK analysis [l]			X	X		
Urine for PK analysis [m]			X	X		
Assess for adverse events [n]			X			
Assess for infusion site reactions [o]			X	X		X
Record all IV catheter site changes and the reason for change [p]			X	X		
Study Drug Administration			X [q]			
Holter Monitoring [r]			X			
Prior and concomitant medications [s]	X					

Abbreviations: BMI = Body mass index; CrCl = creatinine clearance; CRU = clinical research unit; ECG = electrocardiogram; FU = Follow-up; HbsAg = hepatitis B virus surface antigen; HCV = hepatitis C virus; HIV = Human immunodeficiency virus; IV = Intravenous; MAD = multiple-ascending dose; PK= Pharmacokinetic; SAD = single-ascending dose; UA = urinalysis.

Footnotes for Cohort A1, MAD:

a. Screening visit must occur within 28 days before randomization including collection of demographic data (sex, age, race/ethnicity);
b. Participants will be admitted to the CRU on Day -1 and confined to the CRU through 24 hours after the start of the Day 7 final study drug infusion (Day 8); discharge may occur after all assessments and procedures are complete on Day 8.
c. Informed Consent must be obtained prior to initiating any study-related assessments or procedures.
d. Inclusion/Exclusion criteria will be checked pre-dose on Day 1 only.
e. Measure weight, calculate BMI, and estimate CrCl on Day -1 if >24 hours after Screening visit. Measure weight and serum creatinine on same day for estimation of CrCl.
f. Vital signs include supine blood pressure (systolic and diastolic, recorded after lying supine for 5 minutes), heart rate, respiratory rate, and temperature. Vital signs will be obtained at Screening visit, on admission to the CRU on Day -1, within 1 hour before and 1, 2, 4, and 12 hours (±10 minutes each) after the start of the first study drug infusion on each dosing day, at 24 (±2) hours after the start of the first Day 7 study drug infusion (Day 8), and at the FU visit.
g. A complete physical examination will be performed at Screening visit and at the End of Study/FU visit. An abbreviated symptom directed physical examination will be performed on Day -1, within 4 hours before the start of the first study drug infusion on each dosing day, at 24 (±2) hours after the start of the final Day 7 study drug infusion (Day 8).
h. At the Screening visit and Day -1, triplicate ECGs will be obtained within a 15-minute period, separated by at least 1 minute. In addition, a standard single 12-lead safety ECG will be obtained within 1 hour before the start of the first study drug infusion and at 0.5 (±10 minutes), 2, and 6 hours (each ±30 minutes) after the start of the first study drug infusion. On Days 2, 4, 6, and 7, ECGs should be recorded 0.5 hour (± 10 minutes) after start of the first study drug infusion. At the FU visit, triplicate ECGs will be obtained within a 15-minute period, separated by at least 1 minute. All ECG recordings will be taken after at least 5 minutes in a supine, quiet-rest position and prior to obtaining any blood sample. Safety ECGs will be performed using site's 12-Lead equipment.
i. Blood samples will be collected for serum chemistry, hematology, coagulation tests,, and urine samples will be collected for UA (and urine microscopy if UA is positive for red blood cells, white blood cells, or protein and assessed as clinically significant) at Screening visit, upon admission to the CRU on Day -1, on Days 2, 4, 6 and 7 (at 4 hours after start of the study drug infusion on that day [(±10 minutes for blood samples, ±1 hour for urine samples]), on Day 8 (at 24 hours after first Day 7 infusion [±10 minutes for blood samples, ±1 hour for urine samples]), and at the FU visit. At screening, a serum FSH test will be performed to confirm postmenopausal status in women.
j. Females of child-bearing potential must have a negative serum pregnancy test (β–HCG) at screening visit and a negative urine pregnancy test on Day –1, and agree to comply with using a highly effective method of birth control from signing the consent form until 30 days after the last study drug administration. A serum pregnancy test will also be performed at the FU visit for females of child-bearing potential. To be considered not of childbearing potential, a female must have either a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy, or menopause (last menstruation >12 months and follicle-stimulating hormone test in menopausal range unless previous follicle-stimulating hormone result documented in medical history and part of source documentation.
k. Randomize participant on Day 1.
l. Obtain blood (plasma) for PK analyses. Refer to Appendix I for PK timepoint and collection window details.
m. Obtain urine for PK analysis immediately before (within 2 hours before) the start of the first study drug infusion on Day 1; at 0-3, >3-6,>6-8h and >8-12h after the start of the first study drug infusion on Day 1. On Day 7 (1st infusion of Day 7) obtain urine for PK analysis at pre-dose and at 0-3, >3-6,>6-8 and 8-12 hours after the start of the first drug infusion. Note that collection may occur past Day 7. Collect and record urine volumes for each time point.
n. Adverse events are captured after the participant signs the Informed Consent Form up to the FU visit.
o. Infusion site reactions to be assessed during and after each infusion: 15 minutes (±5 minutes), 30 minutes (±5 minutes) (i.e., at end of infusion), and 2 hours (±5 minutes) post start of administration. If infusion site pain or infusion site reactions persist, they should be followed up at regularly scheduled visits until the symptoms resolve.
p. Following dosing, record all IV site (dosing catheter) changes and the reason(s) for change throughout study.
q. BWC0977 dose and dosing frequencies in cohort A1 is 7-days BID.
r. Two continuous ECG recordings (Holter) will be performed for approximately 25 hours each, the first starting at least one hour pre-dose on Day 1, and the second starting at least one hour pre-the first dose on Day 7 (for

respective MAD Cohorts). Holter recordings will continue to approximately 24 hours post- Day 1 and Day 7 dose. A 12-lead safety ECG will be performed if abnormalities are observed during the continuous Holter monitoring. 12-lead ECGs will be extracted by the central ECG laboratory at 3 time points within one hour prior to dosing on Day 1 (Predose 1, 2 and 3) and at the following time points, paired with PK sampling: 5, 15, 30, 60, 75, and 90 minutes and 2, 4, 6, 8, 12, and 24 hours post-dose, and on Day 7 at 15 minutes prior to dosing and then paired with PK at 5, 15, 30, 60, 75 and 90 minutes and 2, 4, 6, 8, 12, and 24 hours post-dose. Subjects will be supinely resting for at least 10 minutes before and 5 minutes after each time point. When ECG extractions coincide with safety ECGs, vital signs assessment and blood draws, procedures will be carried out in said order – safety ECG, vital signs, and PK sampling/extraction ECGs.

s. Prior and concomitant medication history includes all medications taken from Day -28 before the start of the first dose of study drug through the FU visit as per SoA.

Table 6. Cohorts A2, A3 & A4 (MAD) Daily Schedule of Events from Screening through Day 16 ± 1 (10-day dosing)

Protocol Activity	Screen -28 to -2	Day -1	Day 1-10	Day 11	Day 16 ±1 day (FU)	Early Termination
Admission to/Discharge from CRU	Screening[a]	Check-in[b]	→	Check-out	→	X
Visit window (±)	N/A	N/A	0	0	N/A	N/A
Informed consent [c]	X					
Demographics	X					
Inclusion/Exclusion criteria	X	X	X[d]			
Complete medical history	X					
Interim medical history		X				
Height, weight, and BMI calculation	X	X[e]				
Vital signs [f]	X	X	X	X	X	X
Complete physical examination [g]	X				X	X
Symptom-directed physical examination [g]		X	X	X		
12-lead ECG [h]	X	X	X		X	X
Holter recording [i]			X	X		
Blood and urine for laboratory analyses[j]	X	X	X	X	X	X
Blood for HBsAg, HCV, HIV serology	X					
Breath test for alcohol	X	X				
Urine to test for drugs of abuse	X	X				
Cotinine Test		X				
Pregnancy test [k]	X	X			X	X
Estimated CrCl	X	X [e]			X	
Randomization to study drug			X [l]			
Blood for PK analysis [m]			X	X		
Urine for PK analysis [n]			X			
Assess for adverse events [o]			X			
Assess for infusion site reactions [p]			X	X		X
Record all IV catheter site changes and the reason for change [q]			X	X		
Study Drug Administration			X [r]			
Prior and concomitant medications [s]	X					

Abbreviations: BMI = Body mass index; CrCl = creatinine clearance; CRU = clinical research unit; ECG = electrocardiogram; FU = Follow-up; HbsAg = hepatitis B virus surface antigen; HCV = hepatitis C virus; HIV = Human immunodeficiency virus; IV = Intravenous; MAD = multiple-ascending dose; PK= Pharmacokinetic; SAD = single-ascending dose; UA = urinalysis.

Footnotes for Cohorts A2, A3&A4, MAD (10-day dosing):

a. Screening visit must occur within 28 days before randomization including collection of demographic data (sex, age, race/ethnicity);
b. Participants will be admitted to the CRU on Day -1 and confined to the CRU through 24 hours after the start of the Day 10 final study drug infusion (Day 11); discharge may occur after all assessments and procedures are complete on Day 11.
c. Informed Consent must be obtained prior to initiating any study-related assessments or procedures.
d. Inclusion/Exclusion criteria will be checked pre-dose on Day 1 only.
e. Measure weight, calculate BMI, and estimate CrCl on Day -1 if >24 hours after Screening visit. Measure weight and serum creatinine on same day for estimation of CrCl.
f. Vital signs include supine blood pressure (systolic and diastolic, recorded after lying supine for 5 minutes), heart rate, respiratory rate, and temperature. Vital signs will be obtained at Screening visit, on admission to the CRU on Day -1, within 1 hour before and 1, 2, 4, and 12 hours (±10 minutes each) after the start of the first study drug infusion on each dosing day, at 24 (±2) hours after the start of the first Day 10 study drug infusion (Day 11), and at the FU visit.
g. A complete physical examination will be performed at Screening visit and at the End of Study/FU visit. An abbreviated symptom directed physical examination will be performed on Day -1, within 4 hours before the start of the first study drug infusion on each dosing day, at 24 (±2) hours after the start of the final Day 10 study drug infusion (Day 11).
h. At the Screening visit and Day -1, triplicate ECGs will be obtained within a 15-minute period, separated by at least 1 minute. In addition, a standard single 12-lead safety ECG will be obtained within 1 hour before the start of the first study drug infusion and at 1, 2, and 6 hours (each ±30 minutes) after the start of the first study drug infusion. On Days 2, 4, 6, 8 and 10, ECGs should be recorded 1 hour (±30 minutes) after start of the first study drug infusion. At the FU visit, triplicate ECGs will be obtained within a 15-minute period, separated by at least 1 minute. All ECG recordings will be taken after at least 5 minutes in a supine, quiet-rest position and prior to obtaining any blood sample. Safety ECGs will be performed using site's 12-Lead equipment.
i. Two continuous ECG recordings (Holter) will be performed for Cohorts A3 and A4 for approximately 25 hours each, the first starting at least one hour pre-dose on Day 1, and the second starting at least one hour pre-the first dose on Day 10 (for respective MAD Cohorts). Holter recordings will continue to approximately 24 hours post- Day 1 and Day 10 dose. A 12-lead safety ECG will be performed if abnormalities are observed during the continuous Holter monitoring. 12-lead ECGs will be extracted by the central ECG laboratory at 3 time points within one hour prior to dosing on Day 1 (Predose 1,2 and 3) and at the following time points, paired with PK sampling: 30, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 12, and 24 hours post-dose, and on Day 10 at 15 minutes prior to dosing and then paired with PK at 30, 60, 75 and 90 minutes and 2, 2.5, 3, 4, 6, 8, 12, and 24 hours post-dose. Subjects will be supinely resting for at least 10 minutes before and 5 minutes after each time point. When ECG extractions coincide with safety ECGs, vital signs assessment and blood draws, procedures will be carried out in said order – safety ECG, vital signs, and PK sampling/extraction ECGs (Cohort A3 &A4 only).
   Note: 12 hours post-dose sample Holter reading is not applicable for Cohort A4.
j. Blood samples will be collected for serum chemistry, hematology, coagulation tests, and urine samples will be collected for UA (and urine microscopy if UA is positive for red blood cells, white blood cells, or protein and assessed as clinically significant) at Screening visit, upon admission to the CRU on Day -1, on Days 2, 4, 6, 8 and 10 (at 4 hours after start of the study drug infusion on that day [(±10 minutes for blood samples, ±1 hour for urine samples]), on Day 11 (at 24 hours after first Day 10 infusion [±10 minutes for blood samples, ±1 hour for urine samples]), and at the FU visit. At screening, a serum FSH test will be performed to confirm postmenopausal status in women.
k. Females of child-bearing potential must have a negative serum pregnancy test (β–HCG) at Screening visit and a negative urine pregnancy test on Day –1, and agree to and comply with using a highly effective method of birth control from signing the consent form until 30 days after the last study drug administration. A serum pregnancy test will also be performed at the FU visit for females of child-bearing potential. To be considered not of childbearing potential, a female must have either a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy, or menopause (last menstruation >12 months and follicle-stimulating hormone test in menopausal range unless previous follicle-stimulating hormone result documented in medical history and part of source documentation.
l. Randomize participant on Day 1.

m. Obtain blood (plasma) for PK analyses. Refer to Appendix I for PK timepoint and collection window details.
n. Obtain urine for PK analysis immediately before (within 2 hours before) the start of the first study drug infusion on Day 1; at 0-3, >3-6, >6-8h and 8-12 hours after the start of the first study drug infusion on Day 1. On Day 10 (1st infusion of Day 10) obtain urine for PK analysis at pre-dose and at 0-3, >3-6,>6-8 hours and 8-12 hours (8-12 h is applicable for A1-A3). After the start of the first drug infusion. Note that collection may occur past Day 10. Collect and record urine volumes for each time point.
o. Adverse events are captured after the participant signs the Informed Consent Form up to the FU visit.
p. Infusion site reactions to be assessed during and after each infusion: 15 minutes (±5 minutes), 2-hours (±5 minutes) (i.e., at end of infusion), and 4 hours (±5 minutes) post start of administration. If infusion site pain or infusion site reactions persist, they should be followed up at regularly scheduled visits until the symptoms resolve.
q. Following dosing, record all IV site (dosing catheter) changes and the reason(s) for change throughout study.
r. BWC0977 dose and dosing frequencies in the MAD cohorts A2 (750 mg) & A3 (1250 mg) (BID) and A4 (1000 mg) (TID).
s. Prior and concomitant medication history includes all medications taken from Day -28 before the start of the first dose of study drug through the FU visit as per SoA.

Table 7. Part B (SAD) Schedule of Events and Procedures

Protocol Activity	Day -28 to -2	Day -1	Day 1	Day 2	Day 3	Day 4-6	Day 8 ±1 day (FU)	Early Termin-ation
Admission to/Discharge from CRU	Screening[a]	Check-in[b]	→	→	Check out	→	X	X
Visit window (± days)	N/A	N/A	N/A	0	0		1	N/A
Informed Consent [c]	X							
Demographics	X							
Inclusion/Exclusion criteria	X	X	X					
Complete medical history	X							
Interim medical history		X						
Height, weight, and BMI calculation	X	X[d]						
Vital signs [e]	X	X	X	X	X		X	X
Complete physical examination [f]	X						X	X
Symptom-directed physical examination [f]		X	X	X	X			
12-lead ECG [g]	X	X	X	X			X	X
Holter recording [h]			X	X				
Blood and urine for laboratory analyses [i]	X	X		X			X	X
Blood for HBsAg, HCV, HIV serology	X							
Breath test for alcohol	X	X						
Urine to test for drugs of abuse	X	X						
Cotinine Test		X						
Pregnancy test[j]	X	X					X	X
Estimated CrCl	X	X [d]						
Randomization [k]			X					
Blood for PK analyses [l]			X	X	X			
Urine for PK analyses [m]			X	X	X			
Assess for adverse events [n]			X					
Assess for infusion site reactions [o]			X	X				X
Record all IV catheter site changes and the reason for change [p]			X	X				
Study Drug Administration [q]			X					
Prior and concomitant medications [r]	X							

Abbreviations: BMI = Body mass index; CrCl = creatinine clearance; CRU = clinical research unit; ECG = electrocardiogram; FU = Follow-up; HBsAg = hepatitis B virus surface antigen; HCV = hepatitis C virus; HIV = Human immunodeficiency virus; IV = Intravenous; PK= Pharmacokinetic; SAD = single-ascending dose; UA = urinalysis.

Footnotes for Table 7 (Part B SAD Cohorts B1 and B2)

a. Screening visit must occur within -28 days to -2 days before randomization including collection of demographics (age, sex, and race)

b. Participants will be admitted to the CRU on Day -1 and confined to the CRU through 48 hours (Day 3) after the start of study drug infusion (Day 1); discharge may occur after all assessments and procedures are complete on Day 3.

c. Informed Consent must be obtained before initiating any study-related assessments or procedures.

d. Measure weight, calculate BMI, and estimate CrCl on Day -1 if >24 hours after Screening visit. Measure weight and serum creatinine on same day for estimation of CrCl.

e. Vital signs include supine blood pressure (systolic and diastolic, recorded after lying supine for 5 minutes), heart rate, respiratory rate, and temperature. Vital signs will be obtained at Screening visit, on admission to the CRU on Day -1, within 1 hour before and 1, 2, 4, 6, 8, 10, and 12 hours (±10 minutes each) after the start of study drug infusion, at 24 hours (Day 2) and 48 hours (Day 3) (±2 hours each) after the start of study drug infusion, and at the FU visit. In the event of overlap between safety and PK sampling timepoints, then safety will take preference over PK.

f. A complete physical examination will be performed at Screening visit and at the End of Study/FU visit. An abbreviated symptom directed physical examination will be performed on Day -1, within 4 hours before the start of study drug infusion on Day 1; and at 24 hours (Day 2) and 48 hours (Day 3) (±2 hours each) after the start of study drug infusion.

g. At the Screening visit and Day -1, triplicate ECGs will be obtained within a 15-minute period, separated by at least 1 minute. In addition, a standard single 12-lead safety ECG will be obtained within 1 hour before the start of the first study drug infusion on Day 1 and at 1, 2, 6, and at 24 hours (Day 2) (±30 minutes each) after the start of study drug infusion. At the FU visit, triplicate ECGs will be obtained within a 15-minute period, separated by at least 1 minute. Safety ECGs will be performed using site's 12-Lead equipment. All ECG recordings will be taken after at least 5 minutes in a supine, quiet-rest position and prior to obtaining any blood sample.

h. A continuous ECG recording (Holter) will be performed for approximately 25 hours, starting at least one hour pre-dose on Day 1 (SAD Cohorts B1 & B2) and continuing until approximately 24 hours post-dose. 12-lead ECGs will be extracted by the central ECG laboratory at 3 time points within one hour prior to dosing (Predose 1, 2 and 3) and at the following time points, paired with PK sampling: 5, 10, 15, 20, 30, 45, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 10, and 12- and 24-hours post-dose. Subjects will be supinely resting for at least 10 minutes before and 5 minutes after each time point. When ECG extractions coincide with safety ECGs, vital signs assessment and blood draws, procedures will be carried out in said order i.e., safety ECG, vital signs, and PK sampling/extraction ECGs.

i. Blood samples will be collected for serum chemistry (including LFT's), hematology, coagulation tests, and urine samples will be collected for UA (and urine microscopy if UA is positive for red blood cells, white blood cells, or protein and assessed as clinically significant) at Screening visit, upon admission to the CRU on Day -1, and at 24 hours (Day 2) (±10 minutes for blood samples, ±1 hour for urine samples) after the start of study drug infusion, and at the FU visit. At screening, a serum FSH test will be performed to confirm postmenopausal status in women.

j. Females of child-bearing potential must have a negative serum pregnancy test (β–HCG) at Screening visit and a negative urine pregnancy test on Day –1 and agree to and comply with using a highly effective method of birth control from signing the consent form until 30 days after the last study drug administration. A serum pregnancy test will also be performed at the FU visit for females of child-bearing potential. To be considered not of childbearing potential, a female must have either a tubal ligation, hysterectomy, bilateral salpingo-oophorectomy, or menopause (last menstruation >12 months and follicle-stimulating hormone test in menopausal range, unless previous follicle-stimulating hormone result documented in medical history and part of source documentation.

k. Randomize participant on Day 1.

l. Obtain blood (plasma) for PK analyses immediately before (within 60 minutes before) the start of infusion of study drug; at 5, 10, 15, 20, 30, 45, 60, 75, and 90 minutes and 2, 2.5, 3, 4, 6, 8, 10, and 12 hours (Day 1) (window: window: ± 2 minutes of the nominal timepoint up until the 20-minute timepoint and within ±10% of the nominal timepoint thereafter); and at 24 hours (Day 2) & 48 hours (Day 3) (window: within ±10% of the nominal timepoint) after the start of study drug infusion. Refer to Appendix II for PK timepoints and collection windows

m. Obtain urine for PK analysis immediately before (within 2 hours before) the start of study drug infusion and at 0-3, >3-6, >6-8, >8-12, >12-24 hours (Days 1-2), >24-36 and >36-48 hours (Days 2-3) (±10 minutes each) after the start of study drug infusion; collect and record urine volumes for each time point.

n. Adverse events are captured after the participant signs the Informed Consent Form up to the FU visit.

o. Infusion site reactions to be assessed during and after the infusion: 15 minutes (±5 minutes), 2-hours (±5 minutes) (i.e., at end of infusion) and 4 hours (±5 minutes) post start of administration. If infusion site pain or infusion site reactions persist, they should be followed up at regular scheduled visits until the symptoms resolve.

p. Following dosing, record all IV site (dosing catheter) changes and the reason(s) for change throughout the study.

q. Study drug administration on Day 1.

r. Prior and concomitant medication history includes all medications taken from Day -28 before the start of study drug infusion through the FU visit as per SoA.

7.6 Review and Documentation of Medications and Supplements

All medications (including alternate or herbal medications) or supplements participants are taking or have taken within 28 days prior to Day 1 (first dose) through Day 17 of Part A (MAD, at the maximum) will be recorded in the participant's medical record and the prior and concomitant medication.

All medications or supplements participants are taking or have taken within 28 days prior to Day 1 (first dose) through Day 9 of Part B (SAD) will be recorded in the participant's medical record and the prior and concomitant medication.

All medications and supplements (other than study treatment) taken by the participant after screening /Day 1 through Day 17 assessments of Part A (MAD) will be recorded in the participant's medical record and the prior and concomitant medication.

Medications and supplements taken prior to screening that are no longer being taken at screening will be considered "prior" medications and supplements.

Medications and supplements should be recorded according to the generic name when possible. Any medication or supplement used should have an indication recorded, and for concomitant medications and supplements, this indication must be represented as either for the treatment of an AE, for the management of a pre-existing condition, or for prophylaxis or other reasons.

Dosage increases for any concomitant medication or supplement should be noted and the reason for the dosage increase should be recorded. The side effects of concomitant medications will be recorded as AEs.

Any participant whose condition becomes disqualifying during the course of the study may be treated for that condition. If the condition is suspected during Screening, the participant should not be enrolled. Treatment of the condition should be instituted according to the Investigator's/attending physician's judgment.

Medications that have no treatment intent but rather maybe part of supportive routine should also be recorded in the participant's medical record and eCRF. This may include local anesthetics, intravenous solutions to maintain fluid balance and keep access open, medications used for prophylaxis.

8. ADVERSE EVENTS AND SAFETY REPORTING

8.1 Safety and Tolerability Assessments

Safety and tolerability will be assessed on an ongoing basis by review of reported AEs, physical examinations, vital signs (including supine SBP/DBP, temperature, respiratory rate, and heart rate), ECGs, infusion site reactions and clinical safety labs (hematology, serum chemistry, coagulation and urinalysis). Assessments will be performed in accordance with the SoAs. (Section 7.5).

8.2 Definition of Adverse Event

An AE is defined in 21 CFR 312.32(a) as follows:

> Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug and does not imply any judgment about causality.

Worsening of a pre-existing medical condition, (i.e., diabetes, migraine headaches, gout) is to be considered an AE if there is either an increase in severity, frequency, or duration of the condition or an association with significantly worse outcomes.

Adverse events will be recorded from the time of written informed consent through the last follow-up visit, or after the end of the study, if thought to be related to study drug. Any clinically significant observations in results of clinical laboratory, 12-lead ECGs, vital signs, or physical examinations will be recorded as AEs.

An AE which occurs prior to (the first) administration of the study drug will be considered a pre-treatment AE.

A treatment emergent AE (TEAE) is defined as any AE that starts or worsens (in frequency or severity) following exposure to study drug.

The Investigator is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual participant represents a significant change from baseline. In general, abnormal laboratory findings without clinical significance (based on the Investigator's judgment) should not be recorded as AEs; however, laboratory value changes requiring therapy or adjustment in prior therapy are considered AEs.

8.3 Definition of Serious Adverse Event

A SAE is defined in 21 CFR 312.32(a) as follows:

An AE is considered "serious" if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death;
- Life-threatening AE;
- Inpatient hospitalization or prolongation of existing hospitalization;
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Congenital anomaly/birth defect.
- Is a medically important event or reaction

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An AE is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the participant at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

8.4 Eliciting and Reporting of Adverse Events

Participants will be asked in general terms about any AEs at regular intervals during the study. In addition, all AEs reported spontaneously during the course of the study will be recorded.

AE monitoring will start immediately following consent and will continue till end of follow-up. Any participant with a possible study treatment-related AE will be followed until resolution or stabilization of the event. Further, any SAE, whether or not related to study treatment, that occurs until the follow up visit following the last dose of study treatment, will be followed until resolution or stabilization of the event. This may require additional clinical assessments and laboratory tests. The follow-up results will be recorded in the participant 's source documentation and in the eCRF.

Participants will be instructed to report all AEs experienced during the study, and participants will be assessed for the occurrence of AEs throughout the study. At several time points before and after drug administration participants will be asked general, non-leading questions to determine the occurrence of AEs.

Medical conditions existing at Screening should be recorded as medical history. New or worsening pre-existing medical conditions or diseases are considered AEs if they arise or worsen after the Screening visit and should be recorded as AEs.

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required is an AE if it occurs or is detected

following the first dose of study treatment through follow-up visit. Conditions leading to planned surgical procedures are not AEs if the condition(s) was (were) known before study treatment. In the latter case, the condition should be reported as medical history.

8.4.1 Routine Reporting of Adverse Events

All AEs, whether or not associated with the study treatment, that are observed by the Investigator, other Investigational Site personnel, or those reported by the participant will be recorded in the participant's source documentation and on the AE page of the eCRF. Copies of the SAE case report form (CRF) pages or an SAE listing generated based on the eCRF pages will be submitted to the Sponsor at regularly scheduled intervals to allow the Sponsor to meet expedited regulatory reporting requirements under 21 CFR 312.32 (see Section 8.4.3 for further detail) and regular regulatory reporting requirements under 21 CFR 312.33 and any local regulations.

For each AE, the following information will be entered in the eCRF:

- Medical diagnosis of the event in standard medical terminology (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event);
- Date of onset of any new AE or worsening of a previously observed AE;
- Date of resolution of the event (or confirmation ongoing);
- Whether the event is serious (per definition in Section 8.3), and if so, the reason it is considered serious;
- Severity of AE (per definition in Section 8.6);
- Assessment of the attributability of the AE to the study treatment (per definition in Section 8.5);
- Action taken on account of the AE: No action; concomitant medications or therapies required; tests required; hospitalization required (or prolonged); treatment unblinded; and/or change in the study treatment administration or dose (i.e., whether the study treatment was temporarily interrupted or discontinued);
- Outcome of AE (per definition in Section 8.8).

Due to the coronavirus SARS-COV-2 (COVID-19) pandemic, Investigators must also ensure compliance with local governing legislation and reporting requirements associated with COVID-19 infections.

8.4.2 Suspected Adverse Reaction

A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of Investigational New Drug Application (IND) safety reporting, "reasonable possibility" and/or at least possibly related means there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

8.4.3 Reporting of Serious Adverse Events, Including Death

The Sponsor will adhere to all expedited regulatory reporting requirements as per 21 CFR 312.32 and applicable local regulations.

SAEs, including death due to any cause, which occur during this study or within 30 days following the last dose of the study treatment, whether or not related to the administration of study treatment, must be reported by the Investigator or other Investigational Site personnel to the Medical Monitor by telephone or email **within 24 hours of learning of the event**. The contact information for the Medical Monitor is provided below.

Medical Monitor:
Dr. Abhijeeth Chandrasekaran
Clinical Scientist
RxMD
320/1, Lloyds Road, Royapettah,
Chennai 600 014, India
Tel +91.44.2466 2270, Mobile: +91 99417 35679
Email: abhijeeth.chandra@rxmd.com

Sponsor designated safety officer details for SAE reporting:
Pilar Garzon / Elena Bercu / Alexander Lenov
Phone: +61 478 034 138
Email: safety@avancecro.com

Information regarding SAEs will be transmitted to the Sponsor and Sponsor's designated study safety officer using an SAE form as described in the study SAE report form completion guidelines.

If all information is not known at the time of initial reporting, an initial report should still be made. In the event there is a question as to whether the experience is serious, the information should be forwarded to the Medical Monitor for review. The Investigator is responsible for following up on completion of the SAE Form. The Investigator will submit substantiating data in hard copy form, such as diagnostic test reports and progress notes, to the Medical Monitor. In the case of fatality, autopsy reports will be furnished to the Medical Monitor as soon as available. If the Medical Monitor is informed of a SAE via a telephone call, preliminary information will be obtained, and the study site will be instructed to email an SAE Form. New or updated information on the SAE will be recorded on a new SAE form and sent to the Sponsor and Sponsor's designated study safety officer within 24 hours of the information being available.

The initial SAE Form and any subsequent follow-up SAE Forms submitted to provide more accurate, corrected, or new information must be signed by the Investigator. The Investigator and Investigational Site Personnel must make every reasonable effort to obtain, from other institutions,

if necessary, all supporting medical case records as needed to comply with expedited IND safety reporting requirements.

If the SAE involves expedited IND safety reporting (as determined by the Sponsor or designee), all supporting medical records must be submitted to the Sponsor or designee within 4 calendar days for death or life-threatening events, and 10 calendar days for all other events. In cases where medical records and supporting documentation are unobtainable, the Investigator must generate a narrative of the event, utilizing, when necessary, interviews with the participant, their family members and care givers as appropriate.

The Investigator must also promptly inform the governing IRB/IEC of the SAE in accordance with the governing IRB/IEC's requirements. If an SAE is determined by the Sponsor to be reportable to the FDA as an IND Safety Report (as defined in 21 CFR 312.32), it will be reported to FDA by the Sponsor or designee within the specified time frame. All IND Safety Reports will also be promptly provided to the Investigator for submission to their IRB/IEC. Similarly, any SAE that is determined by the Sponsor to require expedited reporting to other regulatory authorities will be reported to the appropriate authorities by the Sponsor or designee within the specified time frames, and will be provided to the Investigator for submission to their IRB/IEC.

The Investigator, Medical Monitor, and Sponsor will review each SAE report and evaluate the relationship of the adverse experience to study treatment and to underlying disease. Based on this assessment, a decision will be made concerning the need for further action. The primary consideration governing further action is whether new findings affect the safety of participants participating in the clinical trial. If the discovery of a new adverse experience related to the study treatment raises concern over the safety of continued administration of study treatment, the Sponsor will take immediate steps to notify the regulatory authorities.

Further action that may be required includes the following:

1. Alteration of existing research by modification of the protocol;
2. Discontinuation or suspension of the study;
3. Alteration of the informed consent process by modification of the existing consent form and informing current study participants of new findings;

8.4.4 Exposure *In Utero* Management and Reporting

In instances of pregnancies or suspected pregnancies identified or reported for any female participant (or male participant's female partner), including a positive pregnancy test regardless of age, following administration of study treatment, the pregnant female participant (or the male participant's female partner) will be advised to notify her healthcare provider. Study drug administration will be discontinued immediately in the event of a reported (or suspected) pregnancy in a female participant.

The Investigator will notify the Sponsor and designated study safety officer of this event and document the pregnancy on the EIU form as described in the study SAE Report Form Completion Guidelines.

Informed consent will be sought from the pregnant female participant (or male participant's female partner) in order to allow for the Investigator to conduct follow-up access and review of relevant medical records throughout the gestational period and on the infant following delivery. The Investigator shall follow-up newborn infants that have been exposed to investigational product (IP) in utero for a minimum of 12 months post-birth. Upon discovery of any congenital anomalies (or neonatal deaths) the Investigator shall submit a follow-up report to the Sponsor (and study safety officer) using an SAE Form (as per study Safety Reporting Plan) including information regarding the status of the newborn. A miscarriage or abortion or any congenital anomaly diagnosed in the infant exposed in utero shall also be reported by the Investigator to the study Safety Officer (and the Sponsor) using an SAE Form as described in the study SAE Report Form Completion Guidelines

8.4.5 Protocol Deviations Due to an Emergency or Adverse Event

Departures from the protocol will be determined as allowable on a case-by-case basis and only in the event of an emergency. The Investigator or other physician in attendance in such an emergency must contact the Medical Monitor as soon as possible to discuss the circumstances of the emergency.

The Medical Monitor, in conjunction with the Investigator, will decide whether the participant should continue to participate in the study. All protocol deviations and reasons for such deviations must be noted in the Clinical Trial Management System.

8.4.6 Reporting of Infusion Site Reactions

Infusion site reactions (ISRs) will be under close monitoring in the current study. Scoring of ISRs will be done through the visual infusion phlebitis (VIP) scale (5& 6). The VIP scale scores can range from 0, indicating no symptoms of phlebitis, to 5, with redness, swelling, pain along path of cannula, and palpable venous cord. Please refer Appendix III for details of the score.

Management depends on the severity. A score of 1 just needs observation. Scores of 2 or above may require resting with symptom-specific treatment. Symptoms such as redness, swelling or pain may be managed by measures which include but are not limited to: use of warm or cold packs as required, checking correct needle placement/length and alternative tapes/dressings to secure needle, and over the counter pain medications. More severe findings such as palpable cord like veins may need use of anti-coagulants. The overall management of ISRs will be as per the discretion of the treating PI.

8.5 Causality Assessment of Adverse Events

For all AEs, the Principal Investigator will provide an assessment of causal relationship to the study treatment (active or placebo). The causality assessment must be recorded in the participant's source documents and, on the AE, eCRF. Causal relationship will be classified according to the following criteria:

Relationship between Study Drug and AE:					
	Category				
AE (is):	None	Unlikely	Possibly	Likely	Definitely
Clearly the result of an external factor	Yes	No	No	No	No
Probable/possibly the result of another factor	No	Yes	Yes	No	No
Has a chronological relationship with the time of administration and /or represents a known reaction to Study Drug	No	No	Yes	Yes	Yes
Disappears or decreases after discontinuation of the Study Drug	NA	NA	NA	Yes	Yes
Recurs on renewed administration (re-challenge)	No	No	NA	NA	Yes or NA**
** A rechallenge is not required; if done, rechallenge would be expected to be positive, NA: Not Applicable					

8.6 Adverse Event Severity Assessment

The severity of each AE will be graded according to the FDA Guidance - Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (FDA Adult adolescent volunteer vaccine guidance). The severity of AEs that are not specifically listed in the FDA Guidance will be categorized according to the general guidelines for systemic illness (i.e., illness or clinical AE) provided in the FDA Guidance, as summarized in the table below.

General Guidelines for Severity Assessment of Clinical Adverse Event (7)

(Mild) Grade 1: No interference with activity
(Moderate) Grade 2: Some interference with activity not requiring medical intervention.
(Severe) Grade 3: Prevents daily activity and requires medical intervention
(Potentially life threatening) Grade 4: ER visit or hospitalization

Note the distinction between the severity and the seriousness of an AE. A severe AE is not necessarily a SAE. For example, a headache may be severe (prevents daily activity or requires use of narcotic pain reliever) but would not be classified as serious unless it met one of the criteria for SAEs, listed above in Section 8.3.

8.7 Expectedness of Adverse Event

An unexpected AE is defined in 21 CFR 312.32(a) as follows:

An AE is considered "unexpected" if it is not listed in the IB or is not listed at the specificity or severity that has been observed; or, if an IB is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

8.7.1 Serious and Unexpected Suspected Adverse Reaction

A suspected unexpected serious adverse reaction (SUSAR) is any event that meets all 3 of the following definitions:

1) suspected adverse reaction (Section 8.4.2);
2) serious (Section 8.3); and
3) unexpected (as described in text above, Section 8.7).

8.8 Assessment of Adverse Event Outcome

Outcome of AEs will be defined according to ICH Topic E2B, ICH Guideline.

- **Recovered/Resolved:** The participant has recovered fully from the AE without any remaining effects or impairment.
- **Recovering/Resolving:** The participant is recovering, but with an after effect possibly due to disease or treatment.
- **Recovered/Resolved with Sequelae**: The participant has recovered, but with an after effect possibly due to disease or treatment.
- **Not Recovered/Not Resolved:** The condition is still present.
- **Fatal:** Fatal should only be used when death is possibly related to the AE.
- **Unknown**: The primary outcome is not known at the time of the final assessment. If an outcome for an AE is not available at the time of the initial report, follow-up will proceed until an outcome is known or followed up to the Final Study Visit. The Investigator must continue to follow all SAEs and non-serious AEs considered to be at least possibly related to study drug either until resolution or the Investigator assesses them as chronic or stable. This follow-up may extend after the end of the study.

8.9 Clinical Findings

Any significant clinical findings will be followed until the condition returns to pre-study status, stabilizes, or can be explained as not being study treatment related. If the clinical finding is reported as an AE (per the criteria outlined in Section 8.2), the follow-up procedures for AEs defined above will apply.

9. STATISTICAL METHODS

This section describes the statistical methods to be used for the analysis and reporting of data collected under Protocol No. C002-2023-01. Additional details will be provided in the statistical analysis plan (SAP). Any major modifications of the primary endpoint definition and/or its analysis will be reflected in a protocol amendment.

A SAP will be prepared and finalized before database lock and analysis of data. Any deviations from the final SAP will be described and justified in the clinical study report. All statistical analyses will be performed using SAS® (SAS Institute Inc. Cary NC USA).

9.1 Healthy Volunteer Disposition

All participants screened and randomized will be accounted for. All post-randomization discontinuations will be summarized by reason for discontinuation. The number of participants screened and not randomized will be presented.

9.2 Analysis Populations

The study analysis populations will consist of:

- Randomized population: All participants will be analyzed who are assigned a randomization number in the Treatment phase.

- Safety population: All randomized participants will be analyzed who receive any study treatment in the Treatment phase.

- Pharmacokinetic Population: All randomized participants who have taken at least one dose of BWC0977 without protocol deviation affecting PK evaluation, and with available PK data to determine plasma concentrations of BWC0977 will be included in the PK data analysis.

9.3 Protocol Deviations

The criteria for protocol deviations considered major with the implication of data exclusions from the Per Protocol (PP) analysis will be determined prior to database lock and unblinding.

9.4 Trial Population

9.4.1 Demographics and other Baseline Characteristics

Baseline and demographic characteristics will be summarized for all participants in the safety population by treatment group and overall. Continuous variables will be displayed via summary statistics (mean, median, sample size, standard deviation, minimum, and maximum). Categorical variables will be summarized via counts and percentages.

9.4.2 Medical History, Concomitant Medication and Other Safety Evaluations

Medical history will be coded by system organ class (SOC) and preferred term (PT) using the currently available version of the Medical Dictionary for Regulatory Activities (MedDRA) and summarized for all participants in the population analysis sets by treatment group.

The number and percentage of participants with medical history conditions will be summarized by SOC and PT.

9.5 Statistical and Analysis methods

9.5.1 Sample Size Determination

This study is exploratory in nature; no pre-planned hypothesis testing is to be performed. The sample size determination is not based on statistical power considerations. The sample size has been selected to provide information on safety, tolerability and PK following single doses of BWC0977. Any *p*-values to be calculated according to the SAP will be interpreted in the perspective of the explorative character of this study. Dose cohort size selected for this study is 8 participants with a 6:2 ratio of active drug to placebo in a randomized manner in both Part A and Part B of the study. Additional participants may be added at a dose level to further evaluate safety and/or tolerability after discussions between the Bugworks study team and the investigator/s.

9.5.2 Pharmacokinetic Analysis

9.5.2.1 Derivation of Pharmacokinetic Parameters

PK parameters following single and multiple-dose administration will be derived from the concentration-time data, as data permits, using non-compartmental methods. Table 8 and Table 9 provide the details of derived plasma PK parameters. Table 10 and Table 11 provide the details of derived urine PK parameters.

The total amount of BWC0977 excreted in urine and dose amount recovered (as a percentage of total dose) will be determined from urine samples collected and amounts listed for each subject and summarized for each dose group.

A Statistical Analysis Plan (SAP) will be drafted and act as a guidance for PK and Statistical analysis. The SAP will override the PK & Statistical analysis methodology provided here.

Table 8. Multiple Dose Plasma PK Parameters

Parameter	Definition	Method of Determination
$AUC_{0-\tau}$	Area under the concentration-time profile from time zero to time tau (τ), the dosing interval	Linear/Log trapezoidal method
$C_{max, ss}$	Maximum plasma concentration at steady state	Observed directly from data
$C\tau$	Concentration at the end of the dosing interval	As observed from concentration data
C_{av}	Average concentration following repeat dosing	$AUC\tau/\tau$
T_{max}	Time for Cmax	Observed directly from data as time of first occurrence
CL	Clearance	Dose / $AUC\tau$
C_{min}	Lowest concentration observed during the dosing interval, following repeat dosing	Observed directly from data
t½	Terminal elimination half-life	Ln(2)/k_{el}, where k_{el} is the terminal phase rate constant calculated by a linear regression of the log-linear concentration-time curve. Only those data points judged to describe the terminal log-linear decline will be used in the regression.
Vdss	Steady-state volume of distribution	CL • MRT, where MRT is the mean residence time calculated as ($AUMC\tau/AUC\tau$ - Infusion duration/2); $AUMC\tau$ is area under the moment curve over the dosing interval.
R_O	Observed accumulation ratio based on AUC	$AUC\tau,ss$ / $AUC\tau$ Day 1
R_O, Cmax	Observed accumulation ratio based on Cmax	Cmax,ss / Cmax Day 1
$AUC_{0-\tau}(dn)$	Dose normalized $AUC_{0-\tau}$	$AUC\tau$ / Dose
$C_{max}(dn)$	Dose normalized Cmax	C_{max} / Dose
$C_{min}(dn)$	Dose normalized Cmax	C_{min} / Dose

Abbreviations: ss = steady state.

Note: Actual PK sampling times will be used in the derivation of PK parameters.

Table 9. Single Dose Plasma PK Parameters at Steady State

Parameter	Definition	Method of Determination
$AUC_{(0-t)}$	Area under the plasma concentration-time profile from time zero to the time of the last quantifiable concentration (Clast)	Linear/Log trapezoidal method
AUC(0-∞)	Area under the plasma concentration-time profile from time zero extrapolated to infinite time	AUClast + (Clast*/kel), where Clast* is the predicted plasma concentration at the last quantifiable time point estimated from the log-linear regression analysis.
C_{max}	Maximum plasma concentration	Observed directly from data
T_{max}	Time for C_{max}	Observed directly from data as time of first occurrence
t½	Terminal elimination half-life	Ln(2)/kel, where kel is the terminal phase rate constant calculated by a linear regression of the log-linear concentration-time curve. Only those data points judged to describe the terminal log-linear decline will be used in the regression.
Vz	Apparent volume of distribution	Dose / (AUCinf * kel)
Vdss	Volume of distribution at Steady state	CL • MRT, where MRT is the mean residence time calculated as (AUMCinf/AUCinf - Infusion duration/2); AUMCinf is area under the moment curve from time 0 extrapolated to infinity
CL	Clearance	Dose / AUCinf
AUClast(dn)	Dose normalized AUClast	AUClast / Dose
AUCinf(dn)	Dose normalized AUCinf	AUCinf / Dose
Cmax(dn)	Dose normalized Cmax	Cmax / Dose

Table 10. Multiple Dose Urine PK Parameters

Parameter	Definition	Method of Determination
$Ae_{0-\tau}$	Cumulative amount of unchanged drug excreted in urine over the entire collection interval	∑(Concentration*volume) Calculated for Days 1 and 10
Fe_{0-tss}	Cumulative fraction of the dose excreted as unchanged in urine over the entire collection interval (at steady state);	100* ($Ae_{0-\tau}$ dose) Calculated for Days 1 and 10
CLr_{ss}	Renal clearance (at steady state)	$Ae_{0-\tau}$/ $AUC\tau$ Calculated for Days 1 and 10

Table 11. Single Dose Urine Parameters

Parameter	Definition	Method of Determination
$Ae_{(0-last)}$	Cumulative amount of unchanged drug excreted in urine	∑(Concentration*volume)
Fe	Cumulative fraction of dose excreted unchanged in urine over the entire collection interval	100* ($Ae_{(0-last)}$/dose)
CLr	Renal clearance	$Ae_{(0-last)}/AUC_{(0-last)}$

9.5.2.2 Statistical Methods of PK analysis

The Plasma PK parameters for BWC0977 will be summarized descriptively by dose level. Plasma concentrations will be listed and summarized descriptively by dose and nominal PK sampling time. Individual participant, summary profiles (mean and median plots) of the Plasma concentration-time data will be plotted by treatment and PK sampling time. For summary statistics and summary plots, the nominal PK sampling time will be used. For individual participant plots, the actual PK sampling time will be used, whilst the pre-dose time will be set to zero. Plots will be presented on both linear-linear and log-linear scales.

Dose normalized parameters for BWC0977 will be plotted against dose and will include individual participant values and the geometric means for each dose. For SAD, AUC_{inf}, AUC_{last}, and C_{max} will be plotted; for MAD, AUC_{τ} and C_{max} (Day 1 and at steady state) and C_{min} (at steady state) will be plotted. These plots will be used to help understand the relationship between the PK parameters and dose.

A Statistical Analysis Plan (SAP) will be drafted and act as a guidance for PK and Statistical analysis. The SAP will override the PK & Statistical analysis methodology provided here.

9.5.2.3 Estimate of Variability

Following log-transformation, AUC_{inf} and C_{max} of BWC0977 for single dosing groups will be analyzed separately by mixed effect models fitting dose as fixed effect term. The repeat dose

groups will be analyzed separately by mixed effect models fitting dose, day, dose-by-day interaction as fixed effect terms and participant as a random effect term. Point estimate and 90% confidence interval (CI) will be constructed using the appropriate error term and then be exponentially back-transformed to provide point estimate and 90% CI of PK parameters at each dose level on Day 1. Model-based within- participant and between- participant coefficients of variations will be calculated based on appropriate error term.

Following log-transformation, AUC(0-τ) of BWC0977 on Day 7 (A1) and 10 (A2, A3 & A4) based on the regimen will be analyzed separately by mixed effect models fitting dose as a fixed effect. Point estimate and 90% CI will be constructed using the appropriate error term and then be exponentially back- transformed to provide point estimate and 90% CI of the PK parameters at each dose level. Model-based between- participant coefficients of variations will be calculated based on appropriate error terms.

9.5.2.4 Dose Proportionality

Dose proportionality of AUC_{inf} and C_{max} on Day 1 and for repeat dose groups AUC(0-τ) and C_{max} of BWC0977 on Day 7 (AI) or Day 10 (A2 to A4) will be assessed separately using the power model as described below:

$y = \alpha * dose^{\beta}$

where y denotes the PK parameter being analyzed and α depends on the random error in the repeat dose phase where participants take the study drug in a parallel-group fashion. Dose proportionality implies that β=1 and will be assessed by estimating β along with its 90% CI. The exponent, β, in the power model will be estimated by regressing the loge-transformed PK parameter on loge-transformed dose. The power model will be fitted by restricted maximum likelihood (REML) using Statistical Analysis Software (SAS) Proc Mixed, with a fixed effect term for dose. The PK parameter endpoint and the factor dose will be loge-transformed prior to the analysis. An estimate of slope (with corresponding 90% CI) will be provided as a measure of potential dose proportionality (i.e., a slope ≈ 1 implies dose proportionality).

In the case where dose proportionality is not established over the entire dosing range, secondary analysis of dose proportionality will be assessed for select doses over the higher dosing range with the power model or by pair-wise analysis of variance (ANOVA) using the SAS Mixed models procedure. If the secondary analysis using the pair-wise ANOVA approach is performed, a reference dose would be chosen based on the lowest clinically relevant dose over which the pharmacokinetics can be adequately described and the other doses would be treated as test doses.

9.5.2.5 Accumulation Ratio

For the repeat dose groups of Part A, the accumulation ratio (Ro) will be calculated as the ratio of AUC(0-τ) on Day 7 or 10 to AUC(0-τ) on Day 1 for each participant.

The dosing interval (τ) will be equal to 8 hours for TID regimen or 12 hours for BID or 24 hours for once daily. The accumulation ratio will be listed and summarized along with other PK parameters.

Following log-transformation, AUC(0-τ) of BWC0977 on Days 1 and 7 (A1), Day 1 and 10 (A2, A3 & A4) will be analyzed by a mixed effect model, fitting dose, day and dose-by-day interaction as fixed effects and subject as a random effect. For each dose, point estimate and 90% CI for the difference "AUC(0-τ) on Day 7 - AUC(0-τ) on Day 1 (A1)" and "AUC(0-τ) on Day-10 - AUC(0-τ) on Day 1 (A2, A3 & A4) will be constructed using the appropriate error term. The point estimate and associated 90% CI will then be exponentially back-transformed to provide point and 90% CI estimates for the ratios "AUC(0-τ) on Day 7: AUC(0-τ) on Day 1 (A1)" and "AUC(0-τ) on Day 10 - AUC(0-τ) on Day 1 (A2, A3 & A4). If dose-by-day interaction is not significant, then a single point estimate and 90% CI pooled across all doses for the ratio AUC(0-τ) on Day 7: AUC(0-τ) on Day 1 (A1) and "AUC(0-τ) on Day 10 - AUC(0-τ) on Day 1 (A2, A3 & A4) may be constructed with all estimates for each dose.

9.5.2.6 Steady State Assessment

To evaluate whether steady state was achieved, statistical analysis of steady-state trough concentrations (Cτ) will be performed after loge-transformation of Cτ on Days 4, 5, 6,7, 8, 9 and 10, as applicable. A mixed effect model will be fitted by dose and day (as a continuous covariate) as a fixed effect term and participant as a random effect term. The coefficients for the slope of the day effect on the loge-scale will be used to evaluate steady-state for each dose group. Using the pooled estimate of variance, the 90% CIs for the slope will be calculated. Distributional assumptions underlying the statistical analyses will be assessed by visual inspection of residual plots. Normality will be examined by normal probability plots, while homogeneity of variance will be assessed by plotting the residuals against the predicted values for the model. Alternative analyses of the data will be performed if any of the model assumptions appear to be violated. Time of occurrence of C_{max} (T_{max}) of BWC0977 will be separately analyzed using non-parametric Wilcoxon rank test to compute point estimates and associated 90% CIs for the median differences.

9.5.3 Cardiodynamic Evaluation

The cardiodynamic ECG endpoints include change-from-baseline in heart rate (HR), QTcF, PR and QRS (ΔHR, ΔQTcF, ΔPR and ΔQRS); placebo-corrected ΔHR, ΔQTcF, ΔPR and ΔQRS (ΔΔHR, ΔΔQTcF, ΔΔPR and ΔΔQRS); categorical outliers for HR, QTcF, PR, QRS; and frequency of treatment-emergent changes for T-wave morphology and U-wave presence.

The primary analysis will be based on concentration-QTc modeling of the relationship between the plasma concentrations of BWC0977 and change-from-baseline QTcF (ΔQTcF) with the intent to exclude an effect of placebo-corrected ΔQTcF (ΔΔQTcF) > 10 ms at clinically relevant plasma concentrations. The effect of BWC0977 on the placebo-corrected ΔQTcF, ΔHR (heart rate), ΔPR, and ΔQRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) will also be evaluated at each post-dosing time

point ('by-time point' analysis). In addition, an analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology and U-wave presence.

More details on cardiodynamic ECG evaluation will be described in a separate SAP.

9.6 Safety Evaluation

All safety data analysis will be performed on the safety analysis set, which includes all enrolled participants who receive at least one dose of study medication. Adverse events, ECGs including continuous cardiac monitoring, vital signs, infusion site reactions, physical examination and safety laboratory data will be presented in tabular and/or graphical format and summarized descriptively, where appropriate.

Summary statistics and data listings will be provided for the following endpoints:

- Incidence of dose limiting or intolerable treatment related AEs.
- Incidence, severity, and causal relationship of treatment emergent AEs (TEAEs).
- Incidence of abnormal laboratory findings (clinical chemistry, hematology, coagulation and urinalysis).
- Changes from baseline in safety laboratory assessments.
- Abnormal and clinically relevant changes in vital signs, ECG parameters.

9.6.1 Adverse Events

AEs will be coded using the most current version of MedDRA. The severity of AEs will be graded according to the schema presented in Section 8.6. TEAEs will be collected starting with Day 1 through Day 13±1 (A1), and 16±1 (A2, A3&A4, 10-day dosing) (Part A, as maximum) and Day 8±1 (Part B). AEs occurring between screening and Day 1 will be regarded as “pretreatment” if they occur before IP administration at Day 1. TEAEs are defined as any AE that starts or worsens (increases in frequency or severity) after the first randomized dose of study IP on Day 1.

The incidence of TEAEs will be tabulated by MedDRA preferred term, system organ class, treatment group, severity, and assigned relationship to study treatment. The incidence for each TEAE will be provided as the total number of participants that experienced the TEAE, as well as the percentage of the population that this represents. If a TEAE is reported more than once for a given participant, the greatest severity and the worst-case attribution will be presented in the summary tables.

TEAEs will be listed for individual participants, along with information regarding onset dates and end dates, onset time where available, severity, seriousness, relationship to study treatment, action taken, and outcome. A similar listing will be prepared for the pretreatment AEs.

Pretreatment AEs and TEAEs that lead to withdrawal from the study will be separately listed and summarized. Similarly, separate tabulations and listings will be prepared for pretreatment and treatment-emergent SAEs.

Descriptive statistics will be generated as appropriate (i.e., frequency for categorical data). Inferential statistical analysis comparing the AE data between active and placebo is not planned. However, crude incidence rates will be provided

9.6.2 Laboratory Evaluations

Individual clinical safety lab (hematology, coagulation, serum chemistry, and urinalysis) values will be listed by treatment and summarized using descriptive statistics as appropriate (i.e., mean, median, range, and standard deviation for continuous data and frequency for categorical data). Individual change from baseline (Screening) in laboratory values will be calculated and summarized descriptively. A clinically significant change from baseline (Screening) will be recorded as an AE if deemed appropriate by the Investigator.

9.6.3 Vital Signs

Individual vital sign measurements (respiratory rate, supine SBP/DBP, temperature and heart rate) will be listed by measurement time and summarized using descriptive statistics as appropriate (i.e., mean, median, range, and standard deviation). Individual change from baseline (Screening) in vital sign measurements will be calculated and summarized descriptively. A clinically significant change from baseline (Screening) will be recorded as an AE if deemed appropriate by the Investigator.

9.6.4 12-lead ECG

Individual 12-lead ECG assessments for each visit will be listed and summarized using descriptive statistics as appropriate (i.e., mean, median, range, and standard deviation). Individual change from baseline (Screening) in ECG measurements will be calculated and summarized descriptively. A clinically significant change from baseline (Screening) will be recorded as an AE if deemed appropriate by the Investigator.

9.6.5 Physical Examination

Individual physical examination findings will be listed for each visit in which a physical examination occurred. A clinically significant change from baseline (Screening) will be recorded as an AE if deemed appropriate by the Investigator.

9.6.6 Prior and Concomitant Medications and Supplements

Medications and supplements will be coded using the most current version of the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system.

All medications and supplements (other than study treatment) taken by the participant from Day 1 through Day 13/16 (Part A, as applicable) and Day 8 (Part B) will be considered “concomitant” medications and supplements. Medications and supplements taken prior to the first dose of BWC0977 on Day 1 that are no longer being taken at the time of the first dose of BWC0977 on Day 1 will be considered “prior” medications and supplements.

Concomitant medications and supplements will be listed for individual participants. A similar listing will be prepared for prior medications and supplements taken within 28 days prior to the first dose of study treatment. The frequency of use of these prior and concomitant medications and supplements will be summarized.

9.6.7 Handling of Missing, Unused, or Spurious Data

No substitution of missing data will be used in any calculations. Data points that appear to be spurious will be investigated and will not be excluded from the listings. An explanation will be given for all missing, unused, and spurious data in the relevant sections of the CSR.

10. DATA MANAGEMENT

10.1 Data Collection

All data required by the study protocol will be collected in a validated database according to the CRO's SOPs.

10.2 Electronic Data Capture

Data from the source documents will be entered into the EDC system by authorized Investigational Site personnel. Data Management staff, using both electronic and manual checks, will systematically check the data. Errors or omissions will result in queries (which can be issued by the Study Monitor or Data Management staff), which will be presented to the Investigational Site within the EDC system. The Investigational Site will resolve the queries within the EDC system. The Study Monitor and Data Management staff will review the responses as part of the query resolution process. The EDC system will track the queries with the corresponding responses.

Medications and supplements entered into the database will be coded in the EDC system using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. AEs and Medical History will be coded in the EDC system using MedDRA terminology.

Clinical safety laboratory blood and urinalysis samples will be processed by Australian Clinical Labs. All lab results will be sent electronically to the clinical site. The clinical laboratory results will be imported into the database.

10.3 Quality Assurance and Database Lock

A 100% critical variable review of all key safety and secondary endpoint data in the database will be performed. Following this review, a data quality control audit, or a random sample equal to the square root plus 1 of the total population will be performed.

When the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement between the Sponsor, the Investigator, the lead data manager, and the study biostatistician.

11. AMENDMENTS/MODIFICATIONS OF THIS PROTOCOL

The Investigator will ensure that the study is conducted in accordance with the procedures and evaluations described in this protocol. As the study progresses it may become necessary to change or modify parts of the protocol. The Sponsor or designee is responsible for submitting protocol amendments to the appropriate government regulatory authorities. The Investigator is responsible for submitting protocol amendments to the appropriate IRB/IEC. Approval by the IRB/IEC must be obtained before changes are implemented.

When an emergency occurs that requires a departure from the protocol for an individual, a departure will be only for that participant. The Investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact the Medical Monitor immediately by telephone. Such contacts will be made as soon as possible to permit a decision as to whether or not the participant (for whom the departure from protocol was affected) is to continue in the study. The eCRF and source documents will completely describe the departure from the protocol and state the reasons for such departure. In addition, the IRB will be notified in writing of such departure from protocol.

12. ETHICAL, LEGAL AND ADMINISTRATIVE CONSIDERATIONS

12.1 Regulatory Documentation

Before the trial starts, Essential Documents as defined in ICH E6 will be generated and placed in both the Investigator's and Sponsor's files. Additional Essential Documents will be added to both files as new information becomes available and at the completion or termination of the trial as defined in ICH E6.

12.2 Protection of Human Participants

12.2.1 Declaration of Helsinki

The Investigator will conduct this study in accordance with the Declaration of Helsinki (1964) including all amendments up to and including the October 2013 revision.

12.2.2 Good Clinical Practice and Regulatory Compliance

The Investigator will conduct this study in accordance with the principles of GCP (current ICH guidelines) and the requirements of all local regulatory authorities regarding the conduct of clinical trials and the protection of human participants.

The study will be conducted as described in the approved protocol, with amendments and in accordance with the obligations of clinical Investigators set forth in the Form FDA 1572 and in 21 CFR 50, 54, 56 and 312 as applicable to Australia.

12.2.3 Independent Ethics Committee / Institutional Review Board

The Investigator is responsible for the submission of the protocol, ICF, and other written materials (such as advertisements and diaries), along with relevant supporting data (e.g., IB), to the appropriate IEC/IRB for review and approval before the study can be initiated. The Investigator is also responsible for submitting amendments to the protocol and ICF to the IRB/IEC for review and approval prior to implementation of the change. The Investigator is responsible for providing the Sponsor with a letter documenting the IEC/IRB approval prior to initiation of the study or implementation of the changes, respectively.

The Investigator will not have authority to implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard to study participants. Any significant deviation from the approved protocol will be documented in the source documents and eCRF.

Any deviation or change to the protocol required to eliminate an immediate hazard prior to obtaining IRB/IEC approval/favorable opinion, will be submitted as soon as possible to:

- IRB/IEC for review and approval/favorable opinion.
- The Sponsor via appropriate designees.
- Regulatory Authorities, if required by local regulations.

Documentation of IRB/IEC approval signed by the chairperson or designee of the IRB/IEC will be provided to the Sponsor via appropriate designees.

If an Amendment substantially alters the study design or increases the potential risk to the participant: (1) the ICF will be revised and submitted to the IRB/IEC for review and approval/favorable opinion; (2) the revised ICF will be used to obtain consent from participants currently enrolled in the study if they are affected by the Amendment; and (3) the new ICF will be used to obtain consent from any new participants prior to enrollment.

The Investigator is responsible for informing the IRB/IEC of all reportable AEs. IND Safety Reports provided by the Sponsor to the Investigator will be promptly forwarded to the IRB/IEC by the Investigator. Updates to the IB provided by the Sponsor to the Investigator will be submitted to the IRB/IEC by the Investigator.

The Investigator is also responsible for informing the IRB/IEC of the progress of the study and for obtaining annual IRB/IEC renewal. The Investigator must inform the IRB/IEC when the study is completed or terminated. After completion or termination of the study, the Investigator will submit the final clinical study report to the IRB/IEC, prepared by the Sponsor (or Sponsor's delegate). The structure and content of the report will meet that described in Structure and Content of Clinical Study Reports E3 (ICH Harmonized Tripartite Guideline, dated November 30, 1995).

12.2.4 Regulatory Notification

The requirements for the conduct of clinical trials in accordance with the applicable regulations of the Australian Therapeutic Goods Administration (TGA) under the Clinical Trial Notification scheme will be met before commencement of this study.

At the end of the study, the IEC and relevant regulatory authorities (TGA) will be notified by the sponsor (or delegate) according to applicable regulatory and IEC requirements.

12.2.5 Clinical Laboratory Certification and Reference Ranges

Before the initiation of this study, the PI, or nominee, will obtain a copy of the certification form, with certification number and expiration date for all clinical laboratories used in the study. Reference ranges for each clinical laboratory test used in this study will be obtained from the appropriate laboratory that will perform the test for the study.

12.2.6 Participant Informed Consent

The Investigator must comply with informed consent regulations (21 CFR Part 50) and relevant country, state, and local regulations.

The informed consent form (ICF) will be prepared by the Sponsor (or Sponsor delegate). The ICF will clearly describe the nature, scope, and potential risks and benefits of the study, in a language that the participant understands. The ICF will conform to all the requirements for informed consent according to ICH GCP and USFDA guidelines (21 CFR 50) and will include any additional elements required by the Investigator's institution or local regulatory authorities. The ICF will adhere to the ethical principles that have their origin in the Declaration of Helsinki.

Prior to the beginning of the study, the Investigator will obtain the IRB/IEC's written approval/favorable opinion of the written ICF. The IRB/IEC approved ICF will be given to each prospective participant. The participants will be given adequate time to discuss the study with the Investigator or site staff and to decide whether or not to participate. Each participant who agrees to participate in the trial and who signs the ICF will be given a copy of the signed, dated, and witnessed document. The original signed ICF will be retained by the Investigator in the study files.

The ICF and any other information provided to participants will be revised whenever important new information becomes available that is relevant to the participant 's consent, and the Investigator will obtain the IRB/IEC's written approval/favorable opinion prior to the use of the revised documents. The Investigator, or a person designated by the Investigator, will fully inform the participant of all pertinent aspects of the study and of any new information relevant to the participant 's willingness to continue participation in the study. Participants will read and sign any and all revised ICFs.

12.3 Pandemic Preparedness

Clinical sites should have in place procedures and strategies to accommodate the current COVID-19 pandemic (or other pandemics/epidemics/outbreaks as appropriate). Such procedures should include requirements in relation to criteria such as:

- Attendance (e.g., who is permitted to be on site during a pandemic, limitations, records of attendance, plans for suppliers and deliveries);
- Physical layout (e.g., physical distancing requirements and signage);

- Flexibility (e.g., procedures for scaling, ability to respond to outbreak);
- Support for remote interactions with Sponsors and study teams (e.g., communication including infrastructure);
- The local environment (e.g., contact with local health authorities, ability to access up-to-date pandemic information);
- Any other site-specific relevant criteria.

COVID-19 testing will be done for any participants who are clinically indicated as per the Investigators discretion and/or as directed by Local Health Authorities.

12.4 Participant Confidentiality

All information obtained during the conduct of the study with respect to the volunteers' state of health will be regarded as confidential. This is detailed in the ICF provided to the participant. An agreement for the use or disclosure of any such Personal health information (PHI) will be obtained from the participant in writing (e.g., HIPAA authorization or country-specific guidelines as applicable) prior to performing any study-related procedures. Disclosure of participant medical information obtained as a result of this study to third parties other than those noted below is prohibited.

Medical information resulting from a participant's involvement in this study may be given to the participant's personal physician or to the appropriate medical personnel responsible for the participant's welfare. Data generated as a result of this study are to be available for inspection on request by FDA or other government regulatory agency auditors, the Sponsor (or designee), and the IRB/IEC.

The information developed in this clinical study will be used by the Sponsor in the clinical development of the Study treatment and therefore may be disclosed by the Sponsor as required for disclosure as a public company to other clinical investigators, to other pharmaceutical companies, to the FDA, and to other government agencies. All reports and communications relating to volunteers in this study will identify each participant only by their initials and participant number.

12.5 Entering Data into EDC

All data required by the study protocol will be recorded in the electronic database provided by the EDC vendor. Data from the source documents will be entered into the EDC system by authorized Investigational Site personnel. The data will be updated at the time of each participant visit. Results of tests performed outside the Investigational Site will be entered as soon as available to the Investigational Site. The Principal Investigator must verify that all data entries are accurate and correct by electronically signing the participant's investigator signature screen.

12.6 Source Documentation

All data entered in the eCRF must be verifiable against source documentation. Source documents may include, but are not limited to, a participant's medical record, hospital charts, clinic charts, the Principal Investigator's study files, as well as the results of diagnostic tests.

12.7 Retention of Records

The Investigator has the responsibility of maintaining a comprehensive and centralized filing system containing all study-related documentation. These files must be available for inspection by the Sponsor or designee, the IRB/IEC, and regulatory authorities (i.e., FDA or international regulatory authorities) at any time and should consist of the Essential Documents as defined in ICH E6, which include, but are not limited to, the following elements:

- Participant files, containing the completed eCRFs, supporting source documentation from the medical record, including laboratory data, and the signed ICF;
- Regulatory files, containing the protocol with all amendments and Sponsor and Investigator signature pages, copies of all other regulatory documentation, and all correspondence between the site and the IRB/IEC, and Sponsor; and
- Drug accountability files, including a complete account of the receipt and disposition of the Study treatment (active and placebo).

The Investigator will retain all study records for at least 2 years after the last approval of a marketing application in an ICH region (i.e., United States, Europe, or Japan), and until there are no pending or contemplated marketing applications in an ICH region. If no application is filed or if the application is not approved for such indication, the Investigator will retain all study records for at least 2 years after the investigation is discontinued and regulatory authorities have been notified. The Sponsor will provide written notification when it is appropriate for the Investigator to discard the study-specific documents referenced above. The minimum retention time will meet the strictest standard applicable to that site for the study, as dictated by any institutional requirements or local laws or regulations, or the study Sponsor's, standards/procedures; otherwise, the retention period will default to 15 years.

The Sponsor will provide written notification when it is appropriate for the Investigator to discard the study-specific documents referenced above. The Investigator will notify the Sponsor prior to destroying any study records. Should the Investigator wish to assign the study records to another party or move them to another location, the Sponsor will be notified in writing in advance.

If the Investigator cannot guarantee this archiving requirement at the Investigational Site for any or all of the documents, special arrangements will be made between the Investigator and the Sponsor for storage. If source documents are required for continued care of the participant, appropriate copies for storage off site will be made.

12.8 Clinical Study Report

After completion or termination of the study, a clinical study report will be prepared. The structure and content of the report will meet that described in Structure and Content of Clinical Study Reports E3 (ICH Harmonized Tripartite Guideline, dated November 30, 1995).

The Principal Investigator and the Sponsor's representative must verify that all information and data in the clinical study report is accurate and correct by signing the clinical study report.

13. STUDY ADMINISTRATION

13.1 Study Monitoring

This study will be monitored by the Sponsor or designee to evaluate the progress of the study, to verify the accuracy and completeness of the eCRFs, to assure that all protocol requirements, applicable laws and/or regulations, and Investigator's obligations are being fulfilled, and to resolve any inconsistencies in the study records.

The Investigator will allow the Study Monitor to periodically review, at mutually convenient times during the study and after the study has been completed, all eCRFs and office, hospital, and laboratory records supporting the participation of each participant in the study.

The Study Monitor will compare the eCRF data against source documentation in order to verify its accuracy and completeness. The Investigator and Investigational Site staff will collaborate with the Study Monitor to resolve any identified data discrepancies in a timely manner.

The Study Monitor will record any protocol deviations identified, including, but not limited to, volunteers that were enrolled even though they did not meet all eligibility criteria, volunteers who took concomitant medications specifically prohibited by the protocol, participants who received the wrong study treatment or incorrect dose, and participants who failed to comply with the protocol-defined dietary restrictions. The Investigator and Investigational Site staff will collaborate with the Study Monitor to identify the reason for each protocol deviation.

The Study Monitor will compare the Investigational Site study treatment accountability record against the study treatment inventory (unused and used) at the site. The Investigator and Investigational Site staff will collaborate with the Study Monitor to resolve any identified discrepancies in a timely manner.

Each issue identified during study monitoring visits will be documented and reported to both the Sponsor and the Investigator.

13.2 On-Site Audits

The TGA, or other regulatory authorities, may request access to all study records for inspection and copying. The Principal Investigator and Investigational Site staff will cooperate with the inspectors and allow access to all source documents supporting the eCRFs and other study-related documents. The Investigator will immediately notify the Sponsor when contacted by any regulatory authority for the purpose of conducting an inspection.

The Sponsor or designee may also request to visit the Investigator's site to conduct an audit of the study. Prior to initiating this audit, the Investigator will be contacted by the Sponsor to arrange a convenient time for this visit. The Principal Investigator and Investigational Site staff will cooperate with the auditors and allow access to all source documents supporting the eCRFs and other study-related documents.

13.3 Data Quality Assurance

All eCRFs must be completed by authorized Investigational Site personnel who have undergone eCRF training. Data will be entered into the eCRF as information becomes available on a visit-by-visit basis. All data recorded on the eCRFs must be supported by source documentation. The Principal Investigator must verify that all data entries in the eCRF are accurate and correct by electronically signing and dating the eCRF.

All eCRF corrections must be made by the Principal Investigator or authorized Investigational Site personnel. The Principal Investigator must authorize changes to the recorded data, and this authorization must be documented in the source documents.

Refer to Section 10.3 for further details regarding Data Management quality assurance, including query generation and resolution, final data review, and database lock.

13.4 Publication Policy

All information and data obtained in the course of the study are the property of the Sponsor and are considered confidential. To avoid disclosures that could jeopardize proprietary rights, the institution and/or the Investigator agree to certain restrictions on publications (e.g., abstracts, speeches, posters, manuscripts, and electronic communications), as detailed in the clinical trial agreement.

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, HIPAA or equivalent country-specific laws.

This trial will be registered in a publicly accessible database (e.g., clinicaltrials.gov or country-specific registries) not later than 21 days after enrollment of the first participant. Results of this trial, including negative and inconclusive, as well as positive results, will be made publicly available.

13.5 Disclosure and Confidentiality

The information in this document is confidential and is not to be disclosed without the written consent of the Sponsor except to the extent that disclosure would be required by law and for the purpose of evaluating and/or conducting a clinical study for the Sponsor. You are allowed to disclose the contents of this document only to your IEC/IRB and study personnel directly involved with conducting this protocol. Persons to whom the information is disclosed must be informed that the information is confidential and proprietary to the Sponsor and that it may not be further disclosed to third parties

14. REFERENCES

1. https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (Accessed on 11 June 2020).
2. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf (Accessed on 11 June 2020).
3. Walia K, Madhumathi J, Veeraraghavan B, Chakrabarti A, Kapil A, Ray P, Singh H, Sistla S, Ohri V C. Establishing Antimicrobial Resistance Surveillance & Research Network in India: Journey so far. Indian J Med Res 2019; 149: 164-79.
4. BWC0977 Investigator's Brochure *(ver2.0 dt 22 Feb 2021).*
5. Jackson A. Infection control--a battle in vein: infusion phlebitis. Nurs Times. 1998;94(4):68, 71.
6. Gallant P and Schultz AA. Evaluation of a visual infusion phlebitis scale for determining appropriate discontinuation of peripheral intravenous catheters. J Infus Nurs. 2006 Nov-Dec;29(6):338-45.
7. FDA Guidance for Industry. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. September 2007.

15. APPENDICES

15.1 APPENDIX I. Pharmacokinetic Sampling Windows (Part A - MAD)

Cohort A1 (7 days BID dosing):

Part A – MAD		
Day	**Timepoint Post-infusion**	**Timepoint window**
1	Pre-dose	*Within 60 minutes prior to infusion*
	0	
	5 minutes	±1 minute
	15 minutes	±1 minute
	30 minutes	± 5 minutes
	60 minutes	± 5 minutes
	75 minutes	± 5 minutes
	90 minutes	± 5 minutes
	2 hours	± 5 minutes
	4 hours	± 15 minutes
	6 hours	± 15 minutes
	8 hours	± 15 minutes
	12 hours (Before start of 2nd infusion)	± 15 minutes
2	24 hours (Before start of 1st infusion of Day 2)	± 15 minutes
3	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
4	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
5	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
6	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
7	***Pre 1st infusion on Day 7*** Pre-dose	*Within 60 minutes prior to infusion*
	Post 1st infusion on Day 7 ***0***	
	5 minutes	±1 minute
	15 minutes	±1 minute
	30 minutes	± 5 minutes
	60 minutes	± 5 minutes
	75 minutes	± 5 minutes
	90 minutes	± 5 minutes
	2 hours (end of infusion)	± 5 minutes
	4 hours	± 15 minutes

Part A – MAD		
Day	**Timepoint Post-infusion**	**Timepoint window**
	6 hours	± 15 minutes
	8 hours	± 15 minutes
	12 hours (Before start of 2nd infusion)	± 15 minutes
8	24 hours	± 15 minutes
Total number of samples		30 Nos.

Cohort A2 & A3 (10-day BID dosing):

Part A - MAD		
Day	**Timepoint Post-infusion**	**Timepoint window**
1	Pre-dose	*Within 60 minutes prior to infusion*
	0	
	30 minutes	± 5 minutes
	60 minutes	± 5 minutes
	75 minutes	± 5 minutes
	90 minutes	± 5 minutes
	2 hours	± 5 minutes
	2.5 hours	± 5 minutes
	3 hours	± 15 minutes
	4 hours	± 15 minutes
	6 hours	± 15 minutes
	8 hours	± 15 minutes
	12 hours (Before start of 2nd infusion)	± 15 minutes
2	24 hours (Before start of 1st infusion of Day 2)	± 15 minutes
3	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
4	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
5	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
6	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
7	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
8	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
9	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*

Part A - MAD		
Day	**Timepoint Post-infusion**	**Timepoint window**
10	***Pre 1st infusion on Day 10***Pre-dose	*Within 60 minutes prior to infusion*
	0	
	30 minutes	± 5 minutes
	60 minutes	± 5 minutes
	75 minutes	± 5 minutes
	90 minutes	± 5 minutes
	2 hours (end of infusion)	± 5 minutes
	2.5 hours	± 5 minutes
	3 hours	± 15 minutes
	4 hours	± 15 minutes
	6 hours	± 15 minutes
	8 hours	± 15 minutes
	12 hours (Before start of 2nd infusion)	± 15 minutes
11	24 Hours	± 15 minutes
Total number of samples		33 Nos.

Cohort A4 (10-day TID dosing):

Part A - MAD		
Day	**Timepoint Post-infusion**	**Timepoint window**
1	Pre-dose	*Within 60 minutes prior to infusion*
	0	
	30 minutes	± 5 minutes
	60 minutes	± 5 minutes
	75 minutes	± 5 minutes
	90 minutes	± 5 minutes
	2 hours	± 5 minutes
	2.5 hours	± 5 minutes
	3 hours	± 15 minutes
	4 hours	± 15 minutes
	6 hours	± 15 minutes
	8 hours (Before start of 2nd infusion)	± 15 minutes
2	24 hours (Before start of 1st infusion of Day 2)	± 15 minutes
3	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
4	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
5	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*

Part A - MAD		
Day	**Timepoint Post-infusion**	**Timepoint window**
6	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
7	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
8	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
9	Pre-dose	*Within 60 minutes prior to start of 1st infusion on every day*
10	***Pre 1st infusion on Day 10*** Pre-dose	*Within 60 minutes prior to infusion*
	0	
	30 minutes	± 5 minutes
	60 minutes	± 5 minutes
	75 minutes	± 5 minutes
	90 minutes	± 5 minutes
	2 hours (end of infusion)	± 5 minutes
	2.5 hours	± 5 minutes
	3 hours	± 15 minutes
	4 hours	± 15 minutes
	6 hours	± 15 minutes
	8 hours (Before start of 2nd infusion)	± 15 minutes
11	24 Hours	± 15 minutes
Total number of samples		31 Nos.

15.2. APPENDIX II. Pharmacokinetic Sampling Windows (Part B - SAD)

Part B – SAD		
Day	**Timepoint Post-infusion**	**Timepoint window**
1	Pre-dose	*Within 60 minutes prior to infusion*
	0	
	5 minutes	± 2 minutes

Part B – SAD		
Day	**Timepoint Post-infusion**	**Timepoint window**
	10 minutes	± 2 minutes
	15 minutes	± 2 minutes
	20 minutes	± 2 minutes
	30 minutes	± 3 minutes
	45 minutes	± 5 minutes
	60 minutes	± 6 minutes
	75 minutes	± 8 minutes
	90 minutes	± 9 minutes
	2 hours	± 12 minutes
	2.5 hours	± 15 minutes
	3 hours	± 18 minutes
	4 hours	± 24 minutes
	6 hours	± 36 minutes
	8 hours	± 48 minutes
	10 hours	± 60 minutes
	12 hours	± 72 minutes (1 hour and 12 minutes)
2	24 hours	± 144 minutes (2 hours and 24 minutes)
3	48 hours	± 288 minutes (4 hours and 48 minutes)

15.3 Infusion Site Reaction (ISR) Evaluation Criteria

VIP Score	Description
0	IV site appears healthy
1	One of the following signs is evident: slight pain near IV site, slight redness near IV site
2	Two of the following are evident: pain at IV site, erythema, swelling
3	All of the following signs are evident: pain along path of cannula, erythema, induration
4	All of the following signs are evident and extensive: pain along path of cannula, erythema, induration, palpable venous cord
5	All of the following signs are evident and extensive: pain along path of cannula, erythema, induration, palpable venous cord, pyrexia