Determination of target dose for Phase II study of 2-Iminobiotin (2-IB) in neonates with perinatal asphyxia

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Summary

A Phase II study is planned to evaluate the efficacy, safety, and pharmacokinetics (PK) of 2-Iminobiotin (2-IB) in neonates of ≥36 weeks gestational age with moderate to severe perinatal asphyxia. For this study a dose will be targeted which is assumed to have an optimal clinical effect and is expected to be safe. The target dose is calculated by combining a predicted clearance value for term neonates obtained by interspecies scaling and an effective target plasma exposure (AUC) observed in piglets.

For the interspecies scaling measurements of plasma clearance in animals and humans in relation to their body weight were obtained from a total of 8 (pre)clinical studies in (adult and juvenile) rats, (adult and juvenile) minipigs and humans (adult healthy volunteers), giving a total of 245 data points. Clearance appeared to increase linearly with body weight across different species and was estimated to be 621 mL/h/kg. Based on this observation it can be concluded that across species and age groups (adult and juvenile), including piglets and humans, the dose/kg can remain constant to achieve similar exposure.

In an inhalational hypoxia study in piglets a dosing range from 0.2 to 1.0 mg/kg/pulse of 2-IB when given every 4 hours was found to be most promising in terms of clinical efficacy. The mean $AUC_{0\text{-}4h}$ value for the piglets with moderate to severe asphyxia that received the 0.2 mg/kg/pulse dose was 365 ng.h/mL. This observed exposure was in line with the expected exposure based on the interspecies scaling. Therefore, for neonates, a dose of 0.2 mg/kg/pulse is considered an appropriate starting dose as well, to reach the target AUC.

Newborns exposed to perinatal asphyxia may have a lower clearance compared to healthy newborns. As 2-IB is predominantly cleared via the renal route, the ratio between creatinine clearance in newborns with and without perinatal asphyxia was taken as estimate for the difference in 2-IB clearance between these two groups. On the basis of the available (limited) literature, renal clearance may be 3-30 fold lower in newborns with exposure to perinatal asphyxia compared to healthy newborns. As exposure is inversely related to clearance, this would result in $AUC_{0\text{-}4h}$ values. As clearance is inversely related to exposure, a 3-30 fold decrease in clearance compared to healthy neonates would result in expected AUC values of 1095 to 10950 ng.h/mL. These estimated AUC values above are still well within the exposure values associated with dose levels that were well tolerated in humans ($AUC_{0\text{-}4h}$ 14358 ng.h/mL after first infusion and 15145 ng.h/mL after last infusion) and associated with the NOAEL in animal models (> 47000 ng.h/mL).

Taken together, based on the interspecies scaling and the observed 2-IB exposure in an efficacy study in piglets, a starting dose of 0.2 mg/kg/pulse is considered a suitable starting dose to reach the target exposure ($AUC_{0\text{-}4h}$) of 365 ng.h/mL. This starting dose also leaves a considerable safety margin compared to exposure associated with dose levels that were well tolerated in humans and exposure associated with the NOAEL in animal models, even in case 2-IB clearance would be lower in asphyxiated neonates compared to healthy neonates.

Introduction

2-Iminobiotin (2-IB) is a biotin (Vitamin H or B7) analogue and a selective nitric oxide synthase (NOS) inhibitor, presently in development to treat and prevent the devastating effects of perinatal asphyxia. Nitric oxide (NO) has been implicated in the pathogenesis of a wide array of conditions, and also plays a critical role in the development of brain cell damage after perinatal asphyxia. Its synthesis occurs through a number of nitric oxide synthase (NOS) iso-enzymes, which can be inhibited selectively by 2-IB.

In in-vitro studies, 2-IB showed a selective inhibition of the neuronal and inducible NOS iso-enzymes whereas no effect has been demonstrated on endothelial nitric oxide synthase (eNOS). This is of major importance, since endothelial NOS action can result in a decreased blood pressure and cerebral perfusion and might cause additional harm after asphyxia.

In-vivo, the efficacy of 2-IB has been demonstrated in piglets and rats in models of perinatal asphyxia both in the short and long term. In piglets, in an inhalational hypoxia model (Study UQCCR/566/09), neuroprotective effects as well as increased survival were observed across the 0.1 to 1.0 mg/kg/pulse range, given every 4 hours. The dose range of 0.2 to 1.0 mg/kg/pulse was found to be most promising.

Currently a Phase II study is planned to evaluate the pharmacodynamics, early efficacy, safety, and pharmacokinetics (PK) of 2-Iminobiotin (2-IB) in term neonates with moderate to severe perinatal asphyxia. For this study a dose will be targeted which is assumed to have an optimal clinical effect and is expected to be safe. The current report describes the methods used to calculate this target dose. Briefly, it is calculated by combining a predicted value for term neonates clearance (CL) obtained by interspecies scaling and an effective target exposure (AUC) observed in piglets. For the interspecies scaling data from juvenile and adult rats, juvenile and adult minipigs, and humans (healthy adult volunteers) were used.

Estimation of 2-IB clearance in newborn infants

Data collection

To make an estimation of the CL of 2-IB in newborn infants, interspecies scaling of preclinical PK data from (adult and juvenile) rats and (adult and juvenile) minipigs was used together with clinical PK data from humans. Studies in which 2-IB was given either intravenously or subcutaneously were processed to perform this scaling, assuming that bioavailability after subcutaneous administration approached 100%. In total, data from 8 studies were used: 3 rat studies (of which 2 in juvenile rats), 4 minipig studies (of which 2 in juvenile minipigs) and 1 human clinical study (see Table 1 for overview of the study designs, and Table 2 for an overview of the available study reports). For minipigs and humans individual data curves were obtained in these studies, while for rats one curve per gender per group was obtained by pooling blood samples for individual time points. In case of multiple dosing, data were used from first and last dose PK, if available. In total, this resulted in a database of 245 data points.

Table 1: Overview of study designs used for interspecies scaling

Study #	Species	Treatment	Route	Dose per administration	N (first dose, last dose)	Individual or pooled curve
NTX 490137	rats	24 pulses of 15 min q4h	IV	6.67 mg/kg	5M, 5F	pooled
NTX 490137	rats	24 pulses of 15 min q4h	IV	13.33 mg/kg	5M, 5F	pooled
NTX 490137	rats	24 pulses of 15 min q4h	IV	27.5 mg/kg	5M, 5F	pooled
NTX 490336	minipig	96 h infusion	IV	400 mg/kg/day	2M,1F	individual
NTX 490336	minipig	24 pulses of 15 min q4h	IV	8.4 mg/kg	2/2M,2/2F	individual
NTX 490336	minipig	24 pulses of 15 min q4h	IV	16.8 mg/kg	2/2M,2/2F	individual
NTX 490982	minipig	24 pulses of 15 min q4h	IV	4.2 mg/kg	7M,7F	individual
NTX 490982	minipig	24 pulses of 15 min q4h	IV	8.4 mg/kg	7M,7F	individual
NTX 490982	minipig	24 pulses of 15 min q4h	IV	16.8 mg/kg	7M,7F	individual
NTX 491904	juv. rats	12 injections q4h	SC	2.07 mg/kg	9/15M,9/15F	pooled
NTX 491904	juv. rats	12 injections q4h	SC	4.14 mg/kg	9/15M,9/15F	pooled
NTX 491904	juv. rats	12 injections q4h	SC	8.25 mg/kg	9/15M,9/15F	pooled
NTX 491905	juv. rats	72 injections q3h	SC	8.25 mg/kg	9/15M, 9/15 F	pooled
NTX 491905	juv. rats	72 injections q3h	SC	8.25 mg/kg	--/15M, 9/15 F	pooled
NTX 491905	juv. rats	72 injections q3h	SC	15 mg/kg	9/15M, 9/15 F	pooled
NTX 491905	juv. rats	72 injections q3h	SC	22.5 mg/kg	9/15M, --/15 F	pooled
NTX 491906	juv. minipig	30 pulses of 45 sec q4h	IV	13.7 mg/kg	10M,11F	individual
NTX 491906	juv. minipig	30 pulses of 45 sec q4h	IV	19.2 mg/kg	12M,10F	individual
NTX 491906	juv. minipig	60 pulses of 45 sec q4h	IV	19.2 mg/kg	6M,6F	individual
NTX 491907	juv. minipig	60 injections q4h	IV	9.7 mg/kg	4/3M, 5/5F	individual
NTX 491907	juv. minipig	60 injections q4h	IV	19.2 mg/kg	3/3M, 4/4F	individual
NTX 491907	juv. minipig	60 injections q4h	IV	19.2 mg/kg	4/4M, 5/5F	individual
NTX 491907	juv. minipig	60 injections q4h	IV	30 mg/kg	4/4M, 5/5F	individual
PRA 90461	human	4h infusion 1x/day	IV	0.6 mg/kg	2	individual
PRA 90461	human	1h infusion 1x/day	IV	0.6 mg/kg	2	individual
PRA 90461	human	15 min infusion 1x/day	IV	0.6 mg/kg	2	individual
PRA 90461	human	15 min infusion 6x/day	IV	0.6 mg/kg	6	individual
PRA 90461	human	15 min infusion 6x/day	IV	2 mg/kg	6	individual

IV: intravenously; SC: subcutaneously; M: male; F: female; 2-IB: 2-Iminobiotin; juv: juvenile; q4h: every 4 hour

Interspecies scaling
All data described above were summarized in an Excel spreadsheet. For each animal or subject, or group of animals (rats), the weight (kg), gender and the dose administered (mg/kg) was listed together with the reported AUC (ng.h/mL) and CL (mL/h/kg), if available, and calculated CL in ml/h. In case the CL was not reported in the original study report, CL was calculated from the exposure using the formula:

CL = Dose / AUC (*in which AUC is the area under the time exposure curve).*

Data in this Excel spreadsheet was verified by an independent data-analyst to assure an accurate and complete reflection of the raw data.

A trend was observed for higher CL after prolonged dosing. The 2 to 3-fold weight increase during the study period observed for the juvenile minipigs will have contributed significantly to this observation (see also Figure 2).

A non-linear weighted least squares fitting method was used to fit the pooled clearance (CL, in mL/h) and weight (WT, in kg) data-pairs to the following equation:

$CL_{predicted} = a * WT^{b}$ *(WT=body weight)*

In this procedure values for CL were predicted for the body weight of each animal/subject and compared with the observed CL values by calculating the residual error.
Subsequently, the sum of the weighted squared residuals of the inidvidual datapoints were calculated, called the 'Costs':

$$\text{Costs: } \sum^{n=245} [(CL_{observed}-CL_{predicted})^2/(CL_{observed})^2]$$

Using the 'Solver' function in Excel, values for *a* and *b* were estimated that resulted in a minimum of Costs. Initially, a value of 0.975 was estimated for *b*. As this value is essentially not different from 1, *b* was omitted from the model. Therefore, in the final model CL increases linearly with weight across the different species.

Final model: $CL_{predicted} = 621.0 * WT$

Figure 1 shows the model fit to the observed data

As CL increases linearly with weight (and CL = Dose/AUC), the dose required to reach a certain exposure across the involved species only needs to be corrected for differences in body weight, i.e. the dose/kg remains the same.

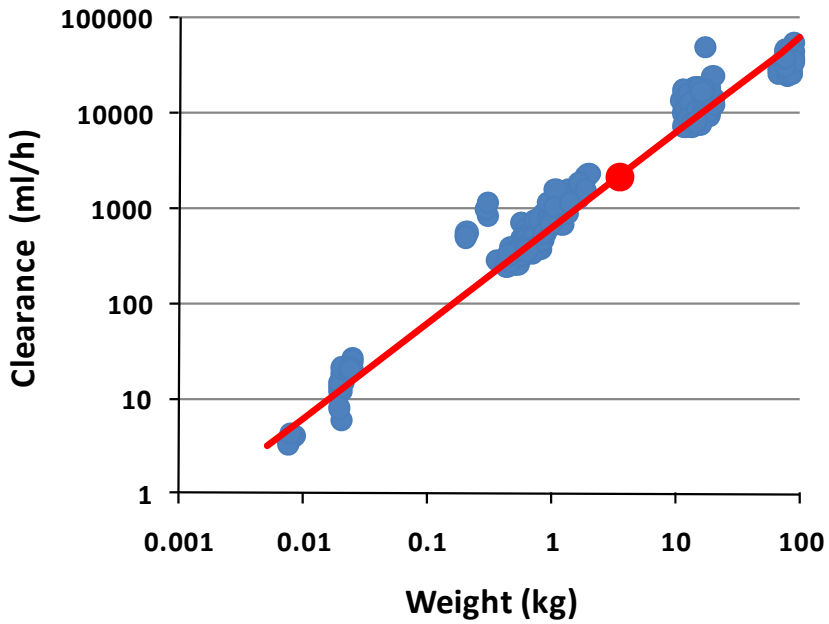

Figure 1: XY scatter plot of clearance (CL, mL/h) vs. body weight (kg). Blue dots represent the observed CL, the red line represents the predicted CL, and the red dot the predicted CL for new born infants with a body weight of 3.5 kg.

Estimation of target exposure

To predict a dose range for new born infants that is assumed to have an optimal clinical effect, a target plasma concentration or exposure (AUC) of 2-IB had to be determined. As mentioned in the introduction, in an inhalational hypoxia study in piglets the dose range from 0.2 to 1.0 mg/kg/pulse given every 4 hours was found to be most promising with respect to efficacy. For the piglets in that study with moderate to severe asphyxia the mean $AUC_{0\text{-}4h}$ after the first infusion for the lowest dose of the effective dose range (0.2 mg/kg/pulse) was 365 ng.h/mL. The mean AUC of all animals in the 0.2 mg/kg/pulse group was 338 ng.h/mL. As the observed plasma level at 4h after the first infusion was already relatively low compared to the observed maximum levels, minimum accumulation to steady-state level is to be expected during repeated dosing every 4 hours. As a result, the value of 365 ng.h/mL can be considered a relevant estimate of steady-state $AUC_{0\text{-}4h}$, assuming time-independent pharmacokinetics. Therefore this value was used as the target exposure.

Target dose for newborn infants

The target dosing range (D) in newborn infants can be calculated from D=CL* AUC. Based on the interspecies scaling clearance is expected to be 621 mL/h/kg across the involved species, resulting in a dose of 0.23 mg/kg/pulse to reach the target AUC of 365 ng.h/mL.

The PK data from the inhalational hypoxia study in piglets were not used in the interspecies scaling and therefore this study can be used as a validation of the interspecies scaling. Based on the interspecies scaling a dose of 0.2 mg/kg/pulse would result in an AUC of 322 ng.h/mL (AUC = Dose /CL), which is close to the actual observed AUC (365 ng.h/mL for the animals with moderate to severe asphyxia and 338 ng.h/mL for all animals) for the 0.2 mg/kg/pulse dose group in the inhalation hypoxia study. This confirms the conclusion from the interspecies scaling that the dose/kg is constant across the species. Therefore for newborns a dose of 0.2 mg/kg is expected to result in similar exposure as in the inhalational hypoxia study in piglets.

As mentioned previously, the interspecies scaling of plasma clearance in relation to body weight includes data of both (healthy) juvenile and adult rats and minipigs. The linearity in this relation suggests that correction for body weight is adequate to cover differences in clearance between adults and (healthy) newborns. It does however not cover possible differences in clearance between healthy newborns vs. newborns exposed to perinatal asphyxia. Asphyxiated neonates may have renal failure caused by acute tubular necrosis. As 2-IB is predominantly cleared via the renal route, differences in creatinine clearance between asphyxiated and healthy neonates may provide an indication of how of 2-IB exposure in newborns exposed to perinatal asphyxia would relate to healthy newborns. Boer et al. (2010) and Aperia et al. (1981) both found a creatinine clearance of 20.8 ml/min/1.73m^2 on Day 1-2 in full-term newborns. Bakr et al. (2005) found a creatinine clearance of 0.62, 0.81, 0.92 ml/min/1.73m^2 on Day 1, 2 and 3 respectively after birth in newborns exposed to perinatal asphyxia. Baht et al. (2006) however found a substantial higher creatinine clearance in this group of newborns i.e. 7.36 ml/min/1.73m^2 at day 2 to 3 after birth. Based on the relation between the creatinine clearance on Day 1, 2 and 3 in Bkar et al (2005), the Day 1-2 figures for newborns exposed to perinatal asphyxia were estimated and compared to those found in full-term newborns. Although the uncertainty in the values is high, these data suggest that the clearance in newborns may be 3-30 times higher compared to newborns exposed to perinatal asphyxia. As clearance is inversely related to exposure, a 3-30 fold decrease in clearance compared to healthy neonates would result in expected AUC values of 1095 to 10950 ng.h/mL. This is based on the assumption that creatinine clearance will be predictive for 2-Iminobiotin clearance.

The estimated AUC values above are still well within the exposure values associated with dose levels that were well tolerated in humans ($AUC_{0\text{-}4h}$ 14358 ng.h/mL after first infusion and 15145 ng.h/mL after last infusion) and associated with the NOAEL in animal models (> 47000 ng.h/mL). In view of the considerable safety margin in 2-IB exposure it is considered unlikely that in asphyxiated neonates exposure levels that exceed levels that have been found to be well tolerated in humans will be observed even when asphyxiated neonates would show reduced clearance of 2-IB compared to healthy neonates. Therefore a starting dose of 0.2 mg/kg/pulse is considered the most appropriate dose to reach 2-IB exposure associated with efficacy in a piglet model, while still providing a considerable safety margin.

Table 2: Overview of available study reports

Study no.	Title	Completion Date
NTX 490137	96 hour toxicity study with 2-IB given by intravenous pulse infusions followed by a 14-day recovery period in wistar rats	8 March 2010
NTX 490336	A preliminary toxicity study of 2-iminobiotin by subcutaneous injection or via intravenous infusion (continuous or pulse) in Gottingen minipigs	18 January 2010
NTX 490982	An intravenous infusion toxicity and toxicokinetic study of 2-iminobiotin in Gottingen minipigs with a 14-day recovery period	29 October 2009
NTX 491904	A preliminary toxicity study of 2-iminobiotin in juvenile rats with 2 cycles of intermittent (every 4 hours) subcutaneous dosing over a 48 hour timepoint starting on PND 3 and PND 10	27 May 2010
NTX 491905	A neurobehavioral, toxicity, reproductive and toxicokinetic study of 2-iminobiotin in juvenile rats with intermittent (every 3 hours) subcutaneous dosing (72 doses) starting on PND 3	20 January 2011
NTX 491906	An intravenous range-finding toxicity and toxicokinetic study of 2-iminobiotin in juvenile Gottingen minipigs	27 May 2010
NTX 491907	An intravenous toxicity and toxicokinetic study of 2-iminobiotin in juvenile Gottingen minipigs with a four-week recovery period	25 November 2010
NOA046EC-090461	Randomized, double-blind, placebo-controlled dose escalation study to evaluate the safety and pharmacokinetics of single and multiple doses of 2-iminobiotin (2-IB) in healthy male subjects	23 June 2011
UQCCR/566/09	Effect of 2-iminobiotin and hypothermia in the perinatal hypoxic brain	Ongoing
Bjorkman ST, Ireland Z, Fan X, Van der Wal W, Leufkens P, Colditz PB, and Peeters-Scholte C.	Dose-response characteristics of 2-Iminobiotin in the neonatal piglet following hypoxia-ischemia.	Manuscript in preparation

Reference list

Aperia A. et al. *Postnatal development of renal function in pre-term and full-term infants*. Acta Paediatr Scand 70: 183-187, 1981.

Bakr A.F. *Prophylactic theophylline to prevent renal dysfunction in newborns exposed to perinatal asphyxia – a study in a developing country*. Pediatr. Nephrol 20: 1249-1252, 2005

Bhat M.A. et al. *Theophylline for renal function in term neonates with perinatal asphyxia: a randomized, placebo-controlled trial*. J. Pediatr 149: 180-184, 2006.

Boer D.P. et al. *Reference values for serum creatinine in children younger than 1 year of age*. Pediatr Nephrol. 25:2107-2113, 2010.