Annex 2

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

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

NOTE

This draft document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). The distribution of this draft document is intended to provide information on a proposed addendum to the previously published WHO *Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases* to a broad audience and to improve transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the ECBS. Written comments proposing modifications to this text MUST be received in English by 31 May 2024 in the Comment Form available separately and should be addressed to the Department of Health Products Policy and Standards, World Health Organization, 1211 Geneva 27, Switzerland. Comments may also be submitted electronically to the Responsible Officer: Dr Eunkyung Kim at: eunkim@who.int

The outcome of the deliberations of the ECBS will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the second edition of the *WHO style guide* (KMS/WHP/13.1).

Annex 2

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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	significant congenital heart disease
CLD	chronic lung disease
DDI	drug-drug interaction
Fc	fragment crystallizable (region)
FcγR	Fc gamma receptor
FI-RSV	formalin-inactivated RSV
GMT	geometric mean titre
IMP	investigational medicinal product
IV	intravenous
LRTI	lower respiratory tract infection
MAAE	medically attended adverse event
mAb	monoclonal antibody
NAAT	nucleic acid amplification testing
NRA	national regulatory authority
PD	pharmacodynamics
PK	pharmacokinetics
RBD	receptor binding domain
RSV	respiratory syncytial virus
RSV-IVIG	intravenous RSV immunoglobulin
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. However, 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 mAb products directed specifically against respiratory syncytial virus (RSV) antigens and intended primarily for pre-exposure prophylaxis in infants and young children. Unless otherwise indicated, the guidance applies to products that are administered parenterally. 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. General considerations

RSV is an Orthopneumovirus of the Paramyxoviridiae family and has two major subtypes, A and B. It consists of a single-stranded, non-segmented negative-sense RNA genome surrounded by a capsid consisting of a lipo-protein envelope derived from the host cell plasma membrane *(3, 4, 5)*. The envelope contains three viral transmembrane surface glycoproteins, a putative attachment glycoprotein G, a fusion glycoprotein F and a small hydrophobic glycoprotein SH. Proteins F and G are considered essential for pathogenesis and induce neutralizing antibodies in the host. The SH protein is a pentameric ion channel analogous to the M2 protein in influenza virus. Although not a major target for neutralising antibodies, anti-SH specific antibodies have been shown to protect through antibody-Fc mediated mechanisms *(6)*. There is also a non-glycosylated matrix protein M present on the inner face of the envelope. Antigenic diversity between and within RSV subtypes mainly reflects variations in glycoprotein G and there is little homology between glycoprotein G of the A and B strains of RSV *(7-9)*.

RSV infection is a major cause of respiratory disease globally, with significant morbidity and mortality in infants, especially those with cardiopulmonary disease *(5, 10)*. It causes both upper and lower respiratory tract infections and is often seasonal in temperate regions, causing epidemics, for example, in winter months. RSV infection also leads to significant disease burden in the elderly with comorbidities such as congestive heart failure or chronic obstructive pulmonary disease, or in the immunocompromised, where infection can lead to severe, sometimes life threatening, lower respiratory tract disease *(5-10)*. The high burden of disease caused by RSV leads to substantial hospitalization and economic costs *(10, 11)*. A link between severe lower respiratory tract RSV infection in infants to asthma and wheezing in childhood is inconclusive *(12)*.

RSV is typically transmitted by direct contact, or to a lesser extent by inhaled droplets, or through the eyes or nose. The virus then spreads through the respiratory tract from the nasopharynx to the distal alveoli *(13)* the hallmark of RSV disease being the formation of syncytia, large multinuclear cells created by many cells fusing together. By two years of age almost all children have had at least one RSV infection. As it does not elicit long lasting sterilizing immunity, repeated upper respiratory tract infections are common *(13)*. Therefore, RSV is a nosocomial threat to young infants as well as to immunocompromised and vulnerable individuals *(5, 10)*, and high mortality rates have been observed in those infected with RSV following bone marrow or lung transplantation *(14, 15)*.

Although there are no established immune correlates of protection for RSV, high concentrations of serum anti-RSV neutralizing antibodies have been shown to be associated with a substantial decrease in the risk of severe lower respiratory tract disease following infection. Clinical trials involving the prophylactic administration of polyclonal (RSV-IVIG) or mAbs against RSV have demonstrated a reduced risk of disease and led to the licensure of the mAbs palivizumab *(16)* and the long-acting nirsevimab *(17-20)*. It should be noted that although mAbs have shown a significant positive prophylactic effect on acute lower respiratory tract RSV infection, no beneficial therapeutic effect has so far been demonstrated once clinical symptoms of RSV infection are evident *(21, 22)*.

Maternal immunization is another option currently being pursued as a means of providing protection against severe RSV disease in young infants from birth through to 6 months of age. Safety signals related to preterm births associated with the use of maternal RSV vaccines , especially in LMICs , are currently being evaluated *(23, 24)*. Even so, there remains a need for mAbs for infants born to unvaccinated mothers, infants born preterm who do not benefit from maternal antibodies and older infants once maternal antibodies have waned. Countries may choose to use vaccines or mAbs, or both products, based on local context. Likewise, although immunization has demonstrated effectiveness against RSV disease in the elderly *(25, 26)*, there remains a need to protect various immunocompromised populations against RSV disease for which mAbs would likely be the method of choice.

Whilst the issue of variants of RSV has not impacted the development or effectiveness of mAbs and vaccines as compared with COVID-19 *(27)*, there has been concern about possible vaccine enhancement of RSV disease. Formalin-inactivated RSV vaccines developed in the 1960s were not protective, but rather primed the recipient to a severe form of the disease upon subsequent infection with RSV *(28-31)*. This phenomenon also occurred with formalin-inactivated vaccines to measles, another important Paramyxovirus. Such immune enhancement has been attributed to the induction of low avidity, poorly neutralizing antibodies, to overactive allergic inflammatory responses affecting lung function and to a low CD8 T-cell response *(21,* *29-31)*. Although safe and effective mAbs against RSV have been produced, attention to the assessment of possible disease enhancement triggered by different products and platforms should be considered during product development.

In 2021, WHO published the Preferred Product Characteristics of mAbs for passive immunization against respiratory syncytial virus disease *(32)*. The document presents considerations for mAb developers and policy makers on preferred, but not required, mAb product characteristics. A WHO SAGE Working Group has also recently been formed to provide further advice on the development and introduction of mAbs to RSV.

4. International reference materials

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. Currently there are no International Reference Materials available specifically for the development of monoclonal antibodies against RSV. However, there is one related WHO international reference standard:

- First WHO International Standard for antiserum to RSV *(33)*.

Although this antiserum is suitable for the standardization of virus neutralization methods to measure antibody levels against RSV A and B in human serum *(34)*, two collaborative studies *(34, 35)* have demonstrated that the mAb Palivizumab behaved differently from human serum samples and the use of this standard did not harmonize data from assays of this mAb.

Further studies are needed to determine whether this International Standard, a polyclonal serum standard, is effective in harmonizing data from neutralization assays of other RSV mAbs.

5. Nonclinical evaluation

5.1 Pharmacodynamics studies

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

5.1.1 Target antigen or epitope

The current available prophylactic mAbs target the RSV fusion (F) glycoprotein which is well conserved between RSV subgroups A and B. As the RSV attachment (G) glycoprotein is highly variable between RSV A and B this makes it a less promising target as compared to the F glycoprotein *(36)*. The targeted epitopes of the mAbs should be identified, and the binding ability against recombinant F proteins from RSV A and B should be demonstrated for both the prefusion and post-fusion conformations. This aims to prevent the fusion of the F protein with the targeted cell membrane and subsequent viral infection.

5.1.2 Virus neutralization assays

The primary antiviral mechanism of mAbs is virus neutralization following its administration. The in vitro virus neutralization activity of mAbs should be assessed against laboratory strains and clinical isolates of RSV A and B. The antiviral activity can be demonstrated by microneutralization assays in HEp-2 cells incubated with RSV virus *(37)*.

5.1.3 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.

5.1.4 Antibody-dependent enhancement of disease assessment

Enhancement of disease was first observed in formalin-inactivated RSV (FI-RSV) vaccination where an increased in hospitalization with severe disease, with two deaths, was observed in children who were immunized with FI-RSV vaccine. It has been suggested that non-neutralizing mAbs to F-protein may have been a contributing factor to enhancement of disease. However, no correlation has been found between disease severity in infants and virus neutralization titers shown to induce antibody-dependent enhancement (ADE) of disease in vitro *(38)*. Complement activation observed in lungs of the two infants suggested that antibody-F protein immune complexes may have been the cause of severe disease, but the relation between complement activation and ADE has yet to be determined. Moreover, ADE of disease has never been demonstrated in vivo *(39, 40)*.

Furthermore, the experience of approved mAbs for the prevention of RSV disease in neonates and infants showed that there is no increase in RSV morbidity with decreases in mAb titers and no risk of severe disease from declining mAb titers. However, no correlate of protection has been identified for RSV infection and the role of mAbs in prevention of disease or contribution to severe disease remains elusive. Although there is no known model predictive of ADE, the potential should be considered during product development and discussed with the NRA.

5.1.5 Virus resistance assessment

Neutralizing mAbs currently available or in development targets the F glycoproteins which is well conserved, showing relative genetic and antigenic stability between RSV A and B. However, there is potential risk from the emergence of antibody resistance escape mutants. This may arise from the emergence of resistance mutation in circulating RSV variants, which was seen in suptavumab phase 3 trial where RSV B isolates with 2 amino acid mutation in the suptavumab targeting epitope led to a loss of neutralizing activity *(41)*. Therefore, the neutralization activity of the mAb binding epitope against the RSV variants should be evaluated using available RSV genetic database analysis and in vitro virus neutralization assays using emerging variants from clinical surveillance, experimentally derived viral escape mutants and/or modelled predicted escape mutants *(42)*. In the event where resistance is observed, genotyping, phenotyping and cross-resistance analyses of the potential escape mutants should be conducted.

5.2 In vivo studies

The cotton rat is the most commonly used and accepted animal model of human RSV infection due to its greater permissiveness to infection as compared to other animals (e.g. mice or 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 acceptance and utility of cotton rat model, the use of other animal models should also be given consideration especially when the RSV infection is reflective of the human infection and of the anticipated mechanism of action of the mAb.

Several animal models have been used for the investigation of treatment against human RSV infection. Each of the following models reflects some aspects of the clinical and pathological features of RSV infection in humans *(43, 44)*.

- Cotton rats have been established as an animal model for human RSV disease and used widely in studies of antibody prophylaxis, vaccine, FI-RSV 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 in other animal models. There is an absence of clinical symptoms but pathological findings such as bronchitis, alveolitis and pneumonitis were 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 as they are semi-permissive to nasal and lungs replication. 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 in the lungs.

- Ferrets, due to their anatomical and respiratory physiological similarities with humans are a common model for studying human respiratory virus infections. Human RSV virus replicates in the nasal tissue of ferrets following intranasal inoculation, but virus replication in lung tissues is only observed in infant ferrets. However, adults 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 virus ($>10^8$ pfu) that leads to upper and lower respiratory tract diseases, with viral replication detected in the lungs. Following intratracheal inoculation, lambs developed mild clinical symptoms including slight fever, wheezing and cough. Pathological findings were similar to observations 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 (NHPs) such as chimpanzees, macaques and African green monkeys have been used as animal models for human RSV infection due to the anatomic and physiological similarities to humans, and their use is mainly for the investigation of vaccine efficacy and safety.

    - Chimpanzees are fully permissive to human RSV infection. Naturally infected chimpanzees display upper respiratory tract disease including clinical symptoms such as coughing, sneezing and nasal discharged, and lower respiratory tract disease with pathological finding that includes pneumonia, high immune cells infiltration, oedema, and detection of viral antigen in the lungs. While experimentally inoculated chimpanzees also display upper respiratory tract disease with virus replication observed in the nasopharyngeal and tracheal passages, no lower respiratory tract disease is observed. Therefore, RSV disease in humans is not fully replicated in chimpanzees in experimental settings.

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

    - African green monkeys are also semi-permissive to human RSV infection. Similar to macaques, African green monkeys do not show signs of clinical symptoms after infection and may only develop minor pathology changes in the lungs.

Based on the differences in clinical and pathological aspects of RSV infection, the selection of animal models for characterizing the potential clinical use of the mAb should be justified. Furthermore, the design of the proof-of-concept study should also reflect the intended clinical use(s) of the mAb.

The characteristics of RSV infection and disease outcome in the above animal models are summarized in Table 1. It should be noted that the summary table is provided for information purposes only and the scientific literature on current animal models of RSV infection should be taken into consideration when designing proof-of-concept studies.

Table 1

RSV infection characteristics and disease outcomes in animal models *(43, 44)*

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

BALB/c Mouse	▪ Widely used for human RSV infection ▪ Semi-permissive to human RSV infection ▪ Viral replication observed in nasal passage and lungs ▪ Disease symptoms observed at high virus inoculation are 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 have viral replication in the upper and lower respiratory tract following intratracheal inoculation only ▪ Infant ferrets have 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 disease after inoculation with high doses of virus ▪ Symptoms include slight fever, wheezing and cough ▪ Lung histopathology changes observed (for example, bronchitis, bronchiolitis, pneumonia, peribronchial lymphocyte infiltration and syncytial cells)
Non-human primate – non-human primates are mainly for vaccine efficacy and safety testing; their use should only be considered as a last resort option and extensively justified	
Chimpanzees	▪ Anatomical, physiological, and genetic similarities to humans ▪ Permissive to human RSV infection ▪ Symptoms include coughing, sneezing and nasal discharge ▪ Lung histopathology changes observed (for example, pneumonia, high immune cell infiltration, oedema, and detection of viral antigens in the lungs) ▪ No lower respiratory tract disease observed following experimental infection
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

Although several animal models have been used for the development of RSV prophylactics, there are no animal models optimized to mimic human RSV infection and disease. The selection of appropriate animal models for proof-of-concept studies should take into consideration the disease outcome of each animal model with regard to the intended study endpoints.

The design of proof-of-concept studies should also ensure the use of a well-characterized virus challenge strain and acceptable route of inoculation. The minimum anticipated biological effect level (MABEL) or biological effective dose (BED) should be used to select dose levels and to optimize the anticipated prophylactic effect.

6. Clinical evaluation

To date, mAbs to RSV have proven to be efficacious for pre-exposure prophylaxis (PreP) in paediatric populations *(45-49)*. However, they have failed to demonstrate clinical efficacy as a therapeutic following the onset of symptoms *(21)*. This guidance therefore 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.

With the availability of RSV mAbs authorized for use in the general paediatric and paediatric at-risk populations, the use of a placebo in efficacy studies is no longer feasible in regions where such mAbs are already introduced. Instead, active controlled clinical studies would be appropriate with either demonstration of non-inferiority or extensive pharmacological characterization of the new products and the control products supported by limited efficacy data in this population. Under certain circumstances, for example when the reference mAb is not available in the region, placebo-controlled double-blind studies could still be justified. In other populations (e.g. immunocompromised adults), a placebo-controlled efficacy study should be considered. Any alternative trial design should be discussed beforehand with the relevant regulatory authorities *(50)*.

The primary objective of early clinical development programmes or mAbs should be to establish its safety and PK, demonstrate its antiviral activity, explore its potential to induce anti-drug antibodies, and to select the right dosing for phase 3 clinical trials *(51, 52)*. In addition, the timing of mAb administration in relation to regional epidemiological activity (RSV outbreaks) will be important as this will differ in temperate climates as compared to regions which have no clear RSV season *(53)*. For clinical studies conducted in regions with little or no prevalent seasonal variation in disease activity, recruitment and dosing should be continuous, and cases collected for at least 6 months or until sufficient cases have accumulated as to allow the primary analysis. In regions with an established RSV disease seasonal pattern, clinical trials should continue for the duration of the season, with extended follow-up to provide information on long-term safety and efficacy.

Participants in phase 2 and 3 clinical studies should be representative of the intended population to receive the mAb. It is expected that the target group will primarily be paediatric patients. Various sub-groups which might benefit include very and moderately preterm infants *(54)*; infants with chronic lung disease (CLD) of prematurity *(55)* or haemodynamically significant congenital heart disease (CHD) *(56)*, as well as healthy children born at term *(57)*. The distribution of sub-populations should be considered and stratified at the time of randomization or, alternatively, enrolled in different trials. Special populations (e.g. those who are immunocompromised, have Down syndrome or cystic fibrosis) may also be considered as appropriate for study *(58)*. In these small populations, only limited data might be obtained. Development programmes geared towards vulnerable adults (e.g. immunocompromised) should ideally be discussed first with regulatory authorities regarding appropriate study design and results required for licensure.

Regardless of the population studied, efficacy endpoints should be based on objective clinical and diagnostic criteria (e.g. lab considerations RT-PCR /NAAT verified and with findings of LRTIs +/ - severity criteria). Case definitions for clinical endpoints to measure have been described elsewhere *(59)*. Symptoms should be evaluated through the RSV season (with methods of surveillance, standardized across study sites).

There has also been considerable interest in the potential of RSV prophylaxis to prevent wheezing episodes and asthma *(12)*. Although showing beneficial effects in these cases is not expected as a requirement for licensure, developers may elect to explore this relationship in the post-authorization setting as a long-term outcome . In such instances, the sponsor should seek advice from regulatory authorities on the selection of the most appropriate endpoints.

To accelerate authorization of novel mAbs to the same antigen as a previously authorized RSV mAb product, comparison of its affinity, avidity, and/or neutralization activity might be considered. However, it is recommended that the relevant NRA be consulted regarding acceptable clinical study design and the use of non-inferiority margins prior to investigating this strategy.

6.1 Inclusion and exclusion criteria

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

Inclusion criteria

- Infants at risk and reflective of the subgroups intended for the study purpose
- Infants who are considered immune-naive to RSV infection at the time of screening (e.g. 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 within 6 months prior to enrolment.
    - CHD: documented haemodynamically significant CHD, unoperated or partially corrected.

Exclusion criteria

- Significant infection or acute illness, including fever ≥ 38°C within 7 days prior to randomization.
- Receipt of another RSV mAb or any RSV vaccine, including passive transfer of RSV specific IgG through maternal RSV vaccination
- Receipt of any investigational product or enrolment in another interventional study
- Any known allergy, including to immunoglobulin products, or history of allergic reaction

- Anticipated survival < 6 months following randomization

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

6.2 Phase I studies

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

Phase 1 clinical studies can be conducted in healthy adults or in infants, based on the mAb product development programme. In general, the principles of direct acting drug development in paediatric populations will be followed. The extrapolation of efficacy results from clinical studies in adults is not possible due to the pathophysiological differences of the disease between adults and children. This difference is particularly evident in infants and toddlers as their airway system is much narrower and more easily compromised by inflammation due to RSV infection. As well, adults are not immune naive to RSV. Nevertheless, adult trials could be useful to establish safety and for a preliminary characterization of the pharmacokinetic properties of the product.

6.3 Clinical pharmacology

Proper bioanalytical and immunogenicity methods need to be developed and validated for determination of the mAb serum concentrations, neutralizing Abs and detection of ADAs.

The primary PD effect of the mAb is shown by an increase in serum anti-RSV neutralizing antibody levels, with the exposure-response model across dose levels to be described. Peak neutralising antibody activity and activity decay curve in trials in the target population, will support proper dose selection.

No formal hypothesis testing for efficacy in some higher-risk infant subgroups might be necessary. Extrapolation of efficacy based on exposure is reasonable, as mAbs have an external target, with exposure-response expected to be comparable between paediatric populations. Appropriate population pharmacokinetic (popPK) models may be developed for extrapolation of efficacy by PK bridging. For modelling, various factors need to be considered (e.g. the effect of baseline body weight, gestational age, organ maturation function). Other factors to be studied may include the influence of race, CHD or CLD 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 might not be warranted. Neither are drug-drug interactions (DDIs) expected due to the nature of the product. Hence, the conduct of DDI studies might be superfluous.

6.4 Phase II and III studies

The primary objective of the phase II trials should be characterizing the safety profile and establishing the proof of concept in the intended target population. The efficacy of the prophylactic mAb studied in phase II/III trials should be to evaluate its ability to prevent the disease.

6.4.1 Efficacy

An emphasis should be placed on designing randomized controlled clinical trials that take account of the study target population, the selected clinical endpoint(s) and case definitions, with methods of assessment to be applied consistently across the pivotal studies. Relevant examples of case definitions for primary, secondary and exploratory endpoints are shown in Table 2.

Active control studies will be suitable in populations for which current RSV prophylaxis is already recommended. However, in populations for which no recommended prophylaxis is currently available as per local standard of care, the conduct of placebo-controlled studies would be appropriate.

6.4.2 Safety

The continual evaluation of mAb product safety is an important component within 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 from a sufficient number of subjects during the clinical trials to characterize and quantify the product safety profile, which can include the type, frequency and severity of adverse drug reactions.

Evaluating the safety and tolerability of anti-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. Local and systemic reactions to first and eventual subsequent doses should be fully captured. Subjects should be followed up for a sufficient period, as determined by the half-life of the RSV neutralizing antibody.

Long-term follow-up should provide special attention to cases suggestive of enhanced respiratory disease (ADE), with such cases to be reported in the safety data. Special attention should be given to the potential of ADA, with immune complex disease and hypersensitivity reactions.

Table 2
Clinical end-points

Objectives	**Estimate description/end-point[a]**
Primary	

Estimate of efficacy of mAb	Incidence of medically attended LRTI (inpatient and outpatient) due to RT-PCR[b]-confirmed RSV, through at least 150 days after dosing (i.e., during a typical 5-month RSV season)
Estimate of safety & tolerability of the mAb	AEs, SAEs and AESIs during study period
Secondary	
Estimate the efficacy of the mAb in preventing *severe* LRTI -RSV (hospitalization)	Incidence of 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	Incidence of the ADA to the mAb in serum
Exploratory	
Estimate the efficacy of the mAb in preventing *very severe* LRTI - RSV	Incidence of hospitalizations with supplementary oxygen or IV fluids due to RT-PCR-confirmed RSV through 150 days after dosing
Estimate the efficacy of the mAb in preventing *severe* LRTI	Incidence of hospitalizations due to any respiratory infection through at least 150 days after dosing
Estimate the efficacy of the mAb in preventing *very severe* LRTI	Incidence of hospitalizations with supplementary oxygen or IV fluids due to any respiratory infection through at least 150 days after dosing
Estimate the efficacy of the mAb in preventing all medically attended RSV	Incidence of medically attended URTI and LRTI (inpatient and outpatient) due to RT-PCR-confirmed RSV, through at least 150 days after dosing (i.e., during a typical 5-month RSV season)

[a] Relevant example of endpoints. In all cases, consultation with the NRA is recommended during trial design and endpoint selection

[b] RT-PCR = reverse transcription-polymerase chain reaction

6.4.3 Post-authorization studies

The potential risk of treatment failure due to the development of RSV variants 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.

Authors and acknowledgements

The first draft of this WHO addendum was prepared by Dr A. Chia (*lead author for the Nonclinical evaluation section*), Health Sciences Authority, Singapore; Dr E. Griffiths (*lead author for the General considerations section*), consultant, United Kingdom; Dr R. Isbrucker (*lead author for the Introduction, and Purpose and scope sections*), Health Canada, Canada; Dr E. Pelfrene (*lead author for the clinical evaluation section*) European Medicines Agency, Netherlands (Kingdom of the). The draft document was then reviewed and revised by a drafting group compromising Dr A. Chia, Dr E. Griffiths, Dr R. Isbrucker, Dr B. Klug, Paul-Ehrlich-Institut, Germany; Dr E. Pelfrene; and by Dr E.K. Kim, Dr I. Knezevic and Dr E.G. Sparrow-Jones, World Health Organization, Switzerland.

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