NON-CLINICAL SAFETY ASSESSMENT OF ALX-0171, A NANOBODY® INTENDED FOR CLINICAL ADMINISTRATION VIA NEBULISATION

Erik Depla, Koen Allosery, Veronique de Brabandere, Sofie Priem, Stefaan Rossenu* , Sandra De Boever, Eline Vanheule, Anne Schoolmeester, Judith Baumeister
Ablynx nv, Zwijnaarde, Belgium

M1034

Abstract

Purpose

To establish non-clinical local and systemic safety and tolerability of ALX-0171, a trivalent Nanobody potently neutralising respiratory syncytial virus. The clinical intended route of administration is inhalation of nebulised Nanobody

Methods

ALX-0171 specifically binds to a viral target that is not expressed in healthy mammals, therefore non-clinical safety assessment relied on rat as a single species. A 14-day toxicity study via inhalation was complemented by a respiratory safety pharmacology study. Nebulisation occurred via a vibrating membrane nebuliser with maximum daily exposure times of 200 min, aiming for a high target dose of 150mg/kg. Nebulised drug was examined as part of stability studies and in-study characterisation. Drug concentrations in bronchoalveolar fluid (BALF) and plasma were measured in satellite animals. PK data from rat were used to predict human systemic and local exposure in first clinical trials. Local safety margins were calculated using an approximated study lung deposition in rat and a conservative 100% lung deposition in man

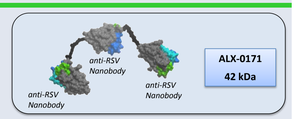
Results

No safety signal was identified in the 14 day-toxicity study after inhalation and no influence on the respiratory system was detected in the safety pharmacology study. ALX-0171 retained quality attributes after nebulisation such as low aggregation (<5% HMW) and potency. In-use study results in the highest dosing scheme yielded: 117 mg/kg achieved dose, 800 µg/l air concentration, MMAD 3.4 µm. Approximated lung concentrations of the highest dose (based on 10% deposition of achieved dose and measured lung weights) in rats were 2.3-3.0 mg/g lung (female/male). Systemic exposure was moderate (F 10%). Predicted maximum human lung exposure was 0.21 mg/g lung, yielding a safety margin >10

Conclusions

Administration of nebulised biotherapeutic drugs is a new frontier with the advantage of high local concentrations and a non-invasive procedure. ALX-0171, a trivalent Nanobody was robustly nebulised with excellent respirability and stability. In toxicity and safety pharmacology studies in rat no safety signal was identified and ALX-0171 was well tolerated after inhalation. Appropriate safety margins to an estimated exposure in man were achieved. ALX-0171 has successfully completed a Phase I clinical trial

ALX-0171: first-in class potential in RSV treatment



- **ALX-0171 - Unique Nanobody format**
  - Nanobodies are derived from naturally occurring heavy chain -only antibodies
  - composed of 3 variable region domains targeting the same epitope
  - trivalent interaction with RSV fusion (F) protein leads to avidity: 2,000 fold increase in potency compared with monovalent building block
- **ALX-0171 potently inhibits respiratory syncytial virus (RSV) *in vitro* and *in vivo***
  - RSV infection can lead to severe respiratory infections and is a leading cause of hospitalisation in infants
  - ALX-0171 potently neutralises a wide range of RSV-A and B strains *in vitro*
  - ALX-0171 prevents RSV infection in a prophylactic RSV-infected cotton rat model and reduces viral load and symptoms when administered therapeutically
- **Clinical experience**
  - well-tolerated in the clinic: clinical phase I double-blind, randomized trial succesfully completed in healthy adult males to investigate safety, tolerability and PK profile of inhaled ALX-0171 (SAD and MAD)
  - no local or systemic treatment emergent immunogenicity observed so far (see poster# T2088)
- **Administration via nebulisation**
  - local delivery leads to high concentrations at the site of disease and rapid onset of action
  - efficient nebulisation without relevant loss in potency, physicochemical properties and pharmacological activity

Methods: design of safety studies in rats

- ALX-0171 binds exclusively to the non-endogenous viral target, therefore non-clinical safety assessment primarily relied on one species (rat) only.
- Tolerability, safety, immunogenicity and possible off-target effects of ALX-0171 when administered via nebulisation were assessed in a 14 day toxicity and a respiratory safety pharmacology study in rats
- Supporting data were derived from a safety/efficacy study (non-GLP) in RSV-infected cotton rats, a 14-day toxicity study in rats after i.v. administration and a cardiovascular safety pharmacology study in dog after i.v. administration. Local tolerability was assessed in the BCOP assay
- Nebuliser: Akita²Apixneb (Activaero GmbH; DE) equipped with Apixneb handset with class 30 aerosol head (PARI Pharma, GmbH, DE)

Study	animals	Administration	Target dose	Objectives/readout
14-day RD toxicity study in rat after inhalation	Crl:CD (SD) rats	Daily snout only inhalation (200 min) 14 days + 14 days recovery	0, 15, 50, 150 mg/kg	Local and systemic safety and tolerability , immunogenicity, PK
Respiratory safety pharmacology study in rat after single dose inhalation	Crl:CD (SD) rats 8 + 5 satellite animals (sacrificed after dosing)	in whole body plethysmograph single dose 60 min baseline, 120 min dosing, 30 min post-dose	0, 15, 50, 150 mg/kg	evaluate effects on respiratory parameters (tidal volume, respiration rate, minute volume)

RD: repeated dose

Methods: assays and calculations

- Drug concentration was measured using validated, target dependent ligand binding assays. Results were subjected to non-compartmental PK analysis (WinNonlin v 5.1, Pharsight)
- Non-linear mixed effect pharmacokinetic modeling (NonMem v 7.1.0, ICON) was employed to fit available rat data from single dose PK (data not shown) and multiple dose toxicity studies. Systemic exposure for FIM after inhalation was predicted by allometric scaling: volumes of distribution for central and peripheral compartment (Vc, Vp) =1, linear clearance (CL) = 0.75, first order absorption from lung =-0.25.
- Calculation of systemic safety margins: [exposure reached in rat safety study after i.v. administration (not shown)] / [systemic exposure in man predicted]
- Calculation lung safety margins using highest tested dose in rat safety studies: [actual rat lung conc[1] /mean lung weight in study] / [theoretical lung concentration assuming 100% deposition of anticipated FIM dose/human lung weight]
- Immunogenicity was determined using bridging ADA assays validated for bronchoalveolar fluid (BALF) and plasma. For results see poster # T2088
- Characterisation of test item included storage stability, F/T and in-study stability (data not shown, criteria were met)
- Compatibility of ALX-0171 with the vibrating membrane nebuliser and the nebulisation procedure was demonstrated by: particle size (NGI and laser diffraction), stability of aerosolised product (potency ELISA, size-exclusion HPLC (SE-HPLC), and concentration (OD280).

[1]Actual rat lung concentration: highest delivered dose* 0.1 fraction deposited (estimated from BALF concentration in alignment with literature: J. Toxicol. Environment. Health, 15: 197-214)

Results I: ALX-0171 was well tolerated

Study	Achieved dose	Local concentrations	Major observations/findings
14-day RD toxicity study in rat after inhalation	• 0, 16.9, 41.7, 117 mg/kg • Particle size in respirable range (MMAD ≤ 3.4 µm) • 800 µg/l air concentration	• Approximated concentration of ALX-0171 in lung (based on 10% deposition of achieved highest dose and measured lung weight): 2.3-3 mg/g (female/male) • ALX-0171 amounts in BALF day 1: low dose 0.3 mg; high dose: 1.2 mg (recovery 32-93%) • Plasma $AUC_{0\text{-}24h}$ (NC): day 1: 1749 ng.h/ml; day 14: 4506 ng.h/ml	• No ALX-0171-related effects on clinical observations, haematology, clinical chemistry, urinalysis, macroscopic examination, organ weights and histopathology (including upper airways) • Treatment emergent ADAs were detected (in plasma and BALF) • Plasma concentrations increase with dose • Limited accumulation after repeated dosing (2.5 fold)
Respiratory SP study in rat after single dose inhalation	• 0, 16, 46, 160 mg/kg • Particle size in respirable range (MMAD ≤ 3.9 µm)	• ALX-0171 amounts in BALF (satellite animals, immediately after exposure): low dose 1 mg; high dose: 1.4 mg (recovery 29-182%) • Plasma concentration (satellite animals, immediately after exposure): low dose: 47 ng/ml; high dose: 68 ng/ml	• No ALX-0171-related effects on clinical observations or respiratory safety enpoints • Plasma concentrations increase with dose, but not proportionally • No immunogenicity assessment performed (single dosing)

MMAD: mass median aerodynamic diameter; BALF: bronchoalveolar fluid; NC: non-compartmental analysis, males: 1.385 g lung weight females: 1.160 g lung weight

Results II: Robust nebulisation properties

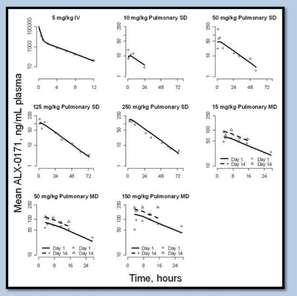
- The influence of nebulisation on drug product used for toxicity studies was studied in dedicated stability studies. Different storage conditions (≤-60°C, -20°C, +5°) up to 24 months were examined, followed by nebulisation. The only effect was some formation of higher molecular weight species (HMWs, approximately + 1%). Exemplary results for long term storage condition are shown.
- In-use characterisation supporting the 14-day toxicity study after inhalation confirmed results from stability assessment. Due to a divergent sampling method HMW formation was noted for information only and was not seen as representative (11%).

Test Method	Reference value based on pre-nebulisation specification	Stability data of ALX-0171 non-GMP material for 14-day toxicity study in rat, post-nebulisation and aerosol measurements (t=24 months), ≤-60°C
Appearance	• Colour < reference standard Y4 • Turbidity < reference standard IV	• Colourless • clear
OD280	• OD280: 50 ±10 mg/ml • Absorbance at 340 nm	• 55.9 mg/ml • 0.004
SE-HPLC	• ≥ 85% main peak • ≤5% HMW	• 98 % main peak • 2.2% HMW
potency	• 100 ± 50% compared to reference standard	• 100%
NGI[a]	• -	• MMAD = 4.32 µm • GSD: 1.57

HMW: high molecular weight, NGI: Next Generation Impactor, MMAD: mass median aerodynamic diameter; GSD: Geometric Standard Deviation
[a] NGI measurement performed at initial stability time point (t=0), Akita2Apixneb equipped with Apixneb handset with class 40 aerosol head .

Results III: Pharmacokinetics

- Basic PK parameters were obtained using NC analysis (see Results I)
- A mixed effects model were employed to describe data in rat and scale to man.
- A ~ 10% bioavailability (lung to systemic) was estimated for rat, dependent on the particle size, confirming estimates from literature



Observed (symbol) and model predicted (solid line for day 1 and dotted line for day 14) plasma concentration-time profiles after i.v. or inhaled administration of ALX-0171 in Sprague-Dawley rats.
Time 0 in this graph refers to the first or last (Day 14) day of dosing. The route of administration was by inhalation (as is indicated by the term Pulmonary).

Open two-compartmental pharmacokinetic model with linear clearance from the central compartment.
CL: linear clearance
V_c: volume of the central compartment
V_p: volume of the peripheral compartment
Q: intercompartmental flow between the central and the peripheral compartment
K_a: first order absorption constant
$F1$: Absolute bioavailability

Study	Ref	Species	Highest dose FIM	AUC_{∞} a,c (µg.h/mL)	AUC_{24} b,c (µg.h/mL) Male/Female	Lung exposure (mg/g lung) Male/Female	Ratio[f] Male/Female
14-day repeated dose toxicity study after daily administration as aerosol		Sprague-Dawley rat	117 mg/kg (achieved)	-	-	**3.0/2.3[e]**	**14/11**
14-day repeated dose toxicity study after daily i.v. administration		Sprague-Dawley rat	50 mg/kg	-	**297/328**	-	**21/24**
Administration of highest dose in Phase I study in human subjects after administration by inhalation	-	Human	3 mg/kg	**13.9**	-	**0.210[d]**	-

AUC_{∞}: calculated as the area under the curve from time zero to infinity
[b] AUC_{24}: calculated as the area under the curve at steady-state for 24h
[c] Calculated lung or systemic exposure ratio with predicted human exposure at 3 mg/kg as reference.
[d] Predicted human exposure based on PK modelling
[e] Calculated as 10% deposited achieved dose times actual mean male/female body weight divided by actual mean male/female lung weight.
[f] Calculated as 100% deposited maximum clinical dose (3 mg/kg) divided by a human lung weight of 1,000 g (Davies and Morris, 1993).

Conclusions

- Excellent tolerability and safety profile was obtained in rat safety studies:
  - no lung or systemic adverse effects observed after inhalation of nebulised ALX-0171
  - no signs of upper airway irritation
  - no adverse effects in respiratory safety pharmacology studies
- Robust nebulisation of ALX-0171
  - independent of the storage time or temperature, the amount of HMW product-related variants, as assessed by SE-HPLC, increased by approximately 1% after nebulisation. The post-nebulisation results remained within the pre-nebulisation specifications for the same time as the results directly obtained on the formulation.
- Pharmacokinetics has been described using non-compartmental analysis and mixed-effect models
  - after daily administration of aerosol, limited accumulation can be observed in rat
  - limited systemic exposure after administration of aerosol
  - acceptable exposure margins >10 obtained
- Immunogenicity has been benign: see poster # T2088

Acknowledgements:

The authors would like to thank in particular **Gilles Hanton (GH Toxconsulting)** in consulting as toxicology expert in this programme. They also thank past and present members of Ablynx RSV project team (Thomas Stohr, Kjell Mortier, Lotte Bohez, Mauro Sergi) ; Activaero GmbH and PARI Pharma GmbH (nebulisers); Huntingdon Life Sciences (rat studies).

*Current affiliation: MSD OSS BV, the Netherlands;