Results of the First In Human Study of Lysin CF-301
Evaluating the Safety, Tolerability and Pharmacokinetic Profile in Healthy Volunteers

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Background: Lysins are recombinantly-produced bacteriophage-derived cell-wall hydrolases that represent a new class of antibacterial agents. CF-301 is a potent and specific lysin being developed as a treatment for serious *Staphylococcus aureus* (*S. Aureus*) infections.[1] CF-301 is an enzymatic protein (MW=26 kDa) that cleaves peptidoglycan structures found in the cell wall, thereby causing the bacterial cell to lyse. CF-301 also exhibits potent anti-biofilm activity, low propensity for resistance, and pronounced synergy with antibiotics. Pre-clinical target attainment studies support a CF-301 dose range of approximately 0.03 to 0.1 mg/kg to have anti-*Staphylococcus* activity in man in combination with standard of care antibiotics. CF-301 is the first lysin to enter clinical trials in the US.

Methods: This was a Phase I, single-center, double-blind, randomized, placebo-controlled, escalating, single-dose study in healthy males and females. There were up to 4 dosing cohorts planned (0.04, 0.12, 0.25 and 0.4 mg/kg/dose), with up to 6 subjects (4 active, 2 placebo) planned for each cohort. Study drug was administered via a single 2-hour intravenous (IV) infusion. Within each cohort, two sentinel subjects (1 active, 1 placebo) were dosed first. An independent Data Safety Monitoring Board (DSMB) reviewed unblinded safety and PK data at pre-specified decision points and determined if dosing should continue as planned. The pre-specified stopping rule for the study was based on attaining C_{max} exposure in the 1.9 to 2.2 µg/mL range.

Subjects remained in the clinic from Day -1 to Day 4. Serial blood and urine samples were collected for clinical laboratory tests and PK evaluation from pre-dose through 72 hours after the start of infusion. Safety assessments included physical exams, vital signs, 12-lead EKGs, Holter monitoring/ telemetry and adverse events (AEs). Subjects returned on Days 5, 8, 14 and 28 for follow-up safety evaluation. All subjects were tested for CF-301 anti-drug antibodies (ADA), immungoglobulin E (IgE), and ex vivo basophil activation (BAT) on Days -1, 5, 8, 14 and 28 and at ongoing long-term follow-up visits on Days 90 and 180; subjects with positive results at screening were excluded. PK parameters for CF-301 were calculated using non-compartmental analysis and all PK calculations were done using SAS® for Windows® Version 9.4. Safety data were summarized descriptively.

Results: A total of 20 healthy subjects were dosed (11 male and 9 female). Subjects were dosed at each of the 4 planned dose levels. There were no serious AEs, no hypersensitivity AEs related to CF-301, and no study stopping rules were met. A total of 5 non-serious AEs were reported through Day 28 by 4 subjects: 2 subjects who received CF-301 reported headache, contact dermatitis, and allergic rhinitis and 2 subjects who received placebo reported viral upper respiratory tract infection and viral infection.

Summary of Treatment Emergent Adverse Events (TEAE)

	CF-301 mg/kg/dose					
	0.04 (N=4)	0.12 (N=4)	0.25 (N=4)	0.4 (N=1)	Total CF-301 (N=13)	Placebo (N=7)
Total # TEAEs	1	2	0	0	3	2
# Subjects with at least one TEAE n (%)	1 (25.0)	1 (25.0)	0 (0)	0 (0)	2 (15.4)	2 (28.6)

All AEs were mild and resolved and no additional AEs were reported through Day 90. There were no clinically relevant changes in vital signs, EKGs or laboratory results.

Nine (9) of the 13 subjects (69%) who received CF-301 generated CF-301-specific ADA titers of variable magnitude. No subjects tested positive for CF-301-specific BAT activation and one subject tested weakly positive for CF-301-specific IgE at Day 28 (0.18 kU/L [>0.1 kU/L = cutoff]) but was IgE negative at Day 90. There did not appear to be a significant relationship between CF-301-specific ADA and positive responses in either the IgE or BAT assays.

After IV infusion of CF-301 at doses ranging from 0.04 to 0.4 mg/kg, plasma concentrations and the PK parameters, C_{max} and AUC, increased in a dose-related but less than dose-proportional manner. However, there was no evidence of a plateau in exposures. There did not appear to be a significant relationship between plasma clearance and either body weight or BMI.

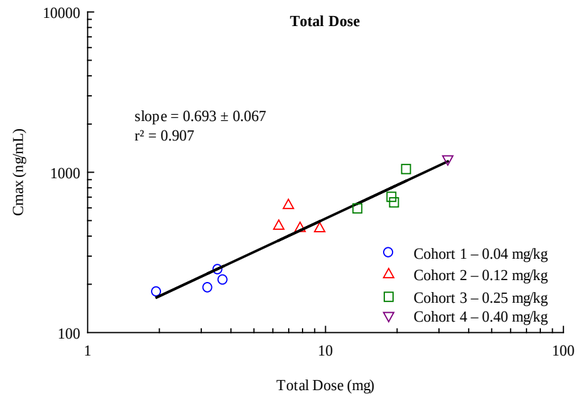
Summary of PK Parameters for CF-301

Parameter*	0.04 mg/kg n=4	0.12 mg/kg n=4	0.25 mg/kg n=4	0.4 mg/kg n=1
C_{max} (ng/mL)	205 (14.2)	489 (16.1)	731 (25.4)	1,212
$AUC_{(0-t)}$ (hr×ng/mL)	498 (12.8)	1,121 (16.0)	1,749 (25.0)	3,311
$AUC_{(inf)}$ (hr×ng/mL)	503 (12.6)	1,126 (16.0)	1,758 (24.8)	3,316
$t_{1/2}$ (hr)	6.58 (52.6)	4.37 (6.79)	6.05 (64.9)	11.3

*Geometric mean (geometric CV)

The geometric mean (gMean) CF-301 $AUC_{(inf)}$ was 1,758 hr x ng/mL and the gMean C_{max} was 731 ng/mL at the 0.25 mg/kg dose.

Individual subject CF-301 C_{max} after IV administration of 0.04, 0.12, 0.25, and 0.4 mg/kg CF-301



Conclusions: CF-301, a first in class IV administered bacteriophage-derived lysin was generally well tolerated at 0.04 to 0.4 mg/kg and no clinical adverse safety signals were observed. While 69% of subjects developed CF-301-specific ADA, no correlation of positive ADA with either IgE or BAT was observed. The increase in plasma AUC and C_{max} of CF-301 across the dose range of 0.04 to 0.4 mg/kg is less than dose-proportional but there was no evidence of a plateau in exposures. Based on PK/PD modeling from animal target attainment studies, when combined with current standard of care antibiotics, CF-301 exposures at the 0.25 mg/kg dose level are anticipated to be effective in treating *S. aureus* infections in humans.

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Reference:
[1] R. Schuch, H. M. Lee, B. C. Schneider, K. L. Sauve, C. Law, B. K. Khan, J. A. Rotolo, Y. Horiuchi, D. E. Couto, A. Raz, V. A. Fischetti, D. B. Huang, R. C. Nowinski, and M. Wittekind, "Combination therapy with lysin CF-301 and antibiotic is superior to antibiotic alone for treating methicillin-resistant staphylococcus aureus-induced murine bacteremia," *J. Infect. Dis.*, vol. 209, no. 9, pp. 1469–1478, 2014.