

Pharmacokinetic / pharmacodynamic modelling of the plasma bactericidal activity of NXL103 against *Streptococcus pneumoniae* and *Staphylococcus aureus* in phase I studies

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ABSTRACT

Objective: NXL103 is a novel oral streptogramin antibiotic associating 2 components RPR202868 (PI) and RPR132552 (PII). The objective of this study was to characterise the relationship between the plasma bactericidal activity against *S. pneumoniae* and *S. aureus* strains, and PI and PII plasma concentrations measured in samples collected in 2 phase I studies.

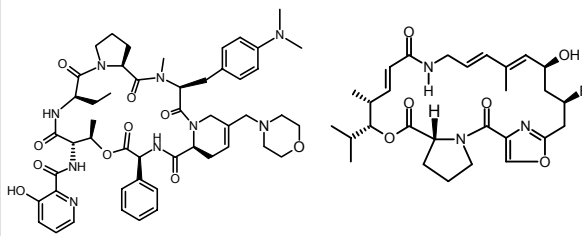
Methods: The dataset for building the PK/PD model was composed of 1067 plasma samples from a phase I repeated (10-day) oral administration study in healthy male volunteers. Four cohorts of 10 subjects (8 received NXL103 and 2 placebo) received 0.5 g bid, 0.75 g bid, 1.5 g oad, or 1 g bid. Blood samples were taken on 7 occasions from day 1 to day 11. PI and PII concentrations were measured in plasma by LC-MS/MS. Separate aliquots of the same samples were serially diluted into a growth medium / control plasma mixture, and incubated with *S. pneumoniae* (030MV1 strain) and *S. aureus* (011HT18 strain) to measure bactericidal activity. For the purpose of PK/PD modelling, cidal activity data were recoded as 0 (no activity) or 1 (cidal activity), irrespective of the intensity of effect. Fits were performed using WinNonlin®.

Results: The model predicted bactericidal activity on *S. pneumoniae* if both PI and PII were more than 0.175 mg/L and 0.523 mg/L, respectively. Breakpoints for *S. aureus* were 0.123 mg/L and 0.081 mg/L, respectively. The descriptive performance of the model was 93.5% and 83.9% accurate against *S. pneumoniae* and *S. aureus*, respectively. The predictive performance of the model was assessed on a separate validation dataset composed of 416 human plasma samples collected in a previous study, where 32 healthy male volunteers received a single oral dose of either 0.5 g, 1 g, 1.5 g or 2 g of NXL103. Plasma concentrations of PI and PII, and bactericidal activity were measured as above. Model-predictions were 91.8% and 93.5% accurate against *S. pneumoniae* and *S. aureus*, respectively, the remainder being evenly distributed between false positive and false negative predictions.

Conclusion: A PK/PD model was built that predicts the plasma bactericidal activity against two pathogens of therapeutic interest. These results should be taken into account in defining dose(s) for phase II trial(s).

INTRODUCTION AND PURPOSE

NXL103 is a novel oral anti-infective agent of the streptogramin group developed so far as an association of two components in a 30/70 dose ratio: RPR202868 (PI) and RPR132552 (PII). PI and PII individually exert only a limited antibacterial effect, but have a strong antibacterial activity (including bactericidal activity) when administered together.



RPR202868 (PI)

RPR132552 (PII)

In vitro NXL103 spectrum of activity includes aerobic Gram-positive cocci (including multi-resistant *S. pneumoniae*, MRSA constitutively resistant to MLSB (MLSB), and vancomycin-resistant *E. faecium*), certain aerobic Gram-negative bacteria responsible for respiratory tract infections (including *H. influenzae* and *M. catarrhalis*) and atypical pathogens. It is rapidly bactericidal, demonstrates post antibiotic effect and shows low potential *in vitro* to select resistant mutants in MSSA, MRSA, susceptible and resistant *S. pneumoniae* and *H. influenzae*.

In vivo NXL103 was found to be active in the different mouse models of infection studied (including infections caused by methicillin and MLSB *S. aureus* and erythromycin-resistant *S. pneumoniae* strains).

In the light of its potency against strains resistant to the other classes of antibiotics, this compound represents an alternative to quinolones, oxazolidinones, macrolides and ketolides.

The objective of this study was to characterise the relationship between the plasma bactericidal activity against *S. pneumoniae* and *S. aureus* strains, and PI and PII plasma concentrations measured in samples collected in two clinical phase I studies.

METHODS

Studies

Study #1 was a phase I, randomised, double-blind, placebo-controlled parallel groups study with 6 different escalating oral doses of NXL103 or placebo, administered in fasting conditions as a single dose to 6 cohorts of 10 healthy adult male subjects (8 receiving NXL103 and 2 receiving placebo). Dose levels of NXL103 (herein expressed as PI+PII) were 0.125 g, 0.25 g, 0.5 g, 1 g, 1.5 g and 2 g. Blood samples were taken at 13 time-points until 24 hours post-dose.

Study #2 was a phase I, randomised, double-blind, placebo-controlled parallel groups study with 4 different escalating oral doses of NXL103 or placebo administered in fed conditions to 4 cohorts of 10 healthy adult male subjects (8 receiving NXL103 and 2 receiving placebo). Dosage regimens were 0.5 g twice a day (bid), 0.75 g bid, 1.5 g once a day (oad), or 1 g bid for 10 days. Serial blood samples were taken at 13 time-points on days 1-2, nine time-points between day 4 and day 8, and at 13 time-points on days 10-11.

Plasma was separated in two aliquots, one for pharmacokinetic (PK) evaluation, one for the determination of bactericidal activity.

Bioanalysis

Plasma concentrations of PI and PII were measured by a validated LC-MS/MS method. The method also quantifies an N-desmethyl metabolite of PI (MET). The lower limit of quantitation was 0.005 mg/L for each analyte.

Plasma bactericidal activity

Strains used

Strains used were selected from the culture collection of Novexel: *S. aureus* 011HT18 (Smith) *S. pneumoniae* 030MV1 were both fully susceptible to penicillins and macrolides.

Susceptibilities of strains to NXL103

Susceptibilities of strains were determined in appropriate liquid medium. Minimum bactericidal concentrations (MBCs) were determined in the absence and in the presence of 50% human serum.

| | MBC _{in vitro} (mg/L) | |
|----------------------|--------------------------------|-----------------------------|
| | <i>S. aureus</i> 011HT18 | <i>S. pneumoniae</i> 030MV1 |
| Without human serum | 0.12 | 0.12 |
| With 50% human serum | 0.25 | 0.5 |

Test medium and inoculum preparation

S. aureus 011HT18 (Smith) was cultured overnight in Mueller Hinton broth and then diluted in growth medium to give inoculum of around 5x10⁵ cfu/ml.

S. pneumoniae 030MV1, overnight culture on blood agar in CO₂ was diluted in Mueller Hinton plus 4% red blood cell extract to give an inoculum or around 5x10⁵ cfu/ml.

Establishment of plasma bactericidal activity test conditions

50% plasma/50% growth broth was used as the test medium. Preliminary experiments with the test strains confirmed that the strains selected tolerated up to 50% plasma in the growth medium. Due to the dilution factor this method results in a limit of detection of 2-fold the MBC_{in vitro}. That is to say, a drug concentration in the original sample plasma equivalent to 1 x MBC_{in vitro} will not be detected.

Preparation of the dilution series

Equal volumes of test plasma were mixed with pooled human control plasma and 2-fold serial dilutions were carried out. An equal volume of growth medium containing the test strain was then added to each well.

Plasma bactericidal activity (PBA)

After overnight incubation all wells were sampled and 10 µl streaked onto solid agar medium. The highest dilution of plasma which suppressed 99.9% of the original inoculum was defined as the PBA. Therefore in relation to the original inocula, growth of 5 colonies or less was considered as bactericidal.

PK/PD modelling

Learning dataset:

PK/PD modelling was conducted on a dataset composed of all subjects under active treatment from study #2. This represented a set of 1067 plasma samples evaluable both for PK (concentrations of PI, MET, and PII) and PD (PBA against *S. pneumoniae* and *S. aureus*).

Candidate PK/PD models:

Separate PK/PD models were built to try and predict the bactericidal activity against *S. pneumoniae* and *S. aureus*. However, models shared one of the following candidate structures: sigmoidal, logistic, or binary.

Sigmoidal model

The sigmoidal model was written as :

$$\text{Prob(PBA=1)} = X^H / (X_50^H + X^H)$$

With H being a Hill coefficient, and X a linear combination of analytes' concentrations in plasma, including terms to allow for possible pharmacological interactions between entities:

$$X = \text{PII} + a \times \text{PI} + b \times \text{MET} + c \sqrt{\text{PI} \times \text{PII}} + d \sqrt{\text{PI} \times \text{MET}} + e \sqrt{\text{PII} \times \text{MET}} + f \sqrt[3]{\text{PI} \times \text{PII} \times \text{MET}}$$

Finally, parameter X₅₀ was the value of variable X associated with 50% probability of bactericidal activity. Part of the modelling exercise consisted in identifying relevant terms in variable X, by stepwise deletion of the terms associated with the least significant coefficients « a » to « f ».

Logistic regression

By analogy with the sigmoidal model, the logistic regression model aimed at predicting the probability of bactericidal activity in a given plasma sample. It was written as:

$$\text{Prob(PBA=1)} = \exp(X) / [1 + \exp(X)]$$

with X being defined and estimated as above.

Binary model

$$\text{If PI} > \text{MBC}_{\text{PI, ex vivo}} \text{ and PII} > \text{MBC}_{\text{PII, ex vivo}} \text{ then} \\ \text{PBA} = 1 \\ \text{else} \\ \text{PBA} = 0$$

Transformation of PK and PD variables:

Plasma concentrations below the limit of quantitation were substituted by zero. Since the purpose of PK/PD modelling was to predict the presence, absence or probability of bactericidal activity in a single plasma sample, PD data were recoded as 0 (no activity) or 1 (cidal activity), irrespective of the bactericidal titer.

Software: Fits were performed using the WinNonlin® software. The minimisation algorithm was the Nelder-Mead simplex.

Assessment of descriptive performance

Candidate models were qualified by the proportion of accurate predictions, versus the incidence of (and balance between) false positive and false negative predictions. Regarding the sigmoidal and logistic models, cidal activity was predicted to be present whenever the calculated probability of activity was greater than 0.70.

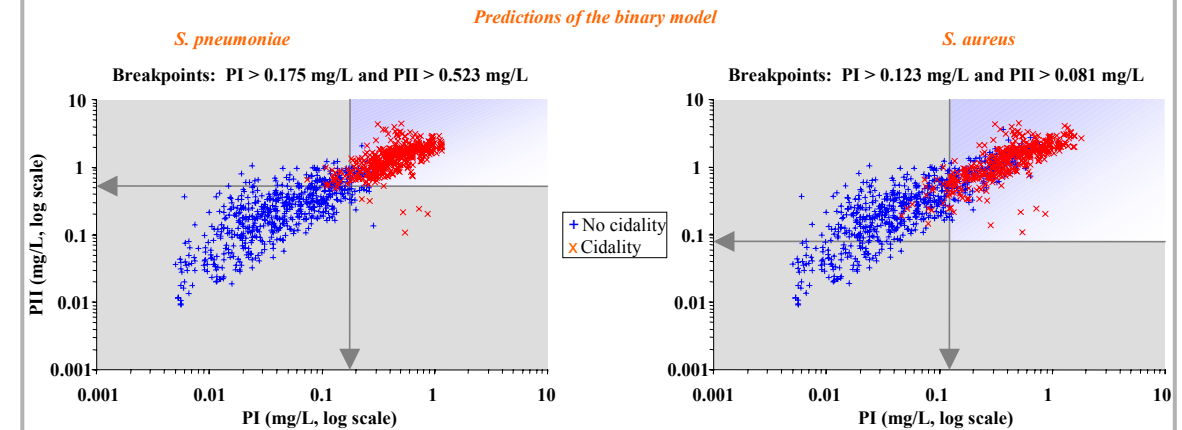
Assessment of predictive performance

The same criteria were applied to the assessment of predictive performance on a separate validation dataset. The dataset was composed of the 32 subjects from study #1 who were treated with NXL103 doses of 0.5 g, 1 g, 1.5 g or 2 g. This represented a total of 416 plasma samples evaluable both for PK and PD. While study #1 actually preceded study #2, the exercise truly mimicked a fully prospective assessment.

RESULTS

Model selection

Both the logistic and sigmoidal models shared a common characteristic: variable X only retained PI and PII concentrations. More explicitly, there was no need considering any significant interaction term, or MET plasma concentrations. However, both these models failed to predict PBA with sufficient accuracy (not shown). The binary model was therefore preferred. It predicted that a given plasma sample should exhibit bactericidal activity on *S. pneumoniae* if both the following conditions are fulfilled: PI > 0.175 mg/L and PII > 0.523 mg/L. A separate combination of breakpoints was estimated for the cidal activity against *S. aureus*: PI > 0.123 mg/L and PII > 0.081 mg/L. The results obtained with the binary model are shown below.



Observed and model-predicted plasma bactericidal activity against *S. pneumoniae* (left) and *S. aureus* (right) as a function of PI and PII concentrations (in mg/L, log-log scale). Blue and red symbols are the plasma samples where bactericidal activity was absent or present, respectively. The top-right blue areas are where the binary PK/PD model predicts cidal activity; blue symbols in these areas correspond to false positive predictions. Conversely, no cidal activity is predicted in the grey areas; red symbols in these areas are false negative predictions.

Assessment of descriptive performance

S. pneumoniae

| Observations N = 1067 | | Model-based predictions | |
|--------------------------|--------------------------------------|----------------------------------|----------------------------------|
| | | No cidal activity | Cidal activity |
| | | + No cidal activity | + Accurate N = 633 (59.3%) |
| x Cidal activity | x False negative N = 27 (2.5%) | x Accurate N = 364 (34.1%) | |

The model on *S. pneumoniae* adequately predicted experimental data with 93.5% of accurate predictions, the remaining being almost balanced between false negative (2.5%) and false positive (4.0%).

S. aureus

| Observations N = 1067 | | Model-based predictions | |
|--------------------------|--------------------------------------|----------------------------------|----------------------------------|
| | | No cidal activity | Cidal activity |
| | | + No cidal activity | + Accurate N = 544 (51.0%) |
| x Cidal activity | x False negative N = 20 (1.9%) | x Accurate N = 351 (32.9%) | |

The descriptive performance on *S. aureus* data dropped down to 83.9% of accurate predictions, and was associated with some imbalance between false negative (1.9%) and false positive predictions (14.2%).

Assessment of predictive performance

S. pneumoniae

| Observations N = 416 | | Model-based predictions | |
|-------------------------|--------------------------------------|---------------------------------|----------------------------------|
| | | No cidal activity | Cidal activity |
| | | + No cidal activity | + Accurate N = 328 (78.8%) |
| x Cidal activity | x False negative N = 15 (3.6%) | x Accurate N = 54 (13.0%) | |

In a predictive mode, the model resulted in 91.8% of accurate predictions on *S. pneumoniae*, in line with the descriptive performance on the learning dataset.

Overall, model-predictions were 91.8% and 93.5% accurate against *S. pneumoniae* and *S. aureus* data, respectively, the remainder being evenly distributed between false positive and false negative predictions.

S. aureus

| Observations N = 416 | | Model-based predictions | |
|-------------------------|-------------------------------------|----------------------------------|----------------------------------|
| | | No cidal activity | Cidal activity |
| | | + No cidal activity | + Accurate N = 275 (66.1%) |
| x Cidal activity | x False negative N = 7 (1.7%) | x Accurate N = 114 (27.4%) | |

The predictive performance of the model on *S. aureus* data was excellent (93.5% of accurate predictions) on this separate dataset, and even better than expected from the learning dataset.

CONCLUSION

A PK/PD model was built that predicts the plasma bactericidal activity against two pathogens of therapeutic interest. These results should be taken into account in defining dose(s) for phase II trial(s).