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Pharmacodynamic Characterization of NVP-LMB415 against *Haemophilus influenzae* in an *In Vitro* Hollow-Fiber System

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ABSTRACT

Purpose. NVP-LBM415 is a peptide deformylase inhibitor with *in vitro* activity against those pathogens commonly associated with community-acquired respiratory tract infections. The purpose of these studies was to determine which pharmacokinetic-pharmacodynamic (PK-PD) measure is most strongly associated with drug response and to examine the relationship between drug exposure and response for NVP-LMB415 against *Haemophilus influenzae*.

Methods. Two wild-type *H. influenzae* strains (MIC 2 and 8 µg/mL) were studied. The hollow-fiber system was inoculated with approximately 10^{7-8} CFU/mL in log-phase growth. Simulating human pharmacokinetics ($t_{½}$ = 2 hours), bacteria were exposed to escalating free drug NVP-LMB415 exposures (AUC ranging from 0 to 200 µg·h/L) using a dose fractionation study design and delivering drug every 12 hours, every 24 hours, and by continuous infusion. Serial samples were collected to determine bacterial counts (CFU/mL) and drug concentrations. Drug effect was quantified as the $\log_{10}$ ratio of the 24-hour area under the bacterial growth/kill curves for drug and growth control ($\log_{10}$ ratio = $\log_{10}$ $AUC_{drug}/AUC_{growth\ control}$).

Results. Overall, the greatest activity (at 6x MIC) was seen with the continuous infusion regimen (continuous infusion > q12 hours >> q24 hours). Due to the short half-life, dosing q24 hours yielded no net kill compared to baseline. Neither time above MIC, AUC:MIC ratio, nor peak:MIC ratio could co-model the q12 hour and q24 hour regimens with continuous infusion results. Separating these into 2 datasets (q12 plus q24 versus continuous infusion), only the AUC:MIC ratio could adequately describe the continuous infusion results (E_{max} $\log_{10}$ ratio of -2 at an AUC:MIC ratio ≥ 50). The q12 plus q24 dataset was reasonably fit by the AUC:MIC ratio and percent time above MIC (E_{max} $\log_{10}$ ratio of -2 to -3 at an AUC:MIC ratio ≥ 120 and percent time above MIC of 50% to 60%, respectively). The peak:MIC ratio was not informative.

Conclusions. For intermittent dosing regimens, percent time above MIC and the AUC:MIC ratio adequately described drug response. Percent time above MIC and the AUC:MIC ratio associated with maximal decline were ≥ 50% to 60% and ≥ 120, respectively. Continuous infusion regimens could not be co-modeled with intermittent regimens, suggesting that neither percent time above MIC nor the AUC:MIC ratio completely described drug effect. Drug effect continued to increase beyond 100% time above MIC. Every 12 hour dosing had more effect than every 24 hour dosing, and would probably be an effective NVP-LBM415 regimen in humans for *H. influenzae*.

BACKGROUND AND OBJECTIVES

- NVP-LBM415 is a potent synthetic peptide deformylase inhibitor and represents the first compound in this novel class of antimicrobial agents.
- NVP-LBM415 has demonstrated *in vitro* activity against key pathogens associated with community-acquired respiratory tract infections, including activity against susceptible and multidrug-resistant strains of *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae*.[1,2]
  - The minimum inhibitory concentrations (MIC) for 90% of strains (MIC_{90}) of *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* have been reported as 0.5-1, 0.5, and 4-8 mg/mL, respectively.
- In animal models of infection, NVP-LBM415 is active against *S. aureus* and *S. pneumoniae* and has been shown to have good target organ penetration in the mouse pneumonia model.[3]
- To date, pharmacokinetic-pharmacodynamic (PK-PD) studies have not been conducted to ascertain the correct target PK-PD parameter, nor the magnitude for which that target is predicative of *in vivo* efficacy for NVP-LBM415 against *H. influenzae*.

BACKGROUND AND OBJECTIVES (cont'd.)

- The objectives of this study were:
  - To evaluate the activity of NVP-LBM415 against two strains of *H. influenzae* in a hollow-fiber infection model in order to determine the impact of dosing and MIC on bacterial eradication;
  - To determine which PK-PD measure was most strongly associated with drug response;
  - To examine the relationship between drug exposure and response for NVP-LMB415 against *H. influenzae*.
  - To confirm that, in the event of high inocula, exposures of NVP-LBM415 are sufficient to prevent the emergence of resistant sub-populations of *H. influenzae*.

METHODS

Bacteria and Antibiotics

- Two wild-type strains of *H. influenzae* were studied (MIC values of 2 and 8 µg/mL)
- NVP-LBM415 was provided by Novartis Pharmaceuticals, Inc.

In vitro Model and Sample Processing

- An *in vitro* hollow-fiber infection model was used to simulate NVP-LBM415 human pharmacokinetics ($t_{½}$ = 2 hours with monoexponential decline).
- *H. influenzae* was grown in Haemophilus test medium at 37°C and 5% CO_2.
  - The hollow-fiber system was inoculated with approximately 10^{7-8} CFU/mL in log-phase growth.
- Simulated dosing regimens provided a wide range of 24-hour area under the concentration-time curve (AUC) to MIC ratios (0 to 220), fractionated into once-daily (q24h), twice-daily (q12h), and continuous infusion (CI) regimens.
  - The q12h and CI regimens were tested with both strains of *H. influenzae*.
  - The q24h regimen was only tested against the strain with a MIC of 8 µg/mL.
- Samples for determination of bacterial counts (colony forming units, CFU/mL) were taken at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 13, 14, and 24 hours after dosing of NVP-LBM415.
- A spiral plater (WASP, DW Scientific) was used to plate samples onto chocolate agar plates, incubated at 37°C for 24 hours, and read using an automated colony counter (Acolyte).
- Samples taken at 12 and 24 hour were plated onto drug-containing agar plates at 4- and 8-times the MIC to test for the emergence of resistance.
- Experiments were conducted in duplicate.

PK-PD Analyses

- The following PK-PD measures were computed for each experiment and evaluated for correlation with drug effect:
  - Free fraction 24-hour AUC:MIC ratio (AUC:MIC)
  - Free fraction peak concentration to MIC ratio (peak:MIC)
  - Free fraction percent time above MIC (%T>MIC)
- Drug effect was quantified as the $\log_{10}$ ratio of the 24-hour area under the bacterial CFU versus time curve (AUCFU) for drug and growth control (**Equation 1**).

$$\text{Log}_{10}\ \text{ratio} = \log_{10}\left[\frac{\text{AUCFU}_{\text{drug}}}{\text{AUCFU}_{\text{Growth Control}}}\right] \quad (1)$$

METHODS (cont'd.)

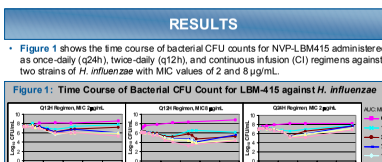
- $\log_{10}$ ratio values of zero indicate no drug effect, with larger negative values indicative of increasing drug effect. For example, a $\log_{10}$ ratio equal to -1 implies 90% less area in the drug containing experiment over 24 hours compared to the area of the growth control.
- Using non-linear regression (SYSTAT, Version 11, Richmond, CA), a Hill-type model was fit to the $\log_{10}$ ratio of the 24-hour AUCFU for each regimen to obtain estimates of E_{max} (fitted maximum $\log_{10}$ reduction in bacteria), $AUC:MIC_m$ (the median effect AUC:MIC ratio or the ratio required to achieve 50% of E_{max}), and H (Hill's constant, which accommodates sigmoidicity) (**Equation 2**).

$$\text{Log}_{10}\ \text{ratio} = \left[\frac{E_{max}\cdot(\text{AUC:MIC})^{H}}{\text{AUC:MIC}_{m}{}^{H}+(\text{AUC:MIC})^{H}}\right] \quad (2)$$

RESULTS

- **Figure 1** shows the time course of bacterial CFU counts for NVP-LBM415 administered as once-daily (q24h), twice-daily (q12h), and continuous infusion (CI) regimens against two strains of *H. influenzae* with MIC values of 2 and 8 µg/mL.

Figure 1: Time Course of Bacterial CFU Count for LBM-415 against *H. influenzae*



- The apparent difference noted for the two strains in the twice-daily regimen (upper left panels) can be explained by the lower initial inocula used in the MIC 8 µg/mL experiment versus the MIC 2 µg/mL experiment ($10^{6.1-6.3}$ versus $10^{7.3-7.5}$, respectively). A fundamental difference between strains was not observed and NVP-LBM415 provided similar antibacterial activity for both strains.
- As measured by the 24-hour maximum decline from baseline and maximum decline versus growth control, the greatest activity was seen with the CI regimen at 6-times the MIC value; slightly less activity was noted with the twice-daily regimen (**Table 1**).

Table 1: Antibacterial Activity of NVP-LBM415

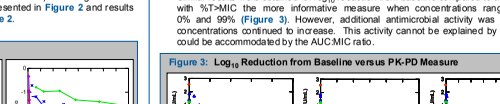
Regimen	Maximum Decline from Baseline at 24 hrs ($\log_{10}$ CFU/mL)	Maximum Decline vs Growth Control at 24 hrs ($\log_{10}$ CFU/mL)
Q12 hours	1.4 – 2.0	3.0 – 4.4
Q24 hours	0.13	1.4
CI at 1xMIC	-1.0 – 0.6	0.9 – 2.0
CI at 2xMIC	1.7 – 2.3	3.1 – 4.1
CI at 4xMIC	2.6 – 2.7	4.1 – 4.5
CI at 6xMIC	3.7 – 3.8	5.1 – 5.5

CI = Continuous Infusion

RESULTS (cont'd.)

- Due to the short half-life of the drug, once-daily dosing yielded no net bacterial killing compared to baseline.
- The emergence of resistance to NVP-LBM415 was not observed in these studies.
- The PK-PD measures (AUC:MIC ratio, peak:MIC ratio, and %T>MIC) for all regimens and both strains of *H. influenzae* versus the $\log_{10}$ ratio are presented in **Figure 2** and results from non-linear regression analyses are presented in **Table 2**.

Figure 2: $\log_{10}$ Ratio versus PK-PD Measure



The **green** line represents the q24h regimen (MIC 2 µg/mL) , the **blue** line represents the q12h regimen (MIC 2 µg/mL), the **red** line represents the CI regimen (MIC 2 µg/mL), the **purple** line represents the CI regimen (MIC 8 µg/mL), and the **aqua** line represents the q12h regimen (MIC 8 µg/mL).

Table 2: Model-fitted Parameter Estimates ($\log_{10}$ Ratio as Effect)

	Q12h MIC=2	Q12h MIC=8[a]	Q24h MIC=2	CI MIC=2	CI MIC=8
E_{max}	-1.98	-3.24	-1.59	-2.06	-2.22
Hill's Constant	0.722	1.49	1.0 [b]	1.34	3.87
$AUC:MIC_m$	11.8	11.2	24.4	13.9	24.8
r^2	0.801	0.998	0.947	0.994	0.986

[a] Initial inocula $10^{6.1-6.3}$
[b] Hill's constant was fixed at 1

- As noted previously, the apparent increased activity caused by the lower initial inocula for the twice-daily regimen and the strain with a MIC of 8 µg/mL (**Figure 2, aqua line**) made comparison between experiments inappropriate. This experiment will be repeated with a higher inocula for appropriate comparison between regimens.
- Neither AUC:MIC ratio, peak:MIC ratio, nor %T>MIC, could co-model the once-daily and twice-daily regimens with the continuous infusion results.
- The AUC:MIC ratio provided a reasonable fit for all curves.
  - Comparable AUC:MIC ratios were noted for both CI regimens, and the experiments co-modeled well (E_{max} $\log_{10}$ ratio of -2 at an AUC:MIC ratio ≥ 50)
  - The once-daily and twice-daily datasets (MIC 2 µg/mL) were reasonably fit by the AUC:MIC ratio (E_{max} $\log_{10}$ ratio of -2 at an AUC:MIC ratio ≥ 120)
- The %T>MIC was also a reasonably informative PK-PD measure for the once-daily and twice-daily datasets ($\log_{10}$ ratio of -2 to -3 at a %T>MIC of approximately 60%), but was uninformative for the CI datasets, as drug effect continued to increase at 100% time above MIC.

RESULTS (cont'd.)

- The peak:MIC ratio was not enlightening for any of the experiments.
- The $\log_{10}$ ratio measure is sensitive to the initial inoculum, and is therefore a less useful gauge of antimicrobial activity across experiments with unequal initial inocula.
- Similar results were obtained for $\log_{10}$ reduction from baseline compared to the $\log_{10}$ ratio, with %T>MIC the more informative measure when concentrations ranged between 0% and 99% (**Figure 3**). However, additional antimicrobial activity was observed as concentrations continued to increase. This activity cannot be explained by %T>MIC but could be accommodated by the AUC:MIC ratio.

Figure 3: $\log_{10}$ Reduction from Baseline versus PK-PD Measure

CONCLUSIONS

- Overall, the greatest antibacterial activity with NVP-LBM415 against *H. influenzae* was observed with the continuous infusion regimen at six times the MIC. The twice-daily regimen produced less bacterial killing than the continuous infusion regimen and the once-daily regimen yielded no net bacterial killing.
- For the once-daily and twice-daily dosing regimens, the AUC:MIC ratio and the percent time above MIC adequately described drug response with NVP-LBM415.
- Continuous infusion regimens with NVP-LBM415 could not be co-modeled with doses given either once-daily or twice-daily, suggesting that neither percent time above MIC nor the AUC:MIC ratio completely described drug effect.

REFERENCES

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