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PHARMACOLOGY

A Phase 1 Study of the Safety, Tolerability, and Pharmacokinetics of Single Ascending Doses of a First-in-Human Engineered Cationic Peptide, PLG0206, Intravenously Administered in Healthy Subjects

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ABSTRACT In this first-in-human study, PLG0206, a novel engineered cationic antimicrobial peptide, was evaluated for safety, tolerability, and pharmacokinetics (PK) when intravenously (i.v.) administered as a single dose to healthy subjects. Six cohorts of 8 subjects each received escalating single i.v. infusions of PLG0206 at 0.05, 0.125, 0.25, 0.5, or 1 mg/kg dose or placebo over 1 to 4 h. Subjects were randomized to receive either PLG0206 (6 per cohort) or placebo (2 per cohort). Serial pharmacokinetic samples were taken prior to infusion and up to 48 h postinfusion. Safety and tolerability were assessed throughout the study. The demographic characteristics of subjects were comparable between those treated with PLG0206 and placebo and between dose groups. The incidence of treatment-emergent adverse events (TEAE) related to PLG0206 was low, and most events were mild in severity and were similar between the PLG0206 treatment and placebo groups. The most common adverse events reported for PLG0206 were infusion-related reactions, which were mitigated with increasing infusion time and volume. There were no severe adverse events (SAEs), life-threatening events, or deaths throughout the study. i.v. PLG0206 exhibited linear pharmacokinetics over the dose range of 0.05 to 1.0 mg/kg. The median terminal half-life ($t_{1/2}$) ranged from 7.37 to 19.97 h. Following a single i.v. infusion to healthy subjects, PLG0206 was safe and well tolerated and exhibited linear PK at doses ranging from 0.05 to 1 mg/kg. These findings support the ongoing development of i.v. PLG0206 as an antimicrobial agent.

KEYWORDS PLG0206, antimicrobial peptides, pharmacokinetics

Antibiotic resistance is one of the biggest threats to global health and food security, and the development of new antibacterial drugs is of high priority (http://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance). Antimicrobial peptides (AMPs) are a novel antibacterial class of drugs that may be able to address antibiotic resistance. Naturally occurring AMPs are antimicrobial effector molecules that serve as a first line of defense against invading pathogens. The interactions of AMPs with their microbial targets are thought to occur electrostatically, mediated by polysaccharide decorations and lipid molecules on bacterial surfaces, resulting in the disruption of the membrane of bacterial cells. Due to their broad antimicrobial properties and unique mechanism of action, synthetic AMPs, which are rapidly bactericidal and broad-spectrum, with potent antibiofilm activity, have the potential to address the ongoing public health threat presented by pathogens that are resistant to standard antimicrobial agents (1, 2). However, to date, AMPs have not met with significant

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The authors declare a conflict of interest. David Huang, Despina Dobbins and Jonathan Steckbeck are employees of Peptilogics.

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TABLE 1 Baseline demographics for all subjects[b]

Cohort or pool	Age in yrs (mean [SD])	Sex (*n* [%])		Race (*n* [%])			Ethnicity (*n* [%])	
		Female	Male	Asian	White	Other	Hispanic or Latino	Not Hispanic or Latino
Cohort 1: PLG0206 0.05 mg/kg i.v. 1-h infusion (*N* = 6)	30 (9.1)	2 (33.3)	4 (66.7)	2 (33.3)	4 (66.7)	0	0	6 (100)
Cohort 2: PLG0206 0.125 mg/kg i.v. 1-h infusion (*N* = 6)	25.5 (5.8)	3 (50.0)	3 (50.0)	3 (50.0)	2 (33.3)	1 (16.7)	0	6 (100)
Cohort 3: PLG0206 0.25 mg/kg i.v. 1-h infusion (*N* = 5)[a]	30.8 (8.7)	2 (40.0)	3 (60.0)	1 (20.0)	4 (80.0)	0	1 (20.0)	4 (80.0)
Cohort 3b: PLG0206 0.25 mg/kg i.v. 2-h infusion (*N* = 6)	27.8 (5.7)	1 (16.7)	5 (83.3)	1 (16.7)	5 (83.3)	0	2 (33.3)	4 (66.7)
Cohort 4: PLG0206 0.5 mg/kg i.v. 2-h infusion (*N* = 6)	25.8 (7.6)	4 (66.7)	2 (33.3)	2 (33.3)	4 (66.7)	0	1 (16.7)	5 (83.3)
Cohort 5: PLG0206 1 mg/kg i.v. 4-h infusion (*N* = 6)	24 (4.9)	3 (50.0)	3 (50.0)	2 (33.3)	2 (33.3)	2 (33.3)	0	6 (100)
Pooled PLG0206 (*N* = 35)	27.2 (7)	15 (42.9)	20 (57.1)	11 (31.4)	21 (60.0)	3 (8.6)	4 (11.4)	31 (88.6)
Pooled placebo (*N* = 12)	25.4 (5.5)	8 (66.7)	4 (33.3)	1 (8.3)	10 (83.3)	1 (8.3)	0	12 (100)
Overall (*N* = 47)	26.8 (6.6)	23 (48.9)	24 (51.1)	12 (25.5)	31 (66.0)	4 (8.5)	4 (8.5)	43 (91.5)

[a]Dosing was halted after 5 subjects based on the Safety Review Committee decision.
[b]Summary statistics are presented as mean no. ± standard deviation for continuous variables and count (%) for categorical variables.

success due to several factors, particularly their toxicity when administered systemically and activity that is highly sensitive to pH and ion concentrations. PLG0206 is an engineered cationic antibiotic peptide that was specifically designed to address the toxicity and activity issues that occur with AMPs (3). This report presents the first-in-human (FIH) results of a phase 1 study to evaluate the safety, tolerability, and pharmacokinetic (PK) profile of PLG0206 when administered as a single intravenous (i.v.) infusion in healthy subjects.

RESULTS

Baseline demographics and characteristics for all subjects are summarized in Table 1.

All enrolled subjects met all inclusion criteria and none of the exclusion criteria for the study. In total, 47 subjects were included in the study; 35 subjects received treatment with PLG0206 and 12 received placebo. Only 7 of 8 subjects were enrolled in the originally planned cohort 3 and received 0.25 mg/kg infusion over 1-h. Only 7 participants were enrolled in cohort 3, as a Safety Review Committee decision was made to halt cohort 3 due to adverse events of infusion-related reactions (none of which were serious or severe) without enrolling all 8 planned participants. The cohort 3 dose level of 0.25 mg/kg was repeated with new subjects who received double the volume (i.e., an infusion at half the concentration by increasing the infusion volume from 50 mL to 200 mL) as a 2-h infusion (Table 1, cohort 3b). In addition, one subject in cohort 3 treated with PLG0206 did not complete the study as planned due to noncompliance with the assessment schedule (did not return for the day 7 follow-up visit); however, data from this subject were still included in both the safety and pharmacokinetic (PK) analysis populations. Two subjects in cohort 3 prematurely discontinued study drug administration (both received PLG0206) due to adverse events (AEs); these 2 subjects partially received the planned dose of PLG0206 and therefore were excluded from the PK population. Because of the adverse events observed in cohort 3, was were originally designed to infuse PLG0206 over 1 h, cohorts 4 and 5 were infused over 2 h and 4 h, respectively. Overall, all 47 (100%) randomized subjects were included in the safety population, and 33 (94.3%) of 35 subjects who received the complete planned dose of PLG0206 were included in the PK population.

Pharmacokinetic results. The PK of a single i.v. infusion of PLG0206 at doses ranging from 0.05 mg/kg to 1 mg/kg (administered over 1 to 4 h) were evaluated in 33 healthy volunteers. The PK of PLG0206 after i.v. administration exhibited linear behavior over the dose range of 0.05 to 1.0 mg/kg (Fig. 1) with a median terminal half-life

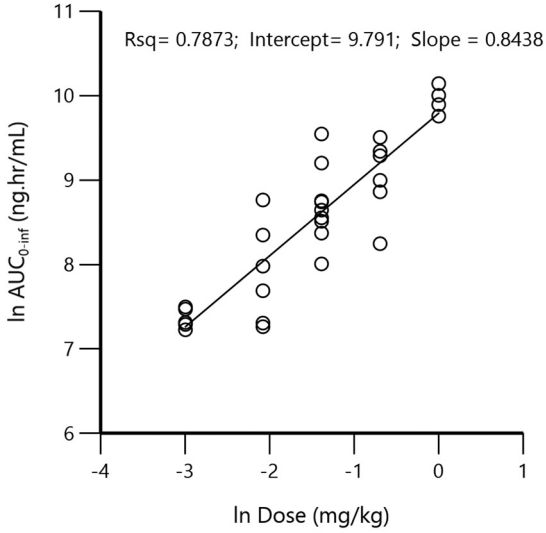


FIG 1 Plasma PLG0206 $AUC_{0-\infty}$ versus dose (natural log scale) and linear regression.

($t_{1/2}$) ranging from 7.37 to 19.97 h. The log of the area under the concentration versus time curve extrapolated to infinite time ($AUC_{0-\infty}$) increased proportionally with increasing log dose over the dose range of 0.05 to 1 mg/kg. The relationship was described by a linear function with a slope not significantly different from 1 (unity line slope), indicating that there was no evidence of significant dose nonproportionality (slope estimate = 0.84 [95% confidence interval (CI) = 0.67 to 1.01]).

Table 2 shows exposure parameters (AUC from time zero to time *t* [last measurable concentration] [AUC_{0-t}] and $AUC_{0-\infty}$) increased with increasing PLG0206 dose, with a mean AUC_{0-t} range between 1,283.74 and 21,612.56 h · ng/mL at 0.05 mg/kg and 1 mg/kg, respectively; $AUC_{0-\infty}$ ranged between 1,581.41 and 21,141.52 h · ng/mL at 0.05 mg/kg and 1 mg/kg, respectively. Mean clearance (CL) across cohorts ranged between 2.4 and 4.2 L/h, and mean apparent volume of distribution (V_z) ranged between 25.5 and 97.7 L. The mean percentage extrapolated AUC was 12.2% or less across cohorts.

The maximum observed plasma concentration (C_{max}) increased from 256 ± 58 ng/mL to 2,653 ± 719 ng/mL from cohort 1 (0.05 mg/kg i.v. 1 h) to cohort 5 (1 mg/kg i.v. 4 h); however, the dose proportionality of the C_{max} could not be determined due to differing infusion durations among dose cohorts. The mean apparent volume of distribution (V_z) increased from 25.49 L in cohort 1 to 94.2 L in cohort 5. The mean clearance (CL) values were similar across all PLG0206 doses and infusion times and ranged from 2.42 to 4.18 L/h. However, it was not possible to draw conclusions by comparing these parameters (C_{max}, time to maximum concentration of drug in serum [T_{max}], CL, and V_z)

TABLE 2 Summary of plasma PLG0206 PK parameters by dose group[a]

Cohort	PLG0206 dose	AUC_{0-t} (ng · h/mL)	$AUC_{0-\infty}$ (ng · h/mL)	C_{max} (ng/mL)	$t_{1/2}$ (hours)	%Extrap.	CL (L/hr)	V_z (L)
1	0.05 mg/kg i.v. 1-h infusion	1,284 (414)	1,581 (189)	256 (58)	7.37 (0.78)	11.2 (6.0)	2.4 (0.3)	25.5 (2.2)
2	0.125 mg/kg i.v. 1-h infusion	2,869 (1,764)	3,109 (1,919)	582 (239)	10.50 (3.26)	8.2 (4.0)	3.5 (1.4)	48.4 (13.5)
3	0.25 mg/kg i.v. 1-h infusion	7,159 (4,400)	8,186 (5,110)	1,216 (596)	19.94 (1.10)	12.2 (0.8)	2.5 (1.3)	70.5 (34.1)
3b	0.25 mg/kg i.v. 2-h infusion	5,434 (2,034)	5,847 (2,283)	943 (278)	13.16 (3.08)	6.5 (2.9)	3.4 (1.1)	60.7 (11.5)
4	0.5 mg/kg i.v. 2-h infusion	8,333 (3,135)	9,094 (3,467)	1,834 (467)	16.20 (4.40)	8.3 (3.9)	4.2 (1.6)	97.7 (48.9)
5	1 mg/kg i.v. 4-h infusion	21,613 (7,966)	21,142 (3,451)	2,653 (719)	19.97 (7.80)	9.8 (5.6)	3.1 (0.6)	94.2 (53.7)

[a]Data are presented as mean ± standard deviation. The statistics are calculated from 6 subjects, except for some parameters that were evaluable only in 5 subjects. Cohort 3 had only 3 subjects who had PLG0206 PK data. AUC_{0-t}, area under the concentration versus time curve from time zero to time *t* (last measurable concentration); $AUC_{0-\infty}$, area under the concentration versus time curve extrapolated to infinite time; C_{max}, maximum observed plasma concentration; $t_{1/2}$, terminal elimination half-life; %Extrap., percentage of $AUC_{0-\infty}$ calculated by extrapolation; CL, clearance; V_z, apparent volume of distribution.

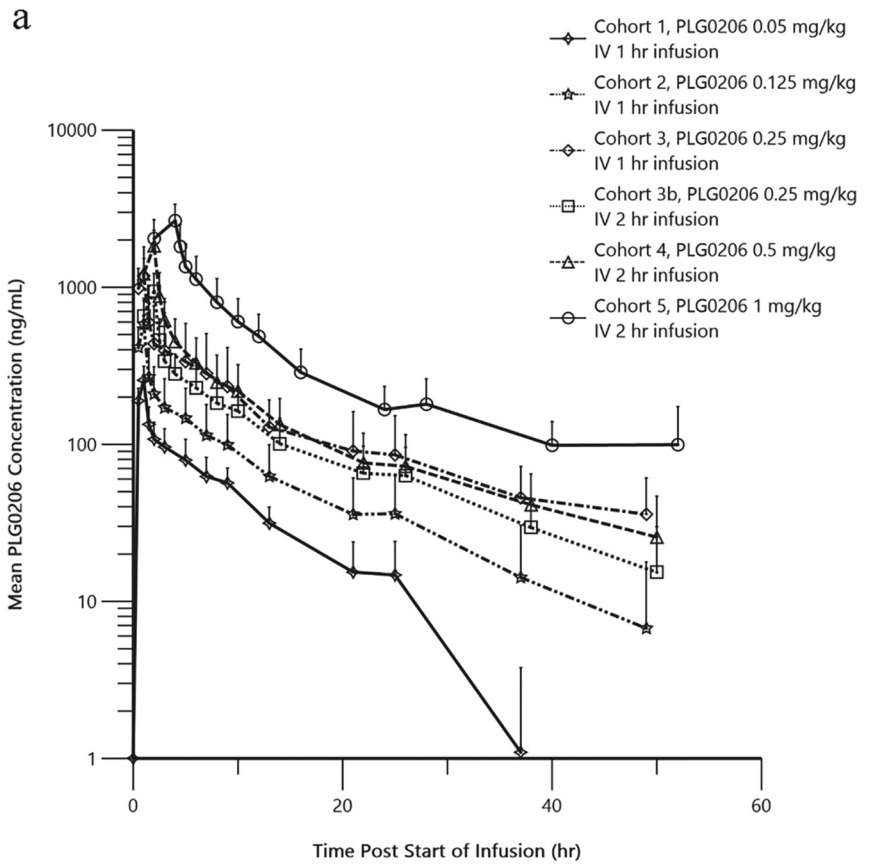
between the dose levels due to the differing infusion durations. Mean coefficient of variation (CV) % values for C_{max} and $AUC_{0-\infty}$ across dose groups were 32.5% and 38.3%, respectively. Figure 2 shows the mean PLG0206 plasma concentrations per dose group in linear and log scales. Most of the drug was cleared by 24 h postdose.

Safety and tolerability results. PLG0206 appeared to be safe and well tolerated when administered to healthy subjects at doses ranging from 0.05 to 1 mg/kg when drug concentration and dose rate remained below 0.5 mg/mL and 25 mg/h, respectively. There were no severe adverse effects (SAEs), life-threatening events, or deaths throughout the study. Overall, the frequencies of treatment-emergent adverse events (TEAEs) occurring in more than one subject was similar between the PLG0206 treatment and placebo groups. The incidence of TEAEs related to study drug administration was low, and most events were mild in severity. Infusion-related reactions (IRRs) were the most reported TEAEs related to PLG0206 and were observed when the concentration of PLG0206 was higher (approximately 0.25 mg/mL) with higher dose rate (i.e., shorter infusion duration). Two of five subjects in cohort 3 (0.25 mg/kg i.v. 1 h in 100 mL 0.9% sodium chloride) experienced treatment-related IRRs of moderate severity, which led to study drug discontinuation. One subject experienced an infusion site reaction, an IRR, and phlebitis (all moderate severity); a second subject experienced phlebitis (moderate severity). The IRRs resolved within hours of discontinuation of PLG0206 administration and receipt of paracetamol. Therefore, the decision was made at a Safety Review Committee meeting to reduce the study drug concentration by increasing both the volume and the infusion time. After reducing the study drug concentration and increasing the infusion time from 1 h to 2 h (i.e., 0.25 mg/kg i.v. 2 h in 200 mL 0.9% sodium chloride; maximum PLG0206 concentration of 0.125 mg/mL), no IRRs were reported at this dose. In cohort 4 (0.5 mg/kg i.v. 2 h in 250 mL 0.9% sodium chloride; maximum PLG0206 concentration of 0.2 mg/mL), all 4 IRRs observed in 3 participants were mild and did not lead to study drug interruption. One participant in the placebo group in cohort 4 (i.e., 200 mL 0.9% saline administered over 2 h) experienced right arm cannula discomfort that commenced at the end of infusion and resolved by 3 h postdose, which suggests the IRRs may also have been procedurally related. In cohort 5 (1 mg/kg administered over 4 h in 250 mL 0.9% sodium chloride; maximum PLG0206 concentration of 0.1 mg/mL), there were 9 IRRs observed in 6 participants; all were reported as mild.

There was a low incidence of concomitant medication use in both the PLG0206 treatment arm and the placebo group; paracetamol was used most frequently to treat mild IRRs. All physical examination findings that were not present at baseline were associated with TEAEs that were unrelated to PLG0206.

An overall summary of treatment-emergent adverse events (TEAEs) is provided in Table 3. In total, 41 (87.2%) of 47 subjects experienced 97 TEAEs across the treatment groups, of which 85 were mild and 12 were moderate in severity. The profile of TEAEs occurring in more than one subject was similar between the between the PLG0206 treatment group (31 [88.6%] of 35 subjects; 72 total events) and the placebo group (10 [83.3%] of 12 subjects; 25 total events). There were no reports of serious TEAEs. Thirteen (37.1%) of 35 subjects in the PLG0206 treatment arm had at least 1 TEAE deemed related to treatment. The most common TEAEs in the PLG0206 treatment arm were infusion site reaction ($n = 4$), infusion site pain ($n = 3$), and phlebitis ($n = 3$). Similarly, in the pooled placebo group, 4 (33.3%) of 12 subjects had at least 1 TEAE deemed related to treatment, including: vessel puncture site pain ($n = 1$), feeling hot ($n = 1$), pain in extremity ($n = 1$), tension headache ($n = 1$), orthostatic hypotension ($n = 1$), constipation ($n = 1$), thrombocytopenia ($n = 1$), and oropharyngeal pain ($n = 1$). All TEAEs were resolved by the end of the study.

Overall, there were no clinically significant patterns of change or trends of concern for laboratory abnormalities (Table 4), vital signs, or electrocardiogram (ECG) patterns of change evident in the PLG0206 cohorts when comparing dose level, infusion time, or between placebo and active treatments.



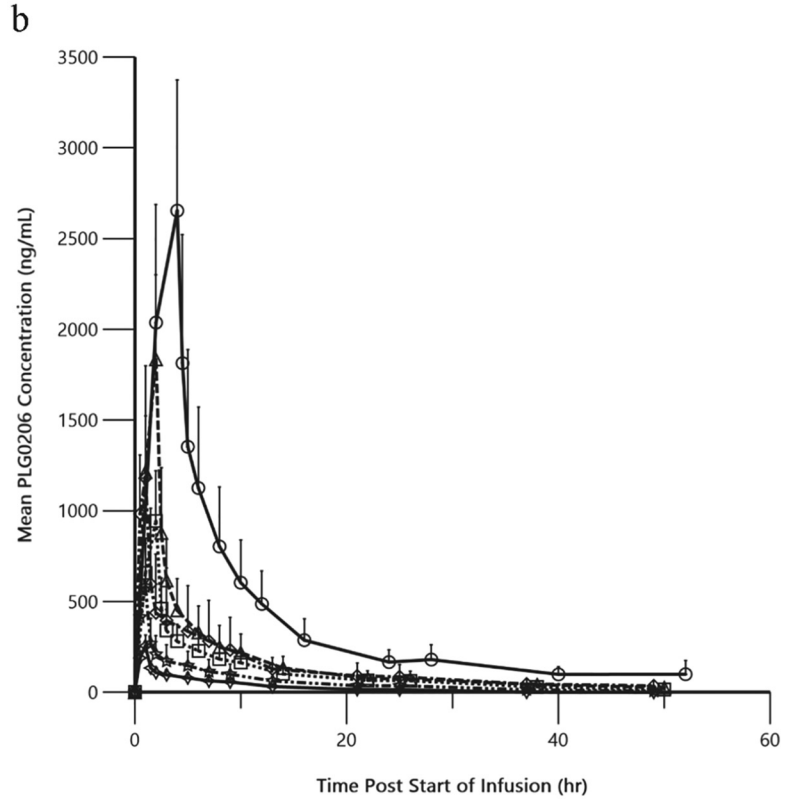


FIG 2 Mean and standard deviation (SD) PLG0206 plasma concentration-time plots by dose group. (a) Concentration in log scale; (b) concentration in linear scale.

TABLE 3 Overall summary of treatment-emergent adverse events

No. of subjects reporting	No. (%) in cohort or pool: 1: PLG0206 0.05 mg/kg i.v. 1-h infusion (*N* = 6)	2: PLG0206 0.125 mg/kg i.v. 1-h infusion (*N* = 6)	3: PLG0206 0.25 mg/kg i.v. 1-h infusion (*N* = 5)	3b: PLG0206 0.25 mg/kg i.v. 2-h infusion (*N* = 6)	4: PLG0206 0.5 mg/kg i.v. 2-h infusion (*N* = 6)	5: PLG0206 1 mg/kg i.v. 4-h infusion (*N* = 6)	Pooled PLG0206 (*N* = 35)	Pooled placebo (*N* = 12)	Overall (*N* = 47)
At least one TEAE[a]	5 (83.3)	4 (66.7)	5 (100)	5 (83.3)	6 (100)	6 (100)	31 (88.6)	10 (83.3)	41 (87.2)
At least one related TEAE	1 (16.7)	0	3 (60.0)	0	3 (50.0)	6 (100)	13 (37.1)	4 (33.3)	17 (36.2)
At least one TEAE leading to study drug discontinuation	0	0	2 (40.0)	0	0	0	2 (5.7)	0	2 (4.3)
TEAE severity									
Mild	5 (83.3)	4 (66.7)	4 (80.0)	5 (83.3)	6 (100)	6 (100)	30 (85.7)	10 (83.3)	40 (85.1)
Moderate	0	0	2 (40.0)	0	2 (33.3)	1 (16.7)	5 (14.3)	3 (25.0)	8 (17.0)

[a]TEAE, treatment-emergent adverse event. TEAEs were defined as adverse events that occurred following the first administration of study medication.

TABLE 4 Select mean (SD) laboratory values before and after (day 7) dosing by treatment group

	Hemoglobin (g/L)		Leukocytes ($\times 10^9$/L)		Platelets ($\times 10^9$/L)		ALT[a] (U/L)		Creatinine (μmol/L)	
Treatment group	Baseline	End of study	Baseline	End of study	Baseline	End of study	Baseline	End of study	Baseline	End of study
PLG0206 0.05 mg/kg i.v. 1 h ($N = 6$)	140.8 (11.8)	142.2 (9.9)	6.05 (0.53)	6.03 (1.61)	249.8 (59.9)	248.8 (49.3)	21.8 (8.1)	19.2 (8.7)	74.8 (11.8)	72.7 (10.3)
PLG0206 0.125 mg/kg i.v. 1 h ($N = 6$)	141.7 (17.0)	138.8 (18.4)	7.48 (1.58)	6.15 (1.27)	265.3 (57.9)	263.3 (40.5)	15.5 (5.3)	16.0 (4.1)	68.3 (16.3)	69.7 (17.4)
PLG0206 0.25 mg/kg i.v. 1 h ($N = 5$)	142.0 (14.6)	139.3 (10.1)	6.42 (0.54)	5.60 (0.97)	242.6 (20.3)	212.0 (16.6)	13.6 (4.8)	18.5 (6.1)	69.6 (14.1)	67.8 (13.1)
PLG0206 0.25 mg/kg i.v. 2 h ($N = 6$)	140.3 (8.5)	138.3 (12.0)	6.47 (1.65)	5.18 (1.44)	283.5 (64.1)	288.3 (79.4)	19.7 (5.3)	17.8 (6.4)	74.2 (10.0)	71.5 (12.1)
PLG0206 0.5 mg/kg i.v. 2 h ($N = 6$)	133.2 (12.0)	135.3 (13.6)	6.37 (1.37)	5.75 (1.62)	263.7 (71.2)	273.5 (66.0)	25.7 (10.8)	29.5 (21.8)	66.2 (6.6)	66.0 (8.0)
PLG0206 1 mg/kg i.v. 2 h ($N = 6$)	142.3 (16.3)	139.7 (15.5)	7.55 (1.20)	5.95 (0.97)	272.7 (66.6)	262.8 (50.5)	21.0 (9.9)	21.6 (10.6)	71.6 (12.9)	63.8 (9.0)
Pooled PLG0206 ($N = 35$)	140.0 (13.0)	138.9 (12.9)	6.73 (1.30)	5.79 (1.30)	263.5 (57.1)	260.9 (55.9)	19.7 (8.2)	20.5 (11.6)	70.8 (11.7)	68.8 (11.5)
Pooled placebo ($N = 12$)	135.3 (14.4)	132.0 (15.9)	6.07 (1.11)	5.67 (1.17)	242.5 (59.9)	244.9 (66.0)	17.7 (6.9)	17.4 (5.6)	61.9 (12.9)	61.5 (10.4)

[a]ALT, alanine aminotransferase.

DISCUSSION

Human cathelicidin LL-37 is the most well characterized of the natural AMPs, and exhibits both antimicrobial activity and host immune regulatory properties that are common among the naturally occurring molecules (4). However, AMPs have not met with significant clinical development success due to several factors, most notably, toxicity when administered systemically. PLG0206 was engineered to mitigate the shortcomings of natural AMPs and provide an improved systemic safety profile.

In this first-in-human study, PLG0206 was safe and well tolerated when intravenously administered to healthy subjects at single doses ranging from 0.05 to 1 mg/kg, provided that drug concentration and dose rates remained below 0.50 mg/mL and 25 mg/hour, respectively. With this proviso, the frequencies of TEAEs related to study drug administration were similar between PLG0206 treatment and placebo groups, and the incidence of TEAEs for PLG0206 was low and most events were mild in severity. IRRs were the most reported TEAEs related to PLG0206 and were observed when the concentration of PLG0206 was higher than 0.25 mg/mL with a higher dose rate. No specific subject risk factors, such as prior allergic reactions or demographic features, were identified in subjects who reported IRRs. Increasing the volume of infusion (thereby decreasing drug infusion concentration) and slowing the rate of infusion (thereby increasing the duration of infusion) mitigated the severity of the IRRs observed.

Intravenous PLG0206 exhibited linear PK (dose-proportional AUC) over the dose range of 0.05 to 1.0 mg/kg, with a median terminal half-life ($t_{1/2}$) ranging from 7.37 to 19.97 h. PLG0206 exposures, over these doses tested, allow for coverage of targeted pathogens based on the MIC_{90} of >900 clinical isolates, including ESKAPE (*Enterococcus faecium*, *S. aureus* [MIC_{90}, 0.12 μg/mL], *Klebsiella pneumoniae*, *Acinetobacter baumannii* [MIC_{90}, 0.5 μg/mL], *P. aeruginosa* [MIC_{90}, 2], and *Enterobacter* species) pathogens (data not shown).

Overall, the results of this FIH phase 1 study support the safety and ongoing development of PLG0206 and will inform dosing formulations and regimens in future studies to investigate its utility as an antimicrobial agent.

MATERIALS AND METHODS

This was a phase 1, randomized, double-blind, placebo-controlled, single-ascending-dose (SAD) FIH study to assess the safety, tolerability, and pharmacokinetics (PK) of PLG0206 in healthy subjects. The study was undertaken at the CMAX Clinical Research Unit in Adelaide, Australia. The protocol was reviewed and approved by an institutional review board (IRB). Subjects could be included if they were aged ≥18 to ≤45 years at screening and had a body mass index (BMI) between 18 and 30 kg/m^2 with weight between 45 and 100 kg. Subjects were excluded if they were pregnant, lactating, or planning pregnancy; had a history of alcohol or

drug abuse; had a history of an allergic diathesis; had any significant past or current cardiac, pulmonary, hepatic, renal, oncological, active infection, or other medical condition; had clinically significant abnormal safety laboratory values; used any prescription or nonprescription medications; had a QT corrected for heart rate by Fridericia's cube root formula (QTcF) of >450 ms; and/or received a vaccination within 3 months of screening.

All subjects who met eligibility criteria and were consented, enrolled and confined to the clinical research unit from day −2 through day 3. Six cohorts of 8 subjects (except cohort 3 only, which included 7 subjects) received escalating single intravenous infusions of PLG0206 at 0.05, 0.125, 0.25, 0.5, and/or 1 mg/kg dose or placebo over 1 to 4-h. Subjects were randomized to receive either PLG0206 (6 per cohort) or placebo (2 per cohort). At each dose level, there were 2 sentinel subjects (1 active and 1 placebo) who were dosed at least 48 h in advance of the other subjects in their group. Subjects underwent safety evaluations, blood samples for safety assessment, a 12-lead electrocardiogram (ECG), and vital sign assessment at specified time points before, during, and following the infusion.

The severity of each AE and laboratory abnormality was assessed by the investigator or medically qualified designee according to the Common Toxicity Criteria for AEs (CTCAE), version 5.0, which grades the severity of clinical AEs and laboratory abnormalities in a five-category system. If an AE or laboratory abnormality was not included in the CTCAE listing, then the investigator determined the intensity of the AE or laboratory abnormality according to the following criteria. Mild, AE or laboratory abnormality that is transient or is easily tolerated on continuation of study drug; moderate, AE or laboratory abnormality that causes the subject discomfort and causes interference with the subject's usual activities; severe, AE or laboratory abnormality that is incapacitating and causes considerable interference with the subject's usual daily activities, and/or may be life-threatening if it worsens; life-threatening, the AE or laboratory abnormality is life-threatening as it exists (i.e., no worsening is required for the abnormality to be life-threatening); and death, death related to the AE. There were no reports of serious treatment-emergent adverse events (TEAEs) In addition, none of the AEs were judged to be severe or higher. Furthermore, there were no reports of treatment-emergent dose-limiting toxicities (DLTs).

Blood samples for PK assessment were collected at predose, at the midpoint of infusion, within 1 min of the end of infusion, and at 0.5, 1, 2, 4, 6, 8, 12, 20, 24, 36, and 48 h after the end of infusion. The blood samples were drawn via an indwelling intravenous catheter or by direct venipuncture from opposite arm to where infusion took place, and the exact times of blood sampling and dosing information were recorded. Approximately 5 mL of blood was collected for each sample into K_2 EDTA vacutainers. Blood samples were spun down into plasma. Samples were processed accordingly before freezing at −70°C or below. Plasma samples were frozen as soon as possible after collection with a target time of within about 1 h. The plasma concentrations of PLG0206 were assayed using validated high-performance liquid chromatography (HLPC)-mass spectrometry with a lower limit of quantification of 5 ng/mL.

Subjects were discharged on day 3 (48 h postdose). Subjects returned to the study center 7 days after dosing during which a full physical examination, vital sign and weight recording, concomitant medications, safety blood analysis, urinalysis, urine drug screening, and a 12-lead ECG were performed. A Safety Review Committee (SRC) oversaw the safety, cohort evaluation, and dose escalation for the study between dose cohorts.

Pharmacokinetic analysis. Plasma concentration data were analyzed to derive the PK parameters using noncompartmental analysis. AUC_{0-t} was determined using trapezoidal linear up and log down method. $AUC_{0-\infty}$ was determined as sum of AUC_{0-t} and extrapolated curve (C_t/λ_z) where C_t was the last observed concentration and λ_z was the apparent terminal elimination rate. C_{max} was determined as the highest concentration observed post start of infusion. $t_{1/2}$ was determined as $\ln(2)/\lambda_z$; CL (clearance) was calculated as dose/$AUC_{0-\infty}$; V_z was calculated as dose/($\lambda_z \times AUC_{0-\infty}$). The noncompartmental analysis was done in WinNonlin software (v8.3).

Statistical analysis. Standard descriptive statistics were reported, including measures of central tendency and variance, as well as frequencies and proportions. No formal sample size calculation was conducted. Six cohorts of 8 subjects each (6 of whom were active drug subjects and 2 of whom were placebo) were deemed suitable to adequately characterize the safety, tolerability and PK of PLG0206 in this phase 1 study. PK parameters were determined from the plasma concentration of PLG0206 using noncompartmental methods via Phoenix WinNonlin software (v8.3).

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