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Efficacy of Fluorocycline TP-434 in the Neutropenic Thigh Infection Model is Predicted by AUC/MIC

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Abstract

Background: TP-434 is a novel broad-spectrum fluorocycline being developed by Tetraphase Pharmaceuticals for a wide range of infections. The current study was performed to determine the pharmacodynamic parameter (PD) that is best predictive of efficacy.

Methods: Female CD-1 mice were rendered neutropenic by IP injection of Cytoxan (150/100 mg/kg at days -4/-1 pre-infection). Infection was established by injection of 10^5 CFU of MRSA (tetracycline-resistant USA300) in the right thigh. Dose fractionation studies (q24h, q12h and q6h) were done with 1-90 mg/kg SC for MRSA. All thighs were removed 26 hrs post-infection and processed for CFU counts. TP-434 was administered SC from 1 to 60 mg/kg to determine PK parameters (Cmax, AUC, T>MIC) in neutropenic, thigh-infected animals. The dose vs change in log CFU/thigh relationship vs untreated controls was determined for each organism and related to the PK parameters at each dose. Protein binding was determined by equilibrium dialysis and size exclusion centrifugation.

Results: The static dose for MRSA was 11.9 mg/kg. The correlation coefficients of the PD parameters to efficacy in the thigh model for the 24 hr AUC/MIC, Cmax/MIC and %T>MIC were 82%, 80% and 58% for MRSA. The 24 hr total AUC/MIC ratios necessary to achieve a static effect and 1 log reduction in CFU were 38.4 and 46.9, respectively. The Cmax/MIC ratio at stasis was 1.64. Protein binding in fresh mouse serum averaged 75% for concentrations from 0.1 to 10 µg/mL and there was good correlation between both methods.

Conclusion: The efficacy of TP-434 in the neutropenic thigh model for a representative MRSA strain, USA300 correlates best to the AUC/MIC, which is similar to other published tetracycline molecules.

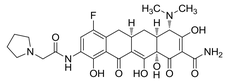
Introduction

TP-434 is designed as a broad spectrum IV antibiotic with the potential for superior efficacy against Gram-negative, Gram-positive, and anaerobic pathogens (see F1-2157-2161). *In vitro* studies with TP-434 have demonstrated greater potency in comparison to currently marketed antibiotics. Preliminary data have shown that TP-434 also has the potential to be developed as an oral therapy (see F1-2163). TP-434 has successfully completed Phase 1 clinical studies (see A1-027-028) and is poised to enter Phase 2 in 2010. The current study was performed to determine the pharmacokinetic/pharmacodynamic parameter that best predicts the efficacy of TP-434 in bacterial infections.

Methods and Materials

Mice: Female 5 - 6 week old CD-1 mice (18-22 gm).
Neutropenia: Female CD-1 mice were rendered neutropenic by IP injection of Cytoxan (cyclophosphamide) 150 mg/kg (-4 days) and 100 mg/kg (-1 day) pre-infection.
Thigh Infection: A fresh overnight culture of a *Staphylococcus aureus* USA300 (MRSA) strain was diluted to approx. 2 x 10^6 CFU/mL and 0.1 mL injected (5x10^5 final cfu) IM into the thighs of the pre-treated mice.
MICs: MICs for TP-434 were determined by microbroth dilution in accordance with CLSI guidelines.
PK: TP-434 was administered SC at 5 selected doses (1 – 60 mg/kg), with 9 time points and N=3 mice in order to determine pharmacokinetic parameters (Cmax, AUC, T>MIC) and their relationship to administered dose. Pharmacokinetics were performed in neutropenic, thigh-infected animals to best predict compound levels in the efficacy studies.
Dose Ranging Study: An initial dose-ranging study (single dose at +1.5 hrs post-infection) was performed over a wide range (0.25 – 60 mg/kg) in thigh-infected animals in order to determine the defined range that will be used in the dose fractionation studies.
Dose Fractionation: TP-434 was administered by the same route used for the PK and dose-ranging study at up to 8 different total daily doses (selected from the dose ranging studies and covering a range from maximal to the no-effect level). Each total dose was given at 3 different regimens; q24hr, q12hr, q6hr. Efficacy in the thigh infection model was compared to calculated PK parameters at each of the dose fractionations.

Panel 1: Chemical Structure TP-434

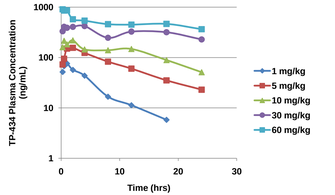


Panel 2: TP-434 Minimum Inhibitory Concentration (MIC)

Organism	MIC (µg/ml) TP-434	Tetracycline
S. aureus MRSA 300	0.13	32

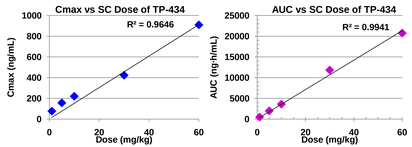
- TP-434 demonstrates excellent activity against the methicillin- and tetracycline-resistant *Staphylococcus aureus* USA300 strain.

Panel 3: Pharmacokinetics of TP-434 following Subcutaneous Administration to Female CD-1 Mice



Parameter	TP-434 Subcutaneous Dose 1 mg/kg	5 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg
C_{max} (ng/mL)	76.2	156	219.7	422.7	908.7
AUC_{0-inf} (ng·hr/mL)	470	1968.7	3591.7	11811.7	20740.2
MRT (hr)	5.5	12.3	13.8	22.1	27.5
Tmax (hr)	1	2	2	4	0.3

- TP-434 exhibits a dose response following subcutaneous administration.
- Correlations of R^2=0.994 and 0.964 were observed for AUC and Cmax vs dose.
- Mean residence times ranged from 5.5 – 27.5 hrs with Tmax values of 0.3 – 4 hrs.



Panel 4: Protein Binding Determination – TP-434

Amicon Centrifuge Filters
Ultracell 10k - regenerated cellulose, 10,000 MWCO
Centrifuged at 3 x 30 min at 4000 x g

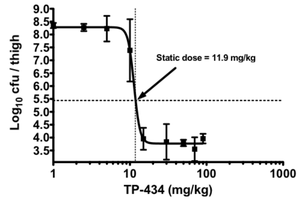
Spike concentration (µg/mL)	Retentate	Filtrate	Calculated % Bound	Range
0.1	NA	NA	NA	
0.5	0.53	0.16	69.81	68.7-80.8%
2.5	2.08	0.40	80.77	
10	9.06	2.84	68.65	
		Mean	73.1	

RED (Rapid Equilibrium Device)
Dialysis membrane 8,000 MWCO
Incubated 4 hrs at 37°C on orbital shaker

Spike concentration (µg/mL)	Sample Chamber	Filter Chamber	Calculated % Bound	Range
0.1	0.08	0.02	75	
0.5	0.28	0.09	67.86	67.9-84.7%
2.5	1.31	0.20	84.73	
10	5.26	1.13	78.52	
		Mean	76.5	

- Protein binding, of TP-434, determined by two different methods, ranged from 67.9– 84.7% (mean of 75%) over the concentration range of 0.1, 0.5, 2.5 and 10 µg/mL.

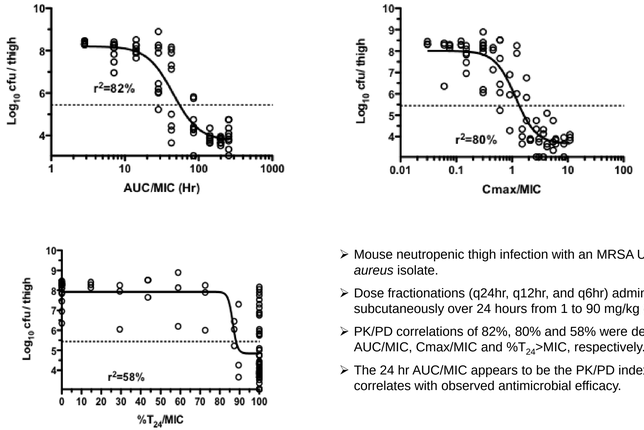
Panel 5: Dose Ranging Thigh Infection: Static Dose Determination



TP-434 (total) Efficacy Ratios

Parameter	Static Effect	1 log reduction
AUC/MIC	38.4	46.9
C_{max}/MIC	1.64	2.00

Panel 6: Dose Fractionation Thigh Infection Study – TP-434



- Mouse neutropenic thigh infection with an MRSA USA300 *S. aureus* isolate.
- Dose fractionations (q24hr, q12hr, and q6hr) administered subcutaneously over 24 hours from 1 to 90 mg/kg (total dose).
- PK/PD correlations of 82%, 80% and 58% were determined for AUC/MIC, Cmax/MIC and $\%T_{24}$>MIC, respectively.
- The 24 hr AUC/MIC appears to be the PK/PD index that best correlates with observed antimicrobial efficacy.

Summary of Results

- TP-434 was active against the methicillin-resistant and tetracycline-resistant MRSA clinical isolate used in this study (see F1-2158 for breadth of spectrum).
- TP-434 exhibits dose-proportional pharmacokinetics following subcutaneous administration with excellent correlations for AUC and Cmax to dose.
- The static dose for TP-434 resulting in no change in the thigh bacterial burden of MRSA USA300 was 11.9 mg/kg.
- The correlation coefficients of the PD parameters to efficacy in the thigh model for the 24 hr AUC/MIC, Cmax/MIC and %T>MIC were 82%, 80% and 58% for MRSA.
- The 24 hr total AUC/MIC ratios necessary to achieve a static effect and 1 log reduction in CFU were 38.4 and 46.9, respectively. The Cmax/MIC ratio at stasis was 1.64.
- Protein binding in fresh mouse serum averaged 75% for concentrations from 0.1 to 10 µg/mL and there was good correlation between the two methods tested.
- The mean $AUC_{(SS)}$ for TP-434 in Phase 1 multiple-ascending dose studies by compartmental analyses for 1.5 mg/kg q24h and 1.0 mg/kg q12h administered intravenously over 1h was 8.670 ± 1.39 and 13.34 ± 1.34 µg·h/mL respectively (see A1-027 and A1-028) giving a total AUC/MIC ratio of 69.4 and 106.7.

Conclusion

- The AUC/MIC predictive of efficacy in a neutropenic thigh model challenged with MRSA USA300 would be comfortably reached by TP-434 administered once daily intravenously at 1.5 mg/kg in humans.

General References

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Acknowledgments

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