Daily intranasal palivizumab to prevent respiratory syncytial virus infection in healthy preterm infants: a phase 1/2b randomized placebo-controlled trial

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Summary

Background Mucosal administration of monoclonal antibodies (mAbs) against respiratory pathogens is a promising alternative for systemic administration because lower doses are required for protection. Clinical development of mucosal mAbs is a highly active field yet clinical proof-of-concept is lacking.

Methods In this investigator-initiated, double-blind, randomized placebo-controlled trial, we evaluated intranasal palivizumab for the prevention of RSV infection in preterm infants (Dutch Trial Register NTR7378 and NTR7403). We randomized infants 1:1 to receive intranasal palivizumab (1 mg/mL) or placebo once daily during the RSV season. Any RSV infection was the primary outcome and RSV hospitalization was the key secondary outcome. The primary outcome was analyzed with a mixed effect logistic regression on the modified intention-to-treat population.

Findings We recruited 268 infants between Jan 14, 2019 and Jan 28, 2021, after which the trial was stopped for futility following the planned interim analysis. Adverse events were similar in both groups (22/134 (16.4%) palivizumab arm versus 26/134 (19.4%) placebo arm). There were 6 dropouts and 168 infants were excluded from the efficacy analyses due to absent RSV circulation during the SARS-CoV-2 pandemic. Any RSV infection was similar in infants in both groups (18/47 (38.3%) palivizumab arm versus 11/47 (23.4%) placebo arm; aOR 2.2, 95% CI 0.7–6.5).

Interpretation Daily intranasal palivizumab did not prevent RSV infection in late preterm infants. Our findings have important implications for the clinical development of mucosal mAbs, namely the necessity of timely interim analyses and further research to understand mucosal antibody half-life.

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Keywords: Intranasal administration; Respiratory syncytial virus infections; Monoclonal antibody; Infant; Preventive medicine

Introduction

Globally, respiratory syncytial virus (RSV) is the second cause of death in the infant period,[1] yet there is no vaccine or treatment available for children in low- and lower-middle income countries (LMICs), where disease burden is highest.[2] Of the vaccine candidates and monoclonal antibodies (mAbs) in late-stage clinical trials—including the recently registered mAb nirsevimab[3]

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Research in context

Evidence before this study

Systemic IgG antibodies lack efficient access to the mucosa. The need for high doses of systemic monoclonal antibodies (mAbs) to reach a therapeutic level in the respiratory tract may be overcome through local administration. Preclinical studies support efficacy of local administration of mAbs against respiratory pathogens yet the highly active clinical development of mucosal mAbs is lacking proof-of-concept. Respiratory syncytial virus (RSV) is the second cause of death in the infant period. Palivizumab, a humanized mAb against the surface F protein of RSV, has been market-approved for more than 20 years, but access is limited to high-risk infants due to prohibitive costs. Administration via monthly intramuscular (i.m.) injections is burdensome and still allows for significant breakthrough infections occurring at low trough antibody levels. Intranasal (i.n.) palivizumab provides full protection against experimental RSV infection in mice in a dose-dependent manner for at least a week after administration.

Added value of this study

This study is the first trial to investigate the efficacy of i.n. administration of antiviral monoclonal antibodies to prevent respiratory infection. We show palivizumab is stable in nose drop formulation and that daily intranasal palivizumab (50 µL per nostril of 1 mg/mL solution) is safe for clinical use. In the planned interim analysis, we do not find efficacy against lab-confirmed RSV infection for this child-friendly and affordable route of administration. Although the sample size was smaller than planned due to lack of RSV circulation, we expect this limitation to have had no impact on the conditional power of the study as the trial was terminated early due to a planned interim analysis.

Implications of all the available evidence

Intranasal antibody drug development is highly active with 11 drug candidates in development for SARS-CoV-2, RSV, and influenza. Our study demonstrates the necessity of a timely interim analysis to evaluate efficacy and avoid wasted time and capital. Further research on pharmacokinetics and medication dosage is needed to understand lack of efficacy of i.n. administration.

and RSV maternal vaccine[4]—none target the LMIC market, where RSV mortality is highest. Palivizumab, a humanized mAb against the surface F protein of RSV, has been market-approved for more than 20 years, but access is limited to high-risk infants due to its prohibitive costs and limited efficacy. Administration via monthly intramuscular (i.m.) injections is burdensome and still allows for significant breakthrough infections occurring at low trough antibody levels.

According to Ku et al.,[5] circulating IgG antibodies lack efficient access to mucosal compartments. Antibody levels in the lung are 200–500 times lower than in blood after intravenous infusion and 30–70 times lower in the nose after i.m. monoclonal antibody injection[6] resulting in the need for high doses of potent neutralizing mAbs with only a small antiviral effect in the respiratory tract. The need for high doses of systemic mAbs to reach a therapeutic level may be overcome through local administration. Mucosal administration of mAbs may offer a key solution for major respiratory pathogens: a dose-sparing highly targeted prevention stopping infection at the site of viral entry. Preclinical studies support efficacy of local administration of mAbs against respiratory pathogens.[7] We demonstrated that intranasal (i.n.) palivizumab provides full protection against experimental RSV infection in mice in a dose-dependent manner for at least a week after administration.[8] Recently, low doses of i.n. hyper-enriched anti-RSV IgG were reported to inhibit infection in mice.[9] Multiple preclinical studies show that i.n. SARS-CoV-2 neutralizing mAbs provide protection in mice.[5,10]

Intranasal antibody drug development is a highly active field with 11 drug candidates in development for SARS-CoV-2, RSV, and influenza. Development is led by pharmaceutical companies, public private partnerships, and non-profit networks with seven preclinical candidates, two candidates in phase I trials, and one candidate in a phase I/II trial (Supplementary Appendix 1). This manuscript reports the first published data from a proof-of-concept trial in infants and the findings are highly relevant to ongoing clinical development.

Intranasal palivizumab offers a child-friendly and affordable alternative for i.m. palivizumab.[11] In the MAKI trial, we have shown 80% efficacy against RSV hospitalization through i.m. administration of palivizumab in late preterm infants 32–35 weeks gestational age.[12] We hypothesized that local administration of palivizumab to the airways prevents RSV infection in infants because it is delivered directly to the main viral point of entry and decreases the chance of breakthrough infection.

Here, we describe product development, a phase I trial and the first report of a phase IIb trial to evaluate the efficacy of daily i.n. administration of palivizumab during the RSV season to prevent RSV infection in otherwise healthy late preterm infants.

Methods

Study design

We conducted a double-blind, randomized, placebo-controlled cross-over phase I safety trial (Narsyn Study

A, Dutch Trial Register NTR7378) in the Netherlands from September to November, 2018. Subsequently, we intended to obtain proof–of-concept that i.n. palivizumab prevents RSV infection in infants. Study B (Dutch Trial Register NTR7403, see Supplementary Appendix for full protocol), a double-blind, randomized, placebo-controlled proof-of-concept phase IIb trial was initiated based on the overall safety profile of Study A upon recommendation of an independent Data Safety and Monitoring Board (DSMB). Recruitment for study B was conducted at 39 hospitals (1 academic, 38 regional) in the Netherlands from November 2018 through January 2021. The trial was approved by the institutional review board of the University Medical Center Utrecht, the Netherlands (NL66735.041.18).

Participants

Study A included 20 healthy adult volunteers between 18 and 60 years of age [Supplementary Figure S1]. Exclusion criteria were nasal obstructions, history of any respiratory symptoms within 4 weeks prior to drug administration, nasal surgery, immunocompromised subjects, simultaneous use of other nasal drops or spray or other nasal drugs ever (including cocaine and tobacco). For study B, we included infants with a gestational age between 32 + 0 and 35 + 6 weeks who were younger than 6 months at the start of the RSV season (October 1st to March 31st). For this reason, recruitment occurred from the end of August to the end of January [Supplementary Figure S2]. To limit the required sample size, the trial was performed in a high-risk population of infants with at least one older sibling under 18 years of age.[13] Exclusion criteria were known cardiac anomalies, Down syndrome or other serious congenital disorders as well as simultaneous use of other nose drops or spray except normal saline drops.

Randomization and masking

Study staff and study participants were blinded to study arm assignments. Study participants were randomized 1:1 in a non-stratified manner using blocks of 2 and 4 using Castor Electronic Data Capture (EDC) platform. Study medication and placebo were identically packaged and indistinguishable by sight or smell.

Procedures

Commercial saline nasal drops (0.9% sodium chloride, Fagron) with a concentration of 1 mg/mL palivizumab were used as study medication and saline nasal drops without palivizumab were used as placebo. We showed the drug formulation is stable at 4 °C (intended use) in the investigational medicinal product dossier (Supplementary Appendix 2). For study B, parents were instructed to administer one drop (50 μL) of study medication in each nostril daily from the beginning of the RSV season (October 1st) or directly post-discharge for children born during the RSV season. Parents were instructed to take a nasal swab (stored in Copan universal transport medium (UTM)) in case of respiratory symptoms lasting more than one day. Parents recorded medication adherence, presence of respiratory symptoms, doctor visits, and the use of airway medication in a daily log. Weekly follow-up calls were performed to minimize missed infections and to maximize study medication compliance.

Nasal swabs were transported in UTM by regular mail to the laboratory and were stored at –80 °C until analysis as done in our previous trial.[12] Polymerase-chain-reaction (PCR) assays were performed to determine the presence of RSV RNA as described previously with minor modifications (Supplementary Appendix 3).[14]

Drug dose

We determined the dose based on best knowledge available from clinical studies of trough levels upon therapeutic efficacy, which were used for intramuscular dose determination for current market approval. Serum trough concentrations are minimally 30 μg/mL and ideally greater than 40 μg/mL (as a margin of safety for person-to-person variability) for clinical efficacy.[15] Dose-dependent increases in concentration of anti-RSV antibodies in bronchoalveolar lavage fluid (BALF) have been observed to be 500–1000x less than steady-state plasma concentrations of antibody.[16,17] Consequently, a protective dose of palivizumab on the airways may be presumed to be 500x less than serum concentration or 0.08 μg/mL for therapeutic efficacy. Nasal epithelial lining fluid (ELF) is estimated to be 800 ul per nostril.[18] Thus, in order to achieve a minimal trough concentration of 0.08 μg/mL in 800 μL, 0.064 μg is needed per nostril as a minimal protective dose. In this study we administer nasal drops with a concentration of 1 mg/mL of palivizumab with 50 μL administered daily per nostril, resulting in a daily dose of 50 μg per nostril, easily above the minimal threshold needed for therapeutic efficacy (780x more than minimal trough concentration). Other therapeutic antibodies that have been used locally utilize doses 1/100th of the required systemic dose to allow for reduced costs and side-effects.[19]

Outcomes

For study A, the primary outcome was self-reported local and systemic adverse events (AEs; Supplementary Appendix 4.1–4.3). In the case of objectifiable symptoms, researchers performed a home visit. An independent DSMB and investigators considered if (S)AEs were treatment related. For study B, the primary outcome was any lab-confirmed RSV infection. The key secondary outcome was lab-confirmed RSV hospitalization. Other secondary outcomes are defined in the Statistical Analysis Plan (Supplementary Appendix 4.4–4.7 and Supplementary File Study Protocol and SAP).

Statistical analysis

Nearly all analyses were performed (DC and PvdV) according to the statistical analysis plan (Supplementary File Study Protocol and SAP); we specified if the analysis was performed post-hoc (Supplementary Appendix 6 and 7). The primary outcome was analyzed with a mixed effect logistic regression including treatment, season, prognostic factors at baseline (having more than one sibling, date of birth between August 14—December 1st, neonatal respiratory support), as fixed effects and a random effect for siblings. The primary analysis was performed on the modified intention-to-treat (mITT) population. The predefined target sample size of 408 infants provided 85% power to detect a relative risk reduction for RSV infection of 62.5%.[12] An interim analysis was performed according to protocol (Supplementary File Study Protocol and SAP) to assess futility or efficacy when 50% of the expected events had been observed. The analyses were performed in R (version 4.0.3 or higher), SAS Enterprise Guide (version 8.2) and SPSS (version 25.0.0.2). All reported effect sizes are for palivizumab relative to placebo.

Role of funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the paper for publication.

Results

In study A, 20 subjects were enrolled in the first-in-human study between October 1–4, 2018 [Supplementary Table S1]. One subject was excluded before any study medication was administered due to symptoms of a respiratory infection. Airway patency after 10 min was 100% in both the palivizumab (10/10) and placebo (9/9) arm. Local and general symptoms were tabulated per arm per study participant [Supplementary Table S2A]. There were no SAEs in either trial arm and no AEs were considered to be treatment-related by the study staff or DSMB [Supplementary Table S2B and C].

In study B, 268 of the 4403 eligible preterm infants were enrolled in the study between January 14, 2019 and January 28, 2021 [Fig. 1]. Eleven infants were enrolled during season 1 (2018–2019), 89 during season 2 (2019–2020) and 168 during season 3 (2020–2021). Infants enrolled during season 3 were excluded from the efficacy analyses due to absence of RSV circulation in the winter of 2020–2021 in the Netherlands during the COVID-19 pandemic.[20] The mITT population (n = 94) used for the interim and final primary analysis consisted of all infants for whom the primary endpoint was known. We excluded 6/100 (6%) infants who discontinued the study early (3 in the intervention group and 3 in the placebo group) for whom the primary endpoint was unknown. One child in the intervention arm who discontinued the trial early, but was hospitalized with RSV before trial discontinuation, was included in the mITT population. The interim analysis performed on May 19th, 2021, showed that the conditional power of the trial was 1%. The trial was stopped on June 7, 2021 after the DSMB confirmed futility (Supplementary Appendix 8).

Baseline characteristics were similar in both study arms [Table 1]. Median age of infants at start of treatment was 2.3 months (interquartile range (IQR) 0.7–4.3) and median gestational age was 34.3 weeks (IQR 33.4-35.1). Twenty-nine (29%) infants were part of a multiple birth. Subjective and objective adherence was high and similar in both study arms [Supplementary Figure S3]. Parents administered nasal drops for a mean duration of 3.9 months (SD: 1.1 months) in the intervention arm and 3.7 months (SD: 1.1 months) in the placebo arm.

Adverse events were determined to be unrelated to study medication in all participants in the intervention arm (n = 3/134, 2.2%) [Supplementary Table S3]. No SAEs were determined to be related to study medication; the number of SAEs was similar in the palivizumab arm and placebo arm (22/134 versus 26/134, non-significant (NS)).

In the mITT population, 29 infants (30.9%) had any laboratory-confirmed RSV infection: 18/47 (38.3%) in the palivizumab arm and 11/47 (23.4%) in the placebo arm [Table 2]. There were more RSV infections in the intervention arm but this was not significantly different (adjusted odds ratio (aOR): 2.2; 95% CI: 0.7–6.5) and per protocol population (aOR: 1.9; 95% CI: 0.6–6.3) [Table 2]. Sensitivity analyses assessing the impact of missing outcome [Table 3], different CT-value cut-offs and adherence [Supplementary Table S4–S5] also showed no statistical significant difference between both trial arms. The key secondary outcome, RSV hospitalization, was similar in the palivizumab arm and placebo arm (7/47 (14.9%) versus 3/47 (6.4%)). Other secondary outcomes were also similar in both trial arms [Table 2, Supplementary Table S6].

There was no difference in any wheezing, fraction of wheezing days [Supplementary Table S7], or wheezing episodes between the trial arms (incidence rate ratio (IRR): 1.2; 95% CI 0.8–1.7) [Supplementary Figure S4 and Supplementary Tables S8–S9]. The proportion of infants with recurrent wheezing and physician-diagnosed wheeze were similar across trial arms [Supplementary Table S8]. Occurrence of any wheezing was similar for RSV-infected and non-infected infants (post-hoc analysis) [Supplementary Table S10].

Discussion

This study is the first trial to investigate the efficacy of mucosal administration of antiviral mAbs to prevent respiratory infection. Development of the investigational

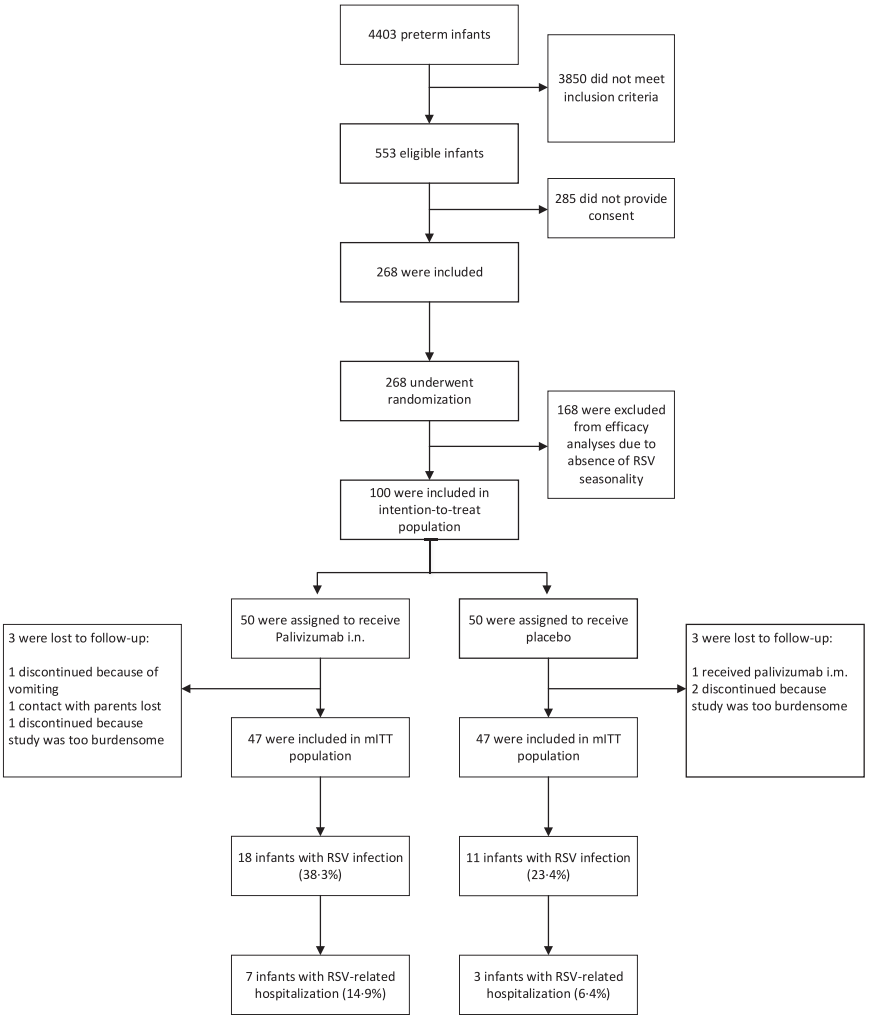

Fig. 1: Study B enrollment.

product was investigator-initiated and was conducted without funding from industry or capital investment, contrary to common practice in late-phase product development. Intranasal prophylaxis in late preterm infants was not effective to prevent lab-confirmed RSV infection despite high rates of adherence. The lack of efficacy has important implications for ongoing and future trials in the rapidly expanding field of mucosal mAb clinical development. However, affordability of RSV prevention may be within reach due to a commitment to ensure access for MK-1654[2] and support of the Bill and Melinda Gates Foundation for affordable maternal PreF vaccination.[21]

The rate of total RSV infection (30.8%) was higher than expected at the time of sample size calculation (expected rate 16%) but similar to the rate reported in a recently published European birth cohort study (26.2%).[22] The proportion of infants with any wheezing in the placebo arm (43.5%) was similar to the MAKI trial (47%).[12]

The observed lack of efficacy may be explained by several non-exclusive mechanisms. First, it is uncertain whether the mucosal half-life of i.n. mAbs is sufficient for protection. At the time of the trial there was no accepted sampling technique to define the half-life of palivizumab in the airways for i.m. administration or measure an effective medication dosage by measuring trough antibody concentrations. The half-life of IgG in the nasal ELF is not well established, but due to mucociliary clearance it is expected to be substantially shorter than in serum. However, it remains unclear whether measurement of half-life corresponds to the clinical outcome of interest, namely, protection against RSV infection. In vivo we previously showed[8] that despite full protection against RSV for at least one week, palivizumab administered (0.5 mg/kg) into the lungs of

	Palivizumab (*N* = 50)	Placebo (*N* = 50)	Total (*N* = 100)
Female, n (%)	22 (44)	26 (52)	48 (48)
Age in months, median (IQR)[a]	2.3 (0.7–4.4)	2.4 (0.6–4.2)	2.3 (0.7–4.3)
Gestational age in weeks, median (IQR)	34.3 (33.1–35.3)	34.5 (33.6–35.1)	34.3 (33.4–35.1)
Birth weight in grams, median (IQR)	2325 (1947–2602)	2364 (2000–2575)	2343 (1954–2590)
Multiple birth, n (%)	16 (32)	13 (26)	29 (29)
Complication(s) during pregnancy, n (%)	23 (46)	27 (54)	50 (50)
Antenatal corticosteroids, n (%)	20 (40)	22 (44)	42 (42)
Complication(s) during delivery, n (%)	26 (52)	29 (58)	55 (55)
Antibiotics during delivery, n (%)	8 (16)	7 (14)	15 (15)
Vaginal delivery, n (%)	24 (48)	27 (54)	51 (51)
Apgar score 5 min, median (IQR)	9 (8–10)	9 (8–10)	9 (8–10)
Respiratory support after birth, n (%)	26 (52)	24 (48)	50 (50)
Received antibiotics after birth, n (%)	19 (38)	22 (44)	41 (41)
Maternal age at birth child, median (IQR)	32 (30–36)	32 (30–35)	32 (30–35)
Exclusive breastfeeding, n (%)[b]	21 (42)	23 (46)	44 (44)
Breastfeeding and formula feeding, n (%)	22 (44)	17 (34)	39 (39)
Exclusive formula feeding, n (%)	7 (14)	10 (20)	17 (17)
Maternal level of education—postgraduate, n (%)	46 (92)	46 (92)	92 (92)
Paternal level of education—postgraduate, n (%)	42 (88)	46 (92)	88/98 (90)
Maternal smoking during pregnancy, n (%)	5 (10)	4 (8)	9 (9)
Smoking inside, n (%)	1 (2)	0 (0)	1 (1)
Total number of persons in household, median (IQR)	4 (4–5)	4 (4–5)	4 (4–5)
More than one older sibling, n (%)	25 (50)	18 (36)	43 (43)
Day care attendance, n (%)[c]	28 (56)	27 (54)	55 (55)
Siblings attending day-care, median (IQR)	1 (1–2)	1 (1–1)	1 (1–1)
Maternal atopy, n (%)	26 (52)	16 (32)	42 (42)
Paternal atopy, n (%)	20/47 (43)	18 (36)	38/97 (39)

Denominator is shown only in case of missing data. [a]Age in months at start of intervention. [b]Breastfeeding was determined based on the question which feeding was given from birth and duration was not taken into account. [c]Day care attendance includes both current and intended day care attendance.

Table 1: **Baseline characteristics of Study B participants.**

	Palivizumab (*N* = 47)	Placebo (*N* = 47)	Adjusted odds ratio[a] (95% CI)	P value	Crude odds ratio (95% CI)	Risk difference % (95% CI)	RRR[b] % (95% CI)
Primary endpoint							
RSV infection, n (%)	18 (38.3)	11[c] (23.4)	2.2 (0.7–6.5)	0.14	2.0 (0.8–5.0)	14.9 (−3.5 to 33.3)	−63.6 (−207.8 to 13.0)
Secondary endpoints							
Hospitalization for RSV infection, n (%)	7 (14.9)	3 (6.4)			2.6 (0.6–10.6)	8.5 (−3.8 to 20.9)	−133.3 (−748.2 to 35.8)
Medically attended RSV infection without hospitalization, n (%)	8 (17.0)	2 (4.3)			4.6 (0.9–23.0)	12.7 (0.1 to 25.0)	−300.0 (−1685.2 to 10.4)
RSV infection without medical attention, n (%)	3 (6.4)	7 (14.9)			0.4 (0.1–1.6)	−8.5 (−20.9 to 3.8)	57.1 (55.8 to 88.2)
All-cause RTI[d]							
Any RTI, n (%)	46 (97.9)	45 (95.7)			2.0 (0.2–23.3)	2.1 (−5.0 to 9.2)	−2.2 (−10.0 to 5.0)
RTI hospitalization, n (%)	9 (19.2)	6 (12.8)			1.6 (0.5–5.0)	6.4 (−8.4 to 21.1)	−50.0 (−288.1 to 43.0)
Medically attended RTI, n (%)	26 (55.3)	22 (46.8)			1.4 (0.6–3.2)	8.5 (−11.6 to 28.7)	−18.2 (−76.1 to 21.7)
Non medically attended RTI, n (%)	43 (91.5)	45 (95.7)			0.5 (0.08–2.7)	−4.3 (−14.1 to 5.6)	4.4 (−6.2 to 14.1)

Abbreviations: RTI: respiratory tract infection; RRR: relative risk reduction; CER: control event rate; EER: experimental event rate. [a]Adjusted analyses using a mixed effect logistic regression with treatment arm, having more than one sibling, date of birth between August 14 and December 1, and neonatal respiratory support as fixed effects and random intercept for family. Adjusted analyses were not performed for secondary outcomes because there was only a very small number of infants with this endpoint. Placebo group is the reference group. [b]The following formula was used to calculate relative risk reduction: RRR = (CER-EER)/CER. [c]One child had an RSV hospitalization (November) followed by a case of non-hospitalized medically-attended RSV (December). One participant had two medically-attended RSV infections and two participants had two non-medically attended RSV infections within one RSV season. [d]Number of children per type of RTI are reported. Children can have several types of RTI during study period.

Table 2: **Efficacy of intranasal prophylaxis against RSV infection and all-cause respiratory tract infections.**

Analysis	Population	Palivizumab	Placebo	aOR (95% CI) or RR (95% CI)
Primary analysis replacing all missing outcomes with no RSV, aOR	ITT (*N* = 100)	18/50 (36.0)	11/50 (22)	2.2 (0.8–6.3)[a]
Primary analysis replacing all missing outcomes with RSV, aOR	ITT (*N* = 100)	21/50 (42.0)	14/50 (28.0)	2.0 (0.7–5.5)[a]
Primary analysis replacing all missing outcomes with RSV in the placebo group and no RSV in the treatment group, aOR	ITT (*N* = 100)	18/50 (36.0)	14/50 (28.0)	1.5 (0.6–4.0)[a]
Primary analysis replacing all missing outcomes with no RSV in the placebo group and RSV in the treatment group, aOR	ITT (*N* = 100)	21/50 (42.0)	11/50 (22.0)	2.8 (1.0–7.8)[a]
Primary analysis in the per protocol population, aOR	Per protocol population (*N* = 86)	16/42 (38.1)	11/44 (25.0)	1.9 (0.6–6.3)[a]
Relative risk	mITT (*N* = 94)	18/47 (38.3)	11/47 (23.4)	1.6 (0.9–3.1)[b]

[a]Mixed effect logistic regression with treatment arm, having more than one sibling, date of birth between August 14 and December 1, and neonatal respiratory support as fixed effects and random intercept for siblings was used to calculate OR for CT-value sensitivity analyses. [b]Crude relative risk for treatment arm.

Table 3: **Sensitivity Analyses including infants with missing outcome.**

naïve wild-type BALB/c mice is detected at low levels or not at all in the nasal airway or lungs on day 7 [unpublished data]. Recent data also suggests that mAbs administered via nasal spray retain neutralizing capacity against SARS-CoV-2 for 24 hours in a small clinical trial.[23] Second, it is known that the eyes and mouth are potential, though less effective, routes of inoculation for RSV[24,25] and i.n. administration unlikely protects via these routes. Third, study medication adherence may have been overestimated due to desirability bias. We minimized this bias by measuring objective adherence (weighing bottles before and after use) as well as subjective adherence (parent-reported). Fourth, inadequate dosing might have contributed to lack of efficacy. However, the trial dosage of i.n. palivizumab is expected to be higher than airway medication concentrations achieved through current market-approved i.m. administration. The daily dose of 50 μg per nostril in this study is easily above the minimal threshold needed for protective efficacy (0.064 μg per nostril) as explained in the methods. In summary, lack of efficacy may most likely be explained by a short half-life of study medication in the nasal cavity although recent trial results show viral neutralization until 24 h after study drug administration.

The strength of this study is the investigator-initiated and industry-independent testing of the "bouncer" hypothesis: i.n. mAb administration stops infection at the site of viral entry and provides a solution to prevent infection of respiratory pathogens with significant cost reduction. It is estimated that 100 μL of 1 mg/mL daily would add up to 15 mg/season, while current i.m. administration of 15 mg/kg for an average weight of 5 kg adds up to 375 mg/season. Therefore, the cost reduction is minimally 20 fold (275 versus 7000 USD per child per season).

Limitations of the study include no circulation of RSV during the third season due to the COVID-19 pandemic. Although the sample size was smaller than planned due to lack of RSV circulation, we expect this limitation to have had no impact on the conditional power of the study as the trial was terminated early due to a planned interim analysis. Baseline incidence of RSV infection was sufficiently high to support our negative trial results. Second, administration of study medication depends on parental adherence. Parent-reported subjective adherence may have been subject to social desirability bias, underreporting missed doses and overestimating adherence. To minimize the impact of this limitation we collected objective adherence data based on weight of dosage. Objective adherence was high, indicating that insufficient adherence is not the main driver of lack of efficacy. Third, nasal swabs were collected by parents as previously done in the MAKI trial.[12] To mitigate the risk of missed outcomes, parents were contacted on a weekly basis by dedicated study staff and general practitioners were contacted to collect information on medical visits and respiratory episodes during the first year of life. Despite intensive follow-up, there were 175 (36.9%) respiratory episodes without a swab. However, we expect limited impact on the primary outcome because these episodes had a shorter median duration of symptoms than episodes with a swab (4 vs 13 days) and were therefore unlikely to be RSV-positive as the median duration of symptoms was 16 days for RSV-positive episodes. Lastly, the nasal drops would ideally coat the large nasal cavity surface for maximum efficacy. Although a spray could increase nasal cavity deposition in adults,[26] nasal drops penetrate the nasal valve to reach the turbinates more effectively than sprays.[27]

Our results show a trend towards higher RSV infection and hospitalization in the intervention arm. We considered four mechanisms that may explain the observed trend. We considered mechanistic damage to the nasal epithelium is unlikely as palivizumab solely targets virus and the two other components present in palivizumab (histidine and glycine) are amino acids.[28] Antibody-dependent enhancement in which suboptimal RSV medication concentrations may enhance viral replication[29] is unlikely as the dosage used was higher than the minimum threshold for protective efficacy

established for i.m. administration. Increased RSV exposure may explain the observed trend as infants who received palivizumab had more siblings than infants who received placebo (50% vs 36% respectively). Finally, an underlying predisposition for RSV infection in the intervention arm is unlikely by study design (randomization).

Several gaps in knowledge make it difficult to confirm the mechanism for lack of efficacy of i.n. palivizumab against RSV infection. First, the mucosal pharmacokinetics of antibodies is unknown. In future trials, it will be important to measure antibody half-life in the nose as improved sampling devices have recently been validated to collect neat mucosal lining fluid.[30] Second, it is important to understand whether the neonatal Fc receptor (FcRn), which is critical to extended half-life of antibodies, is present in the infant's nasal mucosa. Research in vitro and in animal studies suggests this may be the case.[31–34] Intranasal extended half-life mAbs have recently been shown to block COVID-19 infection after experimental infection in mice.[5] Third, an alternative to therapeutic IgG is IgA, which has been associated with protective immunity against RSV.[35] However, we previously showed in mice that re-engineering palivizumab into monomeric and secretory IgA is a less effective i.n. prophylaxis.[36]

In conclusion, daily i.n. palivizumab prophylaxis did not show protection against RSV infection in late preterm infants. Our findings imply that a timely interim analysis is essential to evaluate efficacy to avoid wasted time and capital. Further research is needed on nasal antibody half-life in the highly active field of i.n. drug development. Trials with other therapeutic antibodies are needed to understand whether our findings are specific to palivizumab or generalizable to any mucosal antibody administration.

Contribution

LJB, NIM and YNL were involved in the conceptualization. FS, SN, and JL were involved in methodology. NIM and YNL were involved in investigation and writing of the original draft. JT was involved in writing, review and editing as were all other co-authors. NIM, YNL, and JT accessed and verified the data. DC and PV performed the formal analysis. The manuscript was written in collaboration with the Narsyn study group.

Data sharing statement

Deidentified individual participant data, the analytics code, and other supporting documents collected for our study may be made available upon reasonable request to the corresponding author.

Declaration of interests

UMCU has received major funding (>€100,000 per industrial partner) for investigator initiated studies from AbbVie, MedImmune, AstraZeneca, Sanofi, Janssen, Pfizer, MSD and MeMed Diagnostics. UMCU has received major funding for from the Bill and Melinda Gates Foundation, Gates Medical Research Institute and the Dutch Lung Foundation. UMCU has received major funding as part of the public private partnership IMI-funded RESCEU and PROMISE projects with partners GSK, Novavax, Janssen, AstraZeneca, Pfizer and Sanofi. UMCU has received major funding by Julius Clinical for participating in clinical studies sponsored by MedImmune and Pfizer. UMCU received minor funding (€1000–25,000 per industrial partner) for consultation and invited lectures by AbbVie, MedImmune, Ablynx, Bavaria Nordic, MabXience, GSK, Novavax, Pfizer, Moderna, Astrazeneca, MSD, Sanofi, Janssen. LJB and NIM have regular interaction with pharmaceutical and other industrial partners. They have not received personal fees or other personal benefits. LJB is the founding chairman of the ReSViNET Foundation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102324.

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