

Molecular and phenotypic characteristics of respiratory syncytial virus isolates recovered from medically vulnerable children: An exploratory analysis of a phase 2/3 randomized, double-blind, palivizumab-controlled trial of nirsevimab (MEDLEY)

Kevin M. Tuffy^{a,*}, Bahar Ahani^b, Joseph B. Domachowske^c, Kenji Furuno^d, Hong Ji^a, Shabir A. Madhi^e, Vaishali S. Mankad^f, Ulrika Wählby Hamrén^g, Tonya Villafana^h, Yingyi Wangⁱ, Elizabeth J. Kelly^{a,1,2}, Deidre Wilkins^{a,2}

^a Translational Medicine, Vaccines & Immune Therapies, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD, USA

^b Bioinformatics, Vaccines & Immune Therapies, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD, USA

^c Department of Pediatrics, SUNY Upstate Medical University, Syracuse, NY, USA

^d Department of General Pediatrics and Interdisciplinary Medicine, Fukuoka Children's Hospital, Fukuoka, Japan

^e South African Medical Research Council Vaccines and Infectious Diseases Analytics Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

^f Clinical Development, Respiratory & Immunology, BioPharmaceuticals R&D, AstraZeneca, Durham, NC, USA

^g Clinical Pharmacology and Quantitative Pharmacology, R&D, AstraZeneca, Gothenburg, Sweden

^h Clinical Development, Vaccines & Immune Therapies, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD, USA

ⁱ Biometrics, Vaccines & Immune Therapies, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD, USA

ARTICLE INFO

Keywords:

Respiratory syncytial virus
Nirsevimab
Palivizumab
RSV immunization
Anti-RSV F protein monoclonal antibody

ABSTRACT

Background: Nirsevimab is an extended half-life monoclonal antibody (mAb) licensed for the prevention of respiratory syncytial virus (RSV)-associated lower respiratory tract disease in neonates, infants and medically vulnerable children. We characterized RSV isolates recovered from participants enrolled in MEDLEY: a randomized, palivizumab-controlled phase 2/3 trial of nirsevimab in infants born preterm and/or with congenital heart disease or chronic lung disease of prematurity.

Methods: Participants were assessed in two RSV seasons (Season 1 and 2). Season 1 participants were randomized (2:1) to receive a single dose of nirsevimab (50 mg if weight <5 kg or 100 mg if weight ≥5 kg in Season 1; 200 mg in Season 2) followed by four monthly doses of placebo, or five once-monthly doses of palivizumab (15 mg/kg weight per dose). Season 2 participants continued nirsevimab and placebo (nirsevimab/nirsevimab) or were re-randomized (1:1) to switch to nirsevimab (palivizumab/nirsevimab) or continue palivizumab (palivizumab/palivizumab). Cases of RSV infection were identified by central testing of nasal swabs from participants seeking medical attention for respiratory illnesses. Nirsevimab and palivizumab binding site substitutions were assessed via microneutralization assay.

Results: Twenty-five cases of confirmed RSV infection were observed during the trial and sequenced: 12 in nirsevimab recipients and 10 in palivizumab recipients during Season 1, and 1 case in each Season 2 group.

Abbreviations: AA, amino acid residues; cDNAs, complementary DNAs; CE, Conformité Européenne; CHD/CLD, congenital heart disease or chronic lung disease of prematurity; CT, cycle threshold; EU, European Union; FDA, Food and Drug Administration; GISRS, Global Influenza Surveillance and Response System; IC₅₀, half-maximal inhibitory concentrations; LRTI, lower respiratory tract infection; MA RSV LRTI, medically attended RSV-associated LRTI; mAb, monoclonal antibody; MAF, molecular allele frequency; NGS, next-generation sequencing; NREVSS, National Respiratory and Enteric Virus Surveillance System; PDB, Protein Data Bank; PK, pharmacokinetic; rRSV, recombinant RSV; RSV F, RSV fusion; RSV, respiratory syncytial virus; RT-PCR, reverse transcription-polymerase chain reaction assay; TCID₅₀, tissue culture infectious dose; TESSy, the European Centre for Disease Prevention and Control's European Surveillance System; US, United States..

* Corresponding author at: Translational Medicine, Vaccines & Immune Therapies, BioPharmaceuticals R&D, AstraZeneca, 1 MedImmune Way, Gaithersburg, MD 20878, USA.

E-mail address: Kevin.Tuffy@astrazeneca.com (K.M. Tuffy).

¹ Current affiliation: Global Immunology, Vaccines R&D, Sanofi, Swiftwater, PA, USA.

² Contributed equally.

<https://doi.org/10.1016/j.vaccine.2024.126276>

Received 28 March 2024; Received in revised form 9 August 2024; Accepted 25 August 2024

Available online 5 September 2024

0264-410X/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Molecular sequencing of RSV A ($n = 14$) isolates detected no nirsevimab binding site substitutions, and 3 palivizumab neutralization-resistant substitutions (Lys272Met, Lys272Thr, Ser275Leu). The nirsevimab binding site Ile206Met:Gln209Arg and Ile206Met:Gln209Arg:Ser211Asn substitutions were the only anti-RSV mAb binding site substitutions detected among RSV B isolates ($n = 11$). Nirsevimab neutralized all nirsevimab and palivizumab binding site substitutions in RSV A and B isolates recovered from MEDLEY participants.

Conclusion: No binding site substitution detected during MEDLEY affected RSV susceptibility to nirsevimab neutralization.

1. Introduction

Respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infection (LRTI) and hospitalization in infants and children aged ≤ 24 months worldwide [1,2]. RSV is a negative-sense RNA virus, which circulates through an annual RSV season lasting ~ 5 months each winter in temperate climates [3]. The RSV Fusion (F) protein has been a prominent target for the development of RSV interventions due to its essential roles in host cell entry and broad genetic conservation among RSV A and B subtypes [4–6]. Serological studies have identified six antigenic sites within RSV F: sites Ø (“zero”) and V are exclusively found in its prefusion conformation and sites I–IV have been observed in both prefusion and postfusion conformations [5].

Monthly injections of the humanized murine anti-RSV F protein site II monoclonal antibody (mAb), palivizumab, have been established as a well-tolerated and effective method of preventing RSV-associated LRTI hospitalization in numerous high-risk infant populations (e.g., infants born preterm and/or with hemodynamically significant congenital heart disease or chronic lung disease of prematurity [CHD/CLD]) since its licensure in 1998 [7,8]. Palivizumab was originally licensed for all preterm infants with a gestational age ≤ 35 weeks; however, subsequent local policy decisions by the American Academy of Pediatrics in 2014 restricted palivizumab use to infants with a gestational age ≤ 29 weeks [8,9]. An unmet need for RSV interventions for the benefit of all infants spurred the development of nirsevimab – a highly potent, broadly neutralizing extended half-life (~ 71 days) anti-RSV F protein site Ø mAb, designed to confer protection for a typical 5-month RSV season with a single intramuscular injection [10,11]. Nirsevimab has demonstrated consistently high efficacy against RSV-associated lower respiratory tract disease in placebo-controlled studies throughout its clinical development program, with efficacy rates of 79.5 % against medically attended RSV-associated LRTI (MA RSV LRTI) and 77.3 % against hospitalization for MA RSV LRTI observed over 150 days post dose in a pooled analysis of preterm infants from the pivotal phase 2b (NCT02878330) trial and preterm and term infants from the primary cohort of the phase 3 MELODY (NCT03979313) trial [12–15]. Similarly, nirsevimab was 83.2 % effective at preventing LRTI hospitalization in healthy preterm and term infants during the real-world phase 3b HARMONIE study (NCT05437510) [16]. Further pharmacokinetic (PK) analyses from the phase 2b and the MELODY trials, as well as the phase 2/3 palivizumab-controlled MEDLEY trial (NCT03959488) enabled the extrapolation of nirsevimab efficacy to infants born preterm or born with CHD/CLD [15]. Following approvals in other geographic territories beginning late-2022, nirsevimab was approved for the prevention of RSV lower respiratory tract disease in neonates and infants born during or entering their first RSV season, and in children ≤ 24 months of age who remain vulnerable to severe RSV disease through their second RSV season in the United States (US) in July 2023 [17].

The primary mechanism of nirsevimab and palivizumab resistance is reduced binding to the RSV F protein due to amino acid changes in their respective binding sites [7,10,11,18]. Previous analyses have illustrated that the nirsevimab (amino acid residues [AA] 62–69 and 196–212 in site Ø) and palivizumab (AA 262–275 in site II) binding sites were highly conserved among circulating RSV variants between 1956 and 2021, and that neutralization-resistant variants are rare and have not increased in prevalence between successive RSV seasons [6,7,11]. However, the

inherently error-prone nature of RSV’s RNA-dependent replication cycle could theoretically lead to the emergence of viral variants with reduced neutralization susceptibility, as illustrated by the failure of a phase 3 study of suptavumab – a previous investigational anti-RSV F site V mAb – to meet its primary endpoint due to the emergence of a neutralization-resistant variant of RSV B [3,5,19]. Thus, ongoing viral surveillance is needed to ensure enduring mAb effectiveness between successive RSV seasons.

Analyses of RSV isolates in infants dosed with nirsevimab and palivizumab can support the identification of potential neutralization-resistant variants and complement data from *in vitro* resistance and viral surveillance studies [6,18,20,21]. In this context, we have genotypically evaluated and functionally assessed the impact of amino acid substitutions in the nirsevimab and palivizumab binding sites as observed in participants with confirmed RSV infections from the MEDLEY trial. We also contextualize RSV infections with reference to the geotemporal prevalence of RSV during the trial, and participant serum anti-RSV F mAb concentrations and viral load data.

2. Methods

2.1. MEDLEY trial design

MEDLEY was a phase 2/3 randomized, double-blind, palivizumab-controlled trial evaluating the safety, PK and descriptive efficacy of nirsevimab in infants born preterm or born with CHD/CLD. The trial protocol and statistical analysis plan have been published with the primary analysis [22,23]. The genotypic and phenotypic characterization of RSV isolates with evaluable sequencing data described herein were pre-specified exploratory analyses. The trial was conducted in accordance with the principles of the Declaration of Helsinki and the International Council for Harmonisation Good Clinical Practice guidelines. Each site had approval from an institutional ethics review board or ethics committee, and appropriate written informed consent was obtained for each infant prior to enrollment.

The trial was conducted between July 30, 2019 and January 20, 2023 [24]. Participants were recruited from 126 sites across 25 countries in Europe, North America, Asia and South Africa [25]. Participants were assessed in two 5-month RSV seasons (Seasons 1 and 2, respectively). Participants born during or prior to their first RSV season and who were eligible to receive palivizumab in accordance with local guidelines were enrolled into the trial in two cohorts: infants born preterm (i.e., gestational age of ≤ 35 weeks and 0 days), and infants born with CHD/CLD irrespective of gestational age at birth. Participation in Season 2 was restricted to the CHD/CLD cohort in accordance with local or national guidelines for palivizumab use. Further descriptions of eligibility criteria for both cohorts are detailed in **Supplemental Materials**. Participants were excluded if they had a history of LRTI/RSV infection or active LRTI/RSV infection prior to or at the time of randomization. Baseline characteristics for MEDLEY study participants in Season 1 and Season 2 have been published previously [22,26].

2.2. Study intervention

Participants were randomized 2:1 prior to their first RSV season (Season 1) to receive either a single intramuscular dose of nirsevimab

(weight-banded dosing regimen: 50 mg in infants weighing <5 kg or 100 mg in infants weighing ≥5 kg), followed by four monthly doses of placebo or five monthly intramuscular doses of palivizumab (15 mg/kg weight per dose). Participants who were randomized to nirsevimab in Season 1 received 200 mg nirsevimab followed by four once-monthly placebo doses in Season 2 (nirsevimab/nirsevimab group). Participants who received palivizumab in Season 1 were re-randomized 1:1 to switch to the Season 2 nirsevimab and placebo regimen (palivizumab/nirsevimab group) or continue with five palivizumab once-monthly doses (palivizumab/palivizumab group). Study interventions were administered on Days 1, 31, 61, 91 and 121, and trial participants were followed through to Day 361 (i.e., 360 days post-first dose of study intervention) in their respective seasons.

2.3. Randomization and masking

Randomization was performed using an interactive web response system to assign blinded investigational product kit numbers. Neither the investigators nor the parents or guardians were aware of the trial group assignments. Within each cohort, randomization was stratified by hemisphere (northern, southern) and participant age at the time of Season 1 randomization (<3 months, >3 to ≤6 months, >6 months). Data from the trial were collected by clinical investigators and analyzed by the contract research organization, ClinChoice.

2.4. Confirmation of RSV infection

Nasal swabs were obtained from all trial participants seeking medical attention for a respiratory infection and additionally may have been obtained as part of routine clinical care in the inpatient and outpatient settings. RSV infection was confirmed by a US Food and Drug Administration (FDA)-cleared and Conformité Européenne (CE)-marked *in vitro* diagnostic quantitative real-time reverse transcription-polymerase chain reaction assay (RT-PCR; Lyra RSV+ human metapneumovirus assay, Quidel, San Diego, CA, USA) in a central laboratory (Eurofins-Viracor, MO, USA).

2.5. Categorization of RSV cases

Cases of RSV infection were categorized into one of four sampling periods based on the timing of first dose of study intervention (Season 1 Day 1–151 and Day 152–361, and Season 2 Day 1–151 and Day 152–361) and according to the protocol definition of MA RSV LRTI (Supplementary Table 1). All cases of RT-PCR-confirmed RSV infection occurring through Day 151 that did not meet the protocol definition of MA RSV LRTI, and all confirmed cases of RSV infection occurring during Day 152–361 in their respective seasons were assessed as exploratory RSV infections. Confirmed-RSV cases were also contextualized against participant hospitalization status and the geotemporal prevalence of RSV in northern and southern hemispheres. RSV prevalence data for participant countries were obtained from the following viral surveillance dashboards: the US Centers of Disease Control's National Respiratory and Enteric Virus Surveillance System (NREVSS; <https://www.cdc.gov/surveillance/nrevss/rsv/index.html>), the European Centre for Disease Prevention and Control's European Surveillance System (TESSy; <http://atlas.ecdc.europa.eu/public/index.aspx>) and the World Health Organization's FluNet using data provided by the Global Influenza Surveillance and Response System (GISRS; http://ais.paho.org/phi/viz/ed_who_rsv.asp).

2.6. Genotypic evaluation of RSV

RSV attachment protein (G) Sanger sequencing, RSV A and B subtyping and F protein next-generation sequencing (NGS) sequence assembly and sequence annotation procedures were performed per other viral surveillance studies in the nirsevimab clinical development

program [6,27]. Briefly, following G gene amplification and Sanger sequencing, RSV A and B subtypes were assigned based on alignment of the G hypervariable region 2 against contemporary RSV A and B reference sequences (Netherlands RSV A/13–005275 GenBank accession no. KX858754.1; RSV B/13–001273 GenBank accession no. KX858755.1). RSV genotypes were assigned by phylogenetic clustering using a reference database of 11 RSV A and 23 RSV B genotypes [28].

RSV A and B F gene sequences in FASTA format were translated to amino acid sequences and aligned against RSV A/13–005275 (GenBank accession no. KX858757.1) and RSV B/13–001273 (GenBank accession no. KX858756.1) reference variants. These isolates were selected as reference variants to assess F protein amino acid variation due to their similarities with consensus RSV A and B F sequences at the beginning of nirsevimab development [10]. The full-length RSV F gene was amplified and sequenced using NGS assays for determination of consensus polymorphism(s); major variants were defined as ≥25 % molecular allele frequency (MAF), while minor variant(s) were defined as ≥4 to <25 % MAF for RSV A and ≥5 to <25 % MAF for RSV B. The prevalence of all amino acid sequence variations in the full-length RSV F protein were reported relative to the frequency observed in RSV A and B sequences collected during prospective RSV molecular epidemiology studies conducted between 2015 and 2021 (Supplemental Materials) [6].

2.7. Generation of recombinant RSV variants

Individual and co-occurring F protein amino acid substitutions were engineered using reverse genetics into recombinant RSV (rRSV) A (A2–000; containing the Glu66 residue) or rRSV B (B9320–000; containing the Asn197 residue) complementary DNAs (cDNAs) using molecular cloning techniques as previously described [6,27]. rRSV variants were rescued and plaque purified to obtain sufficient titers for subsequent microneutralization susceptibility assay testing. The sequence of each rRSV stock was confirmed following RNA extraction, RT-PCR, and Sanger sequencing.

2.8. RSV microneutralization assay

Per previous analyses, the effect of binding-site substitutions on the *in vitro* neutralization potency of nirsevimab and palivizumab was assessed in a validated RSV microneutralization assay at Viroclinics Biosciences BV (Rotterdam, Netherlands) [6,27]. Nirsevimab or palivizumab were serially diluted in 96-well plates prior to the addition of a fixed concentration of rRSV reference or test variants at a median tissue culture infectious dose (TCID₅₀) of 50–1700 per well and incubated for 1 h, followed by the addition of HEP-2 cells. After 5 days of incubation at 37 °C, RSV-infected cells were fixed and stained with anti-RSV mAb and a horseradish peroxidase-labeled detection antibody. Tetramethylbenzidine substrate was added and Optical Density_{450nm} was measured. Half-maximal inhibitory concentrations (IC₅₀) were calculated using a four-parameter logistic nonlinear curve fitting and compared to the IC₅₀ values of the rRSV A and B reference variants on the same plate to determine fold-change in IC₅₀ values. Based on statistical power to detect IC₅₀ fold-change values relative to reference viruses, a cut-off of ≥5.0-fold-change IC₅₀ was used to define reduced susceptibility to nirsevimab and/or palivizumab neutralization.

2.9. Visualization RSV F protein amino acid substitutions

RSV A and B F protein structures were obtained from Protein Data Bank (PDB ID 5UDC and 5UDD, respectively) and visualized in PyMol version 2.2.2. Regions of RSV F were defined as follows: signal peptide = AA 1–23; p27 = AA 110–136; nirsevimab binding site = AA 62–69 and 196–212; palivizumab binding site = AA 262–275; and transmembrane and intracellular domains = 525–574 [5,6].

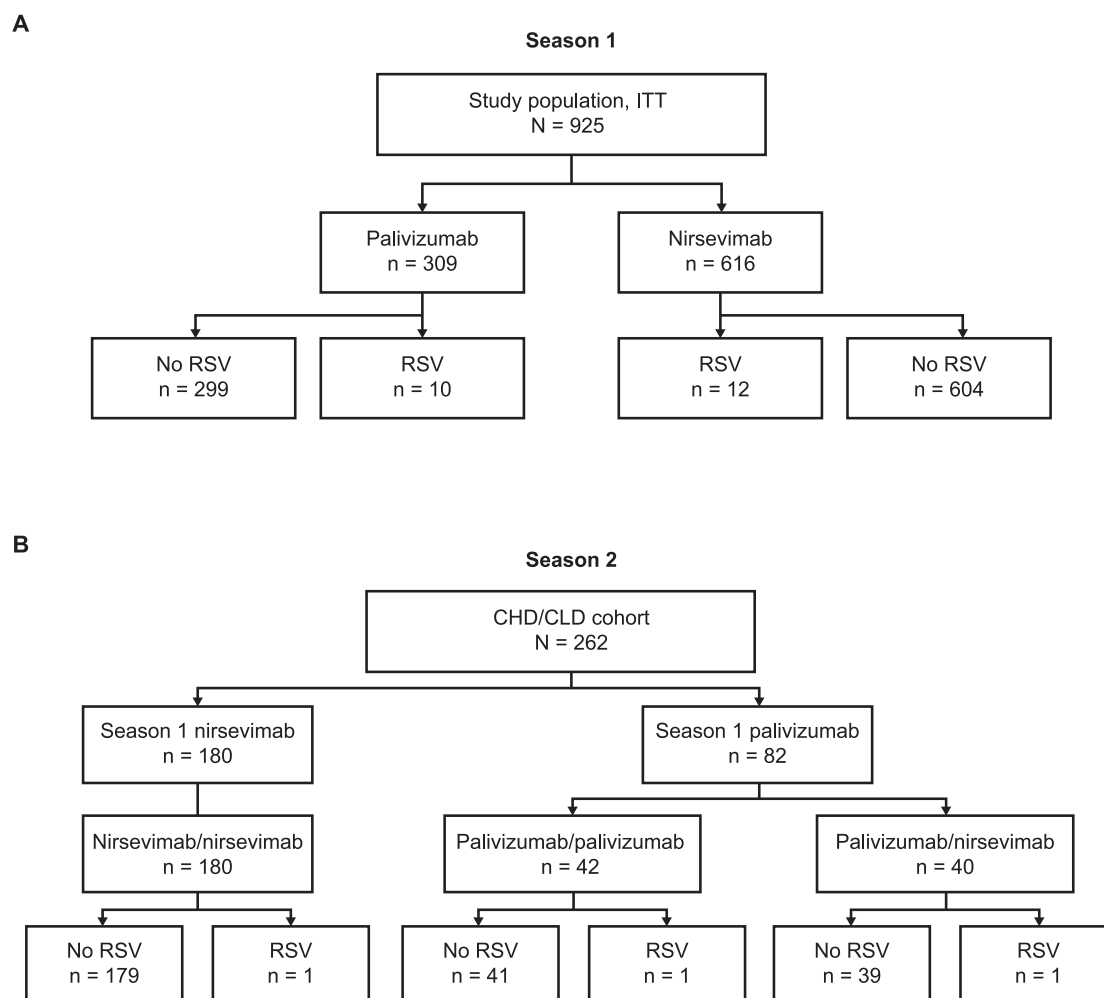


Fig. 1. Overview of participants with confirmed RSV infections in the MEDLEY trial (ITT population). CONSORT diagram indicating number of participants with confirmed cases of RSV infection during MEDLEY (A) Season 1 and (B) Season 2.

Abbreviations: CHD/CLD, congenital heart disease or chronic lung disease of prematurity; ITT, intent-to-treat; RSV, respiratory syncytial virus.

2.10. Pharmacokinetic sampling

Trial participants provided serum samples for PK assessments during their respective study seasons on Days 1 (pre-dose), 8 (Japan only), 15 (European Union [EU] only), 31 (non-EU participants), 151 and 361, and upon seeking medical attention for a respiratory infection. Serum concentrations of nirsevimab were determined using validated colorimetric enzyme-linked immunosorbent assay (AstraZeneca, Gaithersburg, MD, USA). Serum concentrations of palivizumab were measured using a validated Meso Scale Discovery electro-chemiluminescent assay (AstraZeneca, Gaithersburg, MD, USA). Data were analyzed graphically.

2.11. Measurement of viral load

Participant viral loads were measured by semiquantitative RT-PCR during assessments of nasal swabs to confirm the presence of RSV infection. Cycle threshold (CT) values are representative of viral loads.

2.12. Role of the funding source

This trial was supported by AstraZeneca and Sanofi. AstraZeneca employees supported trial design, data collection, data analysis and data interpretation in collaboration with the other authors. The sponsors paid for a professional medical writer who wrote the first draft of the manuscript under the direction of the authors.

3. Results

3.1. MEDLEY trial participants with confirmed RSV infections

A total of 925 infants (preterm cohort $n = 615$; CHD/CLD cohort $n = 310$) were randomized 2:1 to receive nirsevimab ($n = 616$) or palivizumab ($n = 309$) in Season 1 (Fig. 1A). Cases of confirmed RSV infection were observed in 1.9 % ($n = 12/616$) of nirsevimab recipients and 3.2 % ($n = 10/309$) of palivizumab recipients during Season 1. In total, 262 participants from the CHD/CLD cohort continued in the study in Season 2, with 180 participants in the nirsevimab/nirsevimab group, 40 participants in the palivizumab/nirsevimab group and 42 participants in the palivizumab/palivizumab group (Fig. 1B). A single case of RSV infection was observed among each of the three Season 2 groups, representing 0.5 % ($n = 1/180$) of participants in the nirsevimab/nirsevimab group, 2.5 % ($n = 1/40$) of participants in the palivizumab/nirsevimab group and 2.4 % ($n = 1/42$) of participants in the palivizumab/palivizumab group.

RSV cases are depicted according to sampling period and RSV subtype in Fig. 2. Of the 22 cases in Season 1, 9 cases occurred during the Day 1–151 sampling period (4 cases in nirsevimab recipients [all RSV A] and 5 cases in palivizumab recipients [3 RSV A, 2 RSV B]) and 13 cases occurred during the Day 152–361 sampling period (8 cases in nirsevimab recipients [3 RSV A, 5 RSV B] and 5 cases in palivizumab recipients [3 RSV A, 2 RSV B]). Two exploratory cases of RSV A infection were

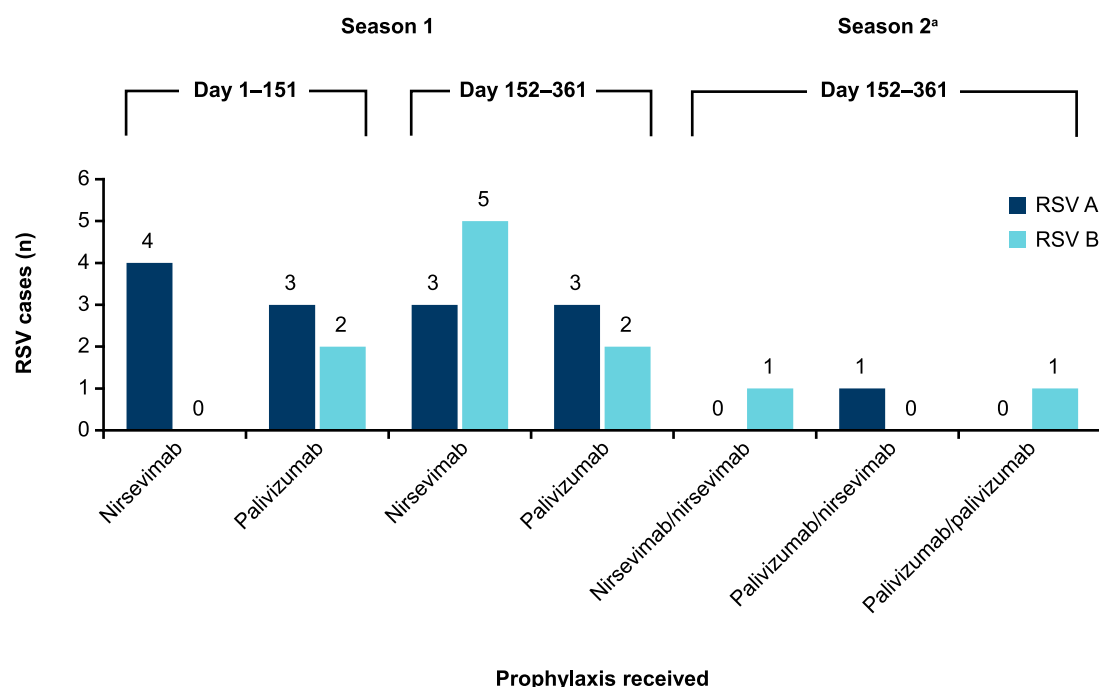


Fig. 2. Overview of confirmed RSV cases according to RSV antigenic subtype, MEDLEY trial sampling period and Season 1 and 2 study interventions.

^aNo cases of RSV infection were observed during the Season 2 Day 1–151 sampling period.

Abbreviations: RSV, respiratory syncytial virus.

observed in palivizumab recipients during Season 1 Day 1–151, with all other cases during this sampling period meeting the protocol definition of MA RSV LRTI (Supplemental Table 2). All cases observed in Season 2 occurred during the Day 152–361 sampling period, with 1 case of RSV A in the palivizumab/nirsevimab group, and 1 case of RSV B in both the palivizumab/nirsevimab and palivizumab/palivizumab groups. Contextualizing RSV cases by timing since first dose study intervention highlighted an increased number of Season 1 cases between Days 230–280 (Supplemental Fig. 1 A). All 3 Season 2 cases occurred between Day 300–360 (Supplemental Fig. 1B).

3.2. Geotemporal contextualization

RSV cases were contextualized against participant hospitalization and the geotemporal prevalence of RSV in the northern and southern hemispheres (Fig. 3). Twenty-one of the 22 Season 1 cases (12 in nirsevimab recipients; 9 in palivizumab recipients) were observed in the northern hemisphere (Fig. 3A). Eight participants – representing 0.81 % ($n = 5/616$) of overall Season 1 nirsevimab recipients and 0.97 % ($n = 3/309$) of Season 1 palivizumab recipients, respectively – in this cohort required hospitalization for RSV LRTI. Notably, 9 of these Season 1 northern hemisphere cases (5 in nirsevimab recipients, 4 in palivizumab recipients) occurred between March 2021 and August 2021, illustrating the impact of the disrupted 2021 RSV season on MEDLEY study participants. The remaining Season 1 case and 3 Season 2 cases occurred within the winter months of their respective geographic territories (Fig. 3B–C). One participant in the palivizumab/palivizumab group required hospitalization.

3.3. Genotypic characterization of RSV A and B isolates during MEDLEY

No nirsevimab binding site substitutions were identified among RSV A isolates recovered during Season 1 (Table 1). Three palivizumab neutralization-resistant substitutions were observed among RSV A isolates recovered from 2 palivizumab recipients during the Season 1 Day 1–151 sampling period (Table 1; Fig. 4A): a major variant (MAF ≥ 25 %) containing a Ser275Leu substitution and a minor variant (≥ 4 % to < 25 %

% MAF in RSV A) containing a mixed population of viruses with the Lys272Met and Lys272Thr substitutions, which comprised 6.8 % and 19.6 % of the respective allele frequencies in the sample [20,21]. No palivizumab binding site variants were observed among RSV A isolates recovered during the Day 152–361 or Season 2 sampling periods (Table 1).

Two clusters of point substitutions in the nirsevimab binding site were observed as major variants (MAF ≥ 25 %) among RSV B isolates recovered throughout both study seasons (Table 1; Fig. 4B). The co-occurring Ile206Met:Gln209Arg polymorphism was identified in 2 palivizumab recipients during the Day 1–151 sampling period. Additionally, the Ile206Met:Gln209Arg polymorphism was also observed in combination with an Ser211Asn substitution in 4 nirsevimab recipients and in 1 palivizumab recipient during the Day 152–361 sampling period, and in both isolates recovered during Season 2 (Table 1). No minor variant (MAF ≥ 5 % to < 25 % in RSV B) nirsevimab binding site substitutions, or major or minor palivizumab binding site variants, were identified among RSV B isolates obtained during either season of the study.

Additional substitutions in the RSV F protein outside the nirsevimab and palivizumab binding sites were observed as major and minor variants among RSV A and B viruses recovered during the study (Supplemental Material; Supplemental Tables 3–7). As depicted in Fig. 4, these substitutions were largely observed in non-extracellular regions of the RSV F protein, such as the signal peptide, p27, and the trans-membrane and intracellular domains.

3.4. Phenotypic characterization of observed RSV F substitutions

Data from rRSV microneutralization assays illustrate that any substitutions observed in the nirsevimab and palivizumab binding sites had minimal impact on the susceptibility of rRSV A and B viruses to nirsevimab neutralization (defined as fold-change in IC_{50} values > 5.0) (Table 1). As expected, the RSV A Lys272Met, Lys272Thr and Ser275Leu substitutions affected susceptibility to palivizumab neutralization (fold-change in IC_{50} : > 179.9 [Lys272Met]; > 213.8 [Lys272Thr]; > 426.95 [Ser275Leu]; Table 1). However, rRSV A viruses containing these

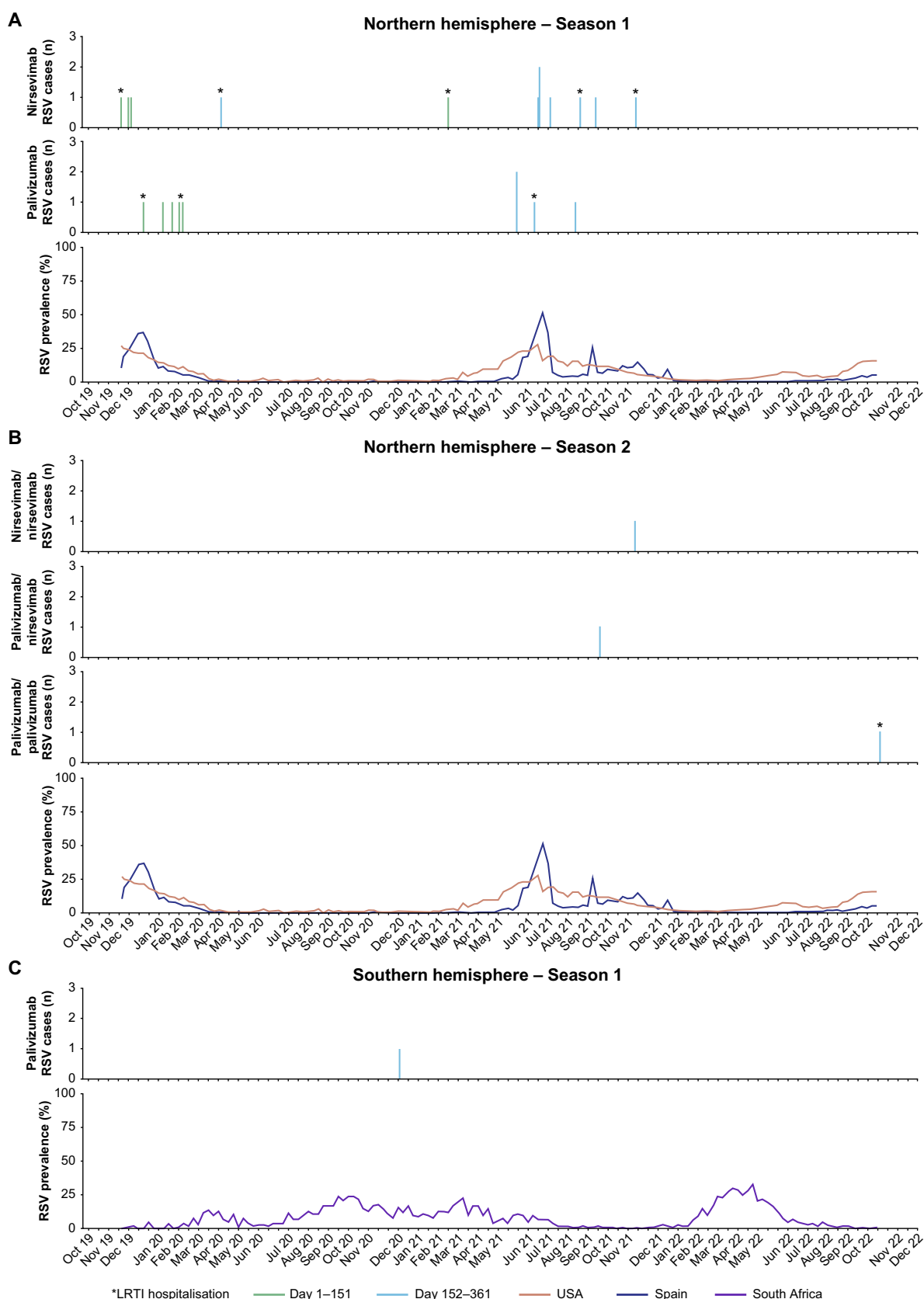


Fig. 3. Contextualization of RSV infections observed among MEDLEY study participants. Cases of confirmed RSV infections among MEDLEY study participants contextualized by sampling period, RSV geotemporal prevalence in the (A–B) northern and (C) southern hemispheres and for LRTI hospitalization. X-axis tick marks indicate 2-week intervals within each calendar month during the conduct of the trial. No cases were observed before November 2019.

Abbreviations: LRTI, lower respiratory tract infection; RSV, respiratory syncytial virus.

Table 1
Nirsevimab and palivizumab binding site changes observed in RSV A or B isolates recovered from MEDLEY Season 1 and 2 participants.

	Anti-RSV F mAb binding site ^a	MAF ^b	Season 1				Season 2 ^b			Fold-change IC ₅₀		IC ₅₀ , ng/mL	
			Day 1–151, n (%)		Day 152–361, n (%)		Day 152–361, n (%)			Nirsevimab	Palivizumab	Nirsevimab	Palivizumab
			Nirsevimab (N = 616)	Palivizumab (N = 309)	Nirsevimab (N = 616)	Palivizumab (N = 309)	Nirsevimab/ Nirsevimab (N = 180)	Palivizumab/ Nirsevimab (N = 40)	Palivizumab/ Palivizumab (N = 42)				
RSV A, n	–	–	4	3	3	3	0	1	0	–	–	–	–
None	N/A	Major	4 (100)	2 (67)	3 (100)	3 (100)	0	1 (100)	0	1	1	–	–
Ser275Leu	Palivizumab	(≥25 % MAF)	0	1 (33)	0	0	0	0	0	2.052	>426.95	1.592	>20,000
None	N/A	Minor (≥4	0	2 (67)	3 (100)	3 (100)	0	1 (100)	0	1	1	–	–
Lys272Met	Palivizumab	% to <25 % MAF)	0	1 (33) ^c	0	0	0	0	0	2.7	>179.9	4.3	>20,000
Lys272Thr			0	1 (33) ^c	0	0	0	0	0	1.1	>213.9	1.3	>20,000
RSV B, n^d	–	–	0	2	5	2	1	0	1	–	–	–	–
None	N/A	Major (≥25 % MAF)	0	0	1 (20)	1 (50)	0	0	0	1	1	–	–
Individual substitutions													
Ile206Met	Nirsevimab	Major	0	2 (100)	4 (80)	1 (50)	1 (100)	0	1 (100)	5.0	2.0	7.0	164.4
Gln209Arg		(≥25 %	0	2 (100)	4 (80)	1 (50)	1 (100)	0	1 (100)	0.5	3.1	0.9	213.7
Ser211Asn		MAF)	0	0	4 (80)	1 (50)	1 (100)	0	1 (100)	1.2	1.9	2.5	148.7
As observed													
Ile206Met: Gln209Arg	Nirsevimab	Major (≥25 %	0	2 (100)	0	0	0	0	0	0.2	1.3	0.4	79.8
Ile206Met: Gln209Arg: Ser211Asn		MAF)	0	0	4 (80)	1 (50)	1 (100)	0	1 (100)	0.5	3.6	0.9	204.4

Abbreviations: AA, amino acid residues; F, fusion; mAb, monoclonal antibody; IC₅₀, half-maximal inhibitory concentration; MAF, molecular allele frequency; N/A, not available; RSV, respiratory syncytial virus.

^a Nirsevimab binding site = AA 62–69 and 196–212; palivizumab binding site = AA 262–275.
^b No cases of RSV infection were observed during the Season 2 Day 1–151 sampling period.
^c A mixed population of viruses were identified in 1 participant.
^d No RSV B minor nirsevimab or palivizumab binding site variants were observed in Season 2 by the time of final database lock (June 6, 2023).

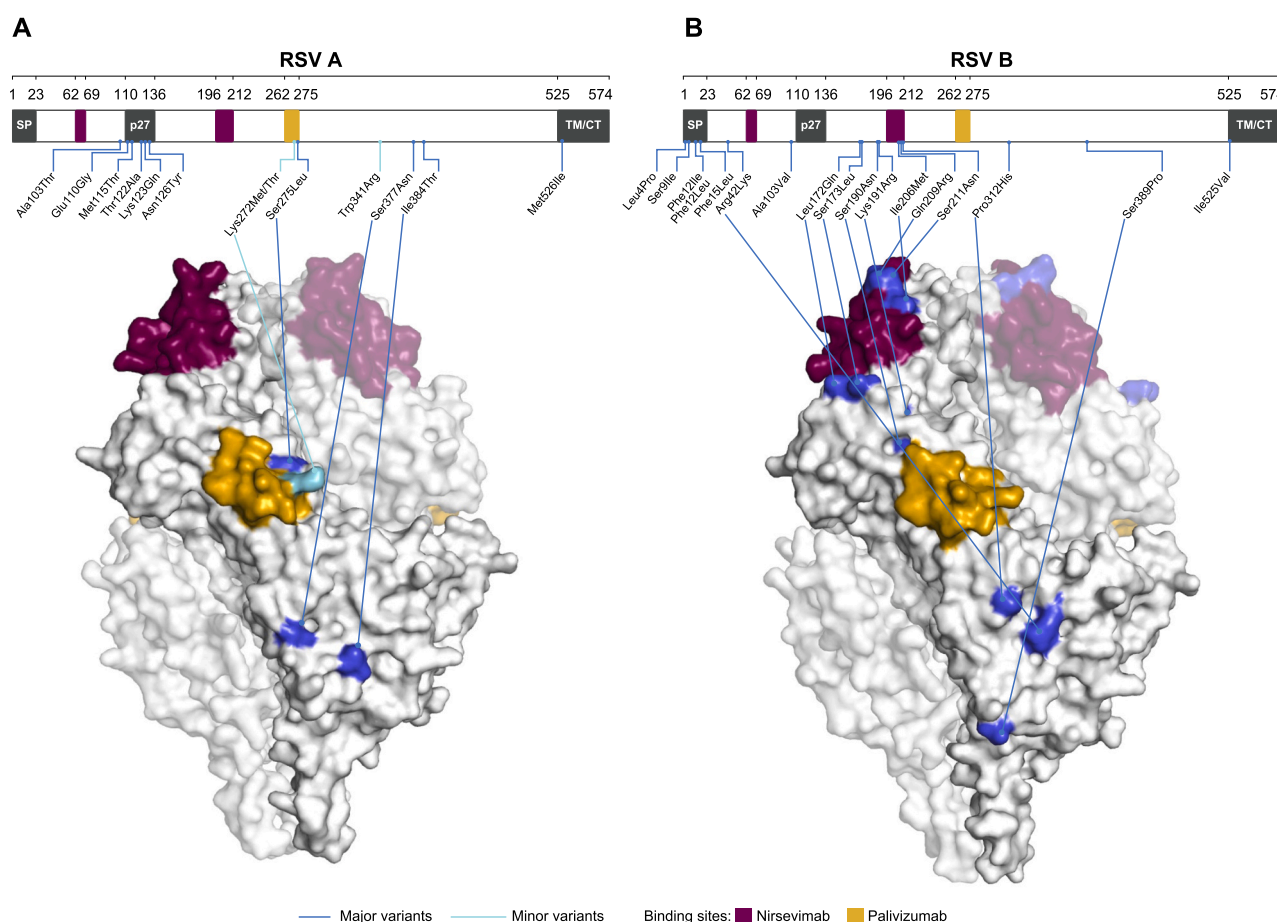


Fig. 4. Major and minor variant RSV A and B F protein substitutions observed during MEDLEY. Major and minor variant substitutions observed in the pre-F protein of (A) RSV A and (B) RSV B in relation to nirsevimab and palivizumab binding sites. The binding sites of nirsevimab (AA 62–69 and 196–212) and palivizumab (AA 262–275) are labeled in purple and yellow, respectively. The grey regions represent the signal peptide (AA 1–23), p27 (AA 110–136), and transmembrane and intracellular domains (AA 525–574) of the RSV F protein. Amino acid substitutions are labeled in dark blue for major variations and light blue for minor variants. These visualizations are depicted as surfaces, as the surface-exposed antigenic sites are the primary targets of antibodies. Thus, only the exposed amino acid residues are visible. Rear protomers are depicted in transparency. For panel A, no density at Ala103Thr and Trp341Arg as these residues are not surface exposed. For panel B, no density at Ala103Val.

Abbreviations: AA, amino acid residues; F, fusion; RSV, respiratory syncytial virus; SP, signal peptide; TM/CT, transmembrane and intracellular domains. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

substitutions remained susceptible to nirsevimab neutralization (fold-change IC_{50} : 2.7 [Lys272Met]; 1.1 [Lys272Thr]; 2.1 [Ser275Leu]).

rRSV B viruses with the double nirsevimab binding site Ile206Met:Gln209Arg substitution demonstrated no loss in susceptibility to nirsevimab or palivizumab neutralization (fold-change IC_{50} : 0.2 [nirsevimab]; 1.3 [palivizumab]; Table 1). A minor shift in susceptibility to nirsevimab neutralization was observed with Ile206Met (fold-change IC_{50} : 5.0) when both substitutions were tested individually. However, these rRSV B viruses with the Ile206Met substitution remained susceptible to palivizumab neutralization (fold-change IC_{50} : 2.0). rRSV B viruses with the Ser211Asn and Ile206Met:Gln209Arg:Ser211Asn substitutions were susceptible to both nirsevimab and palivizumab neutralization.

A summary of all observed RSV F protein substitutions outside the nirsevimab and palivizumab binding sites is provided in **Supplemental Tables 5–6**. We note that 4 of the 6 substitutions (Glu110Gly, Met115Thr, Thr122Ala and Asn126Tyr) observed among RSV A isolates occur in a region of RSV F (AA 110–136) that is cleaved by a host protease during virion assembly [5]. Additionally, 4 of the 11 substitutions (Ala103Val, Leu172Gln, Ser173Leu and Lys191Arg) observed among RSV B isolates have been tested in previous analyses during the nirsevimab clinical development program and have not affected susceptibility to nirsevimab or palivizumab neutralization [29]. Thus, while

investigations of the effects of the remaining substitutions on nirsevimab and palivizumab neutralization are ongoing, we do not anticipate that these remaining substitutions will adversely affect nirsevimab or palivizumab neutralization due to their respective locations within RSV F.

3.5. Serum concentrations of nirsevimab and palivizumab in participants with RSV infections

Serum concentrations of nirsevimab or palivizumab during both study seasons are shown in Fig. 5 by time after dose and are categorized according to the presence/absence of RSV infection. Participants who developed RSV infections during the trial had similar serum levels of nirsevimab or palivizumab compared with participants who remained uninfected during the trial.

3.6. Viral load in participants with RSV infections

Participant viral loads were similar among participants who experienced RSV infection during Season 1 irrespective of whether they received nirsevimab or palivizumab (Fig. 6). However, the low number of cases during Season 2 precludes interpretation. Due to the low overall number of RSV cases during the trial, participant data were compared to the placebo arm of the MELODY study. Although median CT values were

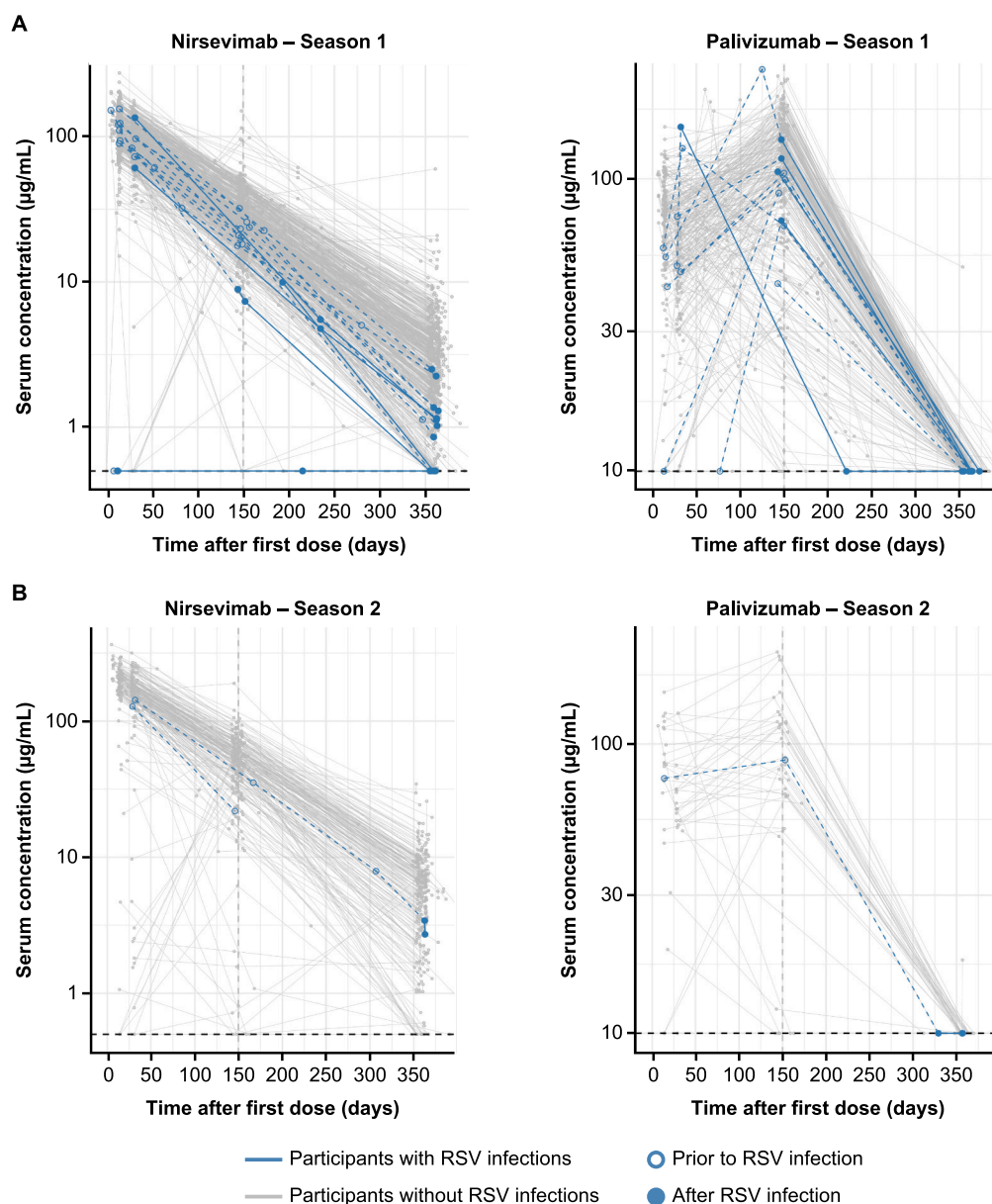


Fig. 5. Serum concentrations versus time after first nirsevimab or palivizumab dose per season in MEDLEY participants. Nirsevimab and palivizumab serum concentrations in MEDLEY study participants during (A) Season 1 and (B) Season 2. Grey lines and circles represent participants who did not develop RSV infections during the trial. Blue lines and circles denote participants who developed RSV infections during the trial; dashed lines/open circles represent samples obtained prior to RSV infection and solid lines/closed circles represent samples obtained after infection. The LLOQ was 0.5 µg/mL for nirsevimab and 10 µg/mL for palivizumab. The vertical dashed grey line denotes Day 151 (i.e., 150 days post first dose study intervention). (A) One participant in the nirsevimab group in Season 1 with a confirmed RSV infection had no measurable levels of nirsevimab at any time point, strongly indicating a dosing error. There were confirmed dosing errors in 3 participants in the palivizumab group in Season 1. One participant received doses on Days 1, 31, 61 and 91, but missed the Day 121 dose, leading to concentrations below the LLOQ by Day 222. No samples were available for this participant between Days 31 and Day 222. A second participant received doses on Days 1, 91 and 121, but missed doses on Days 31 and 61, leading to a concentration below the LLOQ at the first available observation on Day 78. Finally, a third participant did not receive their first Season 1 palivizumab dose, resulting in a concentration below the LLOQ at the first available observation on Day 14. *Abbreviations:* LLOQ, lower limit of quantification; RSV, respiratory syncytial virus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

numerically higher in MEDLEY participants compared with MELODY participants, thereby representing a trend towards lower viral loads compared with placebo recipients, the range of viral titers was similar between both studies.

4. Discussion

Immunoprophylaxis with anti-RSV F protein mAbs has a demonstrated history of reducing the risk of pediatric hospitalization following

RSV LRTI [8,12–15]. As illustrated by *in vitro* and clinical studies, the sustained effectiveness of passive immunization strategies depends on the conservation of the neutralizing epitopes recognized by existing and emerging mAbs [10,18,19]. Although the nirsevimab binding site has been demonstrated to be highly conserved since the 1950s, new RSV variants have the potential to emerge with each replication cycle, necessitating ongoing global surveillance for potential neutralization-resistant mutants [6]. This analysis of confirmed RSV infections from the phase 2/3 MEDLEY trial showed that most naturally occurring

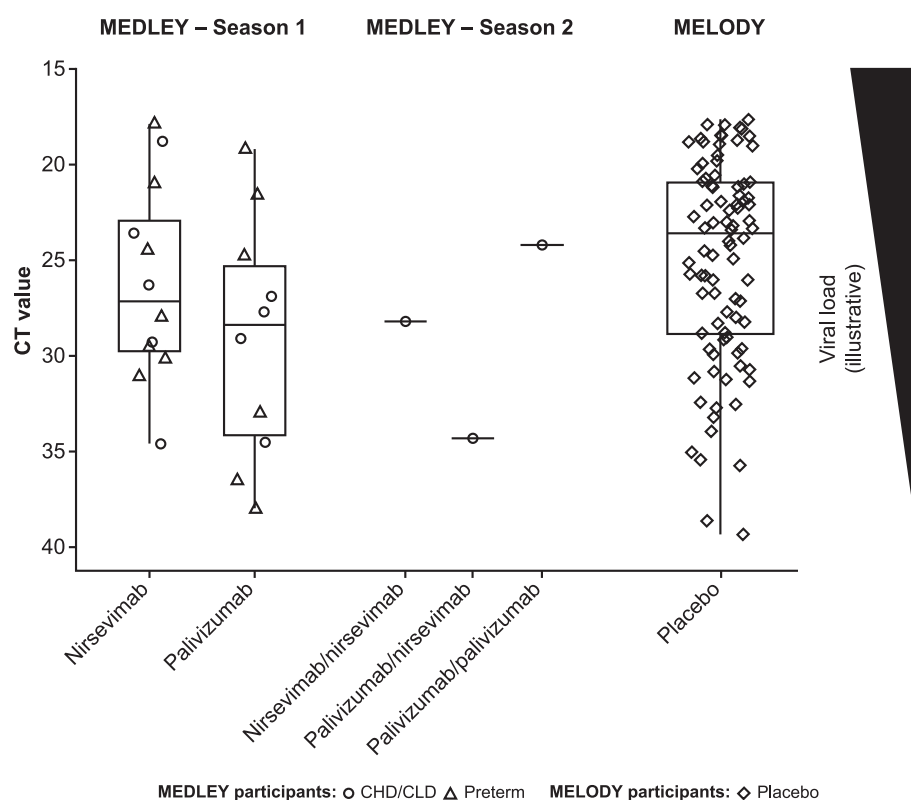


Fig. 6. Viral load in participants who experienced RSV infections during MEDLEY Seasons 1 and 2. Viral load among participants with confirmed RSV infections during MEDLEY per study intervention, compared with preterm and term participants from the placebo arm of the phase 3 MELODY study. Boxes indicate the 25th and 75th percentiles, with the middle line indicating the median value, and the whiskers represent the range.

Abbreviations: CHD/CLD, congenital heart disease or chronic lung disease of prematurity; CT, cycle threshold; RSV, respiratory syncytial virus.

amino acid substitutions in the RSV F protein were located predominantly in non-extracellular regions of the RSV F protein, and that nirsevimab neutralizes a range of RSV A and B viruses with nirsevimab and palivizumab binding site substitutions.

A low rate of RSV infection was observed during the trial, with 2.4 % ($n = 22/925$) of Season 1 and 1.1 % ($n = 3/262$) of Season 2 participants experiencing confirmed RSV infections. This low rate of infection could be attributed to the lack of a placebo arm in the trial design, as both nirsevimab and palivizumab have been shown to be effective RSV prophylaxes for several different populations of infants and children ≤ 24 months of age [8,13–15]. Our data were also influenced by, and reflect the impact of, the non-pharmacological public health measures that were introduced to mitigate the COVID-19 pandemic (i.e., lockdowns, social distancing, facemask mandates, school closures, etc.). The lifting of many of these interventions in 2021 played a partial role in the unusual RSV epidemiology that was seen during the 2020–2021 RSV season, with 40.9 % ($n = 9/22$) of the cases observed in the northern hemisphere during Season 1 occurring during summer months, rather than the typical winter respiratory virus season [3,30]. Both nirsevimab and palivizumab are meant to be administered at the beginning of the RSV season. Therefore, a disrupted RSV seasonality following the COVID-19 pandemic may also explain the increased number of RSV infections observed during our Day 152–361 sampling period (i.e., 13 Day 152–361 cases versus 9 Day 1–151 cases during Season 1), as the timing of dose administration was not optimized to reflect the unexpected and atypical geotemporal prevalence of RSV observed during the 2020–2021 season. The low but similar rate of confirmed RSV cases requiring hospitalization between both trial arms precludes further interpretation; however, we note that nirsevimab retained neutralization activity against all binding site substitutions identified during the study, irrespective of primary or exploratory case definitions.

There were no major nirsevimab binding site variants among RSV A isolates recovered during the trial. The Ser275Leu palivizumab neutralization-resistant substitution was detected as a major RSV A variant in 1 participant [20]. rRSV A viruses containing the Ser275Leu substitution remained susceptible to nirsevimab neutralization. Three individual major variant nirsevimab binding site substitutions – Ile206Met, Gln209Arg and Ser211Asn – were observed among RSV B isolates. While the Ile206Met:Gln209Arg substitution is susceptible to nirsevimab neutralization, the Ile206Met substitution when tested in isolation demonstrated a minor reduction in susceptibility to nirsevimab neutralization (fold-change IC_{50} of 5.02 as compared to reference virus). Previous molecular modeling analyses have illustrated that the Gln209Arg substitution improves electrostatic interactions between RSV F and nirsevimab, thus restoring full neutralization susceptibility [27]. Although the Ile206Met:Gln209Arg polymorphism was observed in RSV B viruses with a prevalence of 65.8 % from 2015 to 2021, the Ile206Met substitution without Gln209Arg has only been observed in 0.6 % of circulating RSV B viruses and has not increased in prevalence in successive RSV seasons [6]. The triple Ile206Met:Gln209Arg:Ser211Asn substitution has been observed at a low (1.1 %) cumulative frequency in ongoing surveillance studies since 2015, but was 28.6 % prevalent during the 2021 RSV season, coinciding in part with the MEDLEY study period [6]. Minor variant polymorphisms were also examined to fully assess the effects of emergent mutations on nirsevimab and palivizumab neutralization. No minor nirsevimab binding site variants were identified among participants' RSV A isolates. However, a mixed population of viruses containing the Lys272Met and Lys272Thr palivizumab neutralization-resistant substitutions were identified as minor RSV A variants in 1 participant's sample [21]. These substitutions have been rarely observed (i.e., prevalence of <0.05 %) during previous nirsevimab surveillance studies and have not increased in geotemporal

prevalence in successive RSV seasons between 2015 and 2021 [6]. rRSV A viruses containing the Lys272Met and Lys272Thr substitutions remained susceptible to nirsevimab neutralization. No minor RSV B variants were detected among participants' samples. Although limited, these data complement previous studies illustrating the broad and potent neutralization activity of nirsevimab across different surveillance studies throughout the clinical development program and that nirsevimab escape mutants are rare [6,27]. Additionally, previously identified nirsevimab neutralization-resistant substitutions (e.g., Lys68Glu in RSV A; Ile64Thr, Lys68Asn, Lys68Gln, Asn201Ser/Thr and Asn208Ser in RSV B) remained susceptible to palivizumab neutralization, illustrating an important absence of cross-resistance between both mAbs [6,11,18,27].

Contextualization of RSV infections against anti-RSV F protein mAb serum concentrations suggests that several cases may have been the result of dosing errors (1 in a nirsevimab recipient in Season 1) or missed doses (3 in palivizumab recipients also in Season 1). A beneficial characteristic of nirsevimab is that it confers protection for the duration of a 5-month RSV season with a single dose, and thus may prevent such missed dosing opportunities that would potentially leave infants vulnerable to RSV infection. Nirsevimab and palivizumab serum concentrations in participants with RSV infections who received all planned doses were similar to those observed in participants who did not develop infection or, feasibly, were asymptomatic or had mild infections not requiring medical attention and were consequently not identified during the trial. Studies of other viruses have illustrated that the emergence of viral resistance is influenced by the balance between the selective pressure exerted by antiviral therapies and the capacity of the virus to generate a pool of viable resistant mutants [31]. However, there was no increase in nirsevimab resistance observed among RSV isolates obtained during the MEDLEY trial, despite a high proportion of cases occurring during the Day 152–361 sampling period during which waning nirsevimab serum concentrations were observed. Participants viral loads were similar among nirsevimab and palivizumab recipients and were similar to the range observed in a larger population of healthy preterm and term infants from the placebo arm of the phase 3 MELODY trial [13,14]. These observations are consistent with previous studies of maternally transferred antibodies and other anti-RSV F protein mAbs, which suggest that although high levels of anti-RSV F protein neutralizing antibodies can reduce the risk of hospitalization from RSV-associated LRTI, they are not sufficient to prevent asymptomatic infection or mild disease [27,32,33]. Moreover, previous studies of both anti-RSV F protein mAbs have shown that nirsevimab and palivizumab administration still allows for the development of host natural immune responses [29,34].

There are several limitations to this analysis. As previously discussed, the use of active RSV interventions in both study arms, alongside an altered RSV circulation due to COVID-19 pandemic control measures during the study period, resulted in a small number of confirmed-RSV cases and did not allow for any between group statistical analyses [30]. Consequently, this analysis does not fully represent the number of cases observed during a typical RSV season and these data should be interpreted within the context of other nirsevimab surveillance studies [6,27]. Additionally, as the nirsevimab viral surveillance program has been designed to evaluate F sequence variations, we are unable to infer whether variations in other surface RSV virion proteins (e.g., G and small hydrophobic proteins) affect nirsevimab or palivizumab binding. In summary, nirsevimab neutralized all nirsevimab binding site substitutions observed during the MEDLEY trial, and no resistance-associated substitutions were identified beyond 150 days post dose as levels of nirsevimab waned. These data complement previous studies from the nirsevimab clinical development program, illustrating that nirsevimab neutralizes >99 % of contemporary RSV variants and that nirsevimab neutralization-resistant variants remain rare, therefore suggesting that highly potent and broadly neutralizing mAbs may limit the potential emergence of neutralization-resistant viral variants [6,27]. At the time of manuscript preparation, nirsevimab has been approved

for the prevention of RSV-associated lower respiratory tract disease in numerous geographic territories and further viral surveillance studies to monitor for nirsevimab resistance are underway [17]. Our observations support the use of nirsevimab for the prevention of RSV-associated LTRIs in infants born during or entering their first RSV season and in children aged ≤24 months who remain vulnerable to severe RSV disease in their second season.

CRedit authorship contribution statement

Kevin M. Tuffy: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization, Data Verification. **Bahar Ahani:** Writing – review & editing, Writing – original draft, Formal analysis. **Joseph B. Domachowske:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Data curation, Data Verification. **Kenji Furuno:** Writing – review & editing, Writing – original draft, Supervision, Investigation. **Hong Ji:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Shabir A. Madhi:** Writing – review & editing, Writing – original draft, Supervision, Investigation. **Vaishali S. Mankad:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Ulrika Wählby Hamrén:** Writing – review & editing, Writing – original draft, Formal analysis. **Tonya Villafana:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Yingyi Wang:** Writing – review & editing, Writing – original draft, Validation. **Elizabeth J. Kelly:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **Deidre Wilkins:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Data curation, Conceptualization, Data Verification.

Declaration of competing interest

Kevin M. Tuffy, Bahar Ahani, Hong Ji, Vaishali S. Mankad, Ulrika Wählby Hamrén, Tonya Villafana, Yingyi Wang and Deidre Wilkins are current employees of AstraZeneca and may hold AstraZeneca stock or stock options. Elizabeth J. Kelly is a former employee of AstraZeneca and a current employee of Sanofi and may hold stock or stock options for both institutions. Ulrika Wählby Hamrén, Tonya Villafana and Deidre Wilkins are named inventors on patents planned, issued or pending related to nirsevimab. Joseph B. Domachowske has received funding to their institution for the conduct of clinical trials from AstraZeneca, Merck and Regeneron, honoraria from Sanofi and has participated in advisory boards for GSK and Sanofi. Kenji Furuno has received honoraria from AstraZeneca and AbbVie. Shabir A. Madhi has received funding from AstraZeneca, Novavax, Providence, Gritstone and ImmunityBio for the conduct of clinical trials; institutional grants from the Bill and Malinda Gates Foundation, GSK, Pfizer and Minervax, honoraria from GSK and MSD and is a member of Data Safety Monitoring Boards for PATH and CAPRISA.

Data availability

Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data sharing policy described at <https://www.astrazenecaclinicaltrials.com/our-transparency-commitments/>.

Data for studies directly listed on Vivli can be requested through Vivli at www.vivli.org. Data for studies not listed on Vivli could be requested through Vivli at <https://vivli.org/members/enquiries-about-studies-not-listed-on-the-vivli-platform/>. AstraZeneca Vivli member page is also available outlining further details: <https://vivli.org/ourmember/astrazeneca/>.

The raw DNA sequence data generated in this study have been deposited in the GenBank® database under accession code PRJNA1154639.

Acknowledgements

We thank MEDLEY trial participants and their families, members of the investigator teams and the full clinical team at AstraZeneca. This research was supported by AstraZeneca and Sanofi. Nirsevimab is being developed and commercialized in partnership between AstraZeneca and Sanofi. The authors acknowledge Rebecca A. Bachmann, PhD, of AstraZeneca, for facilitating author discussion and providing strategic advice and critical review of this manuscript, and Anastasia Aksyuk, PhD, of AstraZeneca, for their support in developing the RSV micro-neutralization assays described in this manuscript. Additionally, the authors would like to acknowledge Rolf Kramer, PhD and Christian Felter, MD, both of Sanofi, for providing the RSV epidemiology data used to contextualize the RSV cases described in this manuscript.

Medical writing support was provided by Craig O'Hare, PhD, of Ashfield MedComms, an Inizio company, which was in accordance with Good Publication Practice (GPP) 2022 guidelines (<https://www.ismpp.org/gpp-2022>; Ann Intern Med. 2022 doi:<https://doi.org/10.7326/M22-1460>) and funded by AstraZeneca.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2024.126276>.

References

- [1] Langley JM, Bianco V, Domachowske JB, Madhi SA, Stoszek SK, Zaman K, et al. Incidence of respiratory syncytial virus lower respiratory tract infections during the first 2 years of life: a prospective study across diverse global settings. *J Infect Dis* 2022;226(3):374–85.
- [2] Hall CB, Weinberg GA, Iwane MK, Blumkin AK, Edwards KM, Staat MA, et al. The burden of respiratory syncytial virus infection in young children. *N Engl J Med* 2009;360(6):588–98.
- [3] Griffiths C, Drews SJ, Marchant DJ. Respiratory syncytial virus: infection, detection, and new options for prevention and treatment. *Clin Microbiol Rev* 2017;30(1):277–319.
- [4] Mazur NI, Terstappen J, Baral R, Bardaji A, Beutels P, Buchholz UJ, et al. Respiratory syncytial virus prevention within reach: the vaccine and monoclonal antibody landscape. *Lancet Infect Dis* 2023;23(1):e2–21.
- [5] Battles MB, McLellan JS. Respiratory syncytial virus entry and how to block it. *Nat Rev Microbiol* 2019;17(4):233–45.
- [6] Wilkins D, Langedijk AC, Lebbink RJ, Morehouse C, Abram ME, Ahani B, et al. Nirsevimab binding-site conservation in respiratory syncytial virus fusion glycoprotein worldwide between 1956 and 2021: an analysis of observational study sequencing data. *Lancet Infect Dis* 2023;23(7):856–66.
- [7] US Food and Drug Administration (FDA). SYNAGIS® (palivizumab) prescribing information. Updated March 2014. Available at, https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/103770s51851bl.pdf. Last accessed 12 July 2024.
- [8] Garegnani L, Styrmsdottir L, Roson Rodriguez P, Escobar Liquitay CM, Esteban I, Franco JV. Palivizumab for preventing severe respiratory syncytial virus (RSV) infection in children. *Cochrane Database Syst Rev* 2021;11(11):CD013757.
- [9] American Academy of Pediatrics Committee on infectious diseases, American Academy of Pediatrics bronchiolitis guidelines committee. Updated guidance for palivizumab prophylaxis among infants and young children at increased risk of hospitalization for respiratory syncytial virus infection. *Pediatrics* 2014;134(2):415–20.
- [10] Zhu Q, McLellan JS, Kallewaard NL, Ulbrandt ND, Palaszynski S, Zhang J, et al. A highly potent extended half-life antibody as a potential RSV vaccine surrogate for all infants. *Sci Transl Med* 2017;9(388):eaaj1928.
- [11] US Food and Drug Administration (FDA). BEYFORTUS™ (nirsevimab-alip) prescribing information. Updated July 2023. Available at, https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/761328s0001bl.pdf. Last accessed 12 July 2024.
- [12] Griffin MP, Yuan Y, Takas T, Domachowske JB, Madhi SA, Manzoni P, et al. Single-dose nirsevimab for prevention of RSV in preterm infants. *N Engl J Med* 2020;383(5):415–25.
- [13] Muller WJ, Madhi SA, Seoane Nunez B, Baca Cots M, Bosheva M, Dagan R, et al. Nirsevimab for prevention of RSV in term and late-preterm infants. *N Engl J Med* 2023;388(16):1533–4.
- [14] Hammit LL, Dagan R, Yuan Y, Baca Cots M, Bosheva M, Madhi SA, et al. Nirsevimab for prevention of RSV in healthy late-preterm and term infants. *N Engl J Med* 2022;386(9):837–46.
- [15] Simoes EAF, Madhi SA, Muller WJ, Atanasova V, Bosheva M, Cabanas F, et al. Efficacy of nirsevimab against respiratory syncytial virus lower respiratory tract infections in preterm and term infants, and pharmacokinetic extrapolation to infants with congenital heart disease and chronic lung disease: a pooled analysis of randomised controlled trials. *Lancet Child Adolesc Health* 2023;7(3):180–9.
- [16] Drysdale SB, Cathie K, Flamein F, Knuf M, Collins AM, Hill HC, et al. Nirsevimab for prevention of hospitalizations due to RSV in infants. *N Engl J Med* 2023;389(26):2425–35.
- [17] Sanofi press release. FDA approves Beyfortus™ (nirsevimab-alip) to protect infants against RSV disease. 2023. Available at, <https://www.sanofi.com/assets/dotcom/pressreleases/2023/2023-07-17-17-00-00-2705911-en.pdf>. Last accessed 12 July 2024.
- [18] Zhu Q, Lu B, McTamney P, Palaszynski S, Diallo S, Ren K, et al. Prevalence and significance of substitutions in the fusion protein of respiratory syncytial virus resulting in neutralization escape from antibody MEDI8897. *J Infect Dis* 2018;218(4):572–80.
- [19] Simoes EAF, Forleo-Neto E, Geba GP, Kamal M, Yang F, Cicirello H, et al. Suptavumab for the prevention of medically attended respiratory syncytial virus infection in preterm infants. *Clin Infect Dis* 2021;73(11):e4400–8.
- [20] Zhu Q, McAuliffe JM, Patel NK, Palmer-Hill FJ, Yang CF, Liang B, et al. Analysis of respiratory syncytial virus preclinical and clinical variants resistant to neutralization by monoclonal antibodies palivizumab and/or motavizumab. *J Infect Dis* 2011;203(5):674–82.
- [21] Oliveira DB, Iwane MK, Prill MM, Weinberg GA, Williams JV, Griffin MR, et al. Molecular characterization of respiratory syncytial viruses infecting children reported to have received palivizumab immunoprophylaxis. *J Clin Virol* 2015: 6526–31.
- [22] Domachowske J, Madhi SA, Simoes EAF, Atanasova V, Cabanas F, Furuno K, et al. Safety of nirsevimab for RSV in infants with heart or lung disease or prematurity. *N Engl J Med* 2022;386(9):892–4.
- [23] Domachowske J, Madhi SA, Simoes EAF, Atanasova V, Cabanas F, Furuno K, et al. Protocol for: safety of nirsevimab for RSV in infants with heart or lung disease or prematurity. *N Engl J Med* 2022;386(9):892–4.
- [24] National Institutes of Health (NIH). NCT03959488: a study to evaluate the safety of MEDI8897 for the prevention of medically attended respiratory syncytial virus (RSV) lower respiratory tract infection (LRTI) in high-risk children. Available at, <https://clinicaltrials.gov/ct2/show/NCT03959488>. Last accessed 12 July 2024. [Clinicaltrials.gov/2023](https://clinicaltrials.gov/2023).
- [25] Domachowske J, Madhi SA, Simoes EAF, Atanasova V, Cabanas F, Furuno K, et al. Supplement to: safety of nirsevimab for RSV in infants with heart or lung disease or prematurity. *N Engl J Med* 2022;386(9):892–4.
- [26] Domachowske JB, Chang Y, Atanasova V, Caba Amp XFAF, Furuno K, Nguyen KA, et al. Safety of re-dosing nirsevimab prior to RSV season 2 in children with heart or lung disease. *J Pediatric Infect Dis Soc* 2023;12(8):477–80.
- [27] Ahani B, Tuffy KM, Aksyuk AA, Wilkins D, Abram ME, Dagan R, et al. Molecular and phenotypic characteristics of RSV infections in infants during two nirsevimab randomized clinical trials. *Nat Commun* 2023;14(1):4347.
- [28] Tabatabai J, Prifert C, Pfeil J, Grulich-Henn J, Schnitzler P. Novel respiratory syncytial virus (RSV) genotype ON1 predominates in Germany during winter season 2012–13. *PLoS One* 2014;9(10):e109191.
- [29] Wilkins D, Yuan Y, Chang Y, Aksyuk A, Núñez B, Wahlby-Hamrén U, et al. Durability of neutralizing RSV antibodies following nirsevimab administration and elicitation of the natural immune response to RSV infection in infants. *Nat Med* 2023;29(5):1172–9.
- [30] Bozzola E, Barni S, Villani A. Respiratory syncytial virus pediatric hospitalization in the COVID-19 era. *Int J Environ Res Public Health* 2022;19(23):15455.
- [31] Focosi D, McConnell S, Sullivan DJ, Casadevall A. Analysis of SARS-CoV-2 mutations associated with resistance to therapeutic monoclonal antibodies that emerge after treatment. *Drug Resist Updat* 2023;71100991. <https://pubmed.ncbi.nlm.nih.gov/37572569/>.
- [32] Walsh EE, Wang L, Falsey AR, Qiu X, Corbett A, Holden-Wiltse J, et al. Virus-specific antibody, viral load, and disease severity in respiratory syncytial virus infection. *J Infect Dis* 2018;218(2):208–17.
- [33] Buchwald AG, Graham BS, Traore A, Haidara FC, Chen M, Morabito K, et al. Respiratory syncytial virus (RSV) neutralizing antibodies at birth predict protection from RSV illness in infants in the first 3 months of life. *Clin Infect Dis* 2021;73(11):e4421–7.
- [34] Claydon J, Sur A, Callejas A, Ladd M, Kwan E, Taylor R, et al. Respiratory syncytial virus-neutralizing serum antibody titers in infants following palivizumab prophylaxis with an abbreviated dosing regimen. *PLoS One* 2017;12(4):e0176152.