2.4. Nonclinical Overview	
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2.4 Nonclinical Overview

BEYFORTUS® (Nirsevimab) for the Prevention of RSV Lower Respiratory Tract Disease in All Infants

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LIST OF ABBREVIATIONS

Abbreviation or Specialized Term	Definition
1G7	non-YTE version of nirsevimab (MEDI8897)
ADA	antidrug antibody
CHMP	Committee for Medicinal Products for Human Use
C_{max}	maximum observed concentration
EC_{90}	90% effective concentration
F	fusion
Fc	fragment crystallisable
FcRn	neonatal Fc receptor
GLP	Good Laboratory Practice
HCl	Hydrochloride
IC_{50}	half-maximal inhibitory concentration
IgG	immunoglobulin G
IgG1κ	immunoglobulin G1 kappa
IM	intramuscular(ly)
IV	intravenous(ly)
K_D	equilibrium dissociation constant(s)
LRTI	lower respiratory tract infection
mAb	monoclonal antibody
MEDI-524	anti-RSV F mAb, motavizumab
MEDI-557	anti-RSV F mAb, YTE modified motavizumab
NOAEL	no-observed-adverse-effect level
PK	pharmacokinetic(s)
RSV	respiratory syncytial virus
RT-PCR	reverse transcriptase-polymerase chain reaction
$t_{1/2}$	terminal half-life
TK	toxicokinetics
YTE	M257Y/S259T/T261E triple amino acid substitution

1 OVERVIEW OF THE NONCLINICAL TESTING STRATEGY

Nirsevimab (also known as MEDI8897) is a recombinant human immunoglobulin G1 kappa (IgG1κ) monoclonal antibody (mAb) that binds the prefusion conformation of the respiratory syncytial virus (RSV) F protein.

All infants, are at risk for severe RSV lower respiratory tract infection (LRTI) with primary RSV infection. RSV LRTI in infants is a potentially serious and life-threatening disease characterised by infection and inflammation in the alveolus and small airways. It is associated with necrosis and sloughing of the epithelium of the small airways, with oedema and increased secretion of mucus. This can lead to airway obstruction and a typical clinical picture of hyperinflation, atelectasis, and wheezing (Hall 2001). The clinical diagnosis being typically of bronchiolitis or pneumonia. It is estimated that RSV causes up to 90% of childhood bronchiolitis and up to 40% of paediatric pneumonias (Hall 2001). In 2015, an estimated 33 million episodes of RSV-associated LRTI occurred worldwide in children < 5 years of age, with 3.2 million episodes necessitating hospitalisation. Approximately 59600 in-hospital deaths were estimated for this age group, of which 43600 were reported in lower-middle income countries (Shi et al 2017). RSV LRTI is the most common reason for admission to hospital in infants < 1 year of age (Hall 2001, 2012, Murray et al 2014, Rha et al 2020).

Respiratory syncytial virus, a member of the *Pneumoviridae* family (Rima et al 2017), is a non-segmented, negative-sense, enveloped RNA virus and is classified into two subtypes, A and B, based on sequence homology of the RSV surface glycoprotein G (Wertz and Moudy 2004). Respiratory syncytial virus entry into host cells and cell-to-cell spread is dependent on the fusion (F) protein, which mediates fusion of the viral and host cell membranes (Walsh et al 1984, Walsh et al 1989, Hall and McCarthy 1995). The F protein is a highly conserved protein across RSV subtypes (Wertz and Moudy 2004) and is synthesized as an inactive precursor (F_0) that is cleaved by host cell proteases into the biologically active, disulfide-linked F1 and F2 subunits. The F protein is expressed on virions in a pre-fusion conformation. Juxtapositioning of the virus and host cell membranes leads to the F protein-mediated fusion event and the accompanying irreversible refolding of F protein into a post-fusion conformation (Chang and Dutch 2012). Six antigenic sites (Ø and I–V) have been identified in prefusion and/or postfusion F proteins (Gilman et al 2016) with target epitopes for prophylactic neutralizing mAbs (Broadbent et al 2015). The majority of the neutralizing activity detected in a human IgG preparation (ie, RespiGam) is directed against the pre-fusion conformation of RSV F (Magro et al 2012).

The F protein is a clinically validated target for immunoprophylaxis of RSV disease. Palivizumab (Synagis®), a humanized mAb directed against the RSV F protein, is effective as passive immunoprophylaxis to prevent RSV hospitalizations, but it is licensed only for infants at higher risk for morbidity and mortality from RSV (ie, premature infants ≤35 weeks gestational age, children with chronic lung disease of prematurity or children with hemodynamically significant congenital heart disease). Therefore, there is a significant

unmet medical need for RSV prevention in otherwise healthy children (Zhou et al 2012, Hall et al 2013). Prevention of RSV illnesses in all infants remains a major public health priority.

Nirsevimab was derived from a human monoclonal antibody D25 (Kwakkenbos et al 2010) that was isolated directly from human B cells and binds the prefusion conformation of the RSV F protein (McLellan et al 2013). D25 was affinity optimized for RSV neutralization potency in vitro to generate 1G7. Nirsevimab differs from 1G7 by a three amino acid substitution, M257Y/S259T/T261E (M252Y/S254T/T256E, according to the EU numbering system), referred to as "YTE" (Dall'Acqua et al 2006a), in the heavy chain CH2 fragment crystallizable (Fc) region of the mAb. The YTE modification was added to the mAb to prolong the terminal half-life of the antibody in humans (Griffin et al 2017, Robbie et al 2013).

Although an increasedT half-life is observed in humans, the YTE modification significantly decreases antibody exposure in rodents resulting in decreased serum antibody levels relative to the native human Fc (Dall'Acqua et al 2002). Since both nirsevimab and 1G7 exhibit equivalent in vitro antiviral activity in cell culture, the nonclinical pharmacology properties of nirsevimab, or its parental mAb 1G7, were studied in non-Good Laboratory Practice (GLP) experiments.

A total of 15 nonclinical studies were performed to characterize mechanism of action, antiviral activity (in vitro and in vivo), antiviral resistance (in vitro), pharmacokinetics, and safety of nirsevimab: 11 nonclinical pharmacologic studies (Table 1); a nonclinical pharmacokinetic study (Table 2); 3 GLP nonclinical toxicologic studies (one toxicity study and two tissue cross-reactivity studies) (Table 3). All pivotal nonclinical safety studies were appropriately designed in compliance with ICH S6 (ref) and were conducted in an Organization for Economic Co-operation and Development (OECD) member country in accordance with OECD GLP guidance. As agreed with the EMA, including the Paediatric Committee, the nonclinical safety package is considered appropriate and sufficient to support registration of nirsevimab to immunise infants from birth entering their first RSV season for the prevention of RSV lower respiratory tract disease (see Module 1; End of Phase 2 Scientific Advice 2019).

Table 1 List of the Nonclinical Pharmacology Studies of Nirsevimab

Study Number	Study Type	Study Title	GLP
ID8897-0001	In vitro	In Vitro Antiviral Activity of MEDI8897 and its Precursor, 1G7, Against Respiratory Syncytial Virus A and B Laboratory Strains.	No
ID8897-0008	In vitro	RSV F mAb Competition Studies	No
ID8897-0010	In vitro	Characterization of the MEDI8897 Binding Site: Crystallographic and Sequence Conservation Analysis	No

Table 1 List of the Nonclinical Pharmacology Studies of Nirsevimab

Study Number	Study Type	Study Title	GLP
ID8897-0005	In vitro	Determination of MEDI8897 Binding Affinity for Human and Cynomolgus Monkey FcRn Receptors	No
ID8897-0002	In vitro	In Vitro Antiviral Activity of the MEDI8897 Precursor, 1G7, Against Respiratory Syncytial Virus Clinical Isolates and Recombinant Variants.	No
ID8897-0031	In vitro / in vivo	In Vitro and In Vivo Characterization of Nirsevimab (MEDI8897) Fc-mediated Effector Functions	No
ID8897-0003	In vitro	Generation and Characterization of MEDI8897 Monoclonal Antibody-Resistant Mutant Viruses	No
ID8897-0011	In vitro	In Vitro MEDI8897 Neutralization Activity Against RSV Variants Containing Polymorphic Amino Acid Changes in the MEDI8897 Binding Site, Contemporary Clinical Isolates and Characterization of Recombinant RSV Variants Encoding Mutations Identified in MEDI8897 MARMs	No
ID8897-0006	In vivo	Antiviral Activity of the MEDI8897 Precursor, 1G7, in a Cotton Rat Model of Respiratory Syncytial Virus A Infection	No
ID8897-0007	In vivo	Antiviral Activity of the MEDI8897 Precursor, 1G7, in a Cotton Rat Model of Respiratory Syncytial Virus B Infection	No
ID8897-0032	In vivo	The Nirsevimab Precursor, 1G7, Does Not Interfere with wt RSV A2 Immunogenicity in the Cotton Rat Model	No

1G7 = non-YTE version of nirsevimab; GLP = Good Laboratory Practice; mAb = monoclonal antibody; RSV = respiratory syncytial virus; wt = wild-type

Table 2 List of the Nonclinical Pharmacokinetic Studies of Nirsevimab

Study Number	Study Title	Species/ROA	GLP
20089726	A Single Dose Non-GLP Lung Biodistribution and Pharmacokinetic Study in Male Cynomolgus Monkeys to compare MEDI8897, MEDI-557, and MEDI-524 Levels	Cynomolgus monkey/IV	No

IV = intravenous injection; GLP = Good Laboratory Practice; MEDI8897 = nirsevimab; MEDI-524 = motavizumab; MEDI-557 = YTE-version of motavizumab; ROA = route of administration

Table 3 List of the Nonclinical Toxicology Studies of Nirsevimab

Study Number	Study Title	Species/ROA	GLP
1468-038	MEDI8897: A 1-month repeat dose IV/IM toxicity study in cynomolgus monkeys with a 25-week recovery period	Cynomolgus monkey/IV and IM	Yes
20046491	A Tissue Cross-Reactivity Study of MEDI8897 in Normal Human Tissues	Human, in vitro/NA	Yes
20060018	A Tissue Cross-Reactivity Study of MEDI8897 in Selected Juvenile, Neonatal and Fetal Human Tissues	Human, in vitro/NA	Yes

IV = intravenous injection; GLP = Good Laboratory Practice; NA = not applicable; ROA = route of administration

2 PHARMACOLOGY

The nonclinical pharmacology of nirsevimab has been investigated in several in vitro and in vivo studies. A list of the individual nonclinical pharmacology studies is provided in Table 1. Written summaries of the pharmacology can be found in Module 2.6.2 and tabulated summaries are presented in Module 2.6.3.

2.1.1 Primary Pharmacodynamics

The YTE modification in nirsevimab increased the affinity for the human neonatal Fc receptor at lower pH (K_D = 161 nM). With the exception of the YTE modification in the Fc region, nirsevimab and 1G7 share the same protein sequence and exhibit equivalent RSV neutralization potency in microneutralization assays, confirming that the YTE modification does not affect neutralization potency.

A co-crystal structure of D25 bound to RSV F revealed that D25 locks RSV F in its prefusion state by binding to a quaternary epitope within antigenic site ∅ at the trimer apex (McLellan et al 2013). Antibody competition studies confirmed that nirsevimab, 1G7, and D25 bind the same region within antigenic site ∅ which does not overlap with previously described anti-RSV F mAbs. In vitro binding kinetics showed that 1G7 binds DS-Cav1 stabilized trimeric prefusion RSV A2 and B9320 F proteins with high affinities with K_D = 0.12 nM and K_D = 1.22 nM, respectively. Finally, additional studies using three-dimensional human airway epithelial (HAE) tissues showed that nirsevimab inhibits the essential membrane fusion step in the viral entry process, neutralising the virus and blocking cell-to-cell fusion.

A crystallographic analysis of nirsevimab Fab in complex with prefusion stabilized DS-Cav1 RSV F protein trimers revealed a discontinuous, well-conserved binding site of 25 amino acid residues in RSV A F and B F, primarily composed of residues 62–69 in the F2 subunit and residues 196–212 in the F1 subunit.

Nirsevimab demonstrated in vitro binding to immobilized human FcγRs (FcγRI, FcγRIIA, FcγRIIB, and FcγRIII) and equivalent neutralization potency compared to parental mAbs,

1G7 and 1G7-TM (Fc region modified to reduce FcR binding and effector function). In a cotton rat model of RSV infection, 1G7 and 1G7-TM exhibited comparable, dose-dependent reduction in RSV replication in the lungs and nasal turbinates, strongly suggesting that protection from RSV infection is dependent on neutralization activity rather than Fc-mediated effector function.

In vitro antiviral activity of nirsevimab against RSV was measured in a dose-response model using cultured HEp-2 cells. Nirsevimab exhibited similar in vitro potency IC_{50} values against RSV A2 (1.6–2.4 ng/mL) and RSV B9320 (1.8–2.2 ng/mL) laboratory strains compared to 1G7 (RSV A2: 1.7–2.2 ng/mL; RSV B9320: 1.8–2.1 ng/mL) and was >150-fold more potent than palivizumab, a licensed monoclonal antibody for the prevention of RSV, in neutralizing both RSV A2 and B9320. Against a diverse panel of global RSV A (N=70) and RSV B (N=49) clinical isolates collected between 2003 and 2017, 1G7 exhibited highly potent and broad antiviral activity with median IC_{50} values of 3.2 ng/mL (range, 0.48 to 15 ng/mL) and 2.9 ng/mL (range, 0.3 to 59.7 ng/mL), respectively.

In vivo antiviral activity and the potential for rapid protection against RSV infection was evaluated in a cotton rat model of RSV infection using 1G7. Intramuscular administration 1 day prior to inoculation with RSV A2 or B9320 provided complete protection from viral replication in the upper and lower respiratory tracts. 1G7 exhibited 11-fold and 9-fold greater potency than palivizumab at reducing pulmonary viral loads by >3-logs in RSV A- and RSV B-infected cotton rats, respectively. Based on the calculated 90% effective concentration (EC_{90}) values for 1G7 against RSV A2 and B9320 in the cotton rat model of RSV infection, a target serum concentration of 6.8 μg/mL of nirsevimab was predicted to provide protection over the 5-month RSV season and was the basis for the Phase 2b study dose selection. Following an exposure-response analysis of the Phase 2b study, a clinically efficacious target exposure (AUC = 13.4 day*mg/mL) was determined (see Module 2.7.2).

Analysis of in vitro selected nirsevimab neutralization escape variants revealed critical residues associated with reduced susceptibility to neutralization at positions 68, 201, and 208 in the RSV F protein. These F sequence variations are all located in the nirsevimab binding site as defined by crystallographic analysis. Substitutions in these residues affected binding of nirsevimab to RSV F, without significant impact on viral replication in vitro.

Finally, the potential for antibody dependent enhancement (ADE) of RSV infection was evaluated in a cotton rat model of RSV infection using 1G7. No evidence of enhancement of RSV infection was observed at any dose evaluated, including sub-efficacious doses down to 0.125 mg/kg. In addition, 1G7 prophylaxed animals mounted a functional immune response after RSV challenge, providing protection from a subsequent RSV infection after 1G7 was no longer present. There was also no evidence of enhanced disease observed in these studies.

2.1.2 Secondary Pharmacodynamics

Secondary pharmacodynamic studies of nirsevimab were not conducted. Nirsevimab is directed against a viral target that is not endogenously expressed in healthy animal or human tissues. In accordance with ICH S6 (R1), a nonclinical safety program including two GLP TCR studies, (one in adult human and cynomolgus monkey tissues and one in foetal human tissues) and a repeat-dose GLP toxicology study in cynomolgus monkeys has been completed ICH S6(R1) 2011). No binding to any human or cynomolgus monkey adult tissues or human foetal tissues was observed.

2.1.3 Safety Pharmacology

In accordance with ICH S6(R1) and ICH S7A ('Safety Pharmacology Studies for Human Pharmaceuticals'), no stand-alone safety pharmacology studies were conducted with nirsevimab. Safety pharmacology of nirsevimab was assessed as a component of the one month repeat dose GLP toxicologic study in cynomolgus monkeys (1468-038). Results are summarized in Module 2.6.6 and Module 2.6.7. In that study, ECG parameters assessed, predose and 24 hours post-dose on Days 1 and 29 and prior to the recovery necropsy, showed no adverse nirsevimab -related effects following 50 mg/kg intravenous (IV), 300 mg/kg IV or 300 mg IM once weekly for 5 total doses. In addition, there were no clinical signs nor microscopic findings indicating any nirsevimab-related effects on the central nervous system (CNS) or respiratory system in the monkeys.

2.1.4 Pharmacodynamic Drug Interactions

Pharmacodynamic drug interaction studies of nirsevimab were not conducted. Based on the high affinity, selectivity, and specificity of nirsevimab, additional pharmacodynamic drug interaction studies were not considered warranted. Nirsevimab is directed against a viral target that is not endogenously expressed in healthy animal or human tissues.

3 PHARMACOKINETICS

3.1.1 Overview of the Nonclinical Pharmacokinetics Program

The nonclinical PK properties of nirsevimab were studied in the repeat-dose one-month GLP toxicology study in cynomolgus monkeys (1468-038) and in a single-dose non-GLP biodistribution study (20089726) in male cynomolgus monkeys, designed to compare levels of nirsevimab to corresponding levels of other anti-RSV F mAbs (motavizumab [MEDI-524] and YTE modified motavizumab [MEDI-557]) in serum, bronchoalveolar lavage (BAL), and nasal wash (NW) samples collected following intravenous (IV) administration. MEDI-557 and MEDI-524 are mAbs that were previously under development for treatment of RSV for which clinical data is available (O'Brien et al 2015). Written summaries of the PK and analytical methods can be found in Module 2.6.4 and tabulated summaries are presented in Module 2.6.5.

3.1.2 Absorption/Exposure

Absorption of nirsevimab at 300 mg following IM administration and 50 or 300 mg/kg following IV administration was evaluated in the repeat-dose one month GLP toxicology study in cynomolgus monkeys (1468-038). Mean peak serum concentration was observed 2.3 days after the first dose, indicating slow absorption from the IM injection site. Nirsevimab exhibited linear PK in cynomolgus monkeys at dose levels of 50 mg/kg and 300 mg/kg IV after the first dose, while exposure increased in a slightly less than dose proportional manner after the last (5^{th}) weekly dose (1468-038). Mean half-life following 5 weekly IV or IM administrations to cynomolgus monkeys was approximately 40 days. No consistent gender related differences in nirsevimab serum exposure were observed between male and female cynomolgus monkeys. The mean concentration ratios of nirsevimab in NW samples compared to nirsevimab serum concentrations was approximately 1:10000.

The C_{max} and cumulative AUC (from Day 1 to Day 31) values after the 5^{th} weekly dose in cynomolgus monkeys (1468-038) is presented in Table 4. No anti-drug antibodies (ADA) were detected in any of the monkeys during the treatment phase. Four of the 12 nirsevimab-treated animals (2 in 300 mg/kg IV and 2 in 300 mg IM) tested positive for ADA during the recovery phase with variable impact on TK. In 2 of the 4 animals that tested positive for ADA, serum concentrations declined faster while PK in the other 2 animals was not impacted.

Table 4 Summary of Mean Pharmacokinetic Parameters of Nirsevimab After 5^{th} Weekly Dose in Cynomolgus Monkeys (1468-038)

Species	No. of animals per group	Dose (ROA)	C_{max} (µg/mL)	AUC_{1-31} (µg·day/mL)	$t_{1/2}$ (days)
Cynomolgus monkey	3M+3F	50 mg/kg (IV)	3772 (910.7)	61590 (7370)	NC
Cynomolgus monkey	6M+6F	300 mg/kg (IV)	13360 (2704)	208500 (43270)	40.45 (14.86) [a]
Cynomolgus monkey	6M+6F	300 mg (IM)	4982 (863.4)	92380 (9428)	39.91 (7.442) [b]

F = female; M = male; NC= not calculated
PK values are presented as mean ± standard deviation.
[a] N=6.
[b] N=5.

3.1.3 Distribution

Classical tissue distribution studies with nirsevimab have not been conducted.

Nirsevimab is an IgG monoclonal antibody and as such the distribution of nirsevimab is likely restricted to the extracellular fluid (Ovacik and Lin 2018).

The biodistribution of nirsevimab into BAL and NW samples, in comparison to two other anti-RSV F mAbs (MEDI-557 and MEDI-524), was however studied by determining ratios of BAL and NW concentration of each mAb relative to the serum concentration of the mAb, following IV dosing to male cynomolgus monkeys (see Section 3.1.7).

3.1.4 Metabolism

In vitro or in vivo metabolism studies with nirsevimab have not been conducted. Nirsevimab is a monoclonal antibody and therefore its expected metabolism is degradation to small peptides and amino acids (Ovacik and Lin 2018). No active metabolite is expected for nirsevimab.

3.1.5 Excretion

Excretion studies with nirsevimab have not been conducted. Nirsevimab is a monoclonal antibody and therefore its expected elimination is through the intrinsic clearance by the reticuloendothelial system in the same way as that for an endogenous IgG. No renal excretion is expected for nirsevimab since the molecular weight is higher than the glomerular filtration threshold (Ovacik and Lin 2018).

3.1.6 Pharmacokinetic Drug Interactions

Pharmacokinetic drug interaction studies of nirsevimab have not been conducted. Based on its mode of action, the likelihood of nirsevimab impacting expression levels of metabolic enzymes, such as cytochrome P450s (CYP450), is considered low. With respect to modulation of metabolism by concomitant medications, pharmacokinetic drug interactions are not anticipated as nirsevimab is likely degraded via normal protein catabolism, which is not dependent on CYP450 enzymes (Ovacik and Lin 2018).

3.1.7 Other Pharmacokinetic Studies

In order to benchmark the distribution of nirsevimab, the biodistribution of nirsevimab was compared to two other anti-RSV F mAbs, MEDI-557 (YTE modified motavizumab) and MEDI-524 (motavizumab), by determining concentrations of the mAbs in serum, BAL, and NW samples in male cynomolgus monkeys (20089726). In this study, animals received a single IV dose (via 30-minute infusion) of nirsevimab at 50 or 150 mg/kg, MEDI-524 at 50 mg/kg, and MEDI-557 at 150 mg/kg. Serum samples were collected at various time points; NW samples were collected at 24 and 72 (± 2) hours post-dose, and BAL samples were collected at 72 (± 2) hours post-dose.

Serum exposure to nirsevimab increased in a dose-proportional manner from 50 to 150 mg/kg IV and nirsevimab serum exposure was higher than for MEDI-524 andMEDI-557 at equivalent doses, respectively. The mean concentration of nirsevimab in BAL samples 72 hours post-dose were within 1- to 2-fold of the comparator mAbs administered at equivalent doses, and the partition ratio (as percent of mean serum concentration) of nirsevimab in BAL was within 2- to 3-fold of the other mAbs. Mean concentration of nirsevimab in NW samples were within 2- to 3-fold of the comparator mAbs at 24 hours and within 2- to 7-fold at 72 hours, although high individual variability was observed.

4 TOXICOLOGY

4.1.1 Overview of the Nonclinical Toxicology Program

Nonclinical safety of nirsevimab was assessed by performing a GLP-compliant repeat-dose toxicity study in cynomolgus monkeys, a GLP-compliant tissue cross-reactivity study using a full panel of frozen human tissues and a GLP-compliant tissue cross-reactivity study using a selected panel of juvenile, neonatal and fetal human tissues (Table 3). Intramuscular injection is the clinical route for the proposed indication, while IV infusion was used in the FTIH adult study.

A discussion of the relevancy of species chosen for the nonclinical toxicology studies and the key findings from these studies are presented below (also see Modules 2.6.6 and 2.6.7).

4.1.2 Justification of the Pharmacologically Relevant Species

The antigen for nirsevimab (RSV F protein) is not endogenously expressed in humans or monkeys. Therefore, the rationale for species selection is based on the FcRn binding affinity of nirsevimab in monkeys and humans. Cynomolgus monkeys represent a pharmacologically relevant model for nonclinical safety assessment based on similar binding of nirsevimab to cynomolgus monkey FcRn compared to human FcRn (Dall'Acqua et al 2006), see Module 2.1.1 and Module 2.6.2.3.7). Rodent species do not represent a relevant model because of the differences in FcRn binding compared to human. The YTE modification in the Fc domain of nirsevimab results in a longer $t_{1/2}$ in humans however, it significantly decreases antibody exposure in rodents resulting in decreased serum antibody levels relative to mAbs containing the native human Fc (Dall'Acqua et al 2002).

4.1.3 Single-dose Toxicity

No formal single-dose toxicity study of nirsevimab was performed. Toxicity following a single dose was evaluated as a component of a one-month repeat IV infusion and IM injection toxicity study in cynomolgus monkeys (1468-038). In that study, based on clinical observations and clinical pathology, no adverse local or systemic effects of nirsevimab were observed.

4.1.4 Repeat-dose Toxicity

Repeat-dose toxicity of nirsevimab was assessed in a GLP-compliant study in cynomolgus monkeys (1468-038). Once weekly IV or IM administration (5 doses total) of nirsevimab to monkeys, up to and including 300 mg/kg/week IV or 300 mg/week IM dose levels followed by a 25-month dose-free recovery phase, was not associated with any treatment-related local or systemic adverse effects. The no-observed-adverse-effect-level (NOAEL) is considered to be 300 mg/kg/week IV and 300 mg/week IM.

4.1.5 Genotoxicity

In accordance with ICH S6(R1), genotoxicity evaluations were not conducted because nirsevimab is not expected to interact with genetic material and such studies are not considered appropriate for an antibody product (ICH S6(R1) 2011).

4.1.6 Carcinogenicity

In accordance with ICH S6(R1), carcinogenicity studies were not conducted and are not considered required given that nirsevimab binds a viral-specific target that is not expressed in nonclinical models or in humans, and the intended intermittent clinical use (ICH S6(R1) 2011).

4.1.7 Reproductive and Developmental Toxicity

In accordance with ICH S6(R1), no studies were conducted to evaluate the effects of nirsevimab on fertility, embryo foetal, and pre/postnatal development because nirsevimab binds a viral-specific target that is not expressed in nonclinical models or in humans, and the intended clinical population (infants and children) does not include women of childbearing potential (ICH S6(R1) 2011). In addition, nirsevimab did not show any adverse effects on reproductive tissues in the repeat-dose toxicity study (1468-038) and did not bind to any evaluated human reproductive tissues (including placenta) in the tissue cross-reactivity study (20046491).

4.1.8 Juvenile Toxicity

Nonclinical safety of nirsevimab in juvenile toxicity studies has not been evaluated. Evaluation of nirsevimab in young cynomolgus monkeys (3.1 to 4.2 years old) in the GLP 1-month repeat-dose study (1468-038) did not identify any potential for test item-related toxicity or pharmacological effects on developing organ systems. In addition, none of the completed nonclinical safety studies have indicated any potential for nirsevimab-related effects on fertility or growth and/or development of offspring. Therefore, nonclinical studies in juvenile animals are not considered necessary as the currently available clinical and nonclinical safety data are sufficient to support the paediatric development and registration of nirsevimab (see Module 1; End of Phase 2 Scientific Advice 2019).

4.1.9 Local Tolerance

Local tolerance was assessed as a component of the one-month repeat dose toxicity study (1468-038). Injection sites (IV and IM) were evaluated for erythema/eschar and edema changes according to the dermal Draize score. There were no nirsevimab-related signs of local irritation.

4.1.10 Other Toxicity

4.1.10.1 Tissue Cross-reactivity

Tissue cross-reactivity of nirsevimab was assessed by immunohistochemical methods in a GLP-compliant study using a full panel of frozen human tissues from 3 normal human donors as recommended in the Points to Consider in the Manufacture and Testing of

Monoclonal Antibodies for Human Use (20046491). Results revealed no staining with nirsevimab, and therefore, no tissue cross-reactivity. Tissue cross-reactivity of nirsevimab was further assessed by immunohistochemical methods in a GLP-compliant study (20060018) against cryosections of selected juvenile, neonatal and fetal human tissues (1 to 3 donors per tissue, as available). Results revealed no staining with nirsevimab, and therefore, no tissue cross-reactivity against a panel of normal human juvenile, neonatal and fetal tissues.

4.1.10.2 Antigenicity

A standalone antigenicity study of nirsevimab was not conducted. Instead, antigenicity of nirsevimab was evaluated as part of the repeat-dose toxicity study in cynomolgus monkeys (1468-038) in accordance with the ICH S8 and ICH S6(R1) guidance documents and the ICH S6 guidance and draft guidance for industry (The Non-clinical Safety Evaluation of the Immunotoxic Potential of Drugs and Biologics [ICH S8 2006, ICH S6(R1) 2011, FDA 2020]). Analysis for immunogenicity in the repeat-dose one-month GLP toxicity study in cynomolgus monkeys showed that none of the animals in the study tested positive for ADA at any time point during the treatment phase. Four of the 18 animals (12 nirsevimab-treated and 6 vehicle/control animals) tested positive for ADA during the recovery phase with variable impact on TK.

4.1.10.3 Immunotoxicity

A standalone immunotoxicity study of nirsevimab was not conducted. Instead, potential immunotoxicity of nirsevimab was evaluated in standard toxicity parameters as part of the repeat-dose toxicity study in cynomolgus monkeys (1468-038) in accordance with the ICH S8 and ICH S6(R1) guidance documents and the ICH S6 guidance and draft guidance for industry (The Non-clinical Safety Evaluation of the Immunotoxic Potential of Drugs and Biologics [ICH S8 2006, ICH S6(R1) 2011, FDA 2020]). There was no evidence of potential nirsevimab-related immunotoxicity in parameters evaluated.

4.1.10.4 Mechanistic Studies

Mechanistic studies of nirsevimab were not conducted as the properties of the molecule (being a monoclonal antibody) and toxicology data generated did not warrant such studies.

4.1.10.5 Dependence

No studies to evaluate the potential for nirsevimab abuse or misuse were conducted consistent with ICH M3(R2) (ICH M3(R2) 2009). In contrast to centrally active small molecules, available data suggests that monoclonal antibodies, as a drug class, are unlikely to cause dependence.

4.1.10.6 Metabolites

No studies to determine nirsevimab metabolites were conducted. Instead of being metabolized, monoclonal antibodies are catabolized and degraded into small peptides and individual amino acids that are taken up in the endogenous amino acid pool.

4.1.10.7 Impurities

No in vivo studies were conducted to assess the potential toxicological impact of nirsevimab-related impurities (eg, process-related impurities, host cell protein or extractables and leachables). The manufacturing strategy for nirsevimab includes robust purification processes to remove impurities and contaminants and rigorous bioanalytical characterization of the drug product. All potential impurities in the nirsevimab drug product were either assessed in the repeat dose toxicology study or were present at levels that were not of toxicological concerns. Therefore, a dedicated nonclinical safety testing program was not deemed warranted for any of these impurities.

5 INTEGRATED OVERVIEW AND CONCLUSIONS

Nonclinical pharmacologic studies demonstrate that nirsevimab, a recombinant human IgG1 kappa monoclonal antibody, binds to the prefusion conformation of the RSV F protein with high affinity and inhibits the essential membrane fusion step in the viral entry process, neutralizing the virus and blocking cell-to-cell fusion. Nirsevimab was derived from parental mAbs 1G7 and D25, optimized to exhibit >150-fold greater potency than palivizumab in vitro, and was engineered with three amino acid substitutions (M252Y/S254T/T256E [YTE]) within its Fc region that enhance binding to FcRn under the acidic conditions of the lysosome to prevent degradation, increase recirculation, and extend serum half-life in humans.

A crystallographic analysis of the nirsevimab Fab in complex with the F protein of RSV A2 and RSV B9320 revealed that nirsevimab binds a conserved discontinuous binding site within antigenic site ∅, comprised of residues 62-69 in the F2 subunit and residues 196-212 in the F1 subunit.

Nirsevimab, 1G7, and 1G7-TM (Fc region modified to reduce FcR binding and effector function) all share the same Fab sequence and as expected, exhibited equivalent potent antiviral activity against RSV A and B laboratory strains in cell culture. Nirsevimab/1G7 further exhibited broad-spectrum antiviral activity against a panel of 70 RSV A and 49 RSV B clinical isolates.

Nirsevimab was shown to bind to immobilized human FcγRs in vitro. However, in a cotton rat model of RSV infection, 1G7 and 1G7-TM exhibited comparable dose-dependent reduction in RSV replication in the lungs and nasal turbinates, strongly suggesting that protection from RSV infection is dependent on neutralization activity and not Fc-mediated effector functions.

Neutralization escape variants were selected following three passages in cell culture of RSV A2 and B9320 strains in the presence of nirsevimab. All resistance-associated substitutions at positions 68, 201, and 208 identified among in vitro neutralisation escape variants were located in the nirsevimab binding site and were shown to reduce binding affinity to RSV F protein without significantly impacting viral replication in vitro.

1G7 provided complete prophylactic protection from viral replication in the upper and lower respiratory tracts in the cotton rat model of RSV A and RSV B infection. 1G7 was 11-fold and 9-fold more potent than palivizumab at reducing pulmonary viral loads by >3-logs in RSV A- and RSV B-challenged cotton rats, respectively. In addition, no evidence of antibody-dependent enhancement of RSV infection was observed at any dose evaluated, including sub-efficacious doses down to 0.125 mg/kg. Prior prophylactic administration of 1G7 in previously challenged cotton rats also resulted in protection from subsequent RSV infection after 1G7 was no longer present in sera. In both acute and secondary infections there was no evidence of enhanced RSV disease.

These data taken together with PK prediction models suggest that the potency of nirsevimab combined with the "YTE" half-life extension technology should enable once-per season dosing for the prevention of RSV disease in all infants entering their first RSV season.

Results from the non-GLP PK and lung biodistribution study showed that nirsevimab serum exposure following IV dosing was higher than for MEDI-524 and MEDI-557 at equivalent doses, respectively, and that all 3 mAbs were detected in both NW and BAL in all animals at all sampled time points. Although absolute mean concentrations of nirsevimab in NW were lower, within 2- to 3-fold of the comparator mAbs at 24 hours and within 2- to 7-fold at 72 hours, results were highly variable and overall, the biodistribution of the 3 mAbs was considered comparable and supported progression into clinical trials in infants.

The nonclinical safety of nirsevimab was assessed in a one month repeat IV and IM dose toxicologic study in cynomolgus monkeys and in two GLP human tissue cross-reactivity studies. Once weekly IV or IM administration (5 doses total) of nirsevimab to monkeys, up to and including 300 mg/kg IV or 300 mg IM dose levels, was not associated with any treatment-related adverse effects both locally and systemically. The NOAEL is considered to be 300 mg/kg/week IV and 300 mg/week IM. Tissue cross-reactivity results showed no staining of any human tissues as expected given the target for nirsevimab is a non-endogenous viral-specific target.

Safety margins based on the NOAEL and predicted PK from the final PopPK model for adults and infants entering their first RSV season were calculated for C_{max} and AUC (see Module 2.6.6, Table 2).

Based on the NOAEL of 300 mg/kg/week IV, calculated safety margins for a dose of 3000 mg IV in adult healthy volunteers was 12-fold based on C_{max} and 3-fold based on AUC.

Based on the NOAEL of 300 mg/week IM calculated safety margins for a dose of 50 mg IM for infants weighing <5 kg and 100 mg IM for those weighing ≥5 kg entering their first RSV season was 44-fold based on C_{max} and 8-fold based on AUC. Given that the toxicity profile did not differ between IM and IV routes in the cynomolgus monkey, safety margins

were also calculated for the NOAEL of 300 mg/kg/week IV, which was 118-fold based on C_{max} and 18-fold based on AUC.

Overall, data from these nonclinical studies did not reveal any nirsevimab-related safety concerns and support the registration of nirsevimab for prevention of RSV in infants at the recommended dose .

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