



# CLINICAL STUDY PROTOCOL

## ALX0171-C201

**A Randomized, Double-Blind, Placebo-Controlled, Multicenter  
Dose-Ranging Study of ALX-0171 in Infants and Young Children  
Hospitalized for Respiratory Syncytial Virus Lower Respiratory Tract  
Infection**

<b>Investigational Product:</b>	ALX-0171 nebulizer solution
<b>EudraCT n°:</b>	2016-001651-49
<b>Sponsor Protocol Code:</b>	ALX0171-C201
<b>Sponsor:</b>	Ablynx NV Technologiepark 21 9052 Zwijnaarde, Belgium
<b>Coordinating Investigator</b>	[REDACTED], Royal Hospital for Sick Children Sciennes Road, Edinburgh, Midlothian EH9 1LF, United Kingdom
<b>Clinical Operations Contract Research Organization:</b>	[REDACTED] [REDACTED] [REDACTED]
<b>Phase of Development:</b>	Phase 2b
<b>Indication:</b>	Respiratory Syncytial Virus Lower Respiratory Tract Infection (RSV LRTI)
<b>Study Center:</b>	Multicenter
<b>Protocol Date:</b>	30 October 2017
<b>Protocol Version:</b>	V3.0
<b>Protocol Status:</b>	Final

*This study will be performed in compliance with the Clinical Study Protocol, the principles of Good Clinical Practice (GCP), and the applicable regulatory requirement(s).*

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## **CONFIDENTIALITY STATEMENT**

The information contained in this document, especially unpublished data, is the property of Ablynx NV (or under its control), and therefore provided to you in confidence as an Investigator, potential Investigator, or consultant, for review by you, your study staff, and applicable Independent Ethics Committee (IEC)/Institutional Review Board (IRB), and Competent Authorities (CA). It is understood that this information will not be disclosed to others without written authorization from Ablynx NV, except to the extent necessary to obtain informed consent from the parent(s)/legal guardian(s) of the young children to whom the study drug may be administered.

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## **APPROVAL OF CLINICAL STUDY PROTOCOL**

The Sponsor and the Investigator(s) agree to conduct the study as outlined in this Clinical Study Protocol. Any modification of the Clinical Study Protocol must be agreed upon by the Sponsor and the Investigator(s), and must be documented in writing.

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**Sponsor:**

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Name:   
Function: Senior Medical Director

Signature – Date: *See signature page at the end of the document*

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**Investigator:**

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I have read Clinical Study Protocol ALX0171-C201 and agree to personally conduct or supervise the clinical study in accordance with this Clinical Study Protocol, with the terms of the clinical trial agreement and with any other study procedures and documents provided by Ablynx NV.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

I confirm that the study team and I will not implement any deviations from this Clinical Study Protocol without agreement of Ablynx NV, except where necessary to eliminate an immediate hazard to the patients.

I confirm that I am thoroughly familiar with the appropriate use of the study drug, as described in this Clinical Study Protocol and any other information provided by Ablynx NV.

I confirm that I am aware of and will comply with ICH-GCP and all applicable national and regional regulations/guidelines.

Hence, I agree to supply Ablynx NV with any necessary information regarding the ownership interest and financial ties, to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study, and that Ablynx NV may disclose any available information about such ownership interest and financial ties to regulatory authorities.

Principal Investigator Name:

Site details/Address:

Signature – Date:

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**Ablynx NV**



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# **SERIOUS ADVERSE EVENT CONTACT INFORMATION**

Contact details of the Sponsor and third parties are available in the "Investigator Site File".

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## LIST OF ABBREVIATIONS

ADA	Anti-drug antibodies
AE	Adverse event
ATC	Anatomical therapeutic chemical
AUC	Area under the curve
BQL	Below the limit of quantification
CA	Competent Authority
CFR	Code of federal regulations
CL/F	Apparent clearance
cPAP	Continuous positive airway pressure
CRA	Clinical research associate
CRO	Contract research organization
CTD	Clinical Trial Directive
DAP	Data analysis plan
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	Electronic case report form
EMA	European Medicines Agency
EOS	End of study
FDA	Food & Drug Administration
FU	Follow-up
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GS	Glycine-serine (linker)
HFOT	High flow oxygen therapy
HIV	Human immunodeficiency virus
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
ICU	Intensive care unit
ID	Identification
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ITT	Intent-to-treat
i.v.	Intravenous
IWRS/IVRS	Interactive web/voice response system
KL-6	Krebs von den Lungen
LBA	Ligand binding assay
Legal guardian(s)	Authorized representative(s) for the subject, according to local legislation
LRTI	Lower respiratory tract infection

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LSLV	Last visit of the last subject
LTRA	leukotriene receptor antagonists
mAb	Monoclonal antibody
MD	Medical Doctor
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified intent-to-treat
NAb	Neutralizing anti-drug antibodies
O <sub>2</sub>	Oxygen
qPCR	Quantitative polymerase chain reaction
PD	Pharmacodynamic
PFU	Plaque forming unit
PK	Pharmacokinetic
PP	Per-protocol
Pre-Ab	Pre-existing antibodies
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RACS	Respiratory Assessment Change Score
RDAI	Respiratory Distress Assessment Instrument
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
SAE	Serious adverse event
SAP	Statistical analysis plan
SpO <sub>2</sub>	Peripheral capillary oxygen saturation
SUSAR	Suspected Unexpected Serious Adverse Reactions
TE	Treatment-emergent
TEAE	Treatment-emergent adverse event
VAS	Visual Analogue Scale
WHO	World Health Organization

## CHANGES COMPARED TO PREVIOUS VERSION(S)

<b>Global Version 2.0 (dated 25 October 2016) compared to Global Version 1.0 (dated 20 June 2016). This amendment is considered substantial due to inclusion of dose-escalation stopping criteria in the study, an update to the instructions for dosing, and changes of in/exclusion criteria.</b>	
Version n° and date were adapted throughout the document (including headers and footers). The Table of Contents was updated and the Section "Changes Compared to Previous Version(s)" was completed.	
Original section	Change/Rationale
Synopsis and Section 3.1.1.	Figures have been updated to ensure consistency.
Synopsis and Sections 1.3.5., 3.1.1., 3.1.2., 3.2.3., 3.3.2. and 3.3.3.	Criteria for stopping dose escalation during the sequential part of the study, as well as specifications for which doses could be used in the parallel part of the study, have been added. The individual discontinuation criteria have been updated to ensure consistency with the dose-escalation stopping criteria.
Synopsis and Section 3.2.1.	Inclusion criterion 2 has been updated to allow subjects weighing up to <15.0 kg to be included in the study.
Synopsis and Section 3.2.2.	For clarity, the exclusion criterion on HIV positivity has been reworded and an additional criterion was added to exclude subjects with a known hypersensitivity to the study drug or any excipient of the study drug from the study.
Sections 3.3.2., 3.3.7. and 3.3.7.1.	Instructions for dosing have been updated with additional weight bands and safety margin calculation has been updated accordingly.
Section 3.4.1.1.	A typographical error has been corrected.
Section 3.4.8.1.	More detailed guidance on early detection and treatment of the potential risks of airway hyperresponsiveness and immediate or delayed adverse drug reactions have been added.
Section 3.5.1.3.	The category "not related/unlikely related" of the causality assessment for AEs has been split to 2 categories; "not related" and "unlikely related".

<p><b>Global Version 3.0 (dated 30 October 2017) compared to Global Version 2.0 (dated 25 October 2016). This amendment is considered substantial as the non-interventional, Follow-Up study that was described in the original version of the Protocol was removed. Furthermore, the method of randomization was corrected. The inclusion and exclusion criteria were further specified and clarified to ensure that a homogenous population of subjects is enrolled across the sites. To allow flexibility and consistency between assessments, certain time windows were adapted in the Schedule of Assessments.</b></p>	
<p>Version n° and date were adapted throughout the document (including headers and footers). The Table of Contents was updated. The section "Changes Compared to Previous Version(s)" was updated with the changes of the current amendment. Minor typographical errors were corrected throughout the document, use of abbreviations and the abbreviations list had minor updates stemming from the amendment changes.</p>	
Section affected	Change/ Rationale
Synopsis, Section 3.1.1	The non-interventional, Follow-up Study ALX0171 C202 was deleted, as it will not be conducted; following interactions between the Sponsor and the European Medicines Agency's Paediatric Committee, it was confirmed the study is not necessary at this stage of development.
Synopsis - Study Design - Study Population Section 3.1.1	The age range for study population was updated for consistency (age range reads: 28 days to <2 years).
Schedule of Assessments; Section 3.4.1.2	The Schedule of Assessments underwent changes for clarity and easier flow in the assessments to be performed: <ol style="list-style-type: none"> <li>1. On Day 1, the time window before study drug administration was extended to 3 hours.</li> <li>2. Time windows of 0.5 hours before and after the 2-hour post-dose assessments were added to allow additional time for the site staff to perform the assessments.</li> <li>3. To enhance the overview, the randomization row was moved.</li> <li>4. Additional guidance was added on the footnote referring to the documentation of sleep disturbance due to night-time coughing.</li> <li>5. The sequence of footnotes was updated.</li> </ol>
Section 1.2.3 (First-in-infant study; Immunogenicity)	The reporting of the first in infant study has been completed; the main conclusions are included in the Protocol. A typographical error was corrected in the code of Phase 1 safety study ALX-0171-1.2/13.
Schedule of Assessments and Section 3.3.3	A clarification was added that, at a minimum, the SpO <sub>2</sub> , feeding, respiratory muscle retractions, and respiratory rate should be evaluated on Day 1 before randomization, unless these assessments were already performed within the last 3 hours before randomization.
Section 3.3.3	The method of stratified randomization per cohort was corrected.
Synopsis, Exclusion criteria, Section 3.3.8	Additional guidance for the Investigators was provided on the use of forbidden medications. It was clarified that initiation of treatment with leukotriene receptor antagonists and sodium cromoglycate is forbidden. It was further clarified that topical corticosteroids to treat ear disorders are permitted. Inosine pranobex, xanthines and expectorants were added to the list of forbidden medications.
Section 3.4.2	An explanation was added that if the nasal swab sample collected before study drug administration on Day 1 was insufficient for adventitious viral screening, the sample collected after the Day 1 study drug administration could be used.

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**Global Version 3.0 (dated 30 October 2017) compared to Global Version 2.0 (dated 25 October 2016). This amendment is considered substantial as the non-interventional, Follow-Up study that was described in the original version of the Protocol was removed. Furthermore, the method of randomization was corrected. The inclusion and exclusion criteria were further specified and clarified to ensure that a homogenous population of subjects is enrolled across the sites. To allow flexibility and consistency between assessments, certain time windows were adapted in the Schedule of Assessments.**

Version n° and date were adapted throughout the document (including headers and footers). The Table of Contents was updated. The section "Changes Compared to Previous Version(s)" was updated with the changes of the current amendment. Minor typographical errors were corrected throughout the document, use of abbreviations and the abbreviations list had minor updates stemming from the amendment changes.

<b>Section affected</b>	<b>Change/ Rationale</b>
Schedule of Assessments, Section 3.4.8.2, Section 3.4.9	The following clarification was added regarding hematology and biochemistry assessments to be performed at screening: If all hematology and biochemistry blood parameters required for the study are already known from analysis done as a routine assessment upon arrival at the hospital, collection of screening clinical laboratory samples does not need to be repeated for the purpose of this study. However, the samples should be analyzed at the same local laboratory that will be used for the remainder of the study.
Section 3.4.3.1	The collection of nasal swabs should be performed in both nostrils using the same swab was added as a clarification.
Synopsis, Section 3.2.1	Methods of feeding support were provided as extra guidance to the Investigator.
Inclusion Criteria: Synopsis and Section 3.2.1	Clarifications were added on the timing study population inclusion criteria must be fulfilled (specified at screening and/or randomization as applicable).
Secondary endpoints: Synopsis and Section 3.6.4	The reference timepoint in the definition of time to clinical response was updated for consistency with other time to event endpoint.
Section 3.4.5.1	The following clarification was added: The blood samples for PK assessments will be collected from a vein, as using capillary blood (e.g., from heel pricks) is not allowed.
Section 3.4.7.1	The following clarification was added: The blood samples for ADA assessments will be collected from a vein, as using capillary blood (e.g., from heel pricks) is not allowed.
Schedule of Assessments and Section 3.4.1.2	Stable oxygen saturation on room air of >92% over a period of at least 4 hours would be adequate to fulfill the criterion for clinical response.
Exclusion Criteria: Synopsis and Section 3.2.2	A sentence specifying that if any of the exclusion criteria were to be fulfilled at screening or randomization, the subject would be excluded. Typographical errors were corrected in Exclusion Criteria 5, 7, 8, and 11.
Synopsis, Section 3.4.8.2	Further details were provided for the analysis of differential white blood cell counts.

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## PROTOCOL SYNOPSIS

**Protocol title:**

A randomized, double-blind, placebo-controlled, multicenter dose-ranging study of ALX-0171 in infants and young children hospitalized for respiratory syncytial virus lower respiratory tract infection

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**Investigational product:**

ALX-0171 nebulizer solution.

The active pharmaceutical ingredient is ALX-0171 Nanobody, a therapeutic protein directed against the fusion protein of the human respiratory syncytial virus (RSV).

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**EudraCT n°:**

2016-001651-49

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**Sponsor protocol code:**

ALX0171-C201

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**Sponsor:**

Ablynx NV  
Technologiepark 21  
9052 Zwijnaarde, Belgium

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**Phase of Development:**

Phase 2b

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**Indication:**

Respiratory Syncytial Virus Lower Respiratory Tract Infection (RSV LRTI)

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**Study centers:**

International multicenter study

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**Objectives:**

Primary objective:

To evaluate the anti-viral effect and safety of different doses of inhaled ALX-0171 in subjects hospitalized for RSV LRTI.

Secondary objective:

To evaluate the clinical activity, pharmacokinetic (PK) properties, pharmacodynamic (PD) effect and immunogenicity of different doses of inhaled ALX-0171.

**Study design:**

This is a randomized, double-blind, placebo-controlled, international, multicenter dose-ranging study. Study drug will be administered along with standard of care treatment, which will be determined by the Investigator (or his/her designee) according to institutional practice\*.

Three dose levels are planned to be evaluated:

- Dose 1: target dose of 3.0 mg/kg
- Dose 2: target dose of 6.0 mg/kg
- Dose 3: target dose of 9.0 mg/kg

The dose range that will be evaluated is expected to provide a potential benefit to the subjects, and is selected to allow assessment of reduction in nasal viral shedding and improvement of clinical symptoms, to support selection of an optimal dose for future development.

Study drug will be administered once daily for 3 consecutive days. The 3-day treatment period is expected to bridge the time needed for the body to mount an effective immune response.

The study is planned to enroll approximately 180 infants and young children (aged 28 days to <2 years) who are diagnosed with and hospitalized for RSV LRTI.

The study consists of a sequential part followed by a parallel part ([Figure 1](#)).

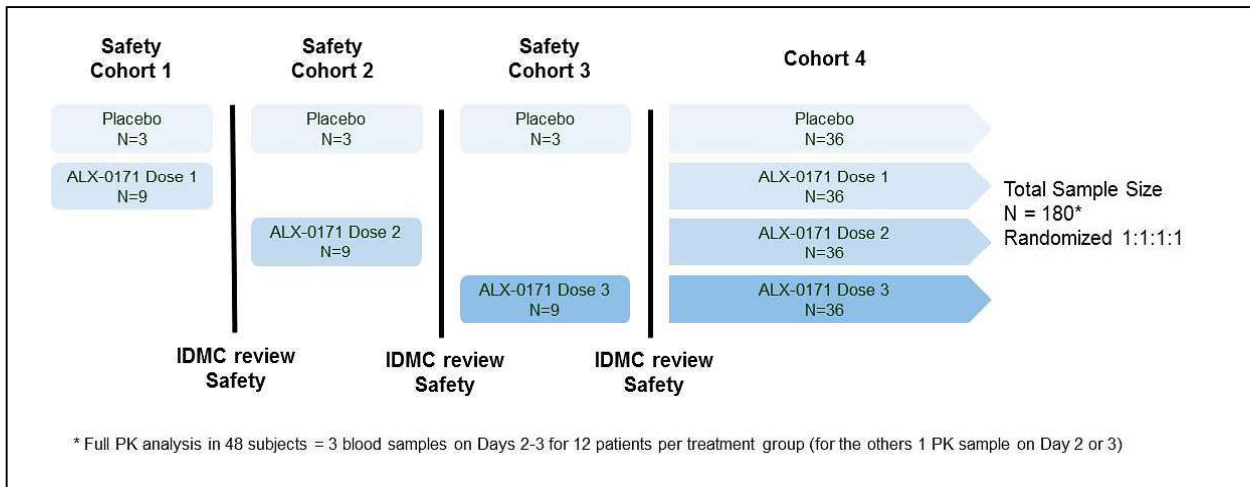
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\* Clinical Practice Guideline: The diagnosis, management and Prevention of Bronchiolitis as described by the American Academy of Pediatrics (2014) may be followed in addition to institutional practice.

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**Figure 1: Overview of study design**

### Sequential Part

In safety Cohorts 1 to 3, sequential dose escalation is used to enable appropriate safety follow-up. Each of the 3 safety cohorts consists of 12 subjects (N=36 in total) randomized 3:1 to ALX-0171 (N=9) or placebo (N=3).

In addition to the individual discontinuation criteria (see section 3.2.3), criteria for stopping or pausing recruitment in a safety cohort and criteria to stop dose escalation will apply in the sequential part of the study (see section 3.1.1). These criteria will also be used to decide on the doses taken forward into Cohort 4 (i.e., parallel part of the study).

In addition to these dose stopping criteria, cumulative unblinded safety data will be reviewed by an IDMC consisting of an independent group of clinical experts not participating in the study (and an independent statistician).

- After each safety cohort (when the last subject in a safety cohort has completed the treatment period), recruitment will be paused while the IDMC reviews the available cumulative safety data (i.e., all data from prior visits that have been done for all included subjects, including preceding cohorts).
- After this comprehensive review, the IDMC will advise the Sponsor on proceeding to the subsequent cohort (i.e., the higher dose), and which planned dose levels can be taken forward into the parallel part.

Throughout the study, SAE information will be communicated to the IDMC in real time, according to the reporting requirements. An ad-hoc meeting can be requested by the IDMC members. During the parallel part, an IDMC review will be done when 72 subjects completed treatment.



*Parallel Part*

If no stopping criteria have been met during the sequential part and if the IDMC issues a positive recommendation after each safety cohort and the Sponsor decides to proceed, the remaining 144 subjects (i.e., Cohort 4) will be randomly assigned in a 1:1:1:1 ratio to one of following treatment groups, yielding an overall randomization ratio of 3:1 active to placebo.

- ALX-0171 dose 1
- ALX-0171 dose 2
- ALX-0171 dose 3
- Placebo

In the event one or two dose groups are discontinued for safety, the remaining groups will enroll the number of subjects initially specified (i.e., 45 per arm).

The Placebo group will serve as comparator group for the 3 ALX-0171 dose groups. To achieve double-blinding across the different groups, each dose will be administered as two serial nebulizations. These two nebulizations will consist either of two nebulizations of ALX-0171, or one of ALX-0171 and one of placebo, or two nebulizations of placebo depending on the assigned treatment group.

Subjects will be screened as soon as possible after arrival to the hospital/emergency unit. After completion of the screening assessments and confirmation of subject's eligibility, randomization should follow as soon as possible but not more than 24 hours after arrival. Study drug administration should start as soon as possible after randomization with a maximum time interval of 3 hours following randomization. Subsequent doses of study drug will be administered at 24-hour intervals ( $\pm 4$  hours) relative to the first dose. No premedication is required or recommended per protocol, but an inhaled  $\beta$ 2-agonist may be administered at the discretion of the treating Investigator.

On the first 2 dosing days, inpatient hospital stay is required. Provided that the clinical response criteria have been met (which are based on adequate oral feeding and oxygen saturation), discharge from the hospital can take place per protocol at the Investigator's discretion from dosing Day 2 onwards after all required assessments of the 5 hours ( $\pm 1$  hour) post-dose time point have been completed. Subjects discharged after the second dose must return to the hospital for the third study drug administration (to be administered  $48 \pm 4$  hours after the first dose by the appropriately trained study personnel), and be monitored for a 2-hour pre-dose and post-dose period. Subjects who are not discharged from the hospital after completion or premature discontinuation of study drug enter an in-hospital post-treatment period with assessments in the morning and evening. A Follow-Up (FU) visit is scheduled on Day 14 ( $\pm 2$  days), and an End of Study (EOS) visit on Day 28

(± 2 days). For subjects prematurely withdrawn from the study, a Withdrawal visit is to be performed on the day of withdrawal.

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**Study population:**

Infants and young children aged 28 days to <2 years with a gestational age of ≥ 33 weeks who are hospitalized for and diagnosed with RSV LRTI.

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**Number of subjects:**

Approximately 180 subjects will be enrolled in the study. Subjects will be randomly assigned to receive ALX-0171 dose 1, ALX-0171 dose 2, ALX-0171 dose 3 or placebo (N=45 per treatment group), yielding an overall allocation ratio of 3:1 active to placebo. Of these, 48 subjects (12 per dose group) will undergo more extensive PK sample analysis (3 PK samples per subject instead of 1 PK sample).

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**Inclusion criteria:**

A subject will be eligible for study participation if he/she meets all of the following criteria at screening and randomization, unless specified otherwise.

1. Subject is a male or female infant or young child aged 28 days to <2 years with gestational age ≥ 33 weeks at screening.
2. Subject weighs between ≥ 3.0 kg and < 15.0 kg at screening.
3. Subject is otherwise healthy, but is hospitalized for and clinically diagnosed with RSV LRTI (bronchiolitis or broncho-pneumonia), i.e., showing typical clinical signs and symptoms such as tachypnea, wheezing, cough, crackles, use of accessory muscles and/or nasal flaring.
4. Subject has a positive RSV diagnostic test at screening.<sup>†</sup>
5. Subject is expected to have to stay in the hospital for at least 24 hours (according to the Investigator's judgment at screening).
6. Symptoms likely related to RSV infection (i.e., the symptoms present need to be probably linked to the current RSV infection according to Investigator's judgment) have appeared within 4 days of screening and are not yet improving at screening and randomization.
7. Subject fulfils at least two of the following RSV disease severity criteria at screening and randomization:
  - Inadequate oral feeding that requires feeding support (i.e., nasogastric tube or intravenous [i.v.] line)

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<sup>†</sup> RSV infection will be confirmed either according to routine site practice (PCR or diagnostic quick test), or using a (Sponsor-provided) commercial kit.

- Inadequate oxygen saturation defined as:
    - Oxygen saturation (SpO<sub>2</sub>) ≤92% on room air or
    - Requiring oxygen supplementation to maintain oxygen saturation >90% with documented pre-supplementation value ≤ 92%
  - Signs of respiratory distress defined as:
    - Respiratory rate ≥ 50 per minute in infants up to 12 months of age, and ≥ 40 per minute in children above 12 months and/or
    - Moderate or marked respiratory muscle retractions
8. Subject has normal psychomotor development.
  9. Parent(s)/legal guardian(s) provide written informed consent in accordance with locally approved consent process at screening.
  10. The parent(s)/legal guardian(s) are able and willing to comply with the study protocol procedures.

**Exclusion criteria:**

Subjects meeting any of the following criteria at screening or randomization will not be eligible for study participation.

1. Subject is known to have significant comorbidities including:
  - Genetic disorders (e.g., trisomy 21, cystic fibrosis),
  - Hemodynamically significant congenital heart disease (e.g., needing corrective therapy or inotropic support),
  - Bronchopulmonary dysplasia,
  - Any hereditary or acquired metabolic (bone) diseases,
  - Hematologic or other malignancy.
2. Subject is known to be human immunodeficiency virus (HIV)-positive. If the subject is < 6 months of age, a known HIV-positivity of the mother is also exclusionary.
3. Subject is known to be immunocompromised.
4. Subject has or is suspected to have an active, clinically relevant concurrent infection (e.g., bacterial pneumonia, urinary tract infection). Concurrent acute otitis media is not exclusionary.
5. Subject has significant oral and/or maxillofacial malformations that would prevent proper positioning of the face mask.
6. Subject received invasive mechanical ventilation or non-invasive respiratory support (i.e., continuous or bilevel positive airway pressure) in the 4 weeks prior to screening.
7. During the current admission, subject is initially hospitalized in an intensive care unit (ICU) setting and/or received invasive mechanical ventilation or non-invasive respiratory support (i.e., continuous or bilevel positive airway pressure).
8. Subject is critically ill and/or is expected to require invasive mechanical ventilation, non-invasive respiratory support (i.e., continuous or bilevel positive airway pressure), or High Flow oxygen therapy (HFOT) at levels not enabling nebulization therapy

according to the Investigator's judgment. High Flow oxygen, with a maximum flow of 2 L/kg/min, is permitted under the following conditions:

- used as Standard of Care outside ICU setting
  - can be removed for study drug administration (Note: oxygen flow at 2 L/min can be provided)
9. Subject has received 1 or more doses of palivizumab or treatment or prophylaxis with any RSV antiviral compound (e.g., ribavirin, i.v. immunoglobulin, or any investigational drug or vaccine for RSV [including subject's mother who has been vaccinated against RSV]) at any time prior to screening.
  10. Subject is required to continue or start systemic corticosteroid therapy. Subject on a maintenance therapy of inhaled corticosteroids will continue this treatment at the usual dose. Topical corticosteroids for skin or ear disorders are permitted.
  11. Subject is a child in care (i.e., a child who has been placed under the control or protection of an agency, organization, institution or entity by the courts, the government or a government body, acting in accordance with powers conferred on them by law or regulation). A child in care can thus include a child cared for by foster parents or living in a care home institution, provided that the arrangement falls within the definition above, but does not include a child who is adopted or has appointed legal representative(s).
  12. Subject is currently participating in any other study with investigational drug or has received an investigational drug within 4 weeks or 5 half-lives of the concerned drug (whichever is longer) prior to screening.
  13. Subject was previously enrolled in a clinical study of ALX-0171 (including the current Study ALX0171-C201).
  14. Subject has a known hypersensitivity to the study drug or any excipient of the study drug.

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**Study drug:**

## Investigational Product

- Name: ALX-0171 (nebulizer solution).
- Active substance: ALX-0171 Nanobody.
- Activity: ALX-0171 Nanobody specifically and potently binds to the RSV F protein, thereby inhibiting an early step in the viral replication cycle.
- Composition: [REDACTED]

## Reference Product

- Name: Matching placebo (nebulizer solution).
- Active substance: None.
- Composition: [REDACTED]

**Device:**

- Name: FOX-Flamingo inhalation system - CE marked.
- Main components: re-usable base unit (containing the electronics), single-use disposable inhalation set (including pediatric face mask in 2 sizes, mask adaptor, vibrating mesh nebulizer with reservoir).
- Description: The FOX-Flamingo inhalation system consists of a battery-operated, hand-held device, intended for single-patient use. The device provides an aerosol with particle size suitable for the intended study population. The nebulizer is always to be used with a flow of 2 L/min additional air or O<sub>2</sub>.

**Standard of care treatment**

The treatment and care provided to each subject are determined by the Investigator (or designee) according to institutional practice. The recommendations on the diagnosis, management, and prevention of bronchiolitis, as described by the American Academy of Pediatrics (2014), may be followed in addition to institutional practice.

Treatment may include (but is not limited to) the following:

- O<sub>2</sub> supplementation through nasal cannula, via face mask or headbox. The initiation, monitoring and weaning of oxygen supplementation will follow local practice. It should be removed for the nebulized study drug administration, during which air or oxygen flow of 2L/min is provided
- Fluid/food supplementation (i.v. or via nasogastric tube, if applicable)
- Antipyretics and/or nonsteroidal anti-inflammatory medication
- Hypertonic saline (but not within 4 hours before start or 4 hours after the end of study drug administration)
- Short acting  $\beta_2$ -agonists
- Antibiotics (in case of secondary bacterial infection)
- Epinephrine
- Anticholinergics

Concomitant medications are permitted, apart from those listed under the prohibited therapies (see below), at the Investigator's discretion (based on medical need).

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**Forbidden medications**

The following treatments are prohibited up to the Follow-up visit (Day 14 ± 2 days):

- Ribavirin, i.v. immunoglobulin and palivizumab
- Inosine pranobex
- Xanthines
- Heliox
- Initiation of leukotriene receptor antagonists (LTRAs, i.e., montelukast) and/or sodium cromoglycate; infants who are on maintenance chronic therapy at screening are to continue their usual dose during the study.
- Exogenous surfactant
- Systemic corticosteroids; topical corticosteroids for treatment of a skin or ear disorder are permitted; initiation of inhaled corticosteroids is not permitted; infants who are on a maintenance therapy of inhaled corticosteroids at inclusion are to continue their usual dose during the study
- Mucolytic and/ or expectorant drugs (e.g., Dornase Alpha, N-Acetylcysteine, Bromhexine)

Subjects whose respiratory condition is deteriorating (e.g., hypercapnia with pCO<sub>2</sub> > 8 kPa/60mmHg, decreased consciousness) and need to start non-invasive respiratory support or invasive ventilation need to discontinue study drug treatment but should remain in the study according to the in-hospital post-treatment period as specified in the [Schedule of Assessments](#), and should also attend the FU and EOS Visits. Subjects on HFOT as standard of care at inclusion and for whom the flow needs to be increased to levels not enabling study drug nebulization and/or above 2 L/kg/min also need to discontinue study drug treatment and be further followed up as described above. SAE reporting is required for these subjects with deterioration of the respiratory condition requiring above mentioned methods of ventilation.

After the end of the subject's participation in the study, each subject is to be treated according to standard clinical practice.

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**Study duration:**

The overall study duration is expected to be approximately 23 months. The planned study duration for each subject is approximately 28 days. The completion of the study is defined as the last visit of the last subject (LSLV) participating in the study.

- Screening period: Within 24 hours after arrival at hospital.
- Study drug treatment period: Subjects will receive once-daily doses of study drug on 3 consecutive days. The first dose will be administered as soon as possible and within 3 hours after randomization. The second dose is to be administered approximately 24 hours (± 4 hours) after administration of the first dose, and the third dose approximately 48 hours (± 4 hours) after administration of the first dose.

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- Ambulatory treatment on dosing Day 3: If the subject's clinical condition has improved sufficiently in the opinion of the Investigator to allow discharge from hospital and if the per protocol-defined clinical response criteria have been met, the subject may be discharge from the hospital after all required assessments of the 5 hours ( $\pm$  1 hour) post-dose time point on dosing Day 2 have been completed. In this case the subject is to return to the investigational site in the hospital for administration of the 3<sup>rd</sup> dose of study drug by appropriately trained study personnel. The subject should remain in the hospital for a 2-hour pre-dose and post-dose monitoring period.
  - In-hospital post-study drug treatment period: Additional post-treatment assessments are foreseen for subjects who are not discharged from the hospital after the completion or discontinuation of study drug treatment.
  - A Follow Up visit will take place on study Day 14 ( $\pm$  2 days).
  - An End of Study visit will take place on study Day 28 ( $\pm$  2 days).
  - For subjects who are prematurely withdrawn from the study, a Withdrawal visit is to be performed on the day of withdrawal unless consent was withdrawn.

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**Outcome Measures:****Viral Load** Outcome Measure

- Quantitative viral titers in the nasal cavity (plaque and qPCR) over time.

**Safety** Outcome Measures

- Treatment-emergent adverse events (TEAEs), as noted by healthcare staff and/or reported by parents/caregivers. The Investigator (or his/her designee) will review at each visit whether AEs/SAEs have occurred since the last visit.
- Physical examination including heart auscultation, examination of abdomen, skin and ears/nose/throat.
- Lung auscultation
- Peripheral capillary O<sub>2</sub> saturation (SpO<sub>2</sub>)
- Vital signs: body weight, body temperature, heart rate, respiratory rate
- Clinical laboratory test results:
  - Clinical chemistry: alanine aminotransferase, aspartate aminotransferase, creatinine, sodium, potassium, chloride, C-reactive protein,  $\gamma$ -glutamyl-transferase, blood urea nitrogen
  - Hematology: hemoglobin, hematocrit, red blood cell count and indices, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count (or estimate), white blood cell count including differential (lymphocytes, neutrophils, monocytes, basophils, eosinophils).

Safety lab assessments are planned two times during the study (at screening and at the FU visit) and will be performed by the local laboratory in order to allow timely availability of the results.

**Clinical Activity** Outcome Measures<sup>‡</sup>

- Heart rate and SpO<sub>2</sub>
- Feeding: type of feeding support, and (time and date of) sufficient feeding to enable hospital discharge, in the opinion of the Investigator (with particular attention to hydration and breathing comfort during feeding)
- Respiratory rate measured over a 1-minute interval
- Wheezing as assessed during lung auscultation
- Cough during the night and during the day
- Respiratory muscle retractions (supraclavicular, intercostal, and subcostal)
- General appearance: activity, irritation, and responsiveness
- Body temperature

Based on the Clinical Activity Outcome Measures, composite scores such as the Respiratory Distress Assessment Instrument (RDAI) score (based on wheezing and respiratory muscle retractions), Respiratory Assessment Change Score (RACS; based on RDAI and change in respiratory rate), and a Global Severity Score (based on respiratory parameters, medical interventions, feeding, general appearance and body temperature) will be calculated.

The feeding and oxygen saturation outcome measures will be used to calculate Time to Clinical Response. Subjects will be considered to have met the Clinical Response criteria when both of the following criteria are fulfilled:

- Stable oxygen saturation on room air, defined as SpO<sub>2</sub> > 92% over a period of ≥ 4 hours. In case of oxygen supplementation, the level of supplementation is to be considered for reduction at least three times per day. Provided oxygen saturation is stable, attempts to remove the supplementation will be done at least three times a day.
- Adequate oral feeding which is sufficient to maintain sufficient hydration, in the judgment of the Investigator.

**Medical Interventions** Outcome Measures:

- Length of hospital stay for RSV infection
- Level, method, and duration of supplemental oxygen therapy
- Initiation of invasive or non-invasive ventilation (i.e., continuous positive airway pressure [cPAP] or HFOT)
- Level, method and duration of invasive or non-invasive ventilation
- Transfer to ICU and duration of stay in ICU

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<sup>‡</sup> The evaluation of several of these parameters will also inform on safety outcome.



**Parent/Caregiver** Outcome Measures:

- Parent(s)/Caregiver(s) assessment of the clinical condition of the child will be done by daily completion of a diary during the hospital stay and up to the EOS (Day 28) visit. This diary includes scoring of respiratory symptoms over the past 24 hours (cough, wheezing, trouble breathing), evaluating the general health of the child by completing a Visual Analogue Scale (VAS), and answering a question whether the child has returned to his/her normal condition from before the onset the RSV infection. For the period after discharge from the hospital, this diary will also allow to capture data on health care utilization and use of medication for respiratory symptoms.

**PK** Outcome Measures

- The systemic concentration of ALX-0171 will be evaluated in serum. A subset (48 subjects, i.e., 12 subjects per dose group) will undergo more extensive PK sample analysis (3 blood samples for PK assessment). In the remainder of subjects, only 1 PK blood sample will be taken.

**PD** Outcome Measures

- Quantification of the biomarker Krebs von den Lungen-6 (KL-6) in serum

**Immunogenicity**

- Systemic presence (serum) of anti-drug antibodies (ADA)
- 

**Endpoints:***Primary Endpoint*

Anti-viral effect as measured by the time needed for the viral load to drop below the plaque assay quantification limit (time-to-BQL) in nasal mid-turbinate swab specimens.

*Secondary Endpoints*

- Safety assessment (assessed by physical examination, AEs, laboratory assessments and vital signs) of different doses of ALX-0171
- Change from baseline in Global Severity Score
- Time to clinical response: defined as the time between first administration of study drug and achieving adequate oxygen saturation and oral feeding
- PK properties of ALX-0171
- Viral load profile over time in nasal swab specimens (qPCR and plaque assay)
- Evaluation of serum ADA

*Other Endpoints*

- Evolution over time in clinical symptoms (SpO<sub>2</sub>, feeding, respiratory rate, wheezing, cough, respiratory muscle retractions, and general appearance)

- Evolution over time in RDAI, RACS
- Length of hospital stay for RSV defined as time between admission and discharge
- Duration of supplemental oxygen therapy
- Initiation of invasive and non-invasive ventilation and duration of respiratory support
- Transfer to ICU and duration of ICU stay
- Parent(s)/Caregiver(s) assessment of the clinical condition of the subject
- Evolution of serum biomarker over time

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**Sample Size:**

With 45 subjects per arm, 85% power is achieved to detect a 50% reduction in median time-to-BQL using a two-sided log-rank test at significance level of 0.05. For each comparison of the ALX-0171 dose groups with placebo, a significance level of 0.05 is assumed. For multiple pairwise comparisons, the family-wise error rate is controlled at 5% through the closed testing principle, where the comparison of the lower dose of ALX-0171 to placebo is only performed if the comparison of the higher dose of ALX-0171 to placebo is significant. The sample size also incorporates a 15% drop-out rate.

**Statistical Analysis:**

For the primary endpoint, the anti-viral effect will be measured as the time-to-BQL in nasal mid-turbinate swab specimens. The median time-to-BQL will be compared between each of the ALX-0171 dose groups and placebo using a log-rank test. The tests will be performed in a sequential way to preserve the family-wise error rate at 0.05. Specifically, dose 3 of ALX-0171 will first be tested against placebo at the 0.05 significance level. Dose 2 of ALX-0171 will only be compared to placebo at the 0.05 significance level if the comparison of dose 3 with placebo is significant. Consequently, dose 1 of ALX-0171 will only be compared to placebo at the 0.05 significance level if the comparison of dose 2 of ALX-0171 with placebo is significant.

The data from the secondary endpoints will be analyzed by descriptive statistics as applicable. For selected secondary endpoints, comparisons of active dose groups with placebo may be performed through appropriate inferential tests. No correction for multiple testing is foreseen for secondary endpoints, and p-values reported for the analyses are to be interpreted accordingly.

Analysis of all parameters will be described in a Statistical Analysis Plan (SAP) or a Data Analysis Plan (DAP; for PK analysis), which will be finalized prior to database lock, and will comprise all methods and tests applied for analysis of the corresponding data.

# SCHEDULE OF ASSESSMENTS

Period	Screening <sup>a, b</sup>	Study Drug Treatment Period <sup>c</sup>									In-hospital Post-Treatment Period <sup>d</sup>	FU visit (Day 14±2) or Withdrawal visit <sup>e</sup>	EOS <sup>e</sup> (Day 28±2)			
		Hospitalized Dosing						Hospitalized or Ambulatory Dosing								
		Day 1			Day 2			Day 3								
Visit	-24h to randomization	-3h to dose	Dose	2h (±0.5h)	5h (±1h)	-2h to dose	Dose	2h (±0.5h)	5h (±1h)	-2h to dose	Dose	2h (±0.5h)	Morning and evening			
Informed consent	X															
Inclusion/exclusion criteria	X															
RSV diagnosis <sup>f</sup>	X															
Demographics and medical history	X															
Hospitalization information/Clinical response information <sup>g</sup>																→
Study drug administration <sup>h</sup>			X				X				X					
Nasal mid-turbinate swab <sup>i</sup>		X			X	X			X	X		X	X <sup>j</sup>	X		
Physical examination <sup>k</sup>	X	X			X	X			X	X		X	X	X	X	X
Body weight	X														X	X
Body height	X														X	X
Body temperature	X	X			X	X			X	X		X	X	X	X	X
General appearance	X	X			X	X			X	X		X	X	X	X	X
Lung auscultation <sup>n</sup>	X	X		X	X	X		X	X	X		X	X	X	X	X
Heart rate	X	X		X	X	X		X	X	X		X	X	X	X	X
SpO <sub>2</sub> l	X	X		X	X	X		X	X	X		X	X	X	X	X
Feeding <sup>m</sup>	X	X			X	X			X	X		X	X	X	X	X
(Respiratory muscle) retractions	X	X		X	X	X		X	X	X		X	X	X	X	X
Respiratory rate	X	X		X	X	X		X	X	X		X	X	X	X	X
Randomization <sup>b</sup>		X														
Daytime coughing	X	X		X	X	X		X	X	X		X	X	X	X	X
(Sleep disturbance from) night-time coughing <sup>o</sup>	X <sup>o</sup>	X <sup>o</sup>				X <sup>o</sup>				X <sup>o</sup>			X <sup>o</sup>	X <sup>o</sup>	X <sup>o</sup>	X
Parent/caregiver assessment <sup>p</sup>																→
Pharmacokinetics (single sample)																← → <sup>q</sup>
Pharmacokinetics (more extensive sampling)						X <sup>r</sup>										← → <sup>r</sup> ← → <sup>r</sup>
Clinical laboratory (hematology and clinical biochemistry)	X <sup>s, t</sup>														X <sup>u</sup>	
Immunogenicity	X <sup>s</sup>														X <sup>u</sup>	
Previous and concomitant medication																→
Adverse events <sup>v</sup>																→

h = hour; FU = Follow-up; EOS = End of Study.

**Timing of assessments:** post-dose time points relate to the end of the 2<sup>nd</sup> of the 2 nebulizations of study drug administration. Pre-dose time points relate to the start of the 1<sup>st</sup> of the 2 nebulizations of study drug administration. Throughout the study, study personnel should make every reasonable effort to follow the timing in the schedule of assessments for each subject.

- a. If the Day 1 pre-dose assessments will be performed within 3 hours after the screening assessments have been performed, the assessments already done at screening do not need to be repeated and only the additional assessments to be performed on Day 1, pre-dose will need to be performed.
- b. At the time of randomization, subjects should be eligible for study participation as defined in the inclusion/exclusion criteria. Therefore, at a minimum, the SpO<sub>2</sub>, feeding, respiratory muscle retractions, and respiratory rate should be evaluated on Day 1 before randomization, unless these assessments were already performed within the last 3 hours before randomization.
- c. Study drug administration should take place within 24 hour intervals ( $\pm$  4 hours) relative to the start of the 1st nebulization of the 1st dose. Provided that the clinical response criteria have been met, discharge from the hospital can take place per protocol at the Investigator's discretion from dosing Day 2 onwards after all required assessments of the 5 hours ( $\pm$  1 hour) post-dose time point have been completed. Subjects who started study drug dosing but were discontinued prematurely from study drug treatment, should be further monitored according to the in-hospital post treatment period (if applicable) and should attend the FU and EOS visits.
- d. The in-hospital post-treatment assessments are to be performed for subjects who are not discharged from the hospital after the completion or discontinuation of study drug treatment.
- e. For subjects who discontinue the study before Day 28, the Withdrawal visit will be conducted on the day of withdrawal as long as there has been no withdrawal of consent. If the Withdrawal visit assessments are performed within 6 hours after the Day 1, Day 2, Day 3, or in-hospital post-treatment Day assessments have been performed, the assessments already done at the prior time point do not need to be repeated and only the additional assessments to be performed on the Withdrawal Visit will need to be performed. No EOS visit needs to be performed after the Withdrawal Visit. In case subjects are hospitalized beyond the Day 14 FU visit, the additional assessments (in addition to the in-hospital post treatment period assessments) of the Day 14 FU visit need to be done on Day 14.
- f. RSV infection will be confirmed either according to routine site practice (PCR or diagnostic quick test), or using a (Sponsor-provided) commercial kit.
- g. Hospitalization information will include occurrence of ICU transfer, apnea episodes, initiation and type of ventilation and hospital/ICU discharge information. Assessment of Clinical Response will be done with collection of date and time of adequate oral feeding to enable discharge as well as adequate stable oxygen saturation on room air of  $>92\%$  over a period of at least 4 hours.
- h. Study drug will be administered via nebulization using the study-specific device. One dose consists of 2 serial nebulizations.
- i. A mid-turbinal nasal swab should be taken using provided kits and following specific instructions in case the subject is re-hospitalized or consults the Investigator for a respiratory condition after hospital discharge.
- j. A nasal swab should only be collected during the in-hospital post-treatment period on the day of hospital discharge.
- k. Physical examination includes examination of abdomen and skin and ears/nose/throat. Lung auscultation is listed separately as it is to be assessed more frequently.
- l. Continuous monitoring of SpO<sub>2</sub> needs to be done until the clinical response criterion for oxygen has been met and/or in case saturation monitoring is to be continued according to the Investigator's judgment.
- m. Type of feeding support, if any, and feasibility of oral feeding.
- n. To assess wheezing, crackles/crepitation and other abnormalities in lung auscultation.
- o. Sleep disturbance from night-time coughing to be documented pre-dose the next day or in the morning during the in-hospital post-treatment period, or at the FU visit, EOS or Withdrawal visit, based on feedback from the nursing staff. In case there is no night between screening and study drug administration on Day 1, the Day 1 assessment should not be performed.
- p. The parent/caregiver assessment should be done every evening from screening until the EOS visit by completing a diary.
- q. Blood draw for PK assessments on Day 2 or Day 3, at any time between 0.5 hours post second dose and start of the third dose. If sufficient serum remains after PK assessment, exploratory biomarker (KL-6) may also be evaluated.

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- <sup>r</sup> 48 subjects will undergo more extensive PK analysis (3 PK samples per subject instead of the 1 sample), i.e., blood draws on Days 2-3 1) pre second dose, 2) at any time between 0.5 hours and 3 hours post second dose, and 3) at any time between 3 hours and 6 hours post second dose (and at least 1 hour apart from the previous sampling); If sufficient serum remains after PK assessment, exploratory biomarker (KL-6) may also be evaluated.
- <sup>s</sup> Blood draw at screening for clinical laboratory (hematology and clinical biochemistry) and immunogenicity. If sufficient serum remains after immunogenicity assessment, exploratory biomarker (KL-6) may also be evaluated. In case the blood sample for immunogenicity was not taken at screening, the sample should be collected at the Day 1 pre-dose timepoint, as far as possible.
- <sup>t</sup> If all hematology and biochemistry blood parameters required for the study are already known from analysis done as a routine assessment upon arrival at the hospital, collection of screening clinical laboratory samples does not need to be repeated for the purpose of this study. However, the samples should be analyzed at the same local laboratory that will be used for the remainder of the study.
- <sup>u</sup> Blood draw on Day 14 for clinical laboratory (hematology and clinical biochemistry) and immunogenicity. If sufficient serum remains after immunogenicity assessment, exploratory biomarker (KL-6) may also be evaluated. No blood sample needs to be taken in case of premature withdrawal from the study for subjects who did not receive any study drug. In case the blood sample for immunogenicity was not taken on Day 14, the sample should be collected at the EOS (Day 28) visit, as far as possible.
- <sup>v</sup> In case of a severe and/or serious hypersensitivity reaction, an additional serum sample should be collected (if reasonably feasible) as soon as possible after the start of the event.

# 1. INTRODUCTION

Respiratory syncytial virus (RSV) lower respiratory tract infection (LRTI) is a leading cause of bronchiolitis in infants and results in over 3 million hospitalizations worldwide per year. ALX-0171 is a therapeutic protein (Nanobody) that is currently being developed for the treatment of RSV LRTI.

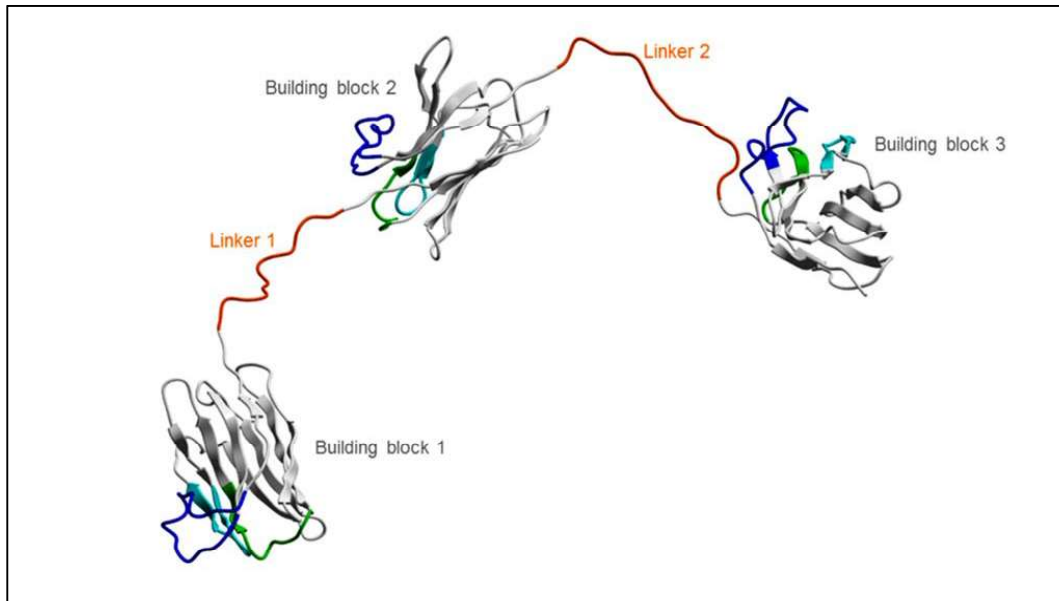
The antiviral effect of ALX-0171 is expected to provide rapid control of the infection, thereby reducing the intensity and duration of severe disease. The favorable *in vitro* potency of ALX-0171 Nanobody and the *in vivo* results obtained in nonclinical studies indicate that ALX-0171 may provide an opportunity for therapeutic intervention in established RSV infection. This is also supported by an initial clinical study in children hospitalized for RSV lower respiratory tract infection, where treatment with inhaled ALX-0171 had an immediate impact on viral replication and reduced viral load compared to placebo.

## 1.1. TREATMENT OF RESPIRATORY SYNCYTIAL VIRUS INFECTION

Nearly all infants are infected with RSV in the first years of life. RSV LRTI is associated with significant morbidity and mortality [1, 2]. RSV infection imposes a significant burden on health care infrastructure and there is a high unmet medical need for treatment options. Evidence-based guidelines indicate that there are no treatments that shorten the course of bronchiolitis or hasten the resolution of symptoms; supportive care remains the cornerstone of clinical management (hydration and oxygen saturation) [1, 3, 4].

## 1.2. ALX-0171

Nanobodies are a novel class of therapeutic proteins, and are based on the smallest functional fragments of single-chain antibodies that occur naturally in the *Camelidae* family. ALX-0171 Nanobody is directed towards the RSV fusion (F) protein, and is intended to interfere with viral entry into host cells. ALX-0171 is a trivalent Nanobody, formed by three identical building blocks connected via two linkers (Figure 2). All three moieties are capable of binding to the homotrimeric RSV F protein.



**Figure 2: Schematic representation of ALX-0171 Nanobody**

The amino acid (15-GS) linkers connect the C-terminus of the first building block with the N-terminus of the second one, and the C-terminus of the second building block with the N-terminus of the third one. The three building blocks (designated RSVNMP1A4) are identical, with the only exception of the E1D mutation in the first building block, which has been introduced to avoid pyroglutamate formation.

The F protein is highly conserved between RSV serotypes, and is therefore considered the main target for development of viral entry inhibitors. Following activation, the RSV F protein causes the membranes of the host cell and the virion to come into close vicinity and fuse together, allowing further infection and viral replication. Glycoprotein F also induces fusion of infected cells with adjacent, uninfected cells (epithelial cell syncytia), which allow for cell-to-cell transmission of the replicated viral RNA and confer additional protection against host immune responses [5].

ALX-0171 Nanobody binds to a well-conserved epitope in antigenic site II of the RSV F protein, close to or overlapping with the epitope of the clinical benchmark palivizumab (trade name Synagis®, MedImmune/AstraZeneca). The latter is a humanized monoclonal antibody (mAb) indicated for the prevention of serious lower respiratory tract disease in high risk children, and is prophylactically administered by monthly intramuscular injections throughout the RSV season.

### 1.2.1. PHARMACEUTICAL PROPERTIES

Since direct delivery and deposition in the respiratory tract is desirable, inhalation was considered the preferred route of administration. ALX-0171 was therefore developed as a solution for nebulization, containing 50 mg/mL ALX-0171 Nanobody dissolved in a formulation buffer suitable for inhalation.

ALX-0171 solution for nebulization is administered using the handheld FOX-Flamingo inhalation system. The FOX-Flamingo is intended for professional use in a clinical environment, in spontaneously-breathing subjects from birth up to 3 years.

## 1.2.2. NONCLINICAL STUDIES

### Pharmacological properties:

- ALX-0171 was characterized using *in vitro* systems, including assessment of affinity, biological activity and specificity. Different cellular systems of RSV replication were used, with cell-line adapted virus strains as well as viruses isolated from clinical cases.
- *In vivo* efficacy was demonstrated in the RSV infection cotton rat model, a standard model used in the field. Intranasal and intratracheal administration and nebulization were used to reflect the clinical route of administration.
- Efficacy and safety were further confirmed in the neonatal lamb model of RSV infection, a disease model that allows the assessment of clinical symptoms, histopathology and viral titres, and more closely mimics the clinical situation in infants.

### Safety pharmacology:

Specific GLP-compliant *in vivo* studies were designed for the evaluation of ALX-0171 effects on respiratory and cardiovascular system, in rats and dogs respectively.

### Pharmacokinetics:

The objective of the pharmacokinetic (PK) program was the characterization of plasma and local (lung) kinetics of ALX-0171 in nonclinical toxicity studies. Additionally, the impact of anti-drug antibodies (ADA) on the PK of ALX-0171 was evaluated. PK parameters were also derived from a study in juvenile rats after single or repeated dose inhalation (starting from post-natal dose 4), as well as from an efficacy study in neonatal lambs. The latter includes information from infected animals.

### Toxicology:

- Two 14-day repeat-dose toxicity studies in rats, after once-daily administration via inhalation and via intravenous (i.v.) administration respectively, showed no treatment-related effects on local tolerance, clinical signs, body weight, food consumption, ophthalmic examination, hematology and coagulation, clinical chemistry, urine analysis, macropathology, histopathology, or organ weights.
- Safety evaluations were included in the studies involving RSV-infected cotton rats and neonatal lambs.



- The risk of accidental exposure of the face, and particularly of the eyes, during nebulization via a face mask, was assessed nonclinically. No local irritant effects of ALX-0171 on bovine cornea were observed. Likewise, in a repeated dose PK study in juvenile rats after inhalation, no clinical signs indicative of eye irritation or histopathology findings were reported.

### **1.2.3. EFFECTS IN HUMANS**

At present, three Phase 1 clinical trials have been conducted in adults, to obtain initial safety and PK data. Following completion of the adult studies, a first-in-infant clinical study was recently completed. Please refer to the Investigator's Brochure (IB) for a detailed overview of each of the individual studies.

- First-in-man Phase 1 study ALX-0171-1.1/11 (N=60) evaluated the safety, tolerability and pharmacokinetics of ALX-0171, administered by inhalation, in healthy male volunteers.
- Phase 1 study ALX-0171-1.3/13 (N=44) was performed to obtain additional information on local and systemic PK of ALX-0171 in healthy male volunteers.
- Phase 1 safety study ALX-0171-1.2/13 (N=24) evaluated the potential occurrence, reversibility and prevention of bronchoconstriction upon inhalation of ALX-0171 by adults with sensitive (hyperresponsive) airways.
- Study ALX0171-C104 (N=53) was a Phase 1/2a multicenter study in infants and toddlers hospitalized for RSV lower respiratory tract infection, to provide an initial evaluation of the safety, tolerability, anti-viral and clinical activity of ALX-0171. A summary of the main conclusions is described below.

#### **Clinical Safety:**

The clinical studies in adults showed that inhalation of ALX-0171 was well-tolerated at all doses evaluated.

- Treatment-emergent adverse events (TEAEs) resolved quickly without further treatment, and were generally of mild to moderate intensity. The most common study drug-related TEAEs reported in the Phase 1 studies in adults included cough, headache, oropharyngeal pain, rhinitis, nasopharyngitis and diarrhea, generally of mild intensity.
- No study drug-related TEAEs leading to withdrawal were reported. Similarly, no study drug-related, clinically significant findings were observed with respect to clinical laboratory parameters, vital signs, electrocardiogram (ECG), physical examinations, or lung auscultations.
- In the studies in healthy volunteers, no meaningful trends in spirometry parameters were observed. The study in adults with hyperresponsive airways demonstrated the

reversibility of bronchoconstriction with bronchodilator treatment ( $\beta$ 2-agonist) and prevention with prophylactic use of bronchodilator thereof.

ALX-0171 also found to be well-tolerated in infants and young children hospitalized for RSV LRTI.

- In line with the underlying disease, the most frequent TEAEs were respiratory, thoracic and mediastinal disorders and infections and infestations. Possibly-related TEAEs occurred in 3 subjects (8.6%) in the ALX-0171 group. These were cough, rhinorrhea, and pyrexia. All were mild and resolved.
- There were no treatment-related serious adverse events (SAEs) in this study. Five SAEs were reported in 4 subjects in the ALX-0171 group. These were hyporesponsiveness and hypotonia (both in 1 subject), pneumonia (2 subjects), and atelectasis. All resolved.
- Two subjects were prematurely discontinued from study treatment (due to SAEs hypotonia and hyporesponsiveness and pneumonia). As noted above, all of these SAEs resolved.

#### **Clinical Pharmacokinetics:**

- In adults, the local (epithelial lining fluid) and systemic exposure levels were above the target for infants and young children, with good tolerability.
- Consistent with lung exposure, inhaled ALX-0171 was quantifiable in the serum of most of the infants and young children in Study ALX0171-C104.

#### **Clinical Activity and Pharmacodynamics:**

Study ALX0171-C104 provided a first indication of anti-viral and clinical activity in a relevant clinical setting, i.e., hospitalized infants with RSV LRTI.

- Data on nasal viral load indicates that ALX-0171 treatment rapidly and sustainably reduces cultivatable virus titers to below the quantification limit.
- Post-hoc analysis of a composite of clinical parameters, the Global Severity Score, led to an encouraging initial indication of a therapeutic effect for infants treated with ALX-0171.

## Immunogenicity

- In adults, pre-existing antibodies (pre-Ab<sup>§</sup>) were found in 7 to 18% of the subjects. In the Phase 1 studies ALX-0171-1.1/11 and ALX-0171-1.3/13, no treatment-emergent (TE) ADA responses were detected, whereas in the Phase 1 safety study ALX-0171-1.2/13, TE ADA were observed in 33% of the subjects (population of subjects with hyperresponsive airways).
- In Study ALX-0171-C104, pre-Ab were found in 45% of the subjects, while TE ADA was detected in 22.9% of the infants. Pre-Ab detected in those children may reflect maternal antibodies transferred to the infant via the transplacental route during gestation or during breastfeeding. As the assay cannot discriminate pre-Ab from drug induced TE ADA, it is not clear to what extent the TE ADA detected may represent fluctuating levels of pre-Ab. Currently it is not known to what extent pre-Ab can fluctuate within the short study period (14 days). No effect of ADA on PK was observed.

TE ADA is not expected to have an impact on PK or efficacy given the short treatment duration, as TE ADA do not emerge to considerable amounts within the first 7 days after initial drug administration.

## **1.3. SAFETY PROFILE AND RISK BENEFIT ASSESSMENT**

The potential benefits and risks for administration of ALX-0171 are assessed from the available nonclinical data and the results of the completed Phase 1 clinical studies in adults and a Phase 1/2a trial in children hospitalized for RSV LRTI.

### **1.3.1. NONCLINICAL RESULTS**

Detailed nonclinical assessment of systemic toxicity as well as local tolerability and respiratory safety revealed no clinically meaningful risks for administration of ALX-0171 to pediatric subjects. The results were consistent across the different nonclinical safety and toxicology studies.

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<sup>§</sup> Pre-existing antibodies, i.e., ADA present in samples from treatment-naïve subjects, have been commonly observed during immunogenicity assessments for various therapeutic proteins.

## 1.3.2. CLINICAL SAFETY RESULTS

At present, three Phase 1 clinical studies have been conducted in adults. In addition, a Phase 1/2a study in children aged 1 month to < 2 years hospitalized for RSV LRTI was recently completed. ALX-0171 was generally well tolerated in all clinical studies.

## 1.3.3. POTENTIAL RISKS

ALX-0171 has no endogenous target and no secondary pharmacology was noted throughout the pre-clinical development program; potential risks related to the pharmacology or to the mechanism of action of the compound are therefore considered unlikely.

More general risks, inherent to administration of inhalation products or therapeutic proteins, cannot be excluded. A continuous further evaluation is included during clinical development to assess if these potential effects would constitute clinically meaningful risks for administration of ALX-0171.

### *Airway Hyperresponsiveness*

Viral respiratory infections are known to transiently increase bronchial reactivity, potentially leading to airway hyperresponsiveness (i.e., an abnormal airway narrowing of the bronchi, triggered by various chemical or physical stimuli).

The occurrence, treatment and prevention of potential bronchoconstriction subsequent to ALX-0171 inhalation was evaluated in a dedicated Phase 1 study in adults with hyperresponsive airways (Study ALX-0171-1.2/13). In total, 24 subjects participated in the study. In this population with bronchial hyperreactivity, 10 subjects (42%) experienced a mild to moderate bronchoconstriction either during the escalating dose or repeated dose part of the study. All events were treated successfully with  $\beta_2$ -agonist (salbutamol). The results of this study demonstrated the immediate reversibility of bronchoconstriction with bronchodilator treatment ( $\beta_2$ -agonist) and prevention with prophylactic use of bronchodilator thereof.

In the first-in-infant Study ALX0171-C104, as a precaution and in order to standardize treatment across the sites an inhaled dose of the short-acting  $\beta_2$ -agonist salbutamol was administered before each administration of study drug. A review of the safety data and respiratory parameters collected in the first-in-infant study did not reveal a bronchoconstrictive risk after study drug inhalation. Therefore, the requirement for prophylactic administration of a  $\beta_2$ -agonist will not be implemented in the current protocol. The use of bronchodilator treatment will be permitted at the Investigator's discretion.

The potential risks of airway hyperresponsiveness and bronchoconstriction will be closely monitored in each of the safety cohorts and the overall study. Independent Data Monitoring Committee (IDMC) review is planned before each increment in dose and on a regular basis thereafter.

### ***Immediate or Delayed Adverse Drug Reactions***

All therapeutic proteins have the potential to elicit antibody or other immune- or non-immune-mediated responses, potentially resulting in hypersensitivity or allergic reactions such as rash, urticaria, angioedema, serum sickness and anaphylactoid or anaphylactic reactions. These responses are complex and could occur following initial or only after repeated exposure, and are very difficult to predict.

Within the context of the planned clinical study, immunogenicity-related AEs as a consequence of TE ADA are not expected, as TE ADA typically do not emerge to considerable amounts earlier than 7 days after first administration of study drug, at times where study drug is expected to be cleared, given the short treatment period of 3 days.

While pre-Ab and TE ADA have been reported in clinical studies with ALX-0171, no obvious correlation with potential immune-related AEs has been established thus far, neither in adults nor in RSV-infected children. No immune response-related toxicological findings were reported during the nonclinical studies.

During Study ALX-0171-1.3/13, a possible mild hypersensitivity reaction (unilateral eyelid oedema) was noticed in one subject approximately 1 hour after 1 single i.v. dose. No pre-Ab nor TE ADA were detected in this subject.

During Study ALX-0171-C104, no correlation between immunogenicity (both pre-Ab or TE ADA) and safety was seen. One subject with a history of acute urticaria presented with a mild exanthema of abdomen, trunk, back and retroauricular area, about 5 hours after the second drug administration. The event resolved without medication and was considered unlikely/not related to the study drug by the Investigator. This subject was pre-Ab positive and TE ADA positive. A possible correlation with immunogenicity (pre-Ab and/or TE ADA) cannot be excluded for this subject.

In line with regulatory and scientific guidance, an immunogenicity risk assessment [6] was prepared for administration of ALX-0171, in support of the currently proposed study [7-9]. This assessment comprised evaluation of the immunogenic potential of ALX-0171 through determination of product-specific, patient- and/or disease-related factors affecting immunogenicity. In addition, the available nonclinical and clinical immunogenicity results were evaluated to come to an appraisal on the potential consequences on safety, PK and efficacy in case immunogenicity would occur in the current pediatric study. The overall risk assessment led to a "lower risk class" designation (the lowest category for a therapeutic protein) [8], with favorable risk/benefit balance for the current pediatric study. Of note, the

immunogenicity risk assessment is a dynamic process, with further evaluations and updates scheduled as data become available [6].

### **Unintentional Contact**

To ensure correct administration of ALX-0171 and handling of the device, detailed instructions for use will be provided. During administration by nebulization, caregivers or other persons close to the nebulizer may nevertheless come into contact with ALX-0171. This may for instance occur due to aerosol escaping through the outlet valves of the facemask, or via particles exhaled by the subject. This is not considered to pose a safety risk. ALX-0171 is highly specific towards the RSV F protein, and lacks an endogenous human target. The *in vivo* safety pharmacology and toxicology studies revealed no meaningful safety risks. As described above, the clinical studies conducted so far showed that ALX-0171 was well tolerated at all dose levels and dosing regimens evaluated. In addition, no teratogenic or oncogenic properties are expected. As ALX-0171 is a protein drug, it is not expected to enter cells and interfere directly with DNA or nuclear proteins.

Furthermore, Investigators, clinical staff, parents, family members or other children in the vicinity would only come in contact with ALX-0171 at a negligible dose<sup>\*\*</sup>. Potential deposition of aerosol particles onto surfaces, or unintentional contact by means other than inhalation, is not of concern. Similar to other proteins or macromolecules, standard hospital or household hygiene procedures are considered sufficient for cleaning of surfaces. There is no added risk for contact with skin, since ALX-0171 does not cause irritation, and is not prone to transdermal delivery (similar to other macromolecules). Exposure to mucosal tissues may be more relevant, but there are no indications that this would be associated with additional safety concerns, systemic uptake, or irritative effects. Finally, in case of accidental oral intake, ALX-0171 would be rapidly degraded in the digestive tract, similar to other proteins.

Routine hygiene conditions and cleaning procedures in the hospital are therefore sufficient for administration of ALX-0171. In view of the above, potential risks associated with unintentional contact are considered negligible. It should also be noted that, while not considered necessary, a standard facemask would be sufficient to further limit unintentional contact with ALX-0171 for the person administering study drug.

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<sup>\*\*</sup> Calculated "worst case" scenarios (e.g., 1-hour stay after administration of ALX-0171 in an examination room of 5 m<sup>2</sup> with poor ventilation, and assuming that the entire dose filled into the nebulizer is dissipated into the room) indicate that inhalation of dissipating aerosol by any person (adult or infant) other than the intended subject would result in an ALX-0171 dose at least 100-fold below the nebulized dose. Of note, in case only incomplete or inefficient administration is achieved (e.g., because of poor fitting of the face mask, or because of intense crying of the subject), nebulization is to be stopped, thereby avoiding unwanted loss of study drug into the room.

### 1.3.4. POTENTIAL BENEFITS

As a respiratory virus, RSV may present as an upper respiratory tract infection (including rhinitis, otitis media and pharyngitis), or, as is more often the case in infants and young children, as a LRTI, (including acute bronchiolitis and/or broncho-pneumonia). RSV LRTI results in hospitalization of about 3% of RSV-infected infants less than 1 year old, and about 0.5% of RSV-infected children aged between 1 and 2 years [1, 2]. Since there are no adequate medications available for treatment of RSV infection, the standard of care for hospitalized infants is mostly supportive (e.g., fluid/feed supplementation, observation, and respiratory support as needed) [1, 3, 4].

ALX-0171 is an antiviral treatment and is intended to neutralize RSV (thereby inhibiting viral infectivity). ALX-0171 was shown to be highly effective in nonclinical *in vitro* and *in vivo* model systems, including RSV-infected cotton rats and neonatal lambs. Results from these studies demonstrate a beneficial effect with regard to viral load, inflammatory signs, and/or symptoms and signs of RSV infection. The studies in RSV-infected neonatal lambs provided proof-of-concept for therapeutic intervention with a 3-day treatment course of ALX-0171.

In the first pediatric study, inhaled ALX-0171 demonstrated pharmacological effect in a relevant clinical setting by an immediate impact on viral titers in nasal swab specimen. Analysis of a composite of clinical parameters, the Global Severity Score, led to an encouraging initial indication of a therapeutic effect for infants treated with ALX-0171 and warrants further assessment of this potential clinical benefit in a larger study.

### 1.3.5. CONCLUSION

The nonclinical and clinical studies performed so far did not reveal clinically meaningful risks for administration of ALX-0171 to pediatric subjects. The results in representative nonclinical models, together with those obtained in first-in-infant Study ALX0171-C104, indicate that ALX-0171 may provide an opportunity for therapeutic intervention in established RSV LRTI. The current benefit/risk assessment is therefore considered favorable and supportive of further clinical development.

The doses that will be administered to subjects in Study ALX0171-C201 are estimated to achieve concentrations at which antiviral and clinical activity can be expected while appropriate safety margins are respected. Regular review of clinical data by the IDMC, including during the pauses in recruitment after enrollment of each safety cohort, together with the predefined stopping criteria defined in section 3.1.1 will enable adequate safety follow-up throughout the study. The clinical study protocol foresees close monitoring of all subjects, and is considered to provide a suitable setting for evaluation of ALX-0171 in infants and young children.

## **2. OBJECTIVES**

Primary objective:

- To evaluate the anti-viral effect and safety of different doses of inhaled ALX-0171 in subjects hospitalized for RSV LRTI.

Secondary objective:

- To evaluate the clinical activity, PK properties, pharmacodynamic (PD) effect and immunogenicity of different doses of inhaled ALX-0171.

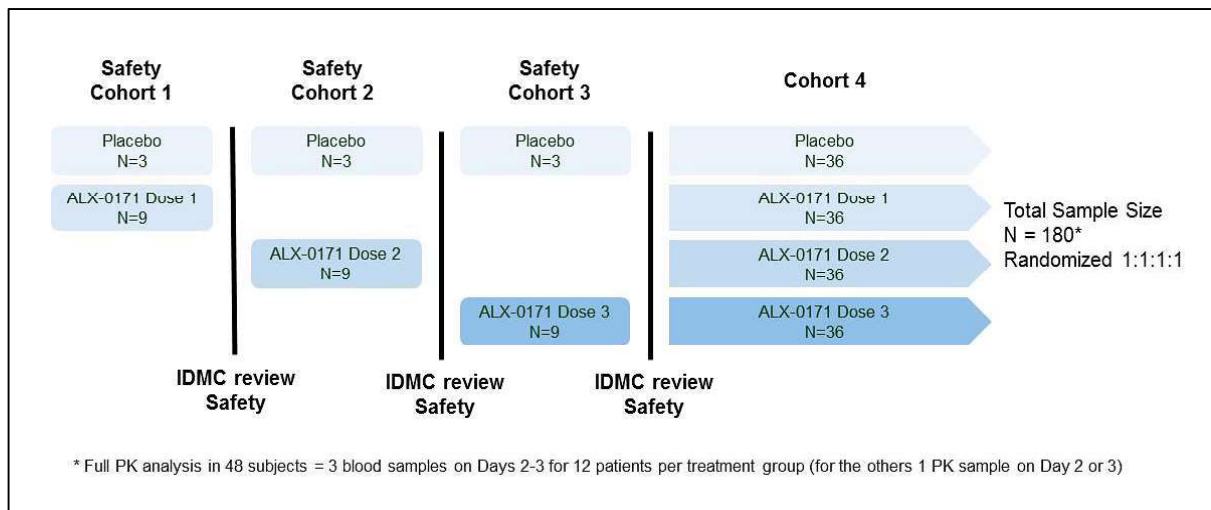


## 3. STUDY DESIGN

### 3.1. OVERALL STUDY DESIGN

#### 3.1.1. STUDY OVERVIEW

This is a randomized, double-blind, placebo-controlled, international, multicenter dose-ranging study (Figure 3). Study drug will be administered along with standard of care treatment, which will be determined by the Investigator (or his/her designee) according to institutional practice<sup>††</sup>.



**Figure 3: Overview of study design**

Three dose levels are planned to be evaluated:

- Dose 1: target dose of 3.0 mg/kg
- Dose 2: target dose of 6.0 mg/kg
- Dose 3: target dose of 9.0 mg/kg

The dose range that will be evaluated is expected to provide a potential benefit to the subjects, and is selected to allow assessment of reduction in nasal viral shedding and improvement of clinical symptoms, to support selection of an optimal dose for future development.

<sup>††</sup> Clinical Practice Guideline: The diagnosis, management and Prevention of Bronchiolitis as described by the American Academy of Pediatrics (2014) may be followed in addition to institutional practice.

Study drug will be administered once daily for 3 consecutive days. The 3-day treatment period is expected to bridge the time needed for the body to mount an effective immune response.

The study is planned to enroll approximately 180 infants and young children (aged 28 days to <2 years) who are diagnosed with and hospitalized for RSV LRTI.

The study consists of a sequential part followed by a parallel part (Figure 3).

#### *Sequential Part*

In safety Cohorts 1 to 3, sequential dose escalation is used to enable appropriate safety follow-up. Each of the 3 safety cohorts consists of 12 subjects (N=36 in total) randomized 3:1 to ALX-0171 (N=9) or placebo (N=3).

In addition to the individual discontinuation criteria (see section 3.2.3), the following criteria apply to stopping or pausing recruitment in a safety cohort (sequential part of the study):

1. Should an SAE indicative of acute respiratory distress or an acute systemic hypersensitivity reaction be considered by the Investigator to be related to administration of ALX-0171, recruitment in that cohort will be stopped and no further dose escalation will be done. This dose will not be taken forward into the parallel part of the study.
2. The reporting of any other SAE considered by the Investigator to be related to administration of ALX-0171 will trigger a pause in recruitment and an immediate review by the IDMC. Confirmation of the causality between the administration of ALX-0171 and the serious adverse reaction by the IDMC will result in the stopping of recruitment in that cohort and no further dose escalation will be done. This dose will not be taken forward into the parallel part of the study.
3. In case 2 SAEs considered by the Investigator to be possibly related to administration of ALX-0171 occur in the same safety cohort, recruitment in that cohort will be paused to allow further investigation by the IDMC. This includes assessment of the index events as well as review of any prior similar events, after which the IDMC will issue a recommendation regarding continuation or stopping of the paused cohort.

The following criteria apply to stopping dose escalation, and will be used to decide on the doses taken forward into Cohort 4 (parallel part of the study):

1. In case recruitment of a safety cohort is stopped due to a related SAE (criteria 1 and 2 above), no further dose escalation will be done and this dose will not be taken forward into the parallel part of the study.
2. In case recruitment is paused due to 2 possibly-related SAEs (criterion 3 above) but reinitiated based on the IDMC recommendation, the assessment for further dose escalation and whether or not the dose is taken forward to Cohort 4, will be performed by

the IDMC during the planned pause after completing the cohort, when the cumulative dataset is available (see below).

3. If 2 severe AEs indicative of acute respiratory distress or an acute systemic hypersensitivity reaction would occur in a particular safety cohort and are considered by the Investigator to be related to administration of ALX-0171, no further dose escalation will be done. This dose will not be taken forward into the parallel part of the study.

In addition to these stopping criteria, cumulative unblinded safety data will be reviewed by an IDMC consisting of an independent group of clinical experts not participating in the study (and an independent statistician).

- After each safety cohort (when the last subject in a safety cohort has completed the treatment period), recruitment will be paused while the IDMC reviews the available cumulative safety data (i.e., all data from prior visits that have been done for all included subjects, including preceding cohorts).
- After this comprehensive review, the IDMC will advise the Sponsor on proceeding to the subsequent cohort (i.e., higher dose) and which planned dose levels can be taken forward into the parallel part.

Please see below for additional information with regard to the IDMC.

Concentrations of ALX-0171 in serum from subjects participating in the safety cohorts will be analyzed after completion of the cohorts to assess if there is a relationship between dose and concentration. The analysis will be performed by an independent statistical analysis team, separate from the statistics and programming staff assigned to the study, and a recommendation will be provided to the Sponsor.

#### *Parallel Part*

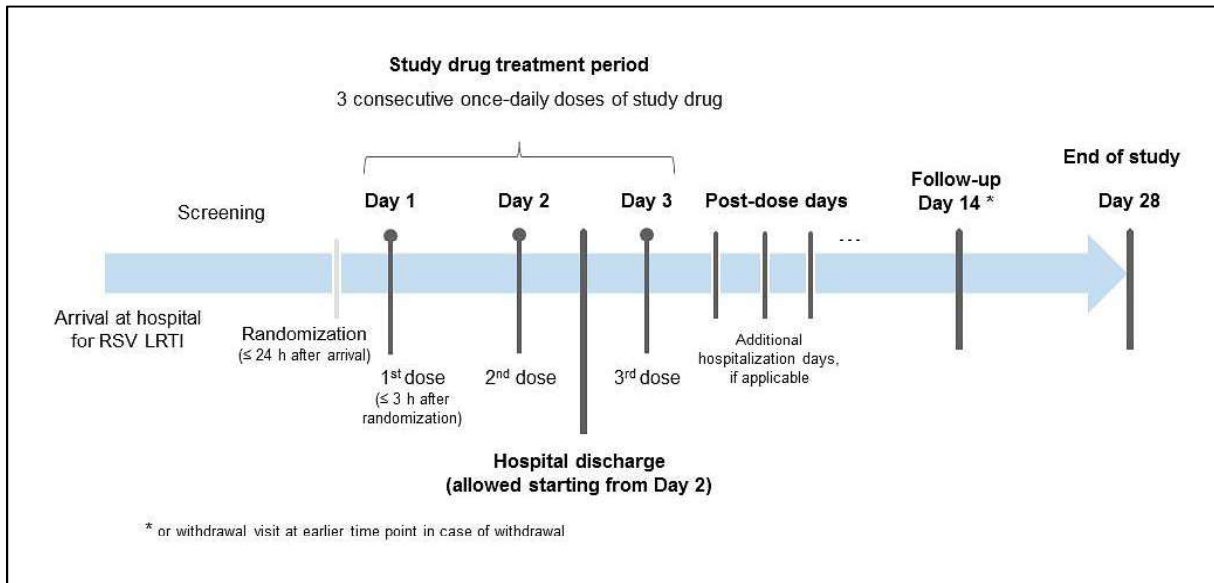
If no stopping criteria have been met during the sequential part and if the IDMC issues a positive recommendation after each safety cohort and the Sponsor decides to proceed, the remaining 144 subjects (i.e., Cohort 4) will be randomly assigned in a 1:1:1:1 ratio to one of following treatment groups, yielding an overall randomization ratio of 3:1 active to placebo.

- ALX-0171 dose 1
- ALX-0171 dose 2
- ALX-0171 dose 3
- Placebo

In the event one or two dose groups are discontinued for safety, the remaining groups will enroll the number of subjects initially specified (i.e., 45 per arm).

The Placebo group will serve as comparator group for the 3 ALX-0171 dose groups. To achieve double-blinding across the different groups, each dose will be administered as two serial nebulizations. These two nebulizations will consist either of two nebulizations of ALX-0171, or one of ALX-0171 and one of placebo, or two nebulizations of placebo depending on the assigned treatment group (also see section 3.3.7).

An overview of the study flow is provided in [Figure 4](#).



**Figure 4: Overview of study flow**

Subjects will be screened as soon as possible after arrival to the hospital/emergency unit. After completion of the screening assessments and confirmation of subject's eligibility, randomization should follow as soon as possible but not more than 24 hours after arrival. Study drug administration should start as soon as possible after randomization with a maximum time interval of 3 hours following randomization. Subsequent doses of study drug will be administered at 24-hour intervals ( $\pm 4$  hours) relative to the first dose. No premedication is required or recommended per protocol, but an inhaled  $\beta 2$ -agonist may be administered at the discretion of the treating Investigator.

On the first 2 dosing days, inpatient hospital stay is required. Provided that the clinical response criteria have been met (which are based on adequate oral feeding and oxygen saturation), discharge from the hospital can take place per protocol at the Investigator's discretion from dosing Day 2 onwards after all required assessments of the 5 hours ( $\pm 1$  hour) post-dose time point have been completed. Subjects discharged after the second dose must return to the hospital for the third study drug administration (to be administered  $48 \pm 4$  hours after the first dose by the appropriately trained study personnel), and be monitored for a 2-hour pre-dose and post-dose period. Subjects who are not discharged

from the hospital after completion or premature discontinuation of study drug enter an in-hospital post-treatment period with assessments in the morning and evening. A Follow-Up (FU) visit is scheduled on Day 14 ( $\pm$  2 days), and an End of Study (EOS) visit on Day 28 ( $\pm$  2 days). For subjects prematurely withdrawn from the study, a Withdrawal visit is to be performed on the day of withdrawal.

The primary endpoint in this study is the evaluation of viral load in samples obtained via nasal mid-turbinate swabs. In particular, the time needed for the viral load (as assessed by plaque assay) to drop below the quantification limit (BQL) is calculated (time-to-BQL).

Secondary endpoints include:

- Safety will be assessed through AE collection, measurements of vital signs, peripheral capillary oxygen saturation (SpO<sub>2</sub>), physical examination and laboratory parameters. Safety lab assessments are planned two times during the study (at screening and at the FU visit) and will be performed by the local laboratory in order to allow timely availability of the results. Furthermore, an IDMC will be involved in the regular review of the available safety data.
- Clinical activity of ALX-0171: assessed through evaluation of clinical symptoms over time (SpO<sub>2</sub>, feeding, respiratory rate, wheezing, cough, respiratory muscle retractions, and general appearance) and further calculation of composite clinical scores such as Global Severity Score, Respiratory Assessment Change Score (RACS) and Respiratory Distress Assessment Instrument (RDAI).
- Time to clinical response, utilizing the criteria of adequate oxygen saturation and oral feeding (see below).

Subjects will be considered to have met the Clinical Response criteria when both of the following criteria are fulfilled:

- Stable oxygen saturation on room air, defined as SpO<sub>2</sub> > 92% over a period of  $\geq$  4 hours. In case of oxygen supplementation, the level of supplementation is to be considered for reduction at least three times per day. Provided oxygen saturation is stable, attempts to remove the supplementation will be done at least three times a day.
- Adequate oral feeding which is sufficient to maintain sufficient hydration, in the judgment of the Investigator.

In addition, data on medical interventions will be captured in order to assess the impact of ALX-0171 on duration of hospitalization and the need for ventilation or transfer to an intensive care unit (ICU) setting.

Parent(s)/Caregiver(s) assessment of the clinical condition of the child will be done by daily completion of a diary during the hospital stay and up to the EOS (Day 28) visit. This diary includes scoring of respiratory symptoms over the past 24 hours (cough, wheezing, trouble breathing), evaluating the general health of the child by making a mark on a Visual

Analogue Scale (VAS) to indicate the child's health, and answering a question whether the child has returned to his/her normal condition from before the onset the RSV infection. For the period after discharge from the hospital, this diary will also allow to capture data on health care utilization and use of medication for respiratory symptoms.

The study will also evaluate pharmacokinetics, pharmacodynamics and immunogenicity:

- The systemic concentration of ALX-0171 will be evaluated in serum, as a surrogate for evaluating local (lung) concentration. A subset (48 subjects, i.e., 12 subjects per dose group) will undergo more extensive PK sample analysis (3 blood samples for PK assessment). The extensive sampling scheme might become mandatory during the study in order to achieve the predefined sample size. Replacement of subjects who are part of the extensive PK sampling will be done for those for whom not all 3 PK samples could be collected successfully and/or who have not completed study drug administration on dosing Day 1 or dosing Day 2. Subjects part of the extensive PK sampling who have not successfully received study drug on Day 1 or Day 2 do not need to undergo the full PK sampling schedule. In the remainder of subjects, only 1 PK blood sample will be taken. The number of subjects undergoing the more extensive PK sample analysis was determined to obtain sufficient precision on the PK parameters as described by Wang et al. [10].
- In addition, PD biomarker (Krebs von den Lungen [KL-6]) will be assessed (serum).
- Potential immunogenicity will also be assessed systemically (serum).

For determination of sample size, please refer to section 3.1.2.

#### *Estimated Study Duration*

The overall study duration is expected to be around 23 months. The planned study duration for each subject is approximately 28 days.

The completion of the study is defined as the last visit of the last subject (LSLV) participating in the study.

#### *Independent Data Monitoring Committee*

In line with applicable guidelines, an IDMC is assigned to monitor the study. The IDMC consists of an independent group of clinical experts not participating in the study and an independent statistician.

After each safety cohort (when the last subject in a safety cohort has completed treatment period), recruitment will be paused while the IDMC reviews the available cumulative safety data (i.e., all data from prior visits that have been done for all included subjects, including preceding cohorts).

After this comprehensive review of safety data, the IDMC will advise the Sponsor on proceeding to the subsequent cohort (i.e., the higher dose) and which planned dose levels

can be taken forward into the parallel part. This recommendation will complement the above-described predefined stopping criteria.

During the parallel part of the study, an IDMC review is planned after 72 subjects completed treatment.

Throughout the study, SAE information will be communicated in real time according to the reporting requirements. An ad-hoc meeting can be requested by the IDMC members.

The composition, objectives, and roles and responsibilities of the IDMC will be described in an IDMC charter, agreed with the IDMC members and Sponsor. The IDMC charter will also define and document the content of the safety summaries, and general procedures (including communications). The first version of the charter will be available prior to the initiation of the study.

### **3.1.2. STUDY RATIONALE AND DISCUSSION OF STUDY DESIGN**

Safety and tolerability of ALX-0171 have been evaluated in 3 Phase 1 studies in adults, and in a Phase 1/2a study in infants and young children hospitalized for RSV LRTI. Study ALX0171-C201 will include a similar population and aims primarily to evaluate the anti-viral effect of three doses of inhaled ALX-0171, as well as to assess the safety and clinical activity (see section 3.3.2 for dose selection rationale). The outcome of this dose ranging study will support the selection of the optimal dose and the most relevant endpoints for further clinical development.

Study ALX0171-C201 will start with a stepwise assessment of cohorts of 12 subjects, each being randomized in a 3:1 ratio to increasing doses of ALX-0171 or placebo (i.e., 9 subjects receiving ALX-0171 and 3 receiving placebo in each cohort). To guide dose escalation, stopping criteria have been included in the protocol (section 3.1.1). In addition, after each of these 3 sequential cohorts, recruitment will be paused in order to allow the IDMC to evaluate available cumulative safety data and recommend whether the subsequent cohort can be initiated and which planned dose levels can be taken forward into the parallel part. When these initial cohorts have been completed and safety has been confirmed, the study will continue with a parallel allocation to ALX-0171 or placebo. 144 eligible subjects will be randomly assigned in a 1:1:1:1 ratio to 1 of the 4 treatment groups (3 active groups and 1 placebo group).

The design of Study ALX0171-C201 allows an approach with a placebo-controlled group as representative comparison for the three active dose levels. The total volume and corresponding inhalation times of study drug per weight category is similar in all dosing groups, by using a combination of placebo and ALX-0171 (if applicable), administered via

2 serial nebulizations. This ensures blinding across the treatment groups (also see section 3.3.7).

The primary objective of the study is evaluation of the anti-viral effect of ALX-0171 compared to placebo.

As pharmacologically active doses will be used, clinical activity of ALX-0171 will be evaluated in order to assess if the pharmacological effect translates in clinical benefit to the subject. Other objectives include evaluation of pharmacokinetics, pharmacodynamics (biomarker), and immunogenicity.

Viral load will be assessed in samples obtained via nasal swabs collecting a mid-turbinate specimen. Based on the results of Study ALX0171-C104, the anti-viral effect of ALX-0171 will be mainly determined by the time to drop BQL in plaque cultures, which is the parameter that best reflects the inhibition of viral replication. Quantification of viral load by RT-qPCR will also be assessed as it is a more sensitive method than culture assay and has a good dynamic range. However, as the mode of action of ALX-0171 is to inhibit viral entry into the target cell, modest effects of ALX-0171 treatment on viral RNA are expected as complete viral particles that are unable to replicate, partially assembled virions, and whole and fragmented viral genome will be quantified by RT-qPCR in addition to fully replication-competent virus.

The clinical impact of treatment by ALX-0171 in infants and young children will be evaluated by changes in clinical symptoms and subsequent calculation of composite scores, the time needed to enable adequate feeding and oxygen saturation, need for medical interventions and length of hospital stay. Based on the clinical activity parameters measured during the study, composite scores that reflect the clinical status of the subject suffering from RSV will be calculated, including the Global Severity Score, RDAI score, and RACS. The Global Severity Score is based on a recent clinical score that allows categorisation of infants with respiratory infections on 7 different parameters: feeding intolerance, medical intervention, respiratory difficulty, respiratory frequency, apnea, general condition and fever [11, 12]. It takes into account all clinically relevant aspects of an RSV infected subject. The RDAI is a scoring system based on the presence and severity of wheezing and respiratory muscle retractions. The RACS is based on the RDAI and adds a standardized score for the change in respiratory rate.

Given the importance of timing of the administration of this anti-viral compound in the time course of the RSV infection, details on the time elapsed between onset of first clinical symptoms and start of treatment with study drug will be collected and analyzed. The need for medical interventions will be documented through collection of data on the need for and level of oxygen supplementation or respiratory support, need for transfer to ICU, duration of stay in ICU and overall hospital stay.



In addition to the assessments performed by healthcare professionals, the study will also collect information on how parents or caregivers perceive the clinical condition of the child through daily completion of a diary. This diary includes scoring of respiratory symptoms over the past 24 hours (cough, wheezing, trouble breathing), evaluating the general health of the child by making a mark on a VAS, and answering to a question if the child has returned to his/her normal condition from before the onset the RSV infection. For the period after discharge from the hospital, this diary will also capture data on health care utilization and use of medication for respiratory symptoms. The diary will be completed during hospital stay and daily thereafter up to the EOS (Day 28) visit as RSV induced bronchiolitis is often associated with continuing respiratory symptoms following hospitalization.

The systemic concentration of ALX-0171 will be evaluated in serum, as a surrogate for local (lung) concentrations. Potential immunogenicity will also be assessed systemically (serum), both pre-dose and post-dose (14 days post initial drug administration) and results will be correlated with PK, PD and safety findings.

Systemic levels of the serum biomarker KL-6 will be determined in left-over serum samples. Serum KL-6 was shown to indicate disease activity in various interstitial lung diseases and is believed to reflect the presence of alveolar damage [13, 14]. High serum KL-6 levels in RSV-infected infants correlate with low SpO<sub>2</sub> and need for O<sub>2</sub> administration [15]. These findings suggest that serum KL-6 is associated with the severity of RSV bronchiolitis, and that it may provide additional insight on potential treatment benefits.

The intended volume of blood to be withdrawn for each of the assessments is based on the minimum required volume for each of the respective analyses, and is in line with the volumes allowed per applicable guidelines [16-20].

#### *Rationale for Sample Size*

The sample size calculation is based on statistical considerations for the analysis of the primary endpoint. With 45 subjects per arm, 85% power is achieved to detect a 50% reduction in median time-to-BQL (0.5 day with ALX-0171 versus 1 day with placebo) for the plaque assay using a two-sided log-rank test at significance level of 0.05. For each comparison of the ALX-0171 dose groups with placebo, a significance level of 0.05 is assumed. For multiple pairwise comparisons, the family-wise error rate is controlled at 5% through the closed testing principle, where the comparison of the lower dose of ALX-0171 to placebo is only performed if the comparison of the higher dose of ALX-0171 to placebo is significant. The sample size calculation also assumes a drop-out rate of 15% to account for subjects dropping out prior to reaching the primary endpoint, or due to a false positive RSV test.

Change from baseline in Global Severity Score is a secondary endpoint of interest in this study. With this sample size, a power of 80% is achieved to detect a difference of 2 points

in the mean change from baseline (to Day 2 post-dose) on the Global Severity Score for each of the ALX-0171 dose groups to placebo. This is based on a two-sided t-test with a significance level of 0.05 and assuming a common standard deviation of 3.2 in each group.

#### *Dosing Rationale*

The study drug will be administered along with standard of care treatment, which will be determined by the Investigator (or his/her designee) according to institutional practice<sup>††</sup>. Study drug will be administered once-daily for 3 consecutive days, similar to the treatment schedule in Study ALX0171-C104. This 3-day treatment period is expected to bridge the time needed for the body to mount an effective immune response. In order to assess a dose response in Study ALX0171-C201, three doses (target dose of 3 mg/kg, 6 mg/kg and 9 mg/kg) will be investigated. This dose range will provide information on the PK, PD and clinical outcomes across a relevant range, is expected to provide a potential benefit to the subjects, and is intended to determine the most optimal dose of ALX-0171 for further clinical development.

Please refer to section [3.3.2](#) for more information on the dose selection.

All doses of study drug will be administered in the hospital setting. Subjects may be discharged from hospital starting from dosing Day 2 onwards, after all required assessments of the 5 hours ( $\pm$  1 hour) post-dose time point have been completed. The decision to discharge is up to the Investigator but can only occur per protocol if the protocol-defined clinical response criteria have been met. Hence, an ambulatory visit will be permitted for the 3<sup>rd</sup> study drug administration (i.e., subjects need to return to the hospital for the administration of the 3<sup>rd</sup> dose by the appropriately trained personnel and have their 2 hour pre- and post-dose monitoring period).

Study drug will be administered via inhalation, using a dedicated vibrating mesh nebulizer.

- Vibrating mesh type nebulizers are considered the most appropriate technology for nebulization of a therapeutic protein such as ALX-0171 Nanobody.
- The FOX-Flamingo nebulizer (Vectura GmbH, Germany) was developed concurrently with the study drug. The nebulizer is a battery-operated, hand-held device, intended for single-patient use. The device provides an aerosol with particle size suitable for the intended study population ( $\sim$ 3  $\mu$ m).
- The nebulizer is always to be used with a flow of 2 L/min additional air or O<sub>2</sub> (to be decided by the Investigator based on oxygen need of the subject).

The formulation of ALX-0171 (and matching placebo) was developed specifically as a liquid solution for inhalation, to ensure rapid delivery of the study drug to the site of RSV infection, and to maintain product quality and stability in combination with the nebulization protocol.

- The formulation buffer is comparable to a normal 0.9% saline solution with respect to salt content but has additional buffering capacity. The osmolality and ion concentration of the (isotonic) solution were chosen to be within the physiological range. The phosphate buffer system helps to maintain a constant pH, and therefore, to guarantee the stability of the drug.
- All excipients comply with the European Pharmacopoeia and the United States Pharmacopoeia.
- The formulation contains no antimicrobial preservatives or tension active agents (surfactants), and no coloring agents, flavors, or sugars/sweeteners are present. Correspondingly, the nebulizer solution does not have a specific taste or odor.

The use of formulation buffer as placebo is considered appropriate, taking into account that formulation buffer is the most representative control for the active study drug, while the minute amounts of phosphate present in the buffer would not be expected to result in effects different from saline. Throughout the clinical studies conducted with ALX-0171 and placebo, there were no consistent findings that would suggest a direct effect of the formulation buffer on respiratory parameters.

Details on initiation, interruptions and completion of study drug administration, will be collected in the electronic case report form (eCRF), as will the acceptability of the face mask and nebulizer and whether or not correct administration was achieved (also see section [3.3.7](#)).

### **3.1.3. BLINDING**

To ensure blinding across dose groups, study drug will be administered via inhalation using 2 serial nebulizations. These two nebulizations will consist either of two nebulizations of ALX-0171, or one of ALX-0171 and one of placebo, or two nebulizations of placebo depending on the assigned treatment group (see section [3.3.7](#)).

In order to protect the integrity of the data, treatment assignment will be kept blinded for investigative sites, subjects, subject's parent(s)/legal guardian(s) and caregiver(s), site monitors, and other members of the study team, until after the final database lock (i.e., after the last subject has completed the final [EOS] visit and all data is considered clean). Identification of Sponsor and contract research organization (CRO) personnel who will have access to unblinded data (e.g., PK sample analysis team) during the course of the study will be documented prior to unblinding. The number of Sponsor personnel having access to the data will be limited.

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**Emergency unblinding procedure**

Code-breaking and unblinding in the event of medical emergencies can be done by the Investigator via the Interactive web/voice response system (IWRS/IVRS), which will be accessible 24 hours per day/7 days per week.

Unblinding by the Investigator should occur only in the event of an AE for which it is necessary to know the study treatment to determine an appropriate course of therapy for the subject. The Investigator should first discuss options with the Medical Monitor if possible with due consideration of the safety of the subject. If the Investigator must identify the treatment assignment of an individual subject, the Principal Investigator/Sub-investigator is to contact the IWRS/IVRS.

Subjects for whom the code has been broken by the Investigator will have to discontinue study drug treatment and subjects will be followed up according to the in-hospital post-treatment period (if applicable), FU visit and EOS visit.

## 3.2. SELECTION OF STUDY POPULATION

Infants and young children of both genders, diagnosed with and hospitalized for RSV LRTI but otherwise healthy, are eligible for this study. Approximately 180 subjects are planned to be enrolled in the study.

### 3.2.1. INCLUSION CRITERIA

A subject will be eligible for study participation if he/she meets all of the following criteria at screening and randomization, unless specified otherwise:

1. Subject is a male or female infant or young child aged 28 days to <2 years with gestational age  $\geq$  33 weeks at screening.
2. Subject weighs between  $\geq$  3.0 kg and < 15.0 kg at screening.
3. Subject is otherwise healthy, but is hospitalized for and clinically diagnosed with RSV LRTI (bronchiolitis or broncho-pneumonia), i.e., showing typical clinical signs and symptoms such as tachypnea, wheezing, cough, crackles, use of accessory muscles and/or nasal flaring.
4. Subject has a positive RSV diagnostic test at screening.<sup>##</sup>
5. Subject is expected to have to stay in the hospital for at least 24 hours (according to the Investigator's judgment at screening).
6. Symptoms likely related to RSV infection (i.e., the symptoms present need to be probably linked to the current RSV infection according to Investigator's judgment) have appeared within 4 days of screening and are not yet improving at screening and randomization.
7. Subject fulfils at least two of the following RSV disease severity criteria at screening and randomization:
  - Inadequate oral feeding that requires feeding support (i.e., nasogastric tube or i.v. line)
  - Inadequate oxygen saturation defined as:
    - Oxygen saturation ( $SpO_2$ )  $\leq$  92% on room air or
    - Requiring oxygen supplementation to maintain oxygen saturation  $>$  90% with documented pre-supplementation value  $\leq$  92%
  - Signs of respiratory distress defined as:
    - Respiratory rate  $\geq$  50 per minute in infants up to 12 months of age, and  $\geq$  40 per minute in children above 12 months and/ or
    - Moderate or marked respiratory muscle retractions

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<sup>##</sup> RSV infection will be confirmed either according to routine site practice (PCR or diagnostic quick test), or using a (Sponsor-provided) commercial kit.

8. Subject has normal psychomotor development.
9. Parent(s)/legal guardian(s) provide written informed consent in accordance with locally approved consent process at screening.
10. The parent(s)/legal guardian(s) are able and willing to comply with the study protocol procedures.

### **3.2.2. EXCLUSION CRITERIA**

Subjects meeting any of the following criteria at screening or randomization will not be eligible for study participation:

1. Subject is known to have significant comorbidities including:
  - Genetic disorders (e.g., trisomy 21, cystic fibrosis),
  - Hemodynamically significant congenital heart disease (e.g., needing corrective therapy or inotropic support),
  - Bronchopulmonary dysplasia,
  - Any hereditary or acquired metabolic (bone) diseases,
  - Hematologic or other malignancy.
2. Subject is known to be human immunodeficiency virus (HIV)-positive. If the subject is < 6 months of age, a known HIV-positivity of the mother is also exclusionary.
3. Subject is known to be immunocompromised.
4. Subject has or is suspected to have an active, clinically relevant concurrent infection (e.g., bacterial pneumonia, urinary tract infection). Concurrent acute otitis media is not exclusionary.
5. Subject has significant oral and/or maxillofacial malformations that would prevent proper positioning of the face mask.
6. Subject received invasive mechanical ventilation or non-invasive respiratory support (i.e., continuous or bilevel positive airway pressure) in the 4 weeks prior to screening.
7. During the current admission, subject is initially hospitalized in an ICU setting and/or received invasive mechanical ventilation or non-invasive respiratory support (i.e., continuous or bilevel positive airway pressure).
8. Subject is critically ill and/or is expected to require invasive mechanical ventilation, non-invasive respiratory support (i.e., continuous or bilevel positive airway pressure), or High Flow oxygen therapy (HFOT) at levels not enabling nebulization therapy according to the Investigator's judgment. High Flow oxygen, with a maximum flow of 2 L/kg/min, is permitted under the following conditions:
  - used as Standard of Care outside ICU setting
  - can be removed for study drug administration (Note: oxygen flow at 2 L/min can be provided).
9. Subject has received 1 or more doses of palivizumab or treatment or prophylaxis with any RSV antiviral compound (e.g., ribavirin, i.v. immunoglobulin, or any investigational

drug or vaccine for RSV [including subject's mother who has been vaccinated against RSV]) at any time prior to screening.

10. Subject is required to continue or start systemic corticosteroid therapy. Subject on a maintenance therapy of inhaled corticosteroids will continue this treatment at the usual dose. Topical corticosteroids for skin or ear disorders are permitted.
11. Subject is a child in care (i.e., a child who has been placed under the control or protection of an agency, organization, institution or entity by the courts, the government or a government body, acting in accordance with powers conferred on them by law or regulation). A child in care can thus include a child cared for by foster parents or living in a care home institution, provided that the arrangement falls within the definition above, but does not include a child who is adopted or has appointed legal representative(s).
12. Subject is currently participating in any other study with investigational drug or has received an investigational drug within 4 weeks or 5 half-lives of the concerned drug (whichever is longer) prior to screening.
13. Subject was previously enrolled in a clinical study of ALX-0171 (including the current Study ALX0171-C201).
14. Subject has a known hypersensitivity to the study drug or any excipient of the study drug.

### 3.2.3. REMOVAL OF SUBJECTS FROM THERAPY OR ASSESSMENT

Every reasonable attempt should be made by the Investigator (or his/her designee) to keep subjects in the study, however, a subject must be withdrawn from the study if:

- Study drug administration cannot be successfully initiated on Day 1 (see section 3.3.7.2).
- The Investigator considers it, for safety reasons, in the best interest of the subject.
- The parent(s)/legal guardian(s) withdraw(s) consent.

A subject may be withdrawn from the study if the subject and/or parent(s)/legal guardian(s) fail to comply with dosing, evaluations, or other requirements of the study.

Investigators (or their designees) must attempt to contact the parent(s) or legal guardian(s) of subjects who fail to attend scheduled visits by telephone or other means, to exclude the possibility of an AE being the cause of withdrawal. Should this be the cause, the AE must be documented, reported, and followed-up as described in section 3.5.

If a subject is prematurely withdrawn from the study, the reason for withdrawal and the date of withdrawal will be recorded in the eCRF. Whenever possible and reasonable, a Withdrawal visit should be conducted at the time of withdrawal (no EOS visit).

A subject must be discontinued from study drug treatment if:

- The randomization code is broken prematurely by the Investigator or Sub-Investigator.
- A severe and/or serious hypersensitivity reaction occurs
- A severe and/or serious acute respiratory distress reaction induced by study drug administration occurs
- The subject needs to start treatment with one of the medications listed as prohibited (see section [3.3.8](#))
- The subject needs to start non-invasive respiratory support, invasive ventilation or HFOT at levels not enabling removal of the cannula for administration of the study drug nebulization and/or levels exceeding 2 L/kg/min. The event will be captured as an SAE and will be monitored until resolution, as described in section [3.5](#).
- If the investigator or the Sponsor / medical monitor deems it is in the subject's best interest

A subject may be discontinued from study drug treatment if an SAE occurs (if considered necessary or appropriate by the Investigator).

Subjects having received at least one study drug administration and remaining in the hospital after study drug treatment discontinuation will remain in the study according to the in-hospital post-treatment period specified in the [Schedule of Assessments](#) and should also attend the FU and EOS visits.

If a subject is discontinued from the study and/or study drug, the study monitor and the Sponsor will be informed immediately.

### **3.2.3.1. REPLACEMENT OF SUBJECTS**

Replacement of subjects will only be done for subjects randomized to the extensive PK sampling schedule. Replacement will be done for those for whom not all 3 PK samples could be collected successfully and/or who have not completed study drug administration on dosing Day 1 or dosing Day 2. Subjects who have not successfully received study drug on Day 1 or Day 2 do not need to undergo the full PK sampling schedule.

### **3.2.3.2. STUDY TERMINATION**

If the Sponsor abandons the study prior to commencement of any protocol activities, and/or after IEC/IRB and CA approvals have been received, the Investigator or Sponsor must notify the IEC/IRB and CA by letter outlining the reasons for abandonment of the study, as required per national regulations.

At any time during the study, the Sponsor may suspend or terminate the study or part of the study for any reason. If the Investigator plans to suspend or terminate participation in



the study, the Investigator will promptly inform the Sponsor and the IEC/IRB and provide them with a detailed written explanation.

Upon study completion, the Sponsor will provide the Investigator, IEC/IRB, and CA with final reports and summaries as required by regulations.

In case of suspension or halt due to safety reasons, the CA and IEC/IRB will be notified immediately and at the latest within the number of days as specified by local regulations after the study is halted, clearly explaining the reasons, and describing follow-up measures, if any, taken for safety reasons.

The IDMC, consisting of an independent group of clinical experts not participating in the study and an independent statistician, will review unblinded safety data. The IDMC will advise the sponsor concerning continuation, modification or potential early termination of the study after every meeting (also see section [3.1.1](#)).

## **3.3. TREATMENT OF SUBJECTS**

### **3.3.1. OVERVIEW OF TREATMENTS ADMINISTERED**

Eligible subjects will be randomly assigned to one of the following treatment groups.

- ALX-0171 dose 1: target dose of 3.0 mg/kg
- ALX-0171 dose 2: target dose of 6.0 mg/kg
- ALX-0171 dose 3: target dose of 9.0 mg/kg
- Placebo

ALX-0171 or matching placebo will be administered via inhalation using the FOX-Flamingo inhalation system (using 2 serial nebulizations; also see section 3.3.7), once daily for 3 consecutive days. The formulation of ALX-0171 (and matching placebo) was developed specifically as a liquid solution for inhalation, to ensure rapid delivery of the study drug to the site of RSV infection, and to maintain product quality and stability in combination with the nebulization protocol.

The first and second study drug administrations require inpatient hospital stay. Provided that the clinical response criteria have been met, discharge from the hospital can take place per protocol at the Investigator's discretion from dosing Day 2 onwards after all required assessments of the 5 hours ( $\pm$  1 hour) post-dose time point have been completed. Subjects must return to the hospital for the third study drug administration (to be administered by the appropriately trained study personnel), and be monitored for a 2-hour pre-dose and post-dose period.

### **3.3.2. SELECTION OF DOSES IN THE STUDY**

For the first-in-infant pediatric study (Study ALX0171-C104), dose determination was based on a modelling approach, complemented by additional experiments that generated data specifically for administration of ALX-0171 with the FOX-Flamingo inhalation system. Physiologically-based PK modelling was considered the best approach to bridge nonclinical, human adult and human pediatric PK parameters, taking into account growth and developmental processes such as organ maturation, changes in blood flow, body composition, and ontogeny of elimination mechanisms [21, 22]. Based on this approach, a dose of 1.2 mg/kg was chosen for evaluation. The results of study ALX0171-C104 indicated that this dose was well tolerated in all age groups (1 to 24 months). Reductions in nasal viral load (obtained from nasal swabs) were noted, confirming that ALX-0171 can exert antiviral activity when relevant concentrations are achieved. Although the first-in-infant

study was not designed to demonstrate clinical effect, first assessments indicated that the desired level of clinical efficacy was not reached with the administered dose. This was in line with the observation of lower than expected serum concentrations of ALX-0171 which could be indicative of insufficient drug concentrations in the lung, an important site of RSV replication and the driver for clinical effect.

In order to assess a dose response in Study ALX0171-C201, three doses (target dose of 3 mg/kg, 6 mg/kg and 9 mg/kg) will be investigated. At the beginning of the study, 3 sequential safety cohorts of 12 subjects each will be enrolled, with predefined stopping criteria and IDMC assessment before proceeding to the next higher dose.

This dose range will provide information on the PK, PD and clinical outcomes across a relevant range, is expected to provide a potential benefit to the patients, and is intended to determine the optimal dose of ALX-0171 for further clinical development. In view of the favorable safety profile of ALX-0171 in nonclinical and clinical studies and the plasma drug concentrations in the prior study, the doses in Study ALX0171-C201 are considered appropriate to further explore clinical effect. Anticipated systemic concentration to ALX-0171 at the highest dose is estimated to be similar or lower than those obtained in human adult volunteer studies, and maintains at least a 10-fold safety margin compared to nonclinical safety studies.

Safety margins were calculated based on results obtained in a pivotal toxicity study conducted in Sprague Dawley rats after 14 day repeated administration via inhalation (see IB). Supportive information from (i) a PK study in juvenile rats after single or repeated inhalation (see IB), and (ii) a neonatal lamb study after single dose in uninfected animals and after repeated dose in RSV uninfected or RSV-infected animals (see IB) did not yield unexpected retention of ALX-0171 in tissue or plasma. Concentrations in lung in juvenile rats were somewhat lower when compared to adult rats, with high variability to be considered in the study. No safety signals were detectable in either study in neonatal or juvenile animals. Based on the available information, a correction of calculations based on the GLP-generated data in the toxicity studies was not considered warranted.

- For calculating the safety margins, in vitro SAINT model data was used to predict the ALX-0171 dose that will come into contact with the infants. The inhaled dose (i.e., the lung dose and the dose deposited in the nasopharyngeal airways) and the dose on the face/oral cavity were taken into consideration and were experimentally shown to be 29.1% of the nebulizer filling dose. This fraction of the nebulizer filling dose is assumed to be totally deposited in the lungs. This was assumed as "maximally expected local exposure".
- The maximally expected local exposure in pediatric subjects was compared to the highest lung exposure achieved in the pivotal nonclinical toxicity study (the 14-day repeated dose toxicity study after daily administration via inhalation in rats; see IB). In this study, no AEs were observed, so the highest dose administered (117 mg/kg) was considered safe. Assuming 10% lung deposition in rat, the highest local dose achieved was

11.7 mg/kg or 2.3 mg/g lung (female numbers were used as these are the lower than males).

- Local safety margins were calculated taking the 29.1% fraction of the nominal dose in the planned clinical study. With a maximal nominal dose of 10.7 mg/kg (see section 3.3.7), and assuming a 1.8% lung weight to body weight ratio [23], the calculated maximal deposited dose is 0.17 mg/g lung in the planned pediatric population.
- The corresponding safety margin was calculated as the ratio between maximal achieved dose in rat (2.3 mg/g lung) to maximal calculated deposited dose in patients (0.17 mg/g lung), which results in a safety margin of  $\geq 13.3$ .

### 3.3.3. RANDOMIZATION

After obtaining written informed consent from the parent(s)/legal guardian(s) or legally acceptable representative(s), subjects will be screened according to the inclusion and exclusion criteria and will receive a unique subject identification (ID) number, assigned by IWRS/IVRS.

At the time of randomization, subjects should be eligible for study participation as defined in the inclusion/exclusion criteria. Therefore, at a minimum, the SpO<sub>2</sub>, feeding, respiratory muscle retractions, and respiratory rate should be evaluated on Day 1 before randomization, unless these assessments were already performed within the last 3 hours before randomization.

At randomization, subjects will be assigned a randomization number by IWRS/IVRS prior to study drug administration according to the randomization scheme.

In safety Cohorts 1 to 3, sequential dose escalation is used to enable appropriate safety follow-up. Each of the 3 safety cohorts consists of 12 subjects (N=36 in total) that will be randomized in a 3:1 ratio to receive either ALX-0171 dose 1 or placebo (Cohort 1), ALX-0171 dose 2 or placebo (Cohort 2), or ALX-0171 dose 3 or placebo (Cohort 3).

After each safety cohort (when the last subject in a safety cohort has completed the treatment period), an IDMC will review the available cumulative safety data and advise the Sponsor on proceeding to the subsequent cohort (i.e., the higher dose) and which planned dose levels can be taken forward into the parallel part. If no stopping criteria have been met during the sequential part and if the IDMC issues a positive recommendation after each safety cohort and the Sponsor decides to proceed, the remaining 144 subjects (i.e., Cohort 4) will be randomly assigned in a 1:1:1:1 ratio to one of the 4 treatment groups, yielding an overall randomization ratio of 3:1 active to placebo. In the event one or two dose groups are discontinued for safety, the remaining groups will enroll the number of subjects initially specified (i.e., 45 per arm).

A separate randomization list will be created for each cohort. A restricted randomization algorithm will be used in cohort 4 to maintain the balance between the different treatment arms overall but also in the PK substudy.

### 3.3.4. IDENTITY OF STUDY DRUG

ALX-0171 and matching placebo will be provided in sterile, preservative-free, masked depyrogenated single-use DIN 2R glass vials, with injection stoppers and aluminium crimped caps. Each vial contains  $\geq 2$  mL nebulizer solution (target fill volume: 2.2 mL).

Instructions for preparation, use and handling of study medication, the appropriate volume to be administered and the materials to be used, are provided in a manual concerning study drug. Instructions for use for the FOX-Flamingo nebulizer are provided as well.

#### *ALX-0171*

- Formulation: nebulizer solution
- Route of administration: inhalation (to be administered via the FOX-Flamingo inhalation system)
- Active substance: ALX-0171 Nanobody
- Activity: ALX-0171 Nanobody specifically and potently binds to the RSV F protein, thereby inhibiting an early step in the viral replication cycle.
- Composition: [REDACTED]

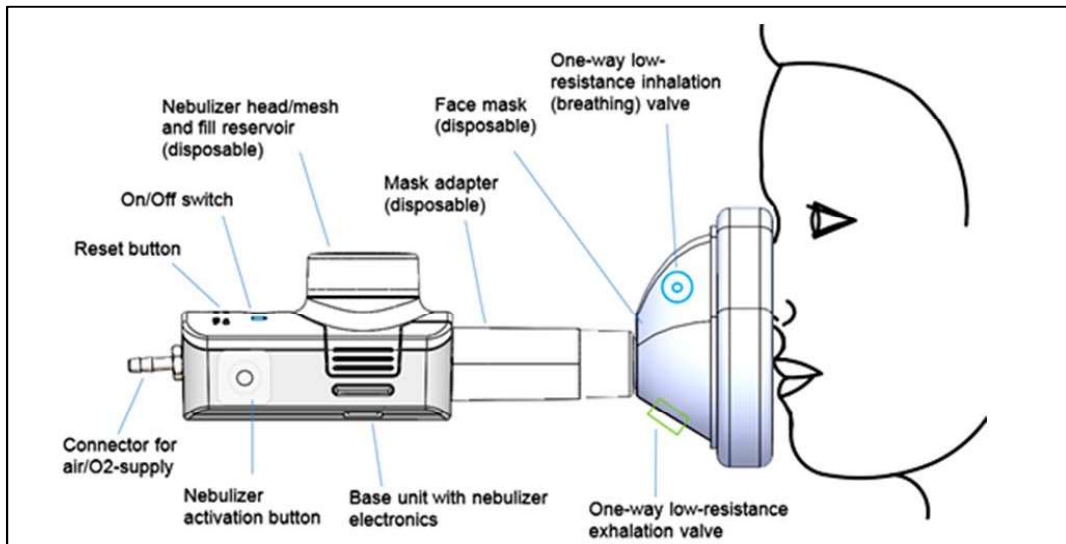
#### *Matching placebo*

- Formulation: nebulizer solution
- Route of administration: inhalation (to be administered via the FOX-Flamingo inhalation system)
- Active substance: None
- Activity: None
- Composition: [REDACTED]

#### *Device:*

- Name: FOX-Flamingo inhalation system - CE marked (Figure 5).
- Main components: re-usable base unit (containing the electronics), disposable inhalation set (including pediatric face mask in 2 sizes, mask adaptor, vibrating mesh nebulizer reservoir).
- Description: Vibrating mesh type nebulizers are considered the most appropriate technology for nebulization of a therapeutic protein such as ALX-0171 Nanobody. The FOX-Flamingo inhalation system consists of a battery-operated, hand-held device, intended for single-patient use. The device provides an aerosol with particle size suitable

for the intended study population. The nebulizer is always to be used with a flow of 2 L/min additional air or O<sub>2</sub>.



**Figure 5: Overview of the FOX-Flamingo inhalation system**

### 3.3.5. DRUG ACCOUNTABILITY

The Pharmacist, the Investigator or his/her designee is responsible for acknowledge receipt of each shipment of study drug/inhalation system and will verify the condition and quantity of the study drug and other clinical supplies related to Investigational Medicinal Product (IMP) administration.

At study site, the study drug will be kept in a locked and secured storage facility accessible only to those authorized by the Investigator to dispense the study drug.

The responsible person will keep an inventory. This will include the quantity of study drug/inhalation system received for the study and a record of the materials that are dispensed, to whom (subject number) and when.

The pharmacist, the Investigator and/or designated personnel will conduct a final inventory of the study drug/inhalation system and will record the results of this inventory. Upon Sponsor approval, all study drug and clinical supplies will be returned to the depot, or will be destroyed locally according to local regulations and site procedures.

Instructions for drug/inhalation system accountability are available in the manual concerning study drug.

### 3.3.6. STUDY DRUG HANDLING

Instructions for study drug receipt, handling, storage and administration are available in the manuals concerning study drug and IWRS/IVRS.

#### Packaging and Labeling

Final packaging and labeling will comply with the regulatory requirements of each country where the study will be conducted.

#### Storage

Study drug will be provided under refrigerated conditions and must be stored in a secure, limited-access location protected from light and under the storage conditions specified by the Sponsor.

Study drug must be refrigerated at 2°C to 8°C (35.6 °F to 46.4 °F) and should be stored in the secondary packaging until administration. It should not be frozen or shaken.

Site storage conditions should be monitored by the site personnel and reviewed by the monitor during site visits. Deviations from the storage requirements must be documented and reported to the Sponsor, according to the instructions provided in the manual concerning study drug.

#### Dispensing

The Investigator or qualified designee(s) will dispense (via IWRS/IVRS) study drug/inhalation system to subjects who have met the entry criteria. Clinical supplies (including study drug) may not be used for any purpose other than that which is stated in this protocol.

#### Product Quality Complaint

Product quality complaints have to be communicated (written or electronically) to the Sponsor and the manufacturer of the device if the device is concerned (as applicable).

### 3.3.7. STUDY DRUG ADMINISTRATION

Three dose levels are planned to be evaluated. The precise dose that will be administered depends on the subject's weight: the drug volume filled into the nebulizer (nominal dose range) is calculated per weight category range (see [Table 1](#)).

- Dose 1: target dose of 3.0 mg/kg (depending on the subject's weight category, the actual nominal dose is between 2.5 and 3.6 mg/kg)
- Dose 2: target dose of 6.0 mg/kg (depending on the subject's weight category, the actual nominal dose is between 5.0 and 7.1 mg/kg)

- Dose 3: target dose of 9.0 mg/kg (depending on the subject's category, the actual nominal dose is between 7.6 and 10.7 mg/kg)

Study drug is administered via inhalation of an aerosol generated by the FOX-Flamingo inhalation system. Detailed instructions for study drug administration are available in the manual concerning study drug.

Study drug administration should start within 3 hours following randomization. Subsequent doses of study drug will be administered at 24-hour intervals ( $\pm$  4 hours) relative to the first dose.

No premedication is required or recommended per protocol, but an inhaled  $\beta$ 2-agonist may be administered at the discretion of the treating Investigator.

Study drug will be administered via inhalation using 2 serial nebulizations. In the placebo group, subjects will receive 2 nebulizations of placebo. In the ALX-0171 dose 1 and 2 groups, subjects will receive 1 nebulization of ALX-0171 and 1 nebulization of placebo. In the ALX-0171 dose 3 group, both nebulizations will contain ALX-0171.

#### Placebo group

**Nebulization 1** with volume 1 of placebo at each dosing day.

**Nebulization 2** with volume 2 of placebo at each dosing day.

#### Dose 1 group

**Nebulization 1** with volume 1 of ALX-0171 at each dosing day.

**Nebulization 2** with volume 2 of placebo at each dosing day.

#### Dose 2 group

**Nebulization 1** with volume 1 of placebo at each dosing day.

**Nebulization 2** with volume 2 of ALX-0171 at each dosing day.

#### Dose 3 group

**Nebulization 1** with volume 1 of ALX-0171 at each dosing day.

**Nebulization 2** with volume 2 of ALX-0171 at each dosing day.

Per body weight category, the total volume (to be administered via the 2 serial nebulizations) will be the same for the 4 treatment groups ensuring blinding across the treatment groups. This approach allows a design with a placebo-controlled group as representative comparison for the 3 dose levels.

For information on nebulizer fill volumes for nebulizations 1 and 2 (in order to achieve the desired dose) to be administered per body weight category, please see section [3.3.7.1](#).



### 3.3.7.1. ADMINISTRATION PROCEDURE

An overview of the appropriate volume to be filled into the nebulizer (per body weight category and per nebulization), is available in [Table 1](#). In line with other inhalation products, the administered dose of ALX-0171 is standardized for body weight categories. This is supported by adequate safety margins (see section [3.3.2](#)), and also takes into account feasibility of accurately measuring and filling the appropriate volume into the nebulizer with a graduated syringe.

**Table 1: Nebulizer filling volumes of study drug**

Weight category	Fill volume	
	Nebulization 1	Nebulization 2
3.0 to <4.0 kg	0.20 mL	0.40 mL
4.0 to <5.0 kg	0.25 mL	0.50 mL
5.0 to <7.0 kg	0.35 mL	0.70 mL
7.0 to <10.0 kg	0.50 mL	1.0 mL
10.0 to <12.0 kg	0.65 mL	1.3 mL
12.0 to <15.0 kg	0.75 mL	1.5 mL

- The FOX-Flamingo nebulizer features a tubing connector for air or O<sub>2</sub> supply, and is always to be used with a fixed 2 L/min flow of air, or if needed, O<sub>2</sub> (to be decided by the Investigator based on O<sub>2</sub> need of the subject).
- During administration, the face mask should always be in contact with the face of the subject: a close fit is essential for appropriate dose administration. Hence, administration by “blow by” technique (i.e., hovering the face mask in front of the child during administration) is not permitted.
- At the start of each study drug nebulization, the face mask (connected to the nebulizer) will be placed on the subject’s face, and must be verified as being in contact with the face. If applicable, the subject’s nasal cannula, face mask, or headbox will need to be removed immediately before administration of the study drug (air or O<sub>2</sub> are provided through the nebulizer). A nasogastric tube, if used, is to remain in place.<sup>§§</sup>
- Once the subject is able to breathe comfortably in the face mask, the nebulization procedure should be started.
- Nebulization should proceed as long as aerosol is visible in the transparent face mask. As a guidance, the estimated duration of nebulization is approximately 4 minutes for a volume of 0.5 mL, approximately 8 minutes for a volume of 1.0 mL and approximately 12 minutes for a volume of 1.5 mL. Note: the FOX-Flamingo inhalation system is

<sup>§§</sup> Presence of the nasogastric tube has a negligible influence on the inhaled dose of study drug.

designed to switch off automatically when the filled volume has been completely nebulized.

### **3.3.7.2. ADDITIONAL GUIDANCE**

If dosing cannot be initiated successfully (e.g., because the subject is so agitated as to prevent adequate contact of the face mask with the face, and/or is crying or coughing intensely), new attempts should be made within a maximum of 1 hour (60 minutes) of nebulizer filling.

- If on Day 1, study drug initiation (first of 2 serial nebulizations) has not been successful after 60 minutes, the subject will be withdrawn from the study (see section 3.2.3).
- If on Day 2 or Day 3, study drug initiation (first of 2 serial nebulizations) has not been successful after 60 minutes, study drug treatment should be discontinued (i.e., in case dose initiation was not successful on Day 2, then no dosing on Day 3 should be done). The subjects should remain in the study and all per protocol visits should be conducted according to the [Schedule of Assessments](#).

If the first of the 2 serial nebulizations is started successfully but cannot be continued successfully and needs to be interrupted (e.g., because the subject starts crying intensely, or if the face mask loses adequate contact with the subject's face), study drug administration is to be stopped and resumed as soon as possible and the nebulization should be completed within 2 hours (120 minutes) of nebulizer filling for this nebulization.

The second of the 2 serial nebulizations should be initiated as soon as possible after the end of the first nebulization and should be completed within 2 hours (120 minutes) of nebulizer filling for this nebulization. In case the study drug administration was started successfully but could not be completed, appropriate documentation is needed in the medical file and eCRF but subsequent daily study drug administration should continue per protocol.

The maximum time window allowed to complete both serial nebulizations is 4 hours.

The exact times of study drug administration (i.e., initiation and end of each nebulization and number of interruptions, if any) will be recorded in the eCRF. In addition, acceptability of the face mask and the nebulizer after each complete or partial administration, and whether or not correct and complete administration could be achieved will be documented in the eCRF.

The product-contacting parts of the nebulizer (i.e., the nebulizer mesh/head and fill reservoir, mask adapter, and face mask) are to be disposed of after each nebulization; only the base unit containing the electronics and the air/O<sub>2</sub> tubing will be reused (per subject and per 3-day treatment window). After completing the study drug treatment period, the base unit of the nebulizer must be safely disposed in a secured environment for further return to the sponsor or designee (e.g., delegated CRO).

### **3.3.7.3. MANAGEMENT OF OVERDOSE**

The intended dose of ALX-0171 should not be exceeded during the study. No antidote is available.

Overdoses that occur during the study should be documented and reported to the Sponsor or designee whether or not it results in an AE/SAE. Overdoses with signs and symptoms need to be reported as an AE/SAE. For information on medication errors, please refer to section [3.5.6.1](#).

### **3.3.8. CONCOMITANT THERAPY**

Any concomitant medication (including over-the-counter medications and herbal supplements) taken during the study (i.e., from signing of ICF until the subject's last visit), must be recorded in the eCRF. Items to be recorded concerning concomitant medication include: dose and units of dose, start and end date, administration frequency, route of administration, therapeutic indication, brand name (or generic name if brand name is not available). Of note, previous medications relevant for eligibility will also be recorded in the eCRF.

#### **Standard of care treatment**

The treatment and care provided to each subject are determined by the Investigator (or designee) according to institutional practice. The recommendations on the diagnosis, management, and prevention of bronchiolitis, as described by the American Academy of Pediatrics (2014), may be followed in addition to institutional practice.

Treatment may include (but is not limited to) the following:

- O<sub>2</sub> supplementation through nasal cannula, via face mask or headbox. The initiation, monitoring and weaning of oxygen supplementation will follow local practice. It should be removed for the nebulized study drug administration, during which air or oxygen flow of 2L/min is provided
- Fluid/food supplementation (i.v. or via nasogastric tube, if applicable)
- Antipyretics and/or nonsteroidal anti-inflammatory medication
- Hypertonic saline (but not within 4 hours before start or 4 hours after the end of study drug administration)
- Short acting  $\beta_2$ -agonists
- Antibiotics (in case of secondary bacterial infection)
- Epinephrine
- Anticholinergics

Concomitant medications are permitted, apart from those listed under the forbidden medications (see below), at the Investigator's discretion (based on medical need).

### **Forbidden medication**

The following treatments are prohibited up to the Follow-up visit (Day 14 ± 2 days):

- Ribavirin, i.v. immunoglobulin and palivizumab
- Inosine pranobex
- Xanthines
- Heliox
- Initiation of leukotriene receptor antagonists (LTRAs, i.e., montelukast) and/ or sodium cromoglycate; infants who are on maintenance chronic therapy at screening are to continue their usual dose during the study.
- Exogenous surfactant
- Systemic corticosteroids; topical corticosteroids for treatment of a skin or ear disorder are permitted; initiation of inhaled corticosteroids is not permitted; infants who are on a maintenance therapy of inhaled corticosteroids at inclusion are to continue their usual dose during the study
- Mucolytic and/ or expectorant drugs (e.g., Dornase Alpha, N-Acetylcysteine, Bromhexine)

Subjects whose respiratory condition is deteriorating (e.g., hypercapnia with  $p\text{CO}_2 > 8$  kPa/60mmHg, decreased consciousness) and need to start non-invasive respiratory support or invasive ventilation need to discontinue study drug treatment but should remain in the study according to the in-hospital post-treatment period as specified in the [Schedule of Assessments](#), and should also attend the FU and EOS Visits. Subjects on HFOT as standard of care at inclusion and for whom the flow needs to be increased to levels not enabling study drug nebulization and/or above 2 L/kg/min also need to discontinue study drug treatment and be further followed up as described above. SAE reporting is required for these subjects with deterioration of the respiratory condition requiring above mentioned methods of ventilation.

After the end of the subject's participation in the study, each subject is to be treated according to standard clinical practice.

### **3.3.9. TREATMENT COMPLIANCE**

Study drug will be administered by a health care professional in hospital, ensuring treatment compliance.

The exact times of study drug administration (i.e., initiation and end of each nebulization and number of interruptions, if any) will be recorded in the eCRF. In addition, acceptability of the face mask and the nebulizer after each complete or partial administration, and whether or not correct and complete administration could be achieved will be documented in the eCRF. Compliance will be further confirmed by bioanalytical assessment of ALX-0171 in blood samples, and could be further confirmed by electronic documentation of the administration on the nebulizer base unit.

## **3.4. ASSESSMENTS**

### **3.4.1. TIMING OF ASSESSMENTS**

Study related procedures will be done after informed consent has been obtained, after which each subject will be assigned a unique subject ID number. Date of obtaining written informed consent will be recorded in the eCRF.

AEs will be recorded from time of obtaining written informed consent to the subject's last visit.

#### **3.4.1.1. ELIGIBILITY PROCEDURES**

A signed and dated ICF must be obtained from the parent(s)/legal guardian(s) or legally acceptable representative(s) before any study-specific procedures are performed.

The screening process begins when written informed consent has been obtained and continues until randomization. Subjects must be randomized within 24 hours of arrival at the hospital/emergency unit.

At screening, subjects will be screened according to the inclusion and exclusion criteria (section 3.2) and have other assessments performed as specified in the [Schedule of Assessments](#). The results of the screening procedures needed to evaluate eligibility must be available prior to randomization.

Note: In case the result of the RSV diagnostic test (see inclusion criterion 4) is negative, the other assessments of the screening visit (see [Schedule of Assessments](#)) do not need to be performed and only the data to be collected in case of "screen failure" needs to be collected in the eCRF.

Data of all subjects screened will be collected in the eCRF in order to assess the numbers and demographic characteristics of the excluded subjects, and the reasons for their exclusion, including documentation of adverse events. In case an SAE occurs, this needs to be reported according to standard process (see section 3.5.2).

For details with regard to randomization, refer to section 3.3.3.

Assessments and procedures should be performed as outlined in the [Schedule of Assessments](#).

Screening and baseline (i.e., Day 1, pre-dose) can be on the same day as long as the above applies.

- Screening
  - Obtain informed consent
  - Review of eligibility criteria (including RSV diagnosis)
  - Demographics and medical history (including RSV-related signs and symptoms)
  - Previous and concomitant medications
  - Body weight, body height, body temperature
  - Physical examination
  - Heart rate and SpO<sub>2</sub>
  - Feeding, general appearance, lung auscultation, respiratory muscle retractions, respiratory rate, daytime coughing, night-time coughing,
  - Hospitalization information: ICU transfer, apnea episodes, ventilation information, discharge from ICU/hospital
  - Parent(s)/caregiver(s) assessment by completion of a diary including scoring of respiratory symptoms and assessing the global condition of the subject
  - Blood sample for clinical laboratory
  - Blood sample for immunogenicity (ADA)

### **3.4.1.2. STUDY DRUG TREATMENT AND IN-HOSPITAL POST-TREATMENT PERIOD**

During the study drug treatment period, assessments should be performed as detailed in the [Schedule of Assessments](#).

If the Day 1, pre-dose assessments will be performed within 3 hours after the screening assessments have been performed, the assessments already done at screening do not need to be repeated and only the additional assessments to be performed on Day 1, pre-dose will need to be performed.

Subjects will receive once-daily administration of study drug on 3 consecutive days. The first dose of study drug is to be administered as soon as possible and within 3 hours after randomization. For all subjects, the exact date and start and end time (and number of interruptions, if any) of the study drug nebulizations will be collected in the eCRF.

Provided that the clinical response criteria have been met, discharge from the hospital can take place per protocol at the Investigator's discretion from dosing Day 2 onwards after all required assessments of the 5 hours ( $\pm 1$  hour) post-dose time point have been completed. Subjects discharged after the second dose must return to the hospital for the third study drug administration (to be administered  $48 \pm 4$  hours after the first dose by the appropriately trained study personnel), and be monitored for a 2-hour pre- and post-dose period.

Subjects who are still hospitalized during the post-treatment period will be followed up twice daily (in the morning and the evening) until the subject is discharged from hospital.

Hospitalization information will be collected during the study drug treatment period and the in-hospital post-treatment period, including occurrence of ICU transfer, apnea episodes, initiation and type of ventilation and hospital/ICU discharge information. Assessment of Clinical Response will be done with collection of date and time of adequate oral feeding to enable discharge as well as adequate stable oxygen saturation on room air of >92% over a period of at least 4 hours. For subjects in the in-hospital post-treatment period, the nasal swab should be collected only on the day of hospital discharge.

All assessments will be performed as specified in the [Schedule of Assessments](#).

Unscheduled visits may be planned to assess, confirm, and follow up on clinically relevant AEs or laboratory abnormalities. Findings made during these unscheduled visits should be reported in the designated sections of the eCRF.

#### *Missed visits:*

If a subject misses a study visit for any reason, the Medical Monitor should be contacted as soon as possible to discuss rescheduling options of the missed visit to be proposed to the subject's parent(s)/legal guardian(s). For subjects who are considered lost to follow-up, reasonable attempts must be made (and documented) to obtain information on the subject.

### **3.4.1.3. FOLLOW-UP AND END-OF-STUDY VISITS**

Subjects who have received at least one dose of study medication should complete a FU visit on Day 14 ± 2 days and an EOS visit on Day 28 ± 2 days.

In case a subject is re-hospitalized or consults the Investigator for a respiratory condition after hospital discharge, an unscheduled visit is to be performed. At a minimum this is to include collection of a mid-turbinate nasal swab, lung auscultation and physical examination.

#### **Premature discontinuation of Study Drug**

A subject who started study drug dosing but was discontinued prematurely from study drug treatment, should be further monitored according to the in-hospital post-treatment period (if they remain hospitalized) and should attend the FU and EOS visits to undergo the assessments as specified in the [Schedule of Assessments](#).

#### **Withdrawal from the Study**

In case a subject is withdrawn from the study for any reason other than lost to follow-up or informed consent withdrawal (including subjects randomized but not dosed), a Withdrawal



Visit should be conducted at the time of withdrawal. The reason for withdrawal should be recorded in the eCRF.

No further assessments/visits will be done in case subjects terminate study participation for reason of informed consent withdrawal.

### **3.4.2. BASELINE CHARACTERISTICS**

Demographic and medical history data (including RSV-related signs and symptoms) will be collected at screening.

Collected data will include (but are not limited to): month and year of birth, age (with indication of week level), gender, race (if allowed), and ethnicity (if allowed).

Data on atopy in the family, environmental exposure to tobacco, pets and breastfeeding will also be collected.

The nasal swab sample collected on Day 1 will be used to test for concurrent viruses and the presence of mycoplasma in nasal swab samples. If the volume of the Day 1 pre-dose sample is insufficient, the sample collected after study drug administration can be used for adventitious viral screening.

### **3.4.3. ASSESSMENT OF VIRAL LOAD**

#### **3.4.3.1. SAMPLE COLLECTION AND HANDLING**

Throughout the study, nasal swabs (mid-turbinate specimen) will be collected for analysis of viral load, according to the time points defined in the [Schedule of Assessments](#).

The swabs that will be used are anatomically-designed for the collection of respiratory specimens which allows improved patient comfort and efficiency in specimen collection. In addition, appropriate training on the nasal swabbing procedure will be provided to the clinical sites to enable standardization across the study. Every effort should be made to perform the nasal swab sampling in both nostrils using the same swab.

For all subjects, the exact date and time of nasal swab sampling will be collected in the eCRF.

Note that a mid-turbinate nasal swab should also be taken in case the subject is re-hospitalized or consults the Investigator for a respiratory condition after hospital discharge up to the EOS (Day 28) visit. This should be considered for AE reporting according to the Investigator's judgment; however in any case, in addition to the nasal swab, a lung auscultation and a physical examination should be performed when a subject returns to the

hospital or consults the Investigator for a respiratory problem. The information on health care utilization for a respiratory condition will be collected through the diary completed by the parent(s)/caregiver(s).

If permitted by local regulations, nasal swab samples that remain after protocol-specific assessments have been performed may be used by the Sponsor for further exploratory work in the context of the development of ALX-0171 or evaluation of RSV (e.g., potential viral resistance monitoring). These samples may be kept for up to 5 years after the end of the study. No human DNA or RNA analysis will be performed.

### **3.4.3.2. BIOANALYSIS**

Viral load will be measured by plaque forming unit (PFU) assay for the primary endpoint and by qRT-PCR for the secondary endpoint(s). Determination of Log<sub>10</sub> PFU/mL and Log<sub>10</sub> viral copies/mL in nasal swabs will be done by validated methods at a designated analytical laboratory. Bioanalytical procedures will be described in a separate Bioanalytical Analysis plan.

## **3.4.4. ASSESSMENTS OF EFFICACY**

### **3.4.4.1. CLINICAL ACTIVITY OUTCOME MEASURES**

The following assessments are performed to evaluate clinical activity of ALX-0171, but may also provide additional safety information:

- Heart rate and SpO<sub>2</sub>
- Feeding: type of feeding support, and (time and date of) sufficient feeding to enable hospital discharge, in the opinion of the Investigator (with particular attention to hydration and breathing comfort during feeding)
- Respiratory rate measured over a 1-minute interval
- Wheezing as assessed during lung auscultation
- Cough during the night and during the day
- Respiratory muscle retractions (supraclavicular, intercostal, and subcostal)
- General appearance: activity, irritation, and responsiveness
- Body temperature

Based on the Clinical Activity Outcome Measures, composite scores such as RDAI score, (based on wheezing and respiratory muscle retractions) (see [Appendix 1](#)), RACS (based on RDAI and change in respiratory rate, see [Appendix 2](#)), and a Global Severity Score (see below and [Appendix 3](#)) will be calculated.

### **Global Severity Score**

The Global Severity Score is a composite score based on the GENVIP scale that allows objective categorization of infants with respiratory infections based on seven different items (i.e., feeding intolerance, medical intervention, respiratory difficulty, respiratory frequency, apnea, general condition, and fever) [11, 12].

Each item is scored from 0 to 3, except for body temperature (fever) that is scored from 0 to 2, resulting in a maximum total score of 20 points.

The collection of the parameters feeding, respiratory muscle retraction, wheezing, respiratory rate, body temperature, apnea episodes, general appearance, information on oxygen supplementation and hospital information (as described in the [Schedule of Assessments](#)) will allow calculation of the Global Severity Score (see [Appendix 3](#)).

### **Clinical Response**

Time to Clinical Response will be calculated based on the feeding and oxygen saturation data. Subjects will be considered to have met the Clinical Response criteria when both of the following criteria are fulfilled:

- Stable oxygen saturation on room air, defined as  $SpO_2 > 92\%$  over a period of  $\geq 4$  hours. In case of oxygen supplementation, the level of supplementation is to be considered for reduction at least three times per day. Provided oxygen saturation is stable, attempts to remove the supplementation will be done at least three times a day.
- Adequate oral feeding which is sufficient to maintain sufficient hydration, in the judgment of the Investigator.

The assessment of the subject having reached Clinical Response will be done daily until achieved and date and time for each item will be documented in the eCRF.

## **3.4.4.2. MEDICAL INTERVENTIONS OUTCOME MEASURES**

The following data will be captured to enable evaluation of the medical interventions outcome measures:

- Length of hospital stay for RSV infection
- Level, method, and duration of supplemental oxygen therapy
- Initiation of invasive or non-invasive ventilation (i.e., continuous positive airway pressure [cPAP] or HFOT)
- Level, method and duration of invasive or non-invasive ventilation
- Transfer to ICU and duration of stay in ICU

These measures also contribute to the calculation of the concerned parameters for the Global Severity Score (see [Appendix 3](#)).

### **3.4.4.3. HEART RATE AND $S_pO_2$**

Continuous monitoring of  $SpO_2$  needs to be done during the study drug treatment period and the in-hospital post-treatment period until the clinical response criterion for oxygen (i.e., saturation on room air was > 92 % for at least 4 hours) has been met and/or in case saturation monitoring is to be continued according to the Investigator's judgment.

The values for these parameters will be regularly captured in the eCRF (together with e.g., oxygen flow delivered and method), according to the [Schedule of Assessments](#).

In addition, the date and time of achieving the clinical response criterion for oxygen will be collected in the eCRF (cfr clinical response information collection).

Clinically significant warnings and measurements during the pulse oximetry monitoring period are to be captured as AEs in the eCRF.

### **3.4.4.4. PARENT/CAREGIVER OUTCOME MEASURES**

Parent(s)/Caregiver(s) assessment of the clinical condition of the subject will be done by daily completion of a diary during the hospital stay and up to the EOS (Day 28) visit. The diary will be used to measure three respiratory symptoms (cough, [audible] wheeze, and trouble breathing), to score the general health of the subject and will ask for use of health care utilization and medication for respiratory symptoms.

The three respiratory symptoms (cough, trouble breathing and wheezing) will be scored from not present over very mild, mild, moderate, and severe up to very severe.

The global rating of the child's health by the parent(s)/caregiver(s) will be captured by a VAS. The parent or caregiver will make a mark between 0 ("very bad") and 100 mm ("perfect") on the scale to indicate the subject's current health. In addition, the parent(s)/caregiver(s) will indicate when the child was back to his/her condition as before the RSV infection started.

## **3.4.5. PHARMACOKINETIC ASSESSMENTS**

### **3.4.5.1. SAMPLE COLLECTION AND HANDLING**

Throughout the study, blood samples will be taken for analysis of ALX-0171 concentrations in serum, according to the time points defined in the [Schedule of Assessments](#).

- Subjects not undergoing the more extensive blood sampling scheme will have 1 blood sample taken on Day 2 or 3, at any time between 0.5 hours after completion of the second dose and initiation of the administration of the third dose.
- Subjects undergoing the more extensive blood sampling scheme will have 3 blood samples taken on Days 2-3: 1) pre second dose, 2) at any time between 0.5 hours and 3 hours after completion of the second dose, and 3) at any time between 3 hours and 6 hours after completion of the second dose (and at least 1 hour apart from the previous sampling).

The exact dates and times of blood sampling will be recorded.

The blood samples for PK assessments will be collected from a vein, as using capillary blood (e.g., from heel pricks) is not allowed. Anesthetic cream or spray can be used to minimize the subject's discomfort and distress. For further details on sample collection, shipment, storage and processing, please refer to a separate Lab Manual.

### **3.4.5.2. BIOANALYSIS**

Determination of ALX-0171 concentrations in serum will be done by a validated Ligand Binding Assay (LBA)-based method at a designated analytical laboratory according to the bioanalytical methodology and procedures described in a separate Bioanalytical Analysis Plan. All PK samples (both from placebo and ALX-0171 treated subjects) will be analyzed for measurement of ALX-0171.

## **3.4.6. PHARMACODYNAMIC ASSESSMENTS**

### **3.4.6.1. SAMPLE COLLECTION AND HANDLING**

Exploratory analysis of KL-6 levels will be done according to the time points defined in the [Schedule of Assessments](#).

KL-6 will be assessed on the remainder (if any) of the blood samples obtained for PK or immunogenicity testing, once the appropriate volume for their intended purpose has been extracted.

### **3.4.6.2. BIOANALYSIS**

Determination of KL-6 levels in serum will be done by validated methods at the Sponsor. Bioanalytical procedures will be described in a separate Bioanalytical Analysis plan.

### **3.4.7. IMMUNOGENICITY**

#### **3.4.7.1. SAMPLE COLLECTION AND HANDLING**

To assess systemic immunogenicity of ALX-0171, blood samples will be collected at the time points defined in the [Schedule of Assessments](#). If ADA are present, a further evaluation of their neutralizing capacity will be performed.

Note: In case no sample was taken at the screening visit, a blood sample for assessment of immunogenicity should be collected at the Day 1 pre-dose timepoint, as far as possible. In case no sample was taken at the FU (Day 14) visit, a blood sample for assessment of immunogenicity should be collected at the EOS (Day 28) visit, as far as possible.

The exact date and time of blood sampling will be recorded.

The blood samples for ADA assessments will be collected from a vein, as using capillary blood (e.g., from heel pricks) is not allowed. Anesthetic cream or spray can be used to minimize the subject's discomfort and distress. For further details on sample collection, shipment, storage and processing, please refer to a separate Lab Manual.

Of note, an additional serum sample should be collected (if reasonably feasible) in case of serious and/or severe hypersensitivity reaction (blood volume: 1 mL and collected as soon as possible after the start of the event) to allow characterization of the reaction if deemed required (also see section [3.4.8.1](#)).

#### **3.4.7.2. BIOANALYSIS**

Determination of ADA will be done using a validated screening, confirmation and titration ADA bridging assay, with further characterization of ADA positive samples by a competitive ligand binding neutralizing antibody assay. The immunogenicity data will be processed according to a dedicated Bioanalytical Analysis plan.

Bioanalytical procedures for the additional sample in case of serious and/or severe hypersensitivity reaction will be described in a separate Bioanalytical Analysis plan.

### 3.4.8. ASSESSMENTS OF SAFETY

Safety assessments consist of AE reports (including SAEs, hypersensitivity reactions), clinical laboratory assessments, heart rate and SpO<sub>2</sub>, respiratory rate, body weight, body temperature, physical examination, and lung auscultation.

The time points are defined in the [Schedule of Assessments](#).

At each of the time points indicated in the [Schedule of Assessments](#), a physical examination should be performed, including heart auscultation, examination of abdomen, skin and ears/nose/throat. Lung auscultation is to be performed more frequently and is therefore listed separately in the Schedule of Assessment.

The parent(s)/legal guardian(s) will receive a subject card, indicating the address and phone number of the Investigator and/or other relevant emergency contact details (including e.g., subject number, medication number). The subject's parent(s)/legal guardian(s) will be advised to carry this card with them throughout the study so that the appropriate actions can be undertaken in case of emergency. They will also be advised to contact the Investigator (or his/her designee) with any medical and/or safety concern.

In order to appropriately document possible events after discharge from the hospital, a diary will be completed by the parent(s) or caregiver(s) and be brought to the site at the FU visit on Day 14 and EOS visit on Day 28.

#### 3.4.8.1. ADVERSE EVENTS

General information on evaluation and reporting of AEs is provided in section [3.5](#).

It is the responsibility of the Investigator to collect all AEs (both serious and non-serious) resulting from spontaneous, unsolicited reports by parents/caregivers and/or by healthcare staff, by observation (by the Investigator and/or healthcare staff), by routine open questioning of the parents/caregivers by the Investigator (e.g., "Is there anything new that you wish to discuss?") and by reviewing the diary with the parent(s)/caregiver(s).

All AEs occurring between the time a signed and dated ICF is obtained until completion of the subject's last visit must be documented in the source documents and the eCRF.

Criteria for determining whether an abnormal objective test finding (e.g., laboratory result), a complication of a protocol mandated procedure (e.g., blood draw), or a change in physical examination findings should be reported as an AE include, but are not limited to:

1. Result/finding is associated with accompanying clinical signs and symptoms (new onset or aggravated in severity or frequency from baseline condition), and/or
2. Result/finding requires extra diagnostic testing (other than diagnostic exclusion tests) or medical/surgical intervention, and/or

3. Result/finding would require a premature discontinuation from the study drug, significant additional concomitant drug treatment or other therapy, and/or
4. Result/finding leads to any of the outcomes included in the definition of an SAE, and/or
5. Result/finding is considered to be an AE by the Investigator.

Any abnormal test result that is determined to be an error and merely repeating an abnormal test does not require reporting as an AE.

Airway hyperresponsiveness and immediate or delayed adverse drug reactions should be managed according to the Investigator's clinical judgment and applicable standard of care.

- If airway hyperresponsiveness would occur, administration of a bronchodilator like the short-acting  $\beta$ 2-agonist salbutamol may be administered (with an appropriate follow-up period) according to the investigator's assessment of the particular reaction and subject's characteristics.
- With regard to immediate or delayed hypersensitivity reactions, measures beyond basic supportive care are not considered necessary for subjects with purely local reactions. Subjects with suspected systemic hypersensitivity, however, should be admitted or treated and observed for a longer period, preferably in the emergency department or another close-observation area, depending on the severity of the reaction and its hemodynamic impact.

In case of severe and/or serious hypersensitivity reactions, an additional serum sample should be collected (if reasonably feasible) as soon as possible after the start of the event (blood volume: 1 mL) to characterize the reaction (if deemed required). As described in section 3.2.3, subjects must be discontinued from study drug treatment in case of a severe and/or serious hypersensitivity reaction, a severe and/or serious acute respiratory distress reaction induced by study drug, or when a subject needs to start non-invasive respiratory support or ventilation.

Occurrence of episodes of apnea will be captured together with the overall hospitalization information and should be reported as an AE. As defined by the American Academy of Pediatrics, apnea is "an unexplained episode of cessation of breathing for 20 seconds or longer, or a shorter respiratory pause associated with bradycardia, cyanosis, pallor, and/or marked hypotonia."

In addition to the events meeting the seriousness criteria mentioned in section 3.5.2, the following events will trigger a SAE reporting:

- Transfer to ICU.
- Initiation of non-invasive respiratory support (including start of HFOT or increasing the flow above the allowed level) or invasive ventilation.
- Episodes of apnea that fulfil the seriousness criteria.



### **3.4.8.2. LABORATORY ASSESSMENTS**

Blood samples for clinical laboratory analyses will be collected at the time points indicated in the [Schedule of Assessments](#).

Safety lab assessments will be performed by the local laboratory in order to allow for timely availability of the results. For local laboratory data, the local normal ranges and laboratory accreditation are to be collected/provided.

If all hematology and biochemistry blood parameters required for the study are already known from analysis done as a routine assessment upon arrival at the hospital, collection of screening clinical laboratory samples does not need to be repeated for the purpose of this study. However, the samples should be analyzed at the same local laboratory that will be used for the remainder of the study.

The blood samples will be collected according to the site's standard practice. Anesthetic cream or spray can be used to minimize the subject's discomfort and distress.

The following tests will be included in the clinical laboratory analysis:

- Clinical chemistry: alanine aminotransferase, aspartate aminotransferase, creatinine, sodium, potassium, chloride, C-reactive protein,  $\gamma$ -glutamyl-transferase, blood urea nitrogen
- Hematology: hemoglobin, hematocrit, red blood cell count and indices, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count (or estimate), white blood cell count including differential (lymphocytes, neutrophils, monocytes, basophils, eosinophils).

Clinical relevance of values outside the local laboratory's normal range will be assessed by the Investigator. All clinically significant laboratory findings will be recorded as AEs in the eCRF (also see section [3.4.8.1](#)).

In the event of unexplained or unexpected clinical laboratory test values, the test(s) may be repeated and followed up until the results have returned to the normal range and/or an adequate explanation for the abnormality is found.

### **3.4.8.3. LUNG AUSCULTATION**

At each of the time points indicated in the [Schedule of Assessments](#), a lung auscultation will be performed to assess wheezing, crackles/crepitation and other abnormalities in lung auscultation.

A new finding or a change of a finding that is judged as an undesirable medical event shall be reported as an AE.

In case the subject is re-hospitalized or consults the Investigator for a respiratory condition after hospital discharge up to the EOS (Day 28) visit, an unscheduled visit is to be performed that will include a lung auscultation assessment (in addition to a physical examination and collection of a nasal swab) (see section 3.4.1.3).

#### **3.4.8.4. PHYSICAL EXAMINATION**

At each of the time points indicated in the [Schedule of Assessments](#), a physical examination should be performed, including heart auscultation, examination of abdomen, skin and ears/nose/throat.

Physical examination will be recorded as "normal", "abnormal, not clinically significant" or "abnormal, clinically significant" at every assessment. A new finding or a change of a finding that is judged as an undesirable medical event (including all findings recorded as "abnormal, clinically significant") shall be reported as an AE.

In case the subject is re-hospitalized or consults the Investigator for a respiratory condition after hospital discharge up to the EOS (Day 28) visit, an unscheduled visit is to be performed that will include a physical examination (in addition to a lung auscultation and collection of a nasal swab) (see section 3.4.1.3).

#### **3.4.8.5. HEART RATE AND $S_pO_2$**

The collection of these parameters is described in section 3.4.4.3 as the evolution of the heart rate and oxygen saturation levels will also inform on clinical activity outcome.

#### **3.4.8.6. BODY WEIGHT, HEIGHT, AND TEMPERATURE**

At the time points indicated in the [Schedule of Assessments](#), the following parameters will be assessed: body height, weight, and temperature.

To obtain the actual body weight, subjects must be weighed lightly clothed.

Temperature is to be measured preferably rectal, oral, or tympanic (axillary method less preferred). For each individual subject, the same method should be used throughout the study as far as possible. The method will be recorded in the eCRF.

### 3.4.9. TOTAL BLOOD VOLUME

The blood samples will be used i) for safety evaluation (clinical laboratory: hematology and clinical biochemistry), ii) to monitor the systemic concentration of ALX-0171 (as a surrogate measure of the local lung concentration), and iii) to assess potential immunogenicity (Table 2).

If all hematology and biochemistry blood parameters required for the study are already known from analysis done as a routine assessment upon arrival at the hospital, collection of screening clinical laboratory samples does not need to be repeated for the purpose of this study. However, the samples should be analyzed at the same local laboratory that will be used for the remainder of the study.

Exploratory biomarker will only be assessed on the remainder of the blood samples once the appropriate volume for their intended purpose has been extracted. An additional serum sample should be collected (if reasonably feasible) in case of serious and/or severe hypersensitivity reaction (volume of 1 mL). Note that no human DNA or RNA analysis will be performed.

The precise assessments that are intended to be performed on each of the blood samples are arranged by priority; when insufficient volume is available (e.g., because of difficulties in or limits to obtaining blood), only the highest priority(ies) will be assessed (see Table 2).

Of note: for subjects with a body weight below 5 kg (irrespective of whether they undergo the more extensive blood sampling scheme or not), the sample for analysis of immunogenicity at screening and on Day 14 should not be collected in case blood volume needed for local assessment of clinical laboratory parameters exceeds 2.5 mL (in order to not exceed the maximum blood volume allowed to be collected at a single time point). Instead and if possible, the blood sample for immunogenicity should be collected at Day 1 pre-dose (replacing the screening sample) and at the EOS (Day 28) visit (replacing the FU visit sample).

In the 48 subjects undergoing more extensive sampling, 5 blood samples will be taken (for evaluation of clinical safety, PK, and immunogenicity) with a total estimated volume of 3.5 mL (not including volumes for local lab assessments). In the remainder of subjects, 3 blood samples will be taken with a total estimated volume of 2.5 mL (not including volumes for local lab assessments).

If permitted by local regulations, blood samples that remain after protocol-specific assessments have been performed may be used by the Sponsor for further exploratory work in the context of the development of ALX-0171 or evaluation of RSV. These samples may be

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kept for up to 5 years after the end of the study. No human DNA or RNA analysis will be performed.

**Table 2: Timing, priority and estimated volume of scheduled blood draws**

<b>Sample</b>	<b>Priority</b>	<b>Maximal Target volume</b>
1 blood draw at Screening <sup>a</sup>	1. Clinical laboratory: - Hematology (whole blood) - Biochemistry (serum) 2. Immunogenicity (serum) <sup>b</sup> 3. Exploratory biomarker <sup>c</sup>	- whole blood for hematology (volume according to local practice) - whole blood to be processed into serum for biochemistry (volume according to local practice) - 1 mL whole blood, to be processed into serum for immunogenicity and biomarker <sup>b</sup> <b>Total: 1 mL whole blood + volume needed for clinical laboratory</b>
1 blood draw on Day 2 or 3 OR 3 blood draws on Days 2-3 (in 48 subjects) 1 blood draw during the Day 14 FU visit	1. PK (serum) 2. Exploratory biomarker <sup>c</sup>	- 0.5 mL whole blood, to be processed into serum for PK and biomarker <sup>b</sup> <b>Total: 0.5 mL whole blood in case of 1 blood draw or 1.5 mL whole blood in case of 3 blood draws</b>
	1. Clinical laboratory: - Hematology (whole blood) - Biochemistry (serum) 2. Immunogenicity (serum) <sup>b</sup> 3. Exploratory biomarker <sup>c</sup>	- whole blood for hematology (volume according to local practice) - whole blood to be processed into serum for biochemistry (volume according to local practice) - 1 mL whole blood, to be processed into serum for immunogenicity and biomarker <sup>b</sup> <b>Total: 1 mL whole blood + volume needed for clinical laboratory</b>

<sup>a</sup>. If all hematology and biochemistry blood parameters required for the study are already known from analysis done as a routine assessment upon arrival at the hospital, collection of screening clinical laboratory samples does not need to be repeated for the purpose of this study. However, the samples should be analyzed at the same local laboratory that will be used for the remainder of the study.

<sup>b</sup>. For subjects with a body weight below 5 kg (irrespective of whether they undergo the more extensive blood sampling scheme or not) and in case blood volume needed for local clinical laboratory exceeds 2.5 mL, the sample for analysis of immunogenicity should not be collected at screening and on Day 14 but at Day 1 pre-dose and Day 28, as far as possible.

<sup>c</sup>. Only if sufficient blood volume (serum) is available after analyzing the samples obtained for PK or immunogenicity.

### **3.4.10. APPROPRIATENESS AND TIMING OF MEASUREMENTS**

The assessments which will be made in this study are standard and generally recognized as reliable, accurate, and relevant.

The timing of all assessments is detailed in the [Schedule of Assessments](#).

Throughout the study, study personnel should make every reasonable effort to follow the timing of assessments and procedures in the Schedule of Assessments for each subject.

## **3.5. ADVERSE EVENT EVALUATION AND REPORTING**

### **3.5.1. ADVERSE EVENTS**

Study-specific information on evaluation and reporting of AEs is provided in section [3.4.8.1](#).

AE definitions will be consistent with the “Note for Guidance on clinical safety data management: definitions and standards for expedited reporting” (International Conference on Harmonization [ICH] topic E2A).

An AE is any untoward medical occurrence in a clinical study subject administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not considered related to that medicinal (investigational or non-investigational) product.

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including laboratory test abnormalities.

In differentiating between medical history and AEs, the following points will be considered:

- Conditions that started before signing of informed consent and for which no symptoms or treatment are present up to the timing of signing of informed consent are recorded as medical history (e.g., seasonal allergy without acute complaints).
- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, but with unchanged severity, are recorded as medical history (e.g., allergic pollinosis).
- Conditions that started or deteriorated after signing of informed consent will be documented as AEs.

All AEs will be reported from the time a signed and dated ICF is obtained until completion of the subject’s last visit.

A TEAE is any AE temporally associated with the use of study drug, whether considered related to the study drug or not. TEAEs are recorded from the start of study drug administration, until completion of the subject’s last visit.

All AEs will be assessed by the Investigator and recorded in the patient medical records and on the AE eCRF page. AE entry should indicate time of onset and end time and rating of the seriousness (see section 3.5.2), severity (see section 3.5.1.1), and outcome (see section 3.5.1.2) of the AEs, relationship to study drug and study procedures (see section 3.5.1.3), action taken regarding study drug (see section 3.5.1.4), and concomitant therapy taken for the AE.

The Investigator will judge upon the severity of the AEs and relation to study drug and study procedures.

### **3.5.1.1. AE SEVERITY**

The severity of AEs will be rated on a 3-point scale:

- Mild: discomfort noticed but no disruption of normal daily activity
- Moderate: discomfort sufficient to reduce or affect normal daily activity
- Severe: incapacitating with performing normal daily activity

It is emphasized that the term severe is a measure of intensity: a severe AE is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

### **3.5.1.2. OUTCOME**

The outcome of the AE is to be documented as follows:

- Recovered/resolved
- Recovering/resolving
- Recovered/resolved with sequelae
- Not recovered/not resolved
- Fatal
- Unknown

### **3.5.1.3. RELATION TO STUDY DRUG OR STUDY PROCEDURES**

The assessment of the causal relationship between an AE and the administration of treatment is a clinical decision based on all available information at the time of the completion of the eCRF.

The assessment is based on the question whether there was a “reasonable causal relationship” to the study treatment in question. Possible answers are:

- Not related
- Unlikely related
- Possibly related
- Related
- Not applicable

Note that only AEs starting after administration of study drug can be assigned a causal relationship between the AE and study drug administration. For AEs starting prior to study drug administration, causal relationship between the AE and study drug should be “not applicable”.

Assessment of causal relationship of any AE to protocol-required procedures can be completed with:

- Yes (specify)
- No

### **3.5.1.4. ACTION TAKEN REGARDING STUDY DRUG**

Any action taken regarding the study drug is to be documented using following categories:

- Dose not changed
- Drug interrupted
- Drug withdrawn
- Not applicable
- Unknown

### **3.5.2. SERIOUS ADVERSE EVENTS**

SAE definitions will be consistent with the “Note for Guidance on clinical safety data management: definitions and standards for expedited reporting” (International Conference on Harmonization [ICH] topic E2A).

An SAE is any untoward medical occurrence that at any dose meets any of the following conditions:

- Results in death.

- Is life-threatening: the subject is at risk of death at the time of the event. It does not refer to an event that hypothetically might cause death if it were more severe.
- Requires in-patient hospitalization or prolongation of existing hospitalization; an AE associated with a hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:
  - The admission results in a hospital stay of less than 12 hours.
  - The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study).
  - The admission is not associated with an AE (e.g., social hospitalization for purposes of respite care).

However, it should be noted that invasive treatment during any hospitalization may fulfill the criterion of "medically important" and as such may be reportable as an SAE dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedence.

- Results in persistent or significant disability/incapacity. Disability means a substantial disruption of a person's ability to conduct normal life's functions.
- Is another medically important serious event as judged by the Investigator, or is defined as requiring intervention to prevent one of the outcomes listed in the definition above (including suspected transmission of an infectious agent by a medicinal product should be reported as an SAE). Other examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. Any AE is considered an SAE if it is associated with clinical signs or symptoms judged by the Investigator to have a significant clinical impact.

In addition, the following events will trigger a SAE reporting:

- Transfer to ICU.
- Initiation of non-invasive respiratory support (including start of HFOT or increasing the flow above the allowed level) or invasive ventilation.
- Episodes of apnea that fulfill the seriousness criteria.

A treatment-emergent SAE is any SAE temporally associated with the use of study drug, whether considered related to the study drug or not.

The Investigator or clinical site personnel should notify the designated CRO of all SAEs, regardless of relationship to the study drug, within 24 hours of clinical site personnel becoming aware of the event (see Investigator Site File). The Investigator will provide the initial notification by sending a completed "SAE Form", which must include the Investigator's assessment of the relationship of the event to study drug, and must be signed by the Investigator.



The first report of an SAE may also be made by telephone to the Medical Monitor or local Clinical research Associate (CRA). At a minimum, the Reporter must provide the following information: reporter identification, study number, year of birth, medication code number, period of intake, nature of the AE, and relation to study drug.

The report of an SAE by telephone must always be confirmed by a written, more detailed report (the SAE Form) to be completed and signed by the Investigator.

Follow-up information, or new information regarding an ongoing SAE, must be provided promptly to the contacts provided in the Investigator Site File.

The SAE should also be recorded in the eCRF. Any medications necessary for the treatment of the SAE must be recorded on the concomitant medication section of the eCRF.

SAEs that begin after the subject's participation in the study is complete, but that the Investigator considers to be related to study drug, should be reported to the CRO/Sponsor at any time.

### **3.5.3. SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTIONS**

Suspected unexpected serious adverse reactions (SUSARs) are serious adverse reactions of which the nature or severity is not consistent with the applicable product information (as described in the Reference Safety Information, provided in the Investigator's Brochure) and have a reasonable suspected causal relationship (i.e., at least possibly related) to the medicinal product.

The vendor(s) will expedite reports of the following SUSARs to the IEC/IRB on behalf of the Sponsor:

- SUSARs that have arisen in the current clinical study that was assessed by the IEC/IRB.
- SUSARs that have arisen in other clinical studies of the same Sponsor and with the same study drug and that could have consequences for the safety of the subjects involved in the current clinical study that was assessed by the IEC/IRB.

The vendor(s) will expedite all reports of SUSARs to the relevant CA on behalf of the Sponsor.

The Sponsor (or the CRO on behalf of the Sponsor) will also report to all concerned Investigators all SAEs that are unlisted (unexpected) and associated with the use of the drug.

The expedited reporting will occur no later than 15 calendar days after the Sponsor (or the CRO on behalf of the Sponsor) has first knowledge of the adverse reactions or as per local regulations.

For fatal or life-threatening cases the term will be maximal 7 calendar days for a preliminary report with another 8 days for completion of the report or as per local regulations.

### **3.5.4. REPORTING OF ADVERSE EVENTS**

AE reporting, including SUSARs, will be carried out in accordance with applicable local regulations. For reported deaths, the Investigator should supply the Sponsor and the IEC/IRB with any additional requested information (e.g., autopsy reports and terminal medical reports).

After termination of the clinical study (last subject last visit in the study), any unexpected safety issue that changes the risk benefit analysis and is likely to have an impact on the subjects who have participated in the study, will be reported by the Sponsor as soon as possible to the competent authority(ies) concerned, together with proposed actions.

### **3.5.5. FOLLOW-UP OF ADVERSE EVENTS**

AEs will be handled according to common clinical practice. If necessary, in order to obtain additional information to ensure safety to the subject, additional blood or other samples may be taken at the discretion of the Investigator. Information relative to other means of investigational diagnostics used in relation to the AE will also be communicated.

AEs are recorded from signing the ICF until completion of the subject's last visit. TEAEs are recorded from the start of study drug administration, until the subject's last visit.

All AEs occurring at any time during the study (including the FU period) will be followed by the Investigator until stable outcome.

### **3.5.6. OTHER REPORTABLE INFORMATION**

#### **3.5.6.1. MEDICATION ERROR**

Medication errors include, but are not limited to, the following:

- Administration of the wrong dosage to the subject (i.e., different from the dose prescribed by the protocol).
- Administration of study drug that has not been assigned to the subject.

- Administration of expired study drug.
- Administration by a route other than inhalation.
- Deviations to the study drug storage conditions when administered to the subject.
- Nasal cannula not removed.
- Air or oxygen tube not connected to the FOX-Flamingo inhalation system, damaged or kinked.
- Air or oxygen flow to the FOX-Flamingo inhalation system too low or too high.
- Re-use of inhalation set (i.e., the face mask, mask adapter, and nebulizer mesh/head and fill reservoir).

Medication errors that occur during the study should be documented and reported to the Sponsor or designee whether or not it results in an AE/SAE. Medication errors with signs and symptoms need to be reported as an AE/SAE.

For information on management of overdoses, please refer to section [3.3.7.3](#).

## 3.6. STATISTICS

### 3.6.1. STUDY POPULATIONS

The following populations will be considered for analysis:

- **Intent-to-treat (ITT) Population:** All randomized subjects.
- **Modified Intent-to-treat (mITT) Population** All subjects who received at least 1 administration of study drug, as randomized (i.e., using the treatment to which the subject was randomized).
- **Safety Population:** All subjects who received at least 1 administration of study drug, as treated (i.e., using the treatment that the subject actually received).
- **PK population:** Subset of the subjects in the safety population for whom the primary PK data are considered to be sufficient and interpretable. For this study, this will correspond to all subjects in the safety population who received at least 1 administration of ALX-0171 and for whom at least one ALX-0171 serum concentration has been determined.
- **Per Protocol (PP) Population:** Consists of a subset of the ITT population, and excludes those subjects who have had a major protocol deviation.

The mITT Population will be the primary study population used for the analysis of efficacy data, the Safety Population for the analysis of safety, PD, and immunogenicity data, and the PK population for analysis of PK data.

### 3.6.2. STATISTICAL AND ANALYTICAL PLAN

Analysis of parameters will be detailed in a Statistical Analysis Plan (SAP). The PK analysis will be described in a Data Analysis Plan (DAP). The SAP and DAP will be generated under responsibility of the Sponsor and will be finalized prior to database lock. Any deviation from the reporting and analysis plans will be reported in the section "Changes in the planned analysis" in the Clinical Study Report.

### 3.6.3. BASELINE CHARACTERISTICS

Baseline characteristics will be summarized using descriptive statistics (including number of observations, mean, standard deviation, median, maximum, and minimum) for continuous variables and counts and percentages for categorical variables.

Prior and concomitant medications will be summarized by treatment group, by the number and percentage of subjects taking each medication, classified using World Health Organization (WHO) Drug Dictionary, anatomical therapeutic chemical (ATC) class and preferred term.

### 3.6.4. ENDPOINTS

#### *Primary Endpoint*

Anti-viral effect as measured by the time needed for the viral load to drop below the plaque assay quantification limit (time-to-BQL) in nasal mid-turbinate swab specimens.

#### *Secondary Endpoints*

- Safety assessment (assessed by physical examination, AEs, laboratory assessments and vital signs) of different doses of ALX-0171
- Change from baseline in Global Severity Score
- Time to clinical response: defined as the time between first administration of study drug and achieving adequate oxygen saturation and oral feeding
- PK properties of ALX-0171
- Viral load profile over time in nasal swab specimens (qPCR and plaque assay)
- Evaluation of serum ADA

#### *Other Endpoints*

- Evolution over time in clinical symptoms (SpO<sub>2</sub>, feeding, respiratory rate, wheezing, cough, respiratory muscle retractions, and general appearance)
- Evolution over time in RDAI, RACS
- Length of hospital stay for RSV defined as time between admission and discharge
- Duration of supplemental oxygen therapy
- Initiation of invasive and non-invasive ventilation and duration of respiratory support
- Transfer to ICU and duration of ICU stay
- Parent(s)/Caregiver(s) assessment of the clinical condition of the subject
- Evolution of serum biomarker over time

### 3.6.5. EVALUATION OF VIROLOGY AND CLINICAL EFFICACY ENDPOINTS

#### **3.6.5.1. ANALYSIS OF PRIMARY ENDPOINT**

For the primary endpoint, the anti-viral effect will be measured as the time-to-BQL in nasal mid-turbinate swab specimens. The median time-to-BQL will be compared between each of the ALX-0171 dose groups and the combined placebo group using a log-rank test. The tests will be performed in a sequential way to preserve the family-wise error rate at 0.05. Specifically, dose 3 of ALX-0171 will first be tested against the combined placebo group at the 0.05 significance level. Dose 2 of ALX-0171 will only be compared to placebo at the 0.05 significance level if the comparison of dose 3 with placebo is significant. Consequently, dose 1 of ALX-0171 will only be compared to placebo at the 0.05 significance level if the comparison of dose 2 of ALX-0171 with placebo is significant.

### **3.6.5.2. EVALUATION OF SECONDARY EFFICACY ENDPOINTS**

The data from the secondary endpoints will be analyzed by descriptive statistics (number of observations, mean, standard deviation, median, minimum and maximum for most continuous data; frequencies and percentages for categorical data, as appropriate) as applicable. For selected secondary endpoints, comparisons of active dose groups with placebo may be performed through appropriate inferential tests. No correction for multiple testing is foreseen for secondary endpoints, and p-values reported for the analyses are to be interpreted accordingly.

Based on the Clinical Activity Outcome Measures, Time to Clinical Response and scores such as the Global Severity Score, RDAI score and RACS will be calculated.

### **3.6.6. EVALUATION OF PHARMACOKINETIC, PHARMACODYNAMIC, AND IMMUNOGENICITY PARAMETERS**

Systemic (serum) concentrations of ALX-0171 will be used as a surrogate for local (lung) concentrations. Individual study drug concentrations will be listed. In addition, a listing of the actual sampling times relative to the study drug administration times will be presented. If considered meaningful, geometric means and standard deviations will be added to the summary tables.

A population PK model (non-linear mixed effect modeling) will be developed based on the ALX-0171 serum concentrations from this study. The dataset might be supplemented with serum concentration data from Study ALX-0171-C104 and/or data from clinical studies in healthy adult volunteers (or a subset to balance between pediatric and adult populations in the dataset) to support model structure. Individual PK parameters will be derived by means of empirical Bayesian estimation. The following individual PK parameters will be provided: apparent clearance (CL/F), area under the curve (AUC) as nominal (filling) dose divided by CL/F and cumulative AUC over 72 hours as an expression of the cumulated exposure during the course of treatment. Individual PK parameters will be summarized with sample size, mean, standard deviation and coefficient of variation. The modeling exercise will be described in a separate analysis plan and will be reported in a separate report. The main findings will be summarized in the Clinical Study Report.

Pharmacodynamic effect will evaluate the evolution over time of (i) viral load, and (ii) exploratory biomarker KL-6.

Analysis of the time needed for the viral load to drop below the plaque assay quantification limit (time-to-BQL) in nasal mid-turbinate swab specimens (i.e., the primary endpoint) will be analyzed as described in section [3.6.5](#).

In addition, viral load will be characterized through parameters including time-to-BQL, AUC, percent of subjects with undetectable RSV (from Day 1 to Day 14) and rate of decline from baseline in viral load.

All PD data (other than the primary endpoint), will be summarized using descriptive statistics and will be listed.

Individual immunogenicity results will be listed, and summarized using descriptive statistics. Subjects will be classified for presence of pre-Ab, TE ADA and neutralizing anti-drug antibodies (NAb). Prevalence of pre-Ab and incidence of TE ADA or NAb, as well as titer levels of the antibody responses, will be reported. Potential correlation between pre-existing antibodies, TE ADA or NAb and safety, pharmacokinetics or efficacy will be assessed.

In case of severe and/or serious hypersensitivity reactions, a blood sample will be collected to further characterize the hypersensitivity reactions (if deemed necessary). Correlation between hypersensitivity reactions and immunogenicity results will be assessed. Results will be summarized through listing of the individual results.

### **3.6.7. EVALUATION OF SAFETY PARAMETERS**

The IDMC will evaluate the cumulative safety data after completion of each safety cohort, and periodically during the parallel dose ranging phase of the study (see section 3.1.1). Further details will be provided in the IDMC charter.

All safety analyses will be performed using the Safety Population.

AEs will be fully described and coded according to the Medical Dictionary for regulatory Activities (MedDRA). A treatment-emergent analysis of AEs will be done. Frequency of subjects presenting with AEs, AEs leading to withdrawal, adverse drug reactions, and SAEs will be tabulated for each treatment group by system organ class and preferred term.

For laboratory parameters, descriptive statistics (mean, median, standard deviation, minimum, and maximum) will be computed on the actual values and the change from baseline for each parameter. All laboratory values will be categorized according to their normal ranges as below, within or above normal.

Other safety variables, including e.g., heart rate and SpO<sub>2</sub> levels, need for O<sub>2</sub> therapy (and when applicable, relevant parameters such as duration of O<sub>2</sub> therapy needed, oxygen delivery mode, flow of inspired oxygen), or any significant worsening requiring immediate or intensive medical intervention, will be fully depicted using descriptive statistics. Where relevant, actual values and changes from baseline, and/or and shift tables according to normal ranges, will be included.

Findings in physical examinations will be listed.

Body height, body weight, and body temperature results will be summarized by descriptive statistics.

### ***3.7. DATA QUALITY ASSURANCE AND DIRECT ACCESS TO SOURCE DATA/DOCUMENTS***

Audits may be conducted to evaluate systems, processes, and expertise for the subcontracted activities and to assess compliance with the contractual agreements, the protocol, applicable Standard Operating Procedures, and regulatory requirements. During or after the conduct of the study, process-related audits may be performed as well. When performed, an audit certificate will be provided in appendix of the final study report.

The clinical research facility will be monitored by the study monitor, to ensure correct performance of the study procedures and to ensure that the study is conducted according to the relevant regulatory requirements.

Regulatory authorities, the IEC/IRB, and/or the Sponsor representative may request access to all source documents, eCRFs and other study documentation for an on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

Quality control principles will be applied throughout the performance of this study.

### ***3.8. DATA PROTECTION***

During this clinical study, all clinical data will be identified only through an ID number in order to protect the rights of the subjects to privacy and to the protection of their personal data in accordance with the European Data Protection Directive 95/46/EC, the Privacy Rule (45 CFR Parts 160 and 164), and Health Insurance Portability and Accountability Act (as applicable). Global principles and standards for Processing Personal Data and for meeting Data Transfer Obligations will be applied.



## **4. ETHICS**

### **4.1. ETHICS COMMITTEES AND COMPETENT AUTHORITIES**

The Clinical Study Protocol(s) and the ICF(s) will be submitted for review and approval by the IEC/IRB prior to the eligibility screening/baseline. The composition of the IEC/IRB is in accordance with the recommendations of the World Health Organization, the ICH E6 Guideline for GCP, the European Union Clinical Trial Directive (CTD) (Directive 2001/20/EC) and/or the USA Code of Federal Regulations (CFR) (21 CFR 56), in line with local regulations.

The Investigator/Sponsor (or CRO on behalf of the sponsor) will keep the IEC/IRB informed about the progress of the study. All changes in research activities and all unanticipated problems involving risks to human subjects will be immediately reported to the responsible persons. The study may be suspended pending further review by the IEC/IRB, unless suspension would jeopardize the subject's health. The Investigator will take care that all subject's parent(s)/legal guardian(s) or the legally acceptable representative(s) are kept informed.

No substantial amendments will be implemented to the study without prior IEC/IRB approval and CA approval (if applicable according to local regulations), except when required to eliminate apparent immediate hazards to subjects.

Notification of the end of the study will be sent to the CA and to the IEC/IRB, within the number of days as specified by local regulations after completion of follow-up for the last subject. In case the study is ended prematurely, the IEC/IRB and the CA will be notified within the number of days as specified by local regulations, including the reasons for the premature termination. Results will be provided to the CA and the EC/IRB according to local requirements for format and timing.

### **4.2. ETHICAL CONDUCT OF THE STUDY**

This study will be conducted in compliance with the Guidance for Industry ICH E6 GCP (including archiving of essential study documents), the Declaration of Helsinki, the applicable regulations of the country(ies) in which the study is conducted, and with the Commission Directives 2001/20/EC and 2005/28/EC.

ICH-adopted guidelines and other relevant international guidelines, recommendations, and requirements will be taken into account as comprehensively as possible, as long as they do not violate local laws.

The Investigator will be responsible for the care of the subjects throughout the study. If the Investigator is not present at the study site, he/she will leave instructions for the staff and a telephone number where he/she can be reached.

### **4.3. SUBJECT INFORMATION AND CONSENT**

As the potential subjects are infants or young children, subjects' parent(s), legal guardian(s) or the legally acceptable representative(s) must provide their written informed consent before enrolment of their child in the clinical study, and before any protocol-specified procedures are performed.

Freely given and written informed consent must be obtained according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used will be approved by both the Sponsor or designee and by the reviewing IEC/IRB. The informed consent will be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Sponsor policy.

Before undertaking any study-related procedure in the study, the Investigator or an authorized member of the investigational staff must explain to the subjects' parent(s), legal guardian(s) or the legally acceptable representative(s) the aims, methods, objectives, potential clinical benefits, and potential hazards of the study, and any discomfort participation in the study may entail.

The subjects' parent(s), legal guardian(s), or the legally acceptable representative(s) will be informed that the participation is voluntary and that they may refuse to participate or withdraw from the study, at any time, without penalty or loss of benefits to which the subject is otherwise entitled, and that all data collected up to the point of withdrawal will be used and reported in an anonymized way. They will be told that the Investigator will maintain a subject identification register for the purposes of long-term follow-up if needed, and that the records may be accessed by health authorities, authorized Sponsor staff, and Sponsor representative without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) or regulations. In addition, insurance coverage provided during the study will be explained.

By signing the ICF the subjects' parent(s), legal guardian(s), or the legally acceptable representative(s) is/are authorizing such access, and agrees to allow the study physician to recontact the subjects' parent(s), legal guardian(s), or the legally acceptable representative(s) for the purpose of obtaining consent for additional safety evaluations, if needed.

If the subjects' parent(s), legal guardian(s), or the legally acceptable representative(s) is/are unable to read, an impartial witness must be present during the entire informed consent discussion. Once the written ICF (and any other written information) is read and explained to the subjects' parent(s), legal guardian(s) or the legally acceptable representative(s), and after the subjects' parent(s), legal guardian(s), or the legally acceptable representative(s) has orally consented to the subject's participation in the study, and, if capable of doing so, has signed and personally dated the ICF, the witness must sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subjects' parent(s), legal guardian(s) or the legally acceptable representative(s), and that informed consent was freely given by the subjects' parent(s), legal guardian(s) or the legally acceptable representative(s).

The language used in the oral and written information about the study, including the ICF, will be as nontechnical as practical and should be understandable to the subjects' parent(s), legal guardian(s) or the legally acceptable representative(s). They will be given sufficient time to read the ICF and given the opportunity to ask questions. After this explanation and before entry into the study, consent will be appropriately recorded by means of the subjects' parent(s), legal guardian(s) or the legally acceptable representative(s) personally dated signature(s) and by the Investigator or an authorized member of the investigational staff who conducted the ICF discussion.

After having obtained the consent, a copy of the signed ICF will be given to the subject's parent(s)/legal guardian(s) or the legally acceptable representative(s). The original of the ICF will be retained by the Investigator in the "Investigator Site File".

## **4.4. PRIVACY**

The study is conducted according to the principles of ICH, including the specifications for privacy and data protection.

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to investigate the safety, quality, and utility of the investigational study drug(s) used in this study. The collected data are adequate, and not excessive in relation to the purposes for which they are collected and processed.

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The data are collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration is in place. The data will be kept in a form, which does not readily permit identification of the subject. Subjects will be identified by his/her assigned unique subject number and his/her year of birth. Personal data will only be collected and processed using these unique identification items. Only the Investigator and his/her study team keeps a list that makes it possible to link unique subject numbers to an individual name. Sponsor personnel whose responsibilities require access to personal data have to agree to keep the identity of study subjects confidential.

The informed consent obtained from the subject's parent(s)/legal guardian(s) or the legally acceptable representative(s) includes explicit consent for the processing of personal data and for the Investigator to allow direct access to his/her original medical records for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities and to other countries.

## **5. DATA HANDLING AND RECORD KEEPING**

### **5.1. DISTRIBUTION OF ACTIVITIES**

Contact details of the Sponsor and third parties are available in the "Investigator Site File".

### **5.2. DOCUMENTATION**

Study documentation required for study start (as specified in the ICH E6 Guideline for GCP [CPMP/ICH/135/95]) shall be exchanged between Ablynx NV and the vendor prior to the administration of study drug.

#### **5.2.1. CASE REPORT FORM COMPLETION**

Case report forms will be completed for each subject (incl. screen failures in order to assess the numbers and demographic characteristics of the excluded subjects, and the reasons for their exclusion).

The Investigator will ensure that data are recorded on the eCRF as specified in the Clinical Study Protocol and in accordance with the instructions in the eCRF completion guidelines. The Investigator will ensure the accuracy, completeness, legibility, and timeliness of the data recorded in the eCRF, and of the provision of answers to data queries according to the Clinical Study Agreement. All eCRF entries, corrections, and alterations must be made by the Investigator or other authorized study-site personnel. The Investigator will sign the completed eCRF. A copy of the completed eCRF will be archived at the study site.

#### **5.2.2. SOURCE DOCUMENTATION**

At a minimum, source documentation must be available for the following: informed consent process, medical history, subject identification, eligibility, and study identification; date of informed consent; dates of visits; results of all efficacy evaluations; results of safety parameters as required by the protocol; record of all AEs; and follow-up of AEs; prior and concomitant medication; study drug receipt records; study drug administration information; any medical notes (original documents, data and records, e.g., laboratory data); date of study completion, and reason for early discontinuation of study procedures or withdrawal from the study (and information on documented attempts to obtain information on the final status of the subject for those who are considered lost to follow-up), if applicable.

In addition, the author of an entry in the source documents should be identifiable.

At a minimum, the type and level of detail of source data available for a study subject should be consistent with that commonly recorded at the site as a basis for standard medical care (Patient's Medical File).

Following the ICH-GCP guidelines, direct access to source documentation (medical records) must be allowed.

### **5.2.3. RECORD RETENTION**

In compliance with the ICH/GCP guidelines, the Investigator/Institution will maintain all eCRFs and all source documents that support the data collected from each subject, as well as all study documents as specified in ICH/GCP section 8, Essential Documents for the Conduct of a Clinical Study, and all study documents as specified by the applicable regulatory requirement(s). The Investigator/ Institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 15 years after completion of the study, at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study drug. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/Institution as to when these documents no longer need to be retained.

If the responsible Investigator retires, relocates or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the Investigator relocate or dispose of any study documents before having obtained written approval from the Sponsor.

If it becomes necessary for the Sponsor or the appropriate regulatory authority to review any documentation relating to this study, the Investigator must permit access to such essential documents and subject data.

The Sponsor and the vendor(s) to whom the Sponsor has transferred duties and functions are responsible for organizing and maintaining a clear documentation of the course of the study.

The Trial Master File maintained during the study by the assigned vendor(s) will be sent back to the Sponsor at the end of the study, after final review and upon Sponsor approval.

## **5.2.4. MONITORING**

The monitor will perform on-site monitoring visits as specified in a monitoring plan to ensure that all aspects of the protocol, contractual agreements and regulatory requirements are followed and that subjects' human rights, safety and well-being are protected. The monitor will record dates of monitoring in a study center visit log that will be kept at the site. At these visits, the monitor will perform source data verification and check the data entered into the eCRF for completeness and accuracy. The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eCRF are known to the Sponsor and investigational staff and are accessible for verification by the Sponsor site contact. If electronic records are maintained at the investigational site, the method of verification must be discussed with the investigational staff.

Direct access to source documentation ([electronic] medical records) must be permitted at all times. Findings from this review of captured data will be discussed with the investigational staff. The Sponsor expects that, during on-site monitoring visits, the relevant investigational staff will be available, the source documentation will be accessible, and a suitable environment will be provided for review of study-related documents. The monitor will meet with the Investigator on a regular basis during the study to provide feedback on the study conduct. The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits will be resolved in a timely manner.

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## 6. FINANCING AND INSURANCE

### *Insurance*

Ablynx NV holds and will maintain an adequate insurance policy covering damages arising from Ablynx-sponsored clinical research studies.

Ablynx NV will indemnify the Investigator in accordance with the provisions as set in a separate written agreement between Ablynx NV (or a vendor on behalf of the Sponsor) and the relevant Investigator/clinical site.

### *Financing*

The financial aspects of the study will be documented in an agreement between the Sponsor (or a vendor on behalf of the Sponsor) and the Investigator/Institution.

Compensation for reasonable expenses made related to the study such as travel costs to visit the study center for assessments related to the study will be foreseen.

### *Financial Disclosure*

Any identified Investigator or sub-investigator directly involved in the treatment or evaluation of research subjects will disclose for the time period during which the Investigator is participating in the study and for 1 year following completion of the study that he/she entered a financial arrangement between the Sponsor and the Investigator/Institution. The Investigator should promptly update this information if any relevant changes occur during this period.



## **7. USE OF INFORMATION AND PUBLICATION**

By signing this protocol, the Investigator reaffirms to the Sponsor that he or she will maintain in confidence all information furnished, or resulting from this study. He/she will only divulge such information as may be necessary to the IEC/IRB and the members of the staff and the parent(s)/legal guardian(s) of subjects who are involved in this study.

All data and records provided by the Sponsor or generated during the study (other than subject's medical records) and all data and inventions covered in the course of conducting the study, whether patentable or not, are the sole and exclusive property of the Sponsor.

The Investigator and all other study team members at any service provider involved will keep strictly confidential all information provided by the Sponsor related to this study and all data and records generated in the course of the study. They will not use the information, data, or records for any other purpose than conducting the study without prior written approval of the Sponsor.

Publication of any results from this study will be according to the principles of the Declaration of Helsinki, and will require prior review and written agreement of the Sponsor.

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## 9. APPENDICES

### Appendix 1: RDAI scoring system

RDAI is a 17-point score based on wheezing and retraction.

Variable	Number of Points					Maximum Points
	0	1	2	3	4	
<b>Wheezing</b>						
Expiration	None	End	1-2	3-4	All	4
Inspiration	None	Part	All	NA	NA	2
Location	None	Segmental ≤ 2-4 lung fields	Diffuse ≥ 3-4 lung fields	NA	NA	2
<b>Retractions</b>						
Supraclavicular	None	Mild	Moderate	Marked	NA	3
Intercostal	None	Mild	Moderate	Marked	NA	3
Subcostal	None	Mild	Moderate	Marked	NA	3

NA = not applicable

The RDAI score is the sum of the row scores, with total range 0 to 17; higher scores indicate more severe disease.

### Appendix 2: RACS score

The RACS score is the sum of the change in the RDAI score and a standardised score for the change in respiratory rate. The change in respiratory rate is assigned 1 point per each 10% change in the respiratory rate.

A decrease in the RDAI or in the respiratory rate during the study period is recorded as a negative RACS, meaning an improvement.

### Appendix 3: Global Severity Score

The Global Severity Score is the sum of the scores of the 7 individual items as described below; total maximum score is 20 points; higher score indicates more severe disease.

Item	Number of Points	Description
Feeding	0	Adequate oral feeding
	1	No adequate oral feeding, occasional breaks during feeding
	2	No adequate oral feeding, frequent breaks during feeding
	3	No adequate oral feeding, unable to feed, feeding support
Medical interventions	0	Not hospitalized
	1	Hospitalization without O <sub>2</sub> supplementation
	2	Conventional oxygen supplementation
	3	Non-invasive respiratory support or invasive ventilation
Respiratory distress	0	No wheezing and no retractions
	1-2-3	Points will be attributed uniformly across the 17 points for RDAI
Respiratory rate	0-1-2-3	Points will be attributed uniformly across the respiratory rate values for each age range
Apnea	0	No apnea episode
	3	Apnea episode
General appearance <sup>a</sup>	0	Active, playing/ content/ interactive and happy
	1	Less active/ mildly irritable/ less interactive or responsive
	2	Less active/ moderately irritable/ less interactive or responsive
	3	Not active/ Severely irritable/ Not interactive or not responsive
Note: In case worst item is less active and/or less interactive: if both are present: 2 points; if only one of both is present and the highest: 1 point		
Body Temperature	0	<37,0°C: (measured axillary); <37,5°C (measured by other method)
	1	≥37,0°C but <38°C (measured axillary); ≥37,5°C but <38,5°C (measured by other method)
	2	>38°C (measured axillary); >38,5°C (measured by other method)

<sup>a</sup> Assessed through activity, irritation and interest in environment (worst of the three items will be used for attributing the points)



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