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Nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention of respiratory syncytial virus disease

Addendum to Annex 2 of WHO Technical Report Series, No. 1048

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Annex 3

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Guidelines and their addenda published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, the parent WHO Guidelines and this addendum may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to the Guidelines and/or this addendum are made only on condition that such modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the guidance set out.

Abbreviations

ADA	anti-drug antibody
ADE	antibody-dependent enhancement (of disease)
AE	adverse event
AESI	adverse event of special interest
CHD	congenital heart disease
CLD	chronic lung disease
DDI	drug-drug interaction
Fc	fragment crystallizable (region)
FcγR	Fc gamma receptor
FI-RSV	formalin-inactivated RSV (vaccine)
LRTI	lower respiratory tract infection
mAb	monoclonal antibody
NRA	national regulatory authority
PD	pharmacodynamics
PK	pharmacokinetics
RSV	respiratory syncytial virus
RT-PCR	reverse transcription-polymerase chain reaction
SAE	serious adverse event
URTI	upper respiratory tract infection

1. Introduction

Evaluating the safety and efficacy of monoclonal antibodies (mAbs) and related products intended for the prevention or treatment of infectious diseases requires different considerations than mAb products that target endogenous proteins, such as those intended for the treatment of noncommunicable diseases. To help address such differences, the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases *(1)* was adopted in 2023 on the recommendation of the WHO Expert Committee on Biological Standardization. These Guidelines outline the general principles applicable to the evaluation of mAbs for use against infectious diseases. Although the document provides guidance on evaluating the safety and efficacy of mAb products regardless of the targeted pathogen, it was recognized that pathogen-specific considerations would potentially affect the interpretation and application of the guidance provided.

2. Purpose and scope

The current addendum provides supplementary considerations when evaluating the safety and efficacy of parenterally administered mAb products directed specifically against respiratory syncytial virus (RSV) antigens, and intended primarily for pre-exposure prophylaxis in infants and young children. Such considerations may also be applicable to mAb products intended for use in immunocompromised individuals. It should be noted that mAbs and related products that target endogenous human antigens are not within the scope of this addendum as these require different considerations for evaluating their safety and efficacy.

Separate and detailed guidance on the production and quality control of mAbs is provided in the WHO Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use *(2)*.

3. Terminology

The terms used in this addendum may have different meanings in other contexts. It should be noted that these and other terms relevant to this addendum are defined in full in the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases *(1)*.

4. General considerations

RSV is an orthopneumovirus of the Pneumoviridae family and occurs as two major subtypes (subtypes A and B). It is a lipid-enveloped virus with a single-stranded, non-segmented negative-sense RNA genome contained within a nucleocapsid *(3–5)*. The envelope contains three viral transmembrane surface glycoproteins – namely, a putative attachment glycoprotein (G), a fusion glycoprotein (F) and a small hydrophobic glycoprotein (SH). The G and F proteins are considered essential for pathogenesis and induce neutralizing antibodies in the host. The SH protein is a pentameric ion channel analogous to the M2 proton channel of influenza viruses. Although not a major target for neutralizing antibodies, anti-SH specific antibodies have been shown to protect through fragment crystallizable (Fc)-mediated mechanisms *(6)*. There is also a non-glycosylated matrix protein (M) present on the inner face of the RSV envelope. Antigenic diversity between and within the two RSV subtypes mainly reflects

variations in the G protein, with little homology observed between the G proteins of RSV subtype A and RSV subtype B strains *(7–9)*.

RSV infection is a major cause of respiratory disease globally, often causing seasonal epidemics in temperate regions, particularly during winter months *(10)*. The emergence of RSV outbreaks is less predictable in warmer, tropical regions. RSV causes both upper and lower respiratory tract infections, with significant morbidity and mortality occurring in infants throughout the first year of life, and especially in those born prematurely or with heart or lung disease *(5, 11, 12)*. Although such infants are at higher risk of morbidity and mortality caused by an RSV infection, the vast majority of infants with RSV-associated morbidity do not have any risk factors *(12, 13)*. It should be noted that there is no conclusive causal link between severe lower respiratory tract RSV infection in infants and the development of either asthma or wheezing *(14)*. RSV infection also leads to a significant disease burden in the elderly, particularly among those with comorbidities such as congestive heart failure or chronic obstructive pulmonary disease, with such infections potentially leading to severe, and sometimes life-threatening, lower respiratory tract disease *(5–9, 11)*. RSV is also a threat to immunocompromised and otherwise vulnerable individuals, including nosocomially, with high mortality rates having been observed in those infected with RSV following bone marrow or lung transplantation *(15, 16)*. The high burden of disease caused by RSV is associated with substantial hospitalization costs and other adverse economic impacts *(11, 17, 18)*.

RSV can be transmitted through the direct inhalation of infectious airborne droplets produced by a symptomatic infected individual. The virus then spreads through the respiratory tract from the nasopharynx to the distal alveoli *(19)*. Other potential routes of transmission include direct deposition of infectious droplets on the eyes or indirect transmission following contact with infectious respiratory secretions on contaminated surfaces or other objects. The hallmark of RSV disease is the formation of large multinuclear cells (syncytia) created by the fusion of numerous cells. By 2 years of age, almost all children have had at least one RSV infection. As RSV infection does not elicit long-lasting sterilizing immunity, repeated respiratory tract infections are common *(19)*. Although the mechanism by which RSV evades long-term immunity is unclear *(19–21)*, the ability of the virus to reinfect children and adults seems not to be due to the emergence of different strains or to virus evolution, suggesting that an RSV mAb could be re-administered in subsequent seasons without loss of efficacy.

Although immune correlates of protection for RSV have not yet been established, evidence suggests that high concentrations of serum anti-RSV neutralizing antibodies are associated with a substantial decrease in the risk of severe lower respiratory tract disease following infection. Clinical trials involving the prophylactic administration of polyclonal intravenous immunoglobulin or mAbs against RSV have demonstrated a reduced risk of disease and led to the licensure of palivizumab *(22)* and the extended half-life mAb, nirsevimab *(10, 22–26)*. Although mAbs have shown a significant positive prophylactic effect against acute lower respiratory tract RSV infection, no beneficial therapeutic effect has so far been demonstrated once the clinical symptoms of RSV infection have become evident *(27, 28)*.

Maternal immunization with an RSV pre-fusion F protein vaccine is another means of providing protection against severe RSV disease in young infants from birth through to 6 months of age, with one such vaccine now authorized in a number of countries *(29, 30)*. However, safety signals related to preterm births associated with the use of a similar candidate maternal RSV vaccine, especially in low- and middle-income countries, led to the cessation of clinical trials *(31)* and a call for a review of the safety signals of such vaccines *(30, 32, 33)*. In addition, despite the ongoing development of RSV vaccines, there remains a need for mAbs for use in infants born to unvaccinated mothers, infants who may not have benefitted from RSV maternal antibodies *in utero* and older infants once such maternal antibodies have waned. Countries may choose to use RSV vaccines or mAbs, or both, based on local context *(10)*.

Furthermore, although immunization has been shown to be effective against RSV disease in the elderly *(34, 35)* there remains a need to protect immunocompromised individuals for whom mAbs may be the method of choice.

To date, the emergence of RSV variant strains has not affected the development or effectiveness of RSV mAbs and vaccines – unlike the situation for COVID-19 products *(36)*. However, concerns have arisen regarding the possibility of vaccine-related enhancement of RSV disease. Formalin-inactivated RSV (FI-RSV) vaccines developed in the 1960s were not protective, but rather primed the recipient to experience a severe form of the disease upon subsequent natural infection with RSV *(37–40)*. This phenomenon also occurred with formalin-inactivated measles vaccines. Such disease enhancement has been attributed to: (a) the induction of low avidity, poorly neutralizing antibodies; (b) overactive allergic inflammatory responses affecting lung function; and (c) a low CD8+ T-cell response and Th2-dominant CD4+ T-helper cell response *(5, 27, 38–41)*. High potency anti-F protein mAbs have not been associated with the mechanisms attributed to the FI-RSV vaccines of the 1960s, and nor have declining mAb titres been associated with disease enhancement. Although safe and effective mAbs against RSV have now been licensed, the potential for disease enhancement triggered by specific products and platforms should be assessed during product development.

The *WHO preferred product characteristics of monoclonal antibodies for passive immunization against respiratory syncytial virus (RSV) disease (42)* presents preferred, but not required, product characteristics for consideration by mAb developers and policy-makers. At its meeting in September 2024, the Strategic Advisory Group of Experts (SAGE) recommended passive immunization for the prevention of severe RSV disease in infants through the use of long-acting mAbs and/or maternal vaccines *(10)*.

5. International reference materials

Currently there are no international reference materials available specifically for the development of RSV mAbs. However, there is one related WHO international reference standard:

- First WHO International Standard for antiserum to respiratory syncytial virus *(43)*.

WHO international reference standards are the primary reference materials used worldwide to support the development of serological assays and to increase the comparability of results obtained by different laboratories. However, although the above material is suitable for the standardization of virus neutralization assays measuring antibody levels against RSV subtypes A and B in human serum, two collaborative studies *(44, 45)* have demonstrated that palivizumab behaved differently to human serum samples, with the international standard failing to harmonize the data obtained from neutralization assays of this mAb. Further studies are needed to determine whether the above WHO polyclonal serum standard is effective in harmonizing data obtained from virus neutralization assays of other RSV mAbs.

6. Nonclinical evaluation

6.1 In vitro pharmacodynamics studies

The pharmacodynamics (PD) of the mAb should be characterized using in vitro assays as follows:

6.1.1 Target antigen or epitope

Currently available prophylactic mAbs against RSV target the F protein, which is well conserved between RSV subtypes A and B. As the G protein of RSV is highly variable both within and between the two subtypes, this makes it a less promising target compared to the F protein *(46)*. The targeted epitope of the mAb should be identified and its binding ability against recombinant F proteins from RSV A and B subtype viruses should be demonstrated for the pre-fusion conformation, with the relative binding ability for pre-fusion versus post-fusion epitopes also assessed. The goal of this evaluation is to confirm the ability of the mAb to prevent fusion of the F protein with the target cell membrane thereby blocking subsequent viral infection.

6.1.2 Virus neutralization assays

The primary antiviral mechanism of mAbs is virus neutralization. The in vitro neutralization activity of the mAb should be assessed against laboratory strains and against clinical isolates of currently circulating RSV strains (subtypes A and B). The antiviral activity can be demonstrated through the use of microneutralization assays in a receptive mammalian cell line (for example, HEp-2, Vero or A549 cells) incubated with RSV *(47)*.

6.1.3 Effector function assays

The secondary antiviral mechanism of mAbs is the effector functions driven by Fc gamma receptor (FcγR) interactions. The effector properties of the mAb, such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC), should be assessed. If the Fc region of the mAb has been engineered, the modified pharmacological effects, such as extending mAb half-life or attenuation of Fc binding activity to Fc receptors, should be assessed and reported.

6.1.4 Virus resistance assessment

RSV neutralizing mAbs currently available or in development target the F protein. This protein is well conserved between the RSV A and B subtypes, and also exhibits similar genetic and antigenic stability. However, there is a potential risk of the emergence of antibody-resistant escape mutations among circulating RSV strains *(48)*. Therefore, the neutralization activity of the mAb against RSV strains should be evaluated using available RSV genetic database analysis, in vitro virus neutralization assays using emerging strains from clinical surveillance or experimentally derived viral escape mutants, and/or modelling of predicted escape mutants *(49)*. Where antibody resistance is observed, genotyping, phenotyping and cross-resistance analyses of the potential escape mutant(s) should be conducted.

6.2 In vivo pharmacodynamics studies

The cotton rat is the most commonly used and accepted animal model of human RSV infection due to its greater permissiveness to infection compared to other animals (such as mice and non-human primates). For this reason, the cotton rat model is usually used in the development of mAbs for the prevention of RSV infection in infants.

Despite the accepted utility of the cotton rat model, consideration should also be given to the use of other animal models that may accurately reflect human RSV infection and the anticipated mechanism of action of the mAb. The following models reflect some aspects of the clinical and pathological features of RSV infection in humans *(50, 51)*.

- Cotton rats have been established as an animal model for human RSV disease and have been used widely in studies of antibody prophylaxis, vaccination, FI-RSV vaccine enhanced respiratory disease and maternally induced immunity. Cotton rats are highly permissive to human RSV infection and allow active viral replication in nasal and lung tissue to a greater extent than other animal models. There is an absence of clinical symptoms but pathological findings such as bronchitis, alveolitis and pneumonitis have been observed.

- Mice have shown variability in their susceptibility to human RSV infection. BALB/c mice are the most widely used strain for human RSV infection studies as they are semi-permissive to virus replication in nasal and lung tissues. However, high doses of virus ($> 10^6$ pfu) are needed to elicit clinical symptoms such as weight loss, reduced activity and piloerection, and pathological findings such as bronchiolitis and infiltration of immune cells into the lungs. The age of the mice should also be considered as ageing leads to altered kinetics of antiviral gene expression, greater susceptibility to RSV infection and greater disease severity *(52, 53)*.

- Ferrets, due to their anatomical and respiratory physiological similarities with humans, are a common model for studying human respiratory virus infections. Human RSV replicates in the nasal tissue of ferrets following intranasal inoculation, but virus replication in lung tissue is only observed in infant ferrets. However, adult ferrets are highly susceptible to human RSV infection following intratracheal inoculation. Virus replication in the upper and lower respiratory tract is observed in this model but the animals do not develop clinical symptoms.

- Lambs are susceptible to high doses of human RSV ($> 10^8$ pfu) leading to upper and lower respiratory tract disease, with viral replication detectable in the lungs. Following intratracheal inoculation, lambs develop mild clinical symptoms, including slight fever, wheezing and cough. Pathological findings are similar to those observed in human infants after RSV infection, including bronchitis, bronchiolitis, pneumonia, peribronchial lymphocyte infiltration and syncytial cells. This makes the preterm and neonatal lamb a useful model for severe RSV disease in preterm and neonatal infants.

- Non-human primates such as macaques and African green monkeys have been used as animal models for human RSV infection due to their anatomical and physiological similarities to humans, with their use having mainly been restricted to the investigation of vaccine efficacy and safety. Non-human primates should only be considered as a last resort when all other models are inadequate, and the use of this model should be extensively justified. Although chimpanzees are fully permissive to human RSV infection, they should no longer be used in the development of products intended to prevent RSV disease due to ethical considerations.

    - Macaque species (rhesus, cynomolgus and bonnet) are only semi-permissive to infection with human RSV, even following the administration of high viral doses. Mild interstitial pneumonia has been observed in juvenile rhesus macaques inoculated with such doses, but these animals display no clinical symptoms.

- African green monkeys are also only semi-permissive to human RSV infection. As with macaques, African green monkeys do not display clinical symptoms after infection and may only develop minor pathological changes in the lungs.

The characteristics of RSV infection and disease outcomes in the above animal models are summarized in Table 1. It should be noted that this summary table is provided for information only and the scientific literature on current animal models of RSV infection should be taken into consideration when designing proof-of-concept studies. The selection of appropriate animal models for such studies should take into consideration the disease outcome of each animal model with regard to the intended study end-points. Furthermore, the design of the proof-of-concept study should reflect the intended clinical use(s) of the mAb.

Although several animal models have been used for the development of RSV prophylactic products, there are no animal models optimized to mimic human RSV infection, transmission or disease. Based on the differences in the clinical and pathological aspects of RSV infection in different species, the selection of animal models for characterizing the potential clinical use of the mAb should always be scientifically justified and the use of animals should adhere to the highest ethical standards.[1]

Nonclinical proof-of-concept studies may be necessary prior to the initiation of clinical testing in infants, as demonstrating the efficacy of an RSV mAb in an adult population may not be possible due to the likelihood of pre-existing immunity. The design of proof-of-concept studies should also ensure the use of a well-characterized virus challenge strain and acceptable route of inoculation. In vivo PD studies and other nonclinical studies can be used to determine the minimum anticipated biological effect level (MABEL) or biological effective dose (BED) to aid the first-in-human study design and dose selection. The analytical methods used to measure mAb serum concentrations and neutralizing antibody activity should ideally be the same as those used in the clinical pharmacology studies (see section 7.3 below).

Table 1
RSV infection characteristics and disease outcomes in potential animal models for RSV mAb development *(50, 51)*

Relevant animal model	Infection characteristics and disease outcome
Rodent	
Cotton rat	▪ Established model for human RSV infection ▪ Highly permissive to human RSV infection ▪ High levels of viral replication in nasal passage and lungs ▪ No overt signs of disease ▪ Lung histopathology changes observed (for example, bronchitis, alveolitis and pneumonitis)

[1] For scientific and ethical reasons, the 3Rs principles of "Replace, Reduce, Refine" should be applied.

BALB/c mouse	▪ Widely used model for human RSV infection ▪ Semi-permissive to human RSV infection ▪ Viral replication observed in nasal passage and lungs ▪ Age-dependent RSV responsiveness ▪ Disease symptoms observed at high-dose virus inoculation (weight loss and reduced activity) ▪ Lung histopathology changes observed (for example, bronchiolitis and infiltration of immune cells in the lungs)
Other	
Ferret	▪ Permissive to human RSV infection ▪ Adult ferrets exhibit viral replication in the upper and lower respiratory tract following intratracheal inoculation only ▪ Infant ferrets exhibit viral replication in the upper and lower respiratory tract following intranasal inoculation ▪ No overt signs of disease
Lamb	▪ Preterm and neonatal lambs are useful models for severe RSV disease in infants ▪ Semi-permissive to human RSV infection ▪ Viral replication observed in the upper and lower respiratory tract after high-dose virus inoculation ▪ Symptoms include slight fever, wheezing and cough ▪ Lung histopathology changes observed (for example, bronchitis, bronchiolitis, pneumonia, peribronchial lymphocyte infiltration and syncytial cells)
Non-human primates – non-human primates are mainly used only for vaccine efficacy and safety testing; their use in RSV mAb development should only be considered as a last resort if other models are inadequate, and should be extensively justified	
Macaque species (rhesus, cynomolgus and bonnet)	▪ Semi-permissive to human RSV infection ▪ No overt signs of disease ▪ Lung histopathology (mild interstitial pneumonia) only observed in juvenile rhesus macaques
African green monkeys	▪ Semi-permissive to human RSV infection ▪ No overt signs of disease ▪ Minor lung histopathology changes observed

6.3 Assessment of antibody-dependent enhancement of disease

The enhancement of RSV disease was first observed following the use of FI-RSV vaccines, and a subsequent increase in hospitalizations due to severe RSV disease, and two deaths, among immunized children. It has been suggested that non-neutralizing antibodies to the F protein may have been a contributing factor. However, no correlation has been found between

disease severity in infants and virus neutralization titres shown to induce antibody-dependent enhancement (ADE) of disease in vitro *(54)*. Although complement activation observed in the lungs of the two fatal cases suggested that antibody-F protein immune complexes may have been the cause of severe disease, the relation between complement activation and ADE has yet to be determined. Moreover, ADE of disease has never been demonstrated in vivo *(55, 56)* or observed following the administration of authorized RSV mAbs.

Furthermore, experience with the use of approved mAbs for the prevention of RSV disease in neonates and infants has indicated no increase in RSV morbidity and no increased risk of severe disease associated with mAb administration or declining mAb titres *(57)*. Although there is no known in vitro or in vivo model predictive of ADE, the potential for its occurrence should be considered during product development and discussed with the NRA.

7. Clinical evaluation

To date, mAbs against RSV have proven to be efficacious for pre-exposure prophylaxis in paediatric populations *(58–62)*. However, they have failed to demonstrate clinical efficacy as a therapeutic following the onset of symptoms *(27, 28)*. Therefore, the following guidance focuses on the clinical development of mAbs for pre-exposure indications, primarily in paediatric populations. As other populations, such as immunocompromised adults, may also benefit from pre-exposure prophylaxis, they could potentially also be the target subjects in clinical trials.

In regions where RSV mAbs are authorized for use in the general paediatric and paediatric at-risk populations, active-controlled clinical trials should be conducted using the appropriate comparator. Placebo-controlled studies would be considered unethical in such circumstances as all study subjects should receive as a minimum the current standard of care for the prevention of RSV infection, regardless of the treatment arm. Placebo-controlled studies may be justified under certain circumstances, for example when the reference mAb is not easily available or authorized in the region *(63)*. Non-inferiority studies using clinical end-points might not be feasible due to sample size requirements. However, should correlates of protection against RSV be identified, this would allow for a smaller Phase III study using the biomarker end-points. Alternative study strategies (such as concentration-time profiles complemented by limited clinical data) might also be considered – however, any alternative study design should be discussed in advance with the NRA.

The primary objectives of early mAb clinical development programmes should be to establish product safety and pharmacokinetics (PK), demonstrate its antiviral activity, explore the potential for induction of anti-drug antibodies (ADAs) and to select the correct dosing for Phase III clinical trials *(64, 65)*. If ADAs are detected, their impact on product safety and PK should be assessed prior to initiating future clinical studies. The timing of mAb administration in relation to regional epidemiological activity (RSV outbreaks) will also be important as this will differ in temperate climates compared to regions experiencing RSV transmission throughout the year, with no clear peak activity *(66)*. For clinical studies conducted in regions with little or no seasonal variations in RSV disease activity, recruitment and dosing should be continuous, and cases recorded for at least 6 months, or until sufficient cases have accumulated to allow for the primary analysis. In regions with an established seasonal pattern of RSV disease, clinical trials should continue for the duration of the season, with extended follow-up to provide information on long-term product safety and efficacy. Participants in Phase II and III clinical studies should be representative of the product's intended target population. Although it is expected that the target group will primarily be the paediatric population, it may be useful to identify particular subgroups which might benefit from RSV mAb prophylaxis. Such subgroups may include very and moderately preterm infants *(67)*, infants with chronic

lung disease (CLD) of prematurity *(68)* or haemodynamically significant congenital heart disease (CHD) *(69)*, as well as healthy children born at term *(13)*. Consideration should be given to the distribution of these subgroups across the different clinical trial groups and to their stratification at the time of randomization or, alternatively, to their enrolment in dedicated trials. While a step-down approach has been recommended for the development of RSV vaccines, this sequential approach may not be relevant for the development of an RSV mAb providing passive immunity. However, should developers wish to take an age step-down approach (starting with healthy infants born at ≥ 35 weeks 0 days gestational age and subsequently descending to premature infants with comorbidities such as CLD), the proposed approach should be discussed in advance with the NRA.

Special populations (for example, those who are immunocompromised, have Down syndrome, neuromuscular disease or cystic fibrosis) may also be appropriate study groups *(70, 71*), but only limited data might be obtained if the population is small. Development programmes geared towards vulnerable adults (for example, those who are immunocompromised) should be discussed with the NRA to ensure appropriate study design and generation of the data required for licensure decisions. Regardless of the population studied, efficacy end-points should be based on objective clinical and diagnostic criteria (such as reverse transcription-polymerase chain reaction/nucleic acid amplification testing verified, and with findings of lower respiratory tract infection (LRTI) +/− severity criteria). Case definitions for appropriate clinical end-points have been described elsewhere *(72)*. Symptoms should be evaluated throughout the RSV season and/or study period, with surveillance methods standardized across study sites.

There has been considerable interest in the potential of RSV prophylaxis to prevent wheezing episodes and asthma – though the link between RSV and wheezing or asthma is uncertain *(14)*. Although demonstrating a beneficial impact of mAb administration on the subsequent occurrence of asthma is not required for licensure, developers may wish to explore this relationship in the post-authorization setting as a long-term outcome and potential benefit. In such cases, the sponsor should seek advice from the NRA on the selection of the most appropriate end-points.

To accelerate authorization of a novel mAb to the same epitope as a previously authorized RSV mAb product, comparisons of their affinity, avidity and/or neutralization activity might be considered. However, it is recommended that the NRA be consulted regarding the acceptability of the clinical study design, and the use of non-inferiority margins or an immunobridging approach, prior to pursuing this strategy.

Attention should also be given to the potential emergence of ADAs, with PK monitoring used as a surrogate for their potential impact on product efficacy.

7.1 Inclusion and exclusion criteria

This section refers to paediatric development programmes. If an indication in adults is sought, the intended population should be reflected in the inclusion and exclusion criteria used.

7.1.1 Inclusion criteria

- Infants representative of the subgroups identified in the study protocol.
- Infants who are likely to be immune naive to RSV infection at the time of screening (for example, entering their first RSV season or prior to a regional outbreak).
- Infants who remain vulnerable to severe RSV disease through their second RSV season.
- For a CLD/CHD cohort:

- CLD – diagnosis of CLD of prematurity requiring medical intervention in the 6-month period prior to enrolment;
- CHD – documented haemodynamically significant CHD, unoperated or only partially corrected.

7.1.2 Exclusion criteria

- Significant infection or acute illness, including fever ≥ 37.8 °C in the 7-day period prior to randomization.
- Receipt of another RSV mAb prior to its washout period, or passive transfer of RSV-specific immunoglobulin G through maternal RSV vaccination.
- Immunization with an RSV vaccine.
- Receipt of any investigational medicinal product or enrolment in another interventional study.
- Known hypersensitivity to the immunoglobulin (active substance) or listed excipients.
- Severe adverse reaction following administration of a mAb.
- Anticipated survival < 6 months following randomization.

It should be noted that the regional availability or use of marketed mAbs to RSV may impact the exclusion criteria.

7.2 Phase I studies

Phase I and first-in-human studies are conducted to determine the initial safety and tolerability of the investigational medicinal product following completion of the essential nonclinical studies. Clinical experience has shown that most human and humanized mAbs are, in general, well tolerated.

Phase I clinical studies can be conducted in healthy adults or in infants depending on the aims of the mAb product development programme. In general, standard clinical study design practices for direct-acting drug development in paediatric populations should be followed. The extrapolation of efficacy results obtained from clinical studies in adults to children is not possible due to the pathophysiological differences in adult and child RSV disease. Such differences are particularly evident in infants and toddlers as their airways are much narrower and more easily compromised by inflammation due to RSV infection. In addition, adults are not immune naive to RSV. Nevertheless, adult trials could be useful for establishing safety and for the preliminary characterization of the PK properties of the product.

7.3 Clinical pharmacology

Appropriate analytical methods should be developed and validated for the determination of mAb serum concentrations, neutralizing antibody activity and detection of ADAs.

The primary PD effect of the mAb will be an increase in serum anti-RSV neutralizing antibody levels, with the dose–response relationship across different dose levels described. Determination of peak neutralizing antibody activity, and activity decay curve, in trials in the target population will support appropriate dose selection. The assays used should be able to differentiate and/or account for the presence of any endogenous antibodies elicited from RSV exposure.

Formal hypothesis testing for product efficacy in some infant subgroups at higher risk and/or older paediatric populations at higher risk might not be feasible. Extrapolation of

efficacy based on exposure is reasonable, as mAbs have an external target, with the dose–response relationship expected to be comparable between paediatric populations. Appropriate population PK models may be developed for the extrapolation of efficacy by PK bridging. Various factors may need to be considered for modelling, such as the effect of baseline body weight, gestational age or organ maturation function. Other relevant factors to consider may include the influence of ethnicity, CLD or CHD, and ADA on the PK.

Since mAbs are not expected to undergo renal elimination or to be metabolized by hepatic enzymes, exploring the effect of renal or hepatic impairment may not be warranted. Drug-drug interactions (DDIs) are not expected due to the nature of the product, and so the conducting of DDI studies will typically not be needed. Similarly, as RSV mAbs are not expected to interfere with current non-RSV childhood vaccines, vaccine coadministration studies involving such vaccines will also not be necessary.

7.4 Phase II and III studies

The primary objectives of the Phase II studies should be to characterize the safety profile and establish proof of concept in the intended target population. The efficacy of the prophylactic mAb should be assessed in Phase III studies to demonstrate the ability of the product to prevent RSV disease. Consultation with the NRA is recommended during study design and end-point selection.

7.4.1 Efficacy

An emphasis should be placed on designing randomized controlled clinical studies that take into account the target population, the selected clinical end-point(s) and case definitions, with the assessment methods applied consistently across the pivotal studies. Relevant examples of case definitions and other criteria that could be used in establishing primary, secondary and exploratory end-points are shown in Table 2.

In regions where RSV prophylaxis is currently recommended, the conducting of placebo-controlled studies would not be appropriate. In this case, active control studies would be needed based on either demonstration of non-inferiority or extensive pharmacological characterization of both the new product and the control product, supported by limited efficacy data in the target population. Although non-inferiority studies using clinical end-points are preferred, conducting such studies may be challenging due to sample size requirements. In populations for which no recommended RSV prophylaxis is currently available as per the local standard of care, the conducting of placebo-controlled studies would be appropriate.

7.4.2 Safety

The continual evaluation of mAb product safety is an important component of all phases of clinical studies. Although mAbs targeting infectious agents generally have a very good safety profile, each product is unique and should be considered independently. Safety data should be obtained during the clinical studies to characterize and quantify the product safety profile, and can include the type, frequency and severity of adverse drug reactions. It is recommended that the size of the database required for licensing be discussed with the NRA.

Evaluating the safety and tolerability of RSV mAbs should include the recording of all adverse events (AEs), serious adverse events (SAEs) and adverse events of special interest (AESIs), such as immediate hypersensitivity (including anaphylaxis) and immune complex disease (Table 2). Local and systemic reactions to first and subsequent doses should be fully captured. Study participants should be followed up for a sufficient period as determined by the half-life of the RSV mAb.

Long-term follow-up should pay special attention to any cases suggestive of ADE, with all such cases reported in the safety data. Attention should also be given to the potential induction of ADAs.

7.4.3 Post-authorization monitoring

The potential risk of treatment failure due to the emergence of RSV strains resistant to the mAb, along with the potential risk of ADE, should continue to be assessed post-authorization. Data monitoring (including systematic and proactive review of the emerging data) should be conducted using all available data sources.

The requirements for a risk-management plan, Phase IV studies and/or use of real-world evidence and data should be discussed with the NRA. Continual monitoring might be helpful in allowing the rapid identification of any emerging RSV escape variants.

Table 2
Examples of clinical end-points

Objectives	**Estimate description/end-point**
Primary	
Estimate of mAb efficacy	Medically attended LRTI (inpatient and outpatient) due to RT-PCR-confirmed RSV, through at least 150 days after dosing (that is, during a typical 5-month RSV season)
Estimate of mAb safety and tolerability	AEs, SAEs and AESIs during study period
Secondary	
Estimate the efficacy of the mAb in preventing *severe* RSV LRTI (hospitalization)	Hospitalizations due to RT-PCR-confirmed RSV through at least 150 days after dosing
Assess the PK of the mAb following administration of an appropriate dose via an appropriate route	Serum concentrations
Evaluate ADA response to the mAb in serum	ADA to the mAb in serum
Exploratory	
Estimate the efficacy of the mAb in preventing *very severe* RSV LRTI	Hospitalizations with supplementary oxygen or intravenous fluids due to RT-PCR-confirmed RSV through at least 150 days after dosing
Estimate the efficacy of the mAb in preventing *severe* LRTI	Hospitalizations due to any respiratory infection through at least 150 days after dosing

Estimate the efficacy of the mAb in preventing *very severe* LRTI	Hospitalizations with supplementary oxygen or intravenous fluids due to any respiratory infection through at least 150 days after dosing
Estimate the efficacy of the mAb in preventing medically attended RSV cases	Medically attended URTI and LRTI (inpatient and outpatient) due to RT-PCR-confirmed RSV, through at least 150 days after dosing (that is, during a typical 5-month RSV season)

Authors and acknowledgements

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