PK-PD Relationship and PK Driver of Efficacy of the Novel Antibacterial Lysin Exebacase (CF-301) in Pre-Clinical Models

P. Ghahramani[1], J. Chiu[1], T. Asempa[2], K. Abdelraouf[2], D. Nicolau[2], W. Abdelhady[3], Y. Xiong[3], A. Bayer[3], T. Carabeo[4], C. Cassino[4], R. Schuch[4], D. Lehoux[4].
[1]Inncelerex, Jersey City, NJ; [2]Hartford Hosp., Hartford, CT, [3]LA Biomed/UCLA Sch. of Med., Los Angeles, CA, [4]ContraFect, Yonkers, NY.

Corresponding author:
Parviz Ghahramani, PhD, PharmD, MSc, MBA
1000 Avenue at Port Imperial, #607,
Tel: +1 201 936 7248
Parviz.Ghahramani@inncelerex.com
http://www.inncelerex.com

Abstract

Background: Exebacase (CF-301) is a novel lysin with rapid bacteriolytic and anti-biofilm activity against S. aureus, pronounced synergy with antibiotics and low propensity for resistance. Exebacase has undergone Phase 1-2 trials. This work was to develop pharmacokinetic (PK) model in animal and determine relationship between exebacase exposure and efficacy in animals.

Methods: PK data in 592 animals (4 species) included in population PK model. A range of linear and nonlinear mammillary models with allometric scaling fitted to the PK data using NONMEM and the most parsimonious model was selected by improvement in objective function value (p< 0.01). To evaluate efficacy, 349 animals with 177 mice (neutropenic thigh infection) and 172 rabbits aortic valve infective endocarditis were treated with exebacase in addition to suboptimal doses of daptomycin (DAP). Full PK profiles were simulated for individual animals. There were 59 dosing regimens of exebacase in mice (0-90 mg/kg) and 18 regimens in rabbits (0-1.4 mg/kg) with q24h, q12h and q8h frequencies. Relationship between AUC/MIC, Cmax/MIC, T> MIC, and log-CFU was examined using a range of functions by comparing residual standard error (RSE).

Results: 3-compartment model with allometric scaling best described the PK data and was validated by bootstrap and Goodness of Fit. Maximum drop in log10CFU/g in target tissues was at AUC/MIC< 0.2 for exebacase when added to DAP that was associated with CFU reduction of -5 logs in rabbits (Figure (a)) with similar magnitudes in cardiac vegetations, kidney and spleen, and -4 logs in mice (Figure (b)). Treatment with DAP alone had log10CFU reduction of -1 in mice; and -2 in rabbits. AUC/MIC was an appropriate predictor of CFU reductions.

Conclusion: PK model adequately described the data for 4 animal species. Exebacase addition to DAP has synergistic effect on efficacy measured by CFU reductions in target tissues in the animal models. Results support previously presented determinations of AUC/MIC as predictor of efficacy. Maximum reductions in CFU in rabbits and mice were observed at AUC/MIC ratios < 0.2. These results further indicate that rabbit is the most appropriate efficacy model with MICs and antibacterial activity reflective of previously reported observations in human serum.

INTRODUCTION

Exebacase is a first-in-class bacteriophage-derived lysin with activity against *Staphylococcus aureus* (*S. aureus*). Exebacase was isolated from *Streptococcus suis* and is produced in *Escherichia coli* (*E. coli*) as a recombinant protein, such that the final product does not contain any bacteriophage material. Exebacase is an enzymatic protein that has 2 functional domains: a binding domain that binds to peptidoglycan structures that comprise the bacterial cell wall and a catalytic domain that cleaves peptidoglycan structures found in the cell wall, thereby causing the bacterial cell to lyse.

Several features distinguish exebacase from small molecule antibiotics, which include: (1) a novel mechanism of action; (2) bactericidal activity against antibiotic-resistant *S. aureus*; (3) rapidity of antibacterial activity both *in vitro* and *in vivo*; (4) narrow lytic spectrum of action; (5) potent activity against biofilms; (6) synergistic activity with standard of care antibiotics (e.g. daptomycin); and (7) low propensity for developing bacterial resistance, because the binding and cleavage sites on the bacteria are in the highly conserved peptidoglycan structures essential for viability. [1]

Scan this QR code to see the video of exebacase in action killing bacteria in real time scale

OBJECTIVES

The objectives of this project were:

a) To update previously developed animal population PK model in NONMEM [2] with the addition of new PK data in mouse and rabbit.

b) Based on PK parameter estimates from the final population PK model, simulate rabbit and mouse exposure indices (C_{max}/MIC, AUC/MIC and T>MIC) for doses corresponding to animal efficacy studies in mouse and rabbit.

c) To perform PK-PD analyses to characterize the PK-efficacy relationship and determine the PK driver of efficacy in animal models to guide selection of efficacious exposures and doses in humans.

DATA

For the population PK analysis, data was pooled from a total of 15 PK studies with 4 animal species (mice, rats, rabbits and dogs) with various routes of administration (IV bolus, IV infusion, subcutaneous (SC) injections) at various dosing regimens ranging from 0.03-50 mg/kg and at administration frequencies ranging from q24h to q8h. Overall, a total of 2,602 PK observations from 592 animals (42 mice, 316 rats, 156 rabbits and 78 dogs) were included in the final population PK model.

For PK-PD analysis, data from studies in mouse and rabbit where efficacy was assessed were included in the analysis. One study used the neutropenic mouse thigh infection model against *S. aureus* (MSSA and MRSA). There were two studies in rabbit with infective endocarditis (IE) due to *S. aureus* (MRSA) where cardiac, kidney and spleen tissue were assessed for bacterial density. In all studies exebacase was administered at various doses in addition to suboptimal doses of daptomycin.

METHODS

Exebacase concentration-time data was analyzed using a non-linear mixed effect modeling approach. The first-order conditional estimation with interaction (FOCE-I) estimation algorithm within NONMEM was applied. One- two- and three-compartmental models with zero-order and first-order absorption dose inputs were explored. The final population PK model was qualified using prediction-corrected visual predictive checks (pcVPC). 1000 replicates of the data were simulated and stratified by species for time after last dose < 25 hours. Percentiles (5th, 50th, and 95th) of the observed data were plotted, along with the 95th percent confidence interval bands (prediction intervals, PI) of the 5th, 50th, and 95th percentiles of the simulated data. Bootstrap was performed for the final population PK model, where 1000 bootstrap runs were executed, and the final model parameter estimates were compared to the medians and 95% CI of the bootstrap estimates. The final population PK model was used to simulate PK profiles in rabbits and mice to predict exposure parameters (C_{max}, $AUC_{0\text{-}24}$ and T>MIC over a 24-hour period). To obtain exposure indices AUC/MIC and C_{max}/MIC, individual predicted exposures (AUC and C_{max}) were divided by their MIC values. PK profiles were simulated at ≤0.1-hour increments.

PK-PD analysis assessed the relationship between exposure indices (AUC/MIC, C_{max}/MIC, T>MIC) and the mean $\log_{10}$CFU/g of tissue corresponding to a wide range of dosing regimens in mouse and in rabbits. In mouse, the dosing regimens ranged from 0-90 mg/kg with q24 to q8 hour dosing frequencies in addition to suboptimal doses of daptomycin. In rabbits, dosing regimens ranged from 0-1.4 mg/kg with q24 to q8 hour dosing frequencies in addition to suboptimal doses of daptomycin.

The $\log_{10}$CFU was plotted against each exposure index (AUC/MIC, C_{max}/MIC, T>MIC). Linear and Hill equations were fitted to the data using NLS function in R. The best exposure index as the predictor of $\log_{10}$CFU was selected by comparing residual standard error (RSE). The model parameter estimates and statistics for the best fit was determined.

The exposure index value at which the maximum reduction in $\log_{10}$CFU occurred was determined by graphical assessment and by numerical prediction from the fitted model. The exposure index value at which reduction in $\log_{10}$CFU was 90% of the maximum reduction was defined as the exposure threshold for efficacy.

Human PK data was collected in a recent study in patients with serious infections and PK parameters including AUC, C_{max} and AUC/MIC ratio for 72 patients were calculated.

RESULTS

Population PK: The final population PK model (Fig. 1) was found to be adequate in characterizing exebacase PK data in animals from various species (mouse, rat, rabbit and dog) and dose inputs (SC, IV bolus, IV injections). Apparent bioavailability was found to be dependent on both route of administration and animal size. The population mean estimate of V2 is large (2642 L) suggesting that exebacase is well distributed in the body.

Based on goodness-of-fit plots, model validation (pcVPC in Fig. 2) and bootstrap results, the final population PK model was deemed suitable for simulation of individual and mean PK profiles in mice and rabbits for use in PK-PD analysis.

PK-PD: Exebacase in addition to daptomycin was associated with additional reduction in CFU in mice by 1.2-$\log_{10}$ (Fig. 3) and in rabbits by 2.3-$\log_{10}$ (Fig. 4). Both animal efficacy models showed exposure to exebacase, in addition to daptomycin, was predictive of efficacy (reduction in $\log_{10}$CFU). AUC/MIC is an adequate exposure index for prediction of CFU reduction. C_{max}/MIC has similar predictive value but provides no additional information compared to AUC/MIC. T>MIC was a poor predictor of CFU reduction (Table 1 and Table2).

The overall PK-PD results show that on average an AUC/MIC ratio>0.10 in rabbits and >0.32 in mouse is associated with 90% of maximum CFU reduction. The MICs determined in mice (serum) were much higher than those determined in rabbits by about an average of about >70-fold. Therefore, higher doses and exposures were required in mice to achieve magnitudes of CFU reduction similar to those in rabbits. This data supports previous findings showing magnitude of effect for a given mg/kg dose depends on species due to differences in native serum activity between species that has potentiating effect on biological activity of exebacase. Rabbit serum has a much larger potentiating effect on exebacase biological activity compared to mice [3]. This is also reflected in MICs determined in rabbit serum that range from 0.5-1 µg/mL compared to mouse serum that range between 16-128 µg/mL. Therefore, higher doses and exposures are needed in mice to achieve similar efficacy in rabbits. These results support the importance of accounting for species difference in serum effect by using the AUC/MIC ratio as the predictor of efficacy where MIC is specific to each species. This means that the MIC must be derived from the media with the serum of the species where the AUC is derived, and the appropriate index for extrapolation into other species and for prediction of human efficacious exposures is the ratio of $AUC_{rabbit}/MIC_{rabbit\ serum}$ or $AUC_{mouse}/MIC_{mouse\ serum}$ and not the AUC per se.

Table 3 shows summary of PK parameters in patients treated with exebacase and confirms that all patients (>99%) achieve an AUC/MIC ratio >0.5 that is the target to achieve or exceed, as established in the animal efficacy studies.

Figure 1. Schematic of the PK model for exebacase (CF-301).

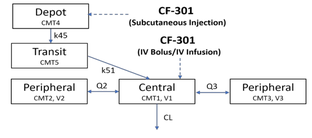
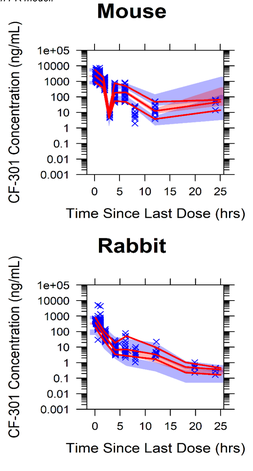


Figure 2. Visual predictive check stratified by species from final population PK model.



× , observations; red lines, percentiles (5th, 50th, and 95th) of the observed data; shaded regions, 95th percent confidence interval bands of the 5th, 50th, and 95th percentiles of the simulated data

Figure 3. The relationship between $\log_{10}$CFU/g of tissue and AUC/MIC (left), C_{max}/MIC (middle) and T>MIC (right) for a range of exebacase dose regimens in addition to suboptimal dose of daptomycin (8% of historical efficacious dose) in mouse thigh infection model

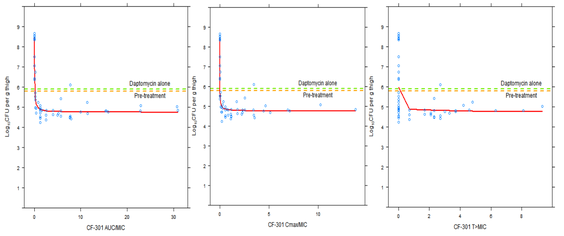
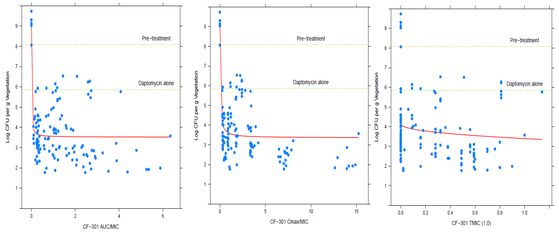


Figure 4. The relationship between $\log_{10}$CFU/g of tissue and AUC/MIC (left) C_{max}/MIC (middle) and T>MIC (right) for a range of exebacase dose regimens in addition to suboptimal dose of daptomycin in rabbit IE model



CONCLUSIONS

- The population PK model adequately described observed PK data of exebacase in mouse and rabbit.
- 3-compartmental linear model with a first-order absorption followed by a delay input reasonably described the pooled data from various animal species (mouse, rat, rabbit and dog)
- Efficacious exposure (AUC) in mice and rabbit is species dependent and not a good index for prediction/translation of efficacy across species.
- AUC/MIC ratio on the other hand is an appropriate exposure index for predicting efficacy regardless of species with the understanding that MIC (as well as AUC) must be determined in the serum of corresponding species. This means either the ratio $AUC_{rabbit}/MIC_{rabbit\ serum}$ or $AUC_{mouse}/MIC_{mouse\ serum}$ provide comparable results and are both appropriate indices for extrapolation to humans.
- An AUC/MIC ratio of 0.10 in rabbits and 0.32 in mice was associated with maximum efficacy (i.e., reduction in CFU).
- Therefore, an AUC/MIC ratio of >0.5 is deemed to be the ratio that should be achieved or exceeded in patients with S. aureus infections. Given exebacase is intended for use in life-threatening S. aureus blood stream infections which can be complicated by difficult to treat metastatic foci, an AUC/MIC ratio of about 5, that is 10-fold of the minimum ratio (0.5 described above), should be considered for use in clinical studies to ensure all or majority of patients receive potentially efficacious exposures.

Human exposure in a recent Phase 2 clinical study demonstrated that an AUC/MIC ratio of 4.5 could be safely achieved or exceeded in all patients (>99%).

Table 1. Parameter estimates from fitting Hill model to the relationship between $\log_{10}$CFU/g of tissue and exposure indices in neutropenic mouse thigh infection model.

Exposure Index	Parameter	Estimate	SE	p-value	Model RSE
AUC/MIC					0.388
	E_0	8.46	0.16	<0.0001	
	E_{max}	3.72	0.18	<0.0001	
	EC_{50}	0.04	0.01	<0.0001	
	p	0.92	0.18	<0.0001	
C_{max}/MIC					0.390
	E_0	8.46	0.16	<0.0001	
	E_{max}	3.71	0.19	<0.0001	
	EC_{50}	0.01	0.00	0.0003	
	p	0.81	0.17	<0.0001	
T>MIC					1.167
	E_0	5.96	0.21	<0.0001	
	E_{max}	2.00	241.11	0.993	
	EC_{50}	0.16	367.10	1.00	
	p	0.10	15.84	0.995	

Table 2. Parameter estimates from fitting Hill model to the relationship between $\log_{10}$CFU/g of tissue and exposure indices in rabbit IE model for cardiac vegetation.

Exposure Index	Parameter	Estimate	SE	p-value	Model RSE
AUC/MIC					1.212
	E_0	9.10	0.50	<0.0001	
	E_{max}	5.58	0.56	<0.0001	
	EC_{50}	0.01	0.05	0.894	
	p	1.00	2.67	0.708	
C_{max}/MIC					1.193
	E_0	9.09	0.49	<0.0001	
	E_{max}	5.73	0.57	<0.0001	
	EC_{50}	0.04	0.10	0.669	
	p	1.00	1.27	0.432	
T>MIC					1.640
	E_0	4.13	0.23	<0.0001	
	E_{max}	8.00	910.50	0.993	
	EC_{50}	100.00	28160.00	0.997	
	p	0.50	3.61	0.890	

Table 3. Summary of the Cmax and AUC0-24 for 72 patients from the clinical Phase 2 Study CF-301-102 predicted using the final Population PK model.

Parameter	N	Mean ± SD	Median
C_{max} (ng/mL)	72	1204 ± 437	1056
$AUC_{0\text{-}24}$ (ng*hr/mL)	72	3407 ± 1436	3108
AUC/MIC	70	6.3 ± 7.0	4.5

REFERENCES

[1] http://www.contrafect.com/

[2] Chiu J, Khariton T, Ghahramani P, at al., Interspecies Scaling in Pre-clinical Population Pharmacokinetics of CF-301. ACoP7; 2016; October 23-26; Washington DC; Abstract W-039.

[3] Indiani C, Sauve K, Raz A, et al .The Antistaphylococcal Lysin, CF-301, Activates Key Host Factors in Human Blood To Potentiate Methicillin-Resistant Staphylococcus aureus Bacteriolysis. Antimicrobial Agents and Chemotherapy, 2019,63(4). pii: e02291-18. doi: 10.1128/AAC.02291-18.