EBioMedicine 73 (2021) 103651

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.elsevier.com/locate/ebiom

Forward and reverse translational approaches to predict efficacy of neutralizing respiratory syncytial virus (RSV) antibody prophylaxis

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ARTICLE INFO

Article History:
Received 20 July 2021
Revised 29 September 2021
Accepted 12 October 2021
Available online xxx

Keywords:
Respiratory Syncytial Virus
Monoclonal Antibody
RSV, Meta-analysis
Modelling and Simulation
Human Challenge Study

ABSTRACT

Background: Neutralizing mAbs can prevent communicable viral diseases. MK-1654 is a respiratory syncytial virus (RSV) F glycoprotein neutralizing monoclonal antibody (mAb) under development to prevent RSV infection in infants. Development and validation of methods to predict efficacious doses of neutralizing antibodies across patient populations exposed to a time-varying force of infection (i.e., seasonal variation) are necessary.

Methods: Five decades of clinical trial literature were leveraged to build a model-based meta-analysis (MBMA) describing the relationship between RSV serum neutralizing activity (SNA) and clinical endpoints. The MBMA was validated by backward translation to animal challenge experiments and forward translation to predict results of a recent RSV mAb trial. MBMA predictions were evaluated against a human trial of 70 participants who received either placebo or one of four dose-levels of MK-1654 and were challenged with RSV [NCT04086472]. The MBMA was used to perform clinical trial simulations and predict efficacy of MK-1654 in the infant target population.

Findings: The MBMA established a quantitative relationship between RSV SNA and clinical endpoints. This relationship was quantitatively consistent with animal model challenge experiments and results of a recently published clinical trial. Additionally, SNA elicited by increasing doses of MK-1654 in humans reduced RSV symptomatic infection rates with a quantitative relationship that approximated the MBMA. The MBMA indicated a high probability that a single dose of $\geq$ 75 mg of MK-1654 will result in prophylactic efficacy ($>$ 75% for 5 months) in infants.

Interpretation: An MBMA approach can predict efficacy of neutralizing antibodies against RSV and potentially other respiratory pathogens.

1. Introduction

Globally, human health is threatened with deadly viral pathogens ranging from localized outbreaks, yearly epidemics, to worldwide pandemics. Neutralizing antibodies, whether elicited by vaccines or introduced by the administration of mAbs, can prevent disease for many respiratory pathogens [1–3]. However, the dose selection process to achieve efficacious titres for vaccine and mAb clinical candidates has historically been performed without the benefit of support from quantitative models. Doses are frequently derived either

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https://doi.org/10.1016/j.ebiom.2021.103651

Research in context

Evidence before this study

Respiratory syncytial virus (RSV) is a common pathogen that causes acute respiratory infection, especially in infants, wherein it is the leading cause of hospitalization. The virus most commonly circulates seasonally, primarily in winter. Novel RSV neutralizing monoclonal antibodies (mAbs) with a long duration of activity (i.e., months), such as MK-1654, are a promising prophylactic approach for the prevention of disease in infants. With a single dose, these antibodies have the potential to prevent disease for an entire winter. Historically, selecting a dose for RSV mAb clinical candidates has relied on animal studies to approximate effective drug levels in humans. This approach does not take into account important factors, such as the duration of protection over time and the amount of drug needed in different patient populations. Thus, more predictive quantitative techniques based on human data are needed to guide clinical dose prediction for antibodies that prevent RSV, as well as other respiratory viruses.

Added value of this study

Here, we report work that uses a mathematical model based on mechanistic understanding to integrate data from previously published RSV studies. This model accounts for the effects of drug, time, and patient population on clinical outcomes. By incorporating decades of qualified published clinical RSV prevention data, the mathematical model enables a quantitative understanding of the relationships between antibody concentrations ("titres") and protection from RSV disease for mAb prophylaxis, as well as for vaccines. Further, by validating our model predictions using animal studies, a published infant trial, and a controlled RSV infection ("challenge") clinical trial of MK-1654 in adults (described here for the first time), we advance the field's ability to accurately predict the prophylactic efficacy of RSV mAbs and vaccines alike. Finally, the model was used to predict the efficacy of MK-1654 across a range of potential infant doses, providing confidence in the degree of protection from RSV infection this antibody can afford.

Implications of all the available evidence

The work described here lays the foundation for an approach that will aid the design and interpretation of clinical trials for RSV and other pathogens. This method enables the prediction of doses and frequencies of administration needed to achieve protection for monoclonal antibodies and can similarly inform the development of vaccines.

empirically or directly from animal models that may not accurately translate to humans [4]. The use of well-validated model-informed approaches for the prediction of clinically efficacious doses can facilitate efficient development of novel antibodies and vaccines by reducing the number of clinical trials that fail due to incorrect dosing [5]. Furthermore, an accurate understanding of the minimal dose necessary for efficacy can translate into dose sparing (e.g., in paediatric populations) and prudent clinical supply implementation in high demand/low supply settings.

For RSV, prophylactic neutralizing antibodies are effective against disease in infants [3,6,7]. The currently licensed mAb, palivizumab, requires multiple doses per season and is generally only recommended for highest-risk infants (e.g., very preterm infants or those with lung or heart disease) [3,8–10] However, there are more healthy infants without specific risk factors who have RSV-associated morbidity and mortality [11]. Thus, most infants lack an option for prophylaxis. To address this need, MK-1654 is under development for the prevention of lower respiratory tract infection (LRTI) caused by RSV for all infants entering their first RSV season and children at high risk in their second year of life. MK-1654 has increased potency compared to palivizumab and binds to the highly conserved site IV of the F glycoprotein [12]. Additionally, it has the potential for single dose coverage of an entire RSV season based on an extended half-life [13] due to three amino acid substitutions referred to as YTE in the fragment crystallizable (Fc) domain that augment recycling though the neonatal Fc receptor, FcRn [14].

Here, to better understand the relationship between MK-1654 dose and potential efficacy in humans, we report a model-based meta-analysis (MBMA) that quantifies the relationship between RSV serum neutralizing activity (SNA) and clinically relevant endpoints in humans, including LRTI in infants. MBMAs integrate data across studies using mathematical models that are based on pharmacological understanding, accounting for the effects of treatment, time, and patient population on clinical outcomes. This quantitative relationship was validated using three complementary approaches, including data from RSV challenge experiments in animals using neutralizing mAbs (including MK-1654), a human RSV challenge trial with MK-1654 (described herein), and the prediction of phase 3 clinical trial results against RSVA for the RSV neutralizing mAb, REGN2222 [15]. Finally, the MBMA was used to predict clinical efficacy in infants across different dose-levels of MK-1654, which recently entered a phase 2b/3 clinical outcome trial in this population (NCT04767373).

2. Methods

2.1. Study design

The objective of our study was to develop and validate a quantitative model based on summary-level literature data to describe the relationship between clinical incidence rates of RSV and SNA titre for vaccines and mAbs across a range of populations. The MBMA model was validated using both in-house and published data from nonclinical viral challenge studies in cotton rats and from published clinical efficacy data in infants. As a proof of concept, model predictions were compared to efficacy data from a human challenge trial using MK-1654, and the validated model was used to predict the efficacy of MK-1654 in a pivotal trial in infants.

2.2. Ethics

The clinical study protocol was approved by a Research Ethics Committee and conducted in conformance with applicable country or local requirements. Specifically, the MHRA (Medicine & Healthcare Product Regulatory Agency) approved the MK-1654-005 study. The ethic committee which approved the MK-1654-005 study is Office for Research Ethics Committees Northern Ireland (ORECNI). Written informed consent was obtained from each participant prior to any study procedure.

The animal studies were approved by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, Institutional Animal Care and Use Committee and conducted in accordance with animal care guidelines.

2.3. Systematic literature review

Several searches were conducted in PubMed to identify all nonclinical and clinical trials evaluating the prevention of RSV. An initial set of searches were conducted between 2016 and 2018 for trials evaluating mAbs and vaccines using predefined search strings. Results from the initial search were supplemented in 2019 with an

additional search to update vaccine results and expand the results from mAb trials. Search strings and dates are provided (**Supplementary Methods**).

A total of 5891 results were identified across all searches. After exclusion of duplicate search results, 1069 abstracts were selected for further review. Abstracts from the identified studies were reviewed by subject matter experts and included if the reference was likely to 1) provide information on anti-RSV efficacy, or 2) provide information either directly on SNA titres or provide adequate information to derive SNA titres following mAb or vaccine administration. A total of 362 abstracts met at least one of these two criteria. The details of these selected studies were reviewed to prevent inclusion of duplicate results, and to ensure eligibility based on the quality of the study and the information reported within. Forty-eight studies were deemed eligible and categorized into two tiers: Tier 1) rigorous, randomised clinical trials, and Tier 2) high-quality epidemiology studies. The dataset was supplemented with publicly available data that was not yet published at the time of search from five additional studies evaluating mAbs that were under development. The final analysis dataset contained data from 53 publications or studies [**Supplementary Table S1**]. A PRISMA (preferred reporting items for systematic reviews) [16] diagram is provided [**Supplementary Fig. S1**].

Data pertaining to study design, treatments, demographics, adherence, SNA titres, and clinical outcomes were extracted from these studies. Reported outcomes were categorized and assigned to one of five nested and hierarchical disease severity levels: asymptomatic RSV infection, symptomatic RSV infection, RSV LRTI, RSV hospitalization, and RSV intensive care unit admission [**Supplementary Fig. S2**]. This categorization structure assumes that all patients at higher disease severity levels are included within lower disease severity levels but not vice versa. For example, all LRTI patients are included in the symptomatic RSV category, but only a subset of symptomatic patients is hospitalized.

2.4. Software

Data from publication figures were digitized using Engauge Digitizer, version 8 or later. MBMA modelling, cotton rat modelling, and trial simulation were performed in R, version 3·3·0 or later (http://www.r-project.org/). Plotting was performed in either R, version 3·3·0 or later, or SAS, version 9·4 or later (SAS Institute; Cary, NC). Supportive modelling activities were performed in Pharsight Phoenix WinNonlin (Certara, L.P., St. Louis, USA) (version 7·0 or higher).

2.5. MBMA modelling approach

Model Structure. A quantitative pharmacometric model using a sigmoidal relationship was fit to SNA titre time course profiles and clinical outcome data. The fundamental assumption of the MBMA is that the probability of RSV disease (of the chosen disease severity level and during a given time period) depends exclusively (to within the precision of the data) on the SNA time course profile. The risk of being infected with RSV over a season in a study, *R*, represents the percentage incidence rate per study, and is calculated via integration of the probability $p(t)$ of contracting RSV at a particular day, *t*, according to the following equation:

$$R_{r,\,i,\,j} = 100 \cdot \int_{t\,=\,t_{last}}^{t=t_{last}} p_{r,j}(t) \cdot FOI(t) \cdot N_{norm}(t) \cdot dt$$

where $R_{r,i,j}$ is the response (incidence rate expressed as %) per study at clinical level *r* for treatment arm *i* in trial/trial stratum *j*, t_{last} is the last day of study, t_{first} is the first day of study (i.e., November 1st for the northern hemisphere), $FOI(t)$ is a weighting function to account for the force of RSV infection over time (described below), and $N_{norm}(t)$ describes the fraction of subjects present at day *t*, $N(t)$, divided by the maximum number of subjects present at any time during the study. $N_{norm}(t) = N(t)/N_{max}$. In Tier 1 studies, $N(t)$ remains constant as all patients were followed throughout the entire study period. $N(t)$ may vary in Tier 2 studies where the number of subjects can vary over the observational period: it was reported for some studies and, for others, it was imputed by distributing the reported number of enrolled subjects based on expected birth rates during the enrollment period, matching reported demographics.

The probability $p(t)$ of RSV infection at time, *t*, is described by the minimum and maximum possible incidence rates at clinical level *r* and the RSV infection suppression effect at time *t*:

$$p_{r,j}(t) = Rmax_{r,j} \cdot e^{\log\left(\frac{Rmin_{r,j}}{Rmax_{r,j}}\right) \cdot Eff(log_2SNA(t))}$$

$$= Rmax_{r,j} \cdot \left(\frac{Rmin_{r,j}}{Rmin_{r,j}}\right)^{Eff(log_2SNA(t))}$$

where $Rmin_{r,j}$ and $Rmax_{r,j}$ are the minimum and maximum possible incidence rates for clinical level *r*, and trial/trial stratum *j*, respectively, and $Eff(log_2SNA(t))$ is the effect (defined below) of SNA on the suppression of RSV infection at time *t*. Since event rates are being modelled (as opposed to event counts), Poisson regression was used.

The maximum incidence rate per clinical level ($Rmax_r$) was estimated in the MBMA model by including a trial-specific exponential random effect on $Rmax_r$ to account for variability in maximum incidence rate between RSV seasons and studies: $Rmax_{r,j} = Rmax_r \cdot e^{\eta_j}$

The suppression effect of mean SNA titre, $Eff(log_2SNA(t))$, at a given time, *t*, is modelled as follows:

$$Eff(log_2SNA(t)) = \frac{log_2SNA^{\gamma}}{log_2SNA^{\gamma} + IT50^{\gamma}}$$

where, $log_2SNA(t)$ is mean log_2SNA at time *t*, γ is the Hill coefficient, and *IT50* is the log_2SNA at which *logR* has been reduced by 50% of the difference between *logRmin* and *logRmax*. At this log_2SNA, $p_{r,j}$ is the geometric average of *Rmin* and *Rmax*.

Weighting Approach. In the MBMA model, the observed incidence rates in the individual study treatment arms were weighted by the precision of the reported incidence rate using a power variance model with a fixed power of 0·5. Weights were calculated using the inverse of the calculated variance (*VAR*) or squared standard (SE) associated with each data point. Variance was calculated as:

$$VAR = \frac{fitted(\cdot)}{total\,exposure}$$

where *fitted*(.) is the model-fitted incidence rate of RSV assuming a Poisson distribution of the incidence rates as represented by the equation of $R_{r,i,j}$ defined above, and *total exposure* is the number of subjects multiplied by the number of RSV seasons spanned by the trial, corrected for the changing number of subjects during the trial, force-of-infection (also described as the strength of RSV exposure over a season), and the RSV test rate (where applicable). A higher *VAR* results in a lower weight of the data point. Upon examination, the large sample size and trial duration of Tier 2 studies resulted in larger relative weights than those for Tier 1 studies. Tier 2 studies were observational in nature, so, to prevent their having a disproportionate influence on model fit relative to the Tier 1 studies (randomised, controlled trials), the weighting of Tier 2 data points was reduced by weighting instead with the square root of *VAR*. This ensured a reasonable balance between the influence of data coming from Tier 1 and Tier 2 trials during model fitting.

Accounting for Seasonality. The daily incidence rate of RSV disease is dependent on the likelihood and strength of the RSV exposure present at a particular point in time, which is described by an empirical force of infection (FOI) function. FOI was obtained by fitting the following Gaussian function plus baseline to data from seasonal incidence rates of RSV:

$$FOI(t) = baseline + amp \cdot \frac{1}{\sigma \cdot \sqrt{2\pi}} \cdot e^{\frac{(t-\mu)^2}{2\sigma^2}}$$

where σ is the standard deviation of the Gaussian curve, μ is the time from August 10th to FOI peak, *amp* is the magnitude between the off-season and peak incidence rates, and *baseline* is the relative off-season incidence rate. This function was fit to digitized epidemiological data from the United States [17], resulting in the following parameter estimates: σ = 48 days, μ = 162 days, *amp* = 2633, and *baseline* = 2·25. *FOI*(t) is scaled to an area under the FOI curve of 1, integrating from the first day of the RSV season (November 1st, northern hemisphere) to 150 days thereafter. An overlay of the fitted function is provided [**Supplementary Fig. S3**].

2.6. Viral challenge studies in cotton rats

Anti-RSV mAbs were evaluated in the cotton rat challenge model and methods have been described previously [12]. Briefly, animals received a single intravenous dose of either RB1 (a MK-1654 parental antibody) or D25 (an RSV neutralizing antibody that binds to site Ø), based on body weight. RB1 or D25 were each administered at five dose levels ranging from 2·5 to 0·3 mg/kg. Untreated (no antibody) animals served as a control group. Neither mAb contained half-life extending YTE mutations. Prophylaxis was administered on Day 0. Blood was sampled on Day 1 to quantify the concentration of mAb in the serum. After pharmcokinetic (PK) sampling, animals were sedated and challenged intra-nasally with 1×10^5 pfu of RSV strain A2. On Day 4, the animals were euthanized to allow for harvesting of nose and lung tissues. Harvested tissues were homogenized, and virus was quantified using plaque assay.

A challenge study with a similar design was conducted for MEDI8897* (a non-YTE containing version of MEDI8897) [18]. These data were extracted and combined with the in-house generated data for RB1 and D25. A sigmoidal model (with a similar structure to the clinical model) was fitted to the data from the three mAbs to establish the relationship between concentration and viral load reduction following inoculation. A covariate on IC50 (the concentration of mAb that resulted in 50% suppression of $\log_2$ viral load) accounted for potential differences between mAbs. Experimental differences in minimum and maximum viral load between studies were also accounted for.

2.7. Clinical trial simulation of REGN2222 efficacy

Clinical trial simulations were conducted using the RSV MBMA model to predict the efficacy of REGN2222 in a published, phase 3 trial [15]. A virtual population matching the demographics reported in the study was created based on trial inclusion and exclusion criteria. The time course of the SNA titres for the virtual population were derived for treatment and placebo groups using the same approach that is described for imputation of SNA titres for mAbs [**Supplementary Methods**]. The population PK model was developed [**Supplementary Methods**] using reported concentration data available from trial results (NCT02325791). Similar to other mAbs, the PK/SNA relationship for REGN2222 was derived based on potency relative to MK-1654 [**Supplementary Table S2**].

A total of 1000 clinical trial simulations were run matching the study design described in the publication. Model parameters for the RSV MBMA were resampled from parameter uncertainty for each replicate. Efficacy for each replicate was calculated as the relative risk (RR) reduction between treatment and placebo arms. Mean predicted efficacies and 95% confidence intervals (CI) values were calculated from parameter estimates obtained from fitting a normal distribution to the log(RR) values for all replicates. For comparison, observed efficacy was calculated as the mean and 95% CI of the RR reduction based on case counts reported in simulated treatment (both one and two doses) and placebo groups

$$\text{Efficacy} = 1 - RR$$
$$95\%\, CI\ for\ RR = e^{\log(RR) - 1.96\, SE\{\log(RR)\}},\ e^{\log(RR) + 1.96\, SE\{\log(RR)\}}$$

where RR corresponds to the calculated risk ratio, and SE represents the corresponding standard error.

2.8. Human challenge study (HCS) Design

This report includes results of a phase 2a double-blind, randomised, placebo-controlled study to evaluate the efficacy and safety of MK-1654 in healthy adult participants inoculated with an experimental respiratory syncytial virus (Protocol MK-1654-005). The study was conducted at a single site in the United Kingdom from October 2019 to August 2020. MK-1654 or placebo (0·9% sodium chloride) was administered in a single IV infusion over ~2 h on study Day 1. On Study Day 29, 70 participants were inoculated with 4 $\log_{10}$ PFU RSV A (Memphis 37b. GenBank Accession KM360090). Participants remained domiciled for 11 days post inoculation and were queried daily for symptoms of RSV infection. Nasal wash samples were collected and tested for RSV viral load by RT-qPCR (quantitative reverse transcription PCR) one or two days before inoculation and then twice daily from Study Days 31 to 39. A single nasal wash sample for RSV viral load by RT-qPCR was collected on Day 40. Nasal wash samples were tested for the presence of virus prior to discharge. Key samples to measure MK-1654 PK (predose, and Days 1, 8, 15, 29, 40, and 57) and SNA (predose and Day 1, two hours postdose, Days 29, 40, and 57) were collected and assays were performed as described in Aliprantis et al [13]. All participants inoculated with RSV A Memphis 37b were followed for safety monitoring for approximately 28 days afterwards (i.e., 56 days post MK-1654 or placebo dosing). In addition, two safety follow-up phone calls were performed for all subjects approximately 33 and 123 days after the poststudy visit on Days 90 and 180, respectively.

2.9. Randomisation and masking

Participants in the HCS study were assigned randomly using a computer-generated allocation schedule to receive a single dose of placebo or of 100, 200, 300, or 900 mg of MK-1654 in a 1:1:1:1:1 treatment ratio (16 participants per group in each of the five groups). There were no stratifications based on age, sex, or other characteristics in the study.

A double-blinding technique was used. MK-1654 and placebo were prepared/dispensed in a blinded fashion by an unblinded pharmacist or other qualified study site personnel. The participant, the investigator, and Sponsor personnel who were involved in the study intervention administration or clinical evaluation of the participants were unaware of the intervention assignments.

2.10. HCS participants

Healthy male and female participants between the ages of 18 through 55 years of age (inclusive) and screened to be in the bottom quartile of screened subjects for immunogenicity to the RSV A Memphis 37b (inoculation strain) were eligible for the study. This screening was performed solely on serological antibody titres. Women who were pregnant or had been pregnant in the six months prior to study were excluded. Other key exclusion criteria included history or evidence of any clinically significant or currently active cardiovascular, respiratory, dermatological, gastrointestinal, endocrinological, haematological, hepatic, immunological (including immune suppression), metabolic, urological, renal, neurological, or psychiatric disease.

2.11. HCS safety assessments

Safety analysis was conducted in the All Participants as Treated population, which consisted of all randomised participants who received a dose of study treatment. All adverse events (non-serious and serious) were collected throughout the duration of the study and reported as descriptive summaries.

2.12. RT-qPCR and area under the curve (AUC) analysis

RSV viral load AUC (VL-AUC) was determined by RT-qPCR from Day 2 to 11 after intranasal inoculation. The lower limit of quantification (LLOQ) of the PCR assay used was 2·8 $\log_{10}$ copies/mL. PCR results of "detected (< LLOQ)" and "not detected" were imputed as 1·4 and as 0·7 for the analysis, respectively. The primary endpoint of VL-AUC between Days 2 and 11 after intranasal inoculation was computed for each participant in $\log_{10}$ copies/ml · days.

2.13. RSV quantitative culture assay and AUC analysis

Participant nasal wash samples that had a detectable RT-qPCR result were also tested by a quantitative culture assay according to the following methodology. The day prior to addition of test samples, Human epithelial type 2 (HEp-2) cells were seeded into 24-well assay plates at 5×10^5 cells/mL and incubated at 37°C, 5% CO_2. Samples were added to the assay plates in triplicate wells at four different dilutions, followed by incubation for one hour at 37°C, 5% CO_2 before addition of methyl cellulose media. After six further days of incubation at 37°C, 5% CO2 the cells were fixed by removal of methylcellulose and addition of 10% neutral buffered formalin. Plaque counting was facilitated by staining with haematoxylin solution (0·06% v/v) and eosin Y solution (1%) v/v). The virus titre, in $\log_{10}$ Plaque Forming Units (PFU)/ml, for each dilution was calculated from the average number of plaques obtained for the three replicate wells with the titre given for the sample calculated by the mean of all valid dilution replicates.

The samples that had undetectable or negative RT-qPCR results were not tested using the quantitative culture assay, and results for these samples were imputed as zero. VL-AUC by quantitative culture in $\log_{10}$ PFU/ml · days from Day 2 through Day 11 after intranasal inoculation was summarized by treatment group.

2.14. Analysis of symptomatic RSV infection in HCS

Participants were provided a Symptom Diary Card to record any RSV-infection-related symptoms starting from the day prior to inoculation to 11 days after inoculation using a categorical score Grade 0–3 for each symptom. Symptomatic RSV infection was defined as presence of at least two quantifiable RT-qPCR (≥ LLOQ) on two or more consecutive days, plus symptoms of either any Grade 1 and above from two different symptoms from the subject symptom card, or at least one Grade 2 and above symptom from one or more respiratory categories (runny nose, stuffy nose, sneezing, sore throat, cough, and shortness of breath). The number of participants with infection (both symptomatic and asymptomatic) were also recorded.

2.15. Statistical methods for the HCS

The primary endpoint of VL-AUC between Days 2 and 11 after intranasal inoculation was analysed using an analysis of variance (ANOVA) model with treatment group as a fixed categorical effect. The mean VL-AUC in each group and the differences in mean VL-AUC between each MK-1654 dose group and placebo and the corresponding two-sided 90% confidence intervals (CI) were computed based on the ANOVA model. The primary hypothesis was that at least one of four dose levels would result in a reduction in viral load after intranasal RSVA inoculation compared to IV placebo. The primary hypothesis was tested using a stepwise procedure with an assumption that there is an increasing relationship between viral load reduction and MK-1654 doses. The statistical criteria required that the upper limit of the 90% CI (equivalent to the upper bound of a one-sided 95% CI) for the difference in mean VL-AUC between the highest MK-1654 dose and placebo is < 0 (indicating a reduction). If the hypothesis was supported in the previous step, then the same procedure was applied to the next lower dose. The procedure continued in this stepwise fashion until the upper limit of the 90% CI at a particular dose is > 0. A sample size of 13 per dose group (assuming an attrition rate of ~12% between dosing and inoculation and ~6% after inoculation through completion of the 11-day post inoculation follow-up), provided ~80% power to detect a decrease in viral load AUC of 70% in the highest MK-1654 dose group versus the placebo group assuming a coefficient of variation (CV) in viral load AUC of 0·7 with a 1-sided alpha = 0·05 test. Analyses were conducted in the Full Analysis Set population, which consisted of all randomised participants who received one dose of treatment and the RSV viral inoculation. For the secondary endpoint of symptomatic RSV infection, the number of participants in each treatment group with symptomatic RSV infection was reported with a 95% CI based on the binomial method [19]. For the exploratory endpoint of VL-AUC by quantitative culture, summary statistics were provided by treatment group.

2.16. Clinical trial simulations for efficacy prediction of MK-1654

The efficacy of MK-1654 in the setting of a hypothetical, late-stage clinical trial was predicted using clinical trial simulation and the RSV MBMA. A virtual population of both preterm [29 to 35 weeks gestational age (GA)] and full-term (35 weeks GA and later) infants was created. Distributions of GA were resampled from distributions that were derived from epidemiological natality data in the United States (CDC WONDER tool; https://wonder.cdc.gov/). Infant body weight over the duration of the trial was projected using standard curves to account for growth in term (CDC growth charts; www.cdc.gov/growthcharts/who_charts.htm) and preterm infants [20]. The hypothetical trial randomised infants from the virtual population to MK-1654 or placebo in a 2:1 ratio. Doses of MK-1654 that were evaluated included 10, 30, 50, 75, 100, and 125 mg. Infants up to the age of eight months were 'enrolled' prior to the start of the RSV season and dosed approximately one month prior to season onset. Neonates were also enrolled during the season but prior to the peak and were dosed within 28 days following birth.

Serum concentrations versus time profiles of MK-1654 were predicted for the infants in the virtual population using a population PK model that was developed using data from healthy adults and scaled down to paediatrics using weight-based allometric scaling. Study design and results in a phase I study in healthy adults have previously been described [13]. Briefly, single ascending doses of MK-1654 were administered to healthy volunteers by intramuscular injection (up to 300 mg) or intravenous infusion (up to 3000 mg). Blood samples were serially collected out to 365 days and used to measure serum concentration and SNA titre. The pharmacokinetics of MK-1654 were best characterized using a two-compartment model with first-order absorption [**Supplementary Fig. S4**]. Body weight was included as a covariate on clearance and volume parameters. Allometric exponents for the purposes of scaling the model to paediatrics were estimated based on infant and adult pharmacokinetic data from MEDI8897, another anti-RSV mAb with the same YTE mutations [21,22]. The relationship between MK-1654 concentration and SNA titre was described using the following linear slope and intercept function:

$$SNA_{total} = SNA_{base} + Conc_{MK-1654} * Slope$$

where SNA_{total} is the total SNA titre in the sample, SNA_{base} is the endogenous SNA titre at baseline, $Conc_{MK\text{-}1654}$ is the concentration of

Table 1
Approach to developing an integrated RSV MBMA based on published clinical data.

Step	Field Clinical Trials	Challenge Clinical Trials
1. Literature Review	Conduct review of all published and in-house RSV mAb and vaccine field studies in infants, adolescents, adults, and older adults Review studies and rank based on quality of publication and reporting of results Extract data on study design, treatments, adherence, clinical endpoints, and demographics	Conduct review of all published RSV vaccine human challenge studies in adults Review studies and rank based on quality of publication and reporting of results Extract data on study design, treatments, clinical endpoints, and demographics
2. Imputation of Missing Data	For each study arm in a vaccine trial: • Derive SNA titre versus time profile based on SNA titre at Day 29 and SNA titre decay For each study arm in a mAb trial: • Derive a virtual trial population to match reported demographics • Simulate PK vs. time profile • Derive PK vs. SNA titre relationship based on preclinical relative potency • Impute SNA titre vs. time profile • Account for study drug adherence and subject dropout	For each study arm in a vaccine trial: • Derive SNA titre at the time of viral challenge
3. Data Integration via Quantitative Modelling	Combine analysis datasets from field clinical trials and challenge clinical trials Simultaneously fit incidence rate versus SNA titre using a sigmoid relationship Investigate which parameters can be shared across field clinical trials and challenge clinical trials Evaluate and control for covariates (e.g., disease severity, population, modality, adjuvant), as needed	
4. Validation	Compare model predictions to observed efficacy from cotton rat challenge experiments Compare model predictions to observed efficacy from MK-1654 in an adult human challenge study Compare model predictions to observed efficacy from REGN2222 in infants	

SNA, serum neutralizing antibody titre; PK, pharmacokinetics.

MK-1654 in the sample, and *Slope* is the amount of SNA titre produced per concentration unit of MK-1654. Additional model details are provided [**Supplementary Table S3**].

A total of 1000 clinical trial simulation replicates were conducted to predict the efficacy of MK-1654 for the prevention of RSV LRTI in the hypothetical late-stage trial. Consistent with the imputation approach described for trials evaluating mAbs, SNA titres following administration of MK-1654 were predicted as the sum of endogenous SNA titre (derived using the endogenous SNA model, **Supplementary Methods**) and SNA titre contributed from study drug (derived using the population PK model scaled to paediatrics and the PK/SNA relationship established in healthy adults). Body weight was included as a time-varying covariate to account for the impact of infant growth on the PK of MK-1654 over the duration of the simulated trial. For each replicate, model parameters for both the population PK model and the RSV MBMA were resampled including parameter uncertainty. Efficacy for each replicate was calculated as the RR reduction between treatment and placebo groups. Mean predicted efficacies and 95% CI values were calculated from parameter estimates obtained from fitting a normal distribution to the log(RR) values of all replicates.

2.17. Role of funding source

The authors from Merck & Co., Inc., Kenilworth, NJ, USA participated in study design, data collection, analysis, interpretation, and writing of the report. The funders reviewed the penultimate draft of the manuscript. All authors had full access to the data in the study and approved the decision to submit for publication.

3. Results

3.1. Development of a MBMA for RSV prevention

An MBMA was developed using published data to establish the relationship between clinical incidence rates (IRs) of RSV and SNA titre, a putative correlate of protection, using the approach in Table 1. First, a systematic literature review was conducted to survey all available RSV prevention data from both field clinical trials (trials where participants are naturally exposed to virus through contact with infected individuals in their daily lives) and challenge clinical trials (trials where healthy adults are intentionally exposed to virus under medical supervision). Summary-level data including demographics, treatment, and clinical endpoints were extracted and stored in an analysis database. Clinical endpoints were categorized into one of five disease severity levels ranging from asymptomatic infection to severe (e.g., intensive care unit admission) disease (**Supplementary Fig. S2**). Data from all trials used in the model included some imputed data. For vaccine trials, the SNA titre time-course was imputed using reported titres on Day 29 and first-order decay. For mAb trials, the SNA titre time-course was imputed using simulated PK profiles and potency relative to MK-1654 [**Supplementary Methods**]. All data were integrated via a quantitative pharmacometric model relating SNA titre to incidence rate for each level of disease severity. The model accounts for differences between populations and trial types (i.e., field versus challenge). The model was validated using both clinical and nonclinical data. Additional detail is provided in Materials and Methods.

Observed data from field clinical trials identified that the primary modality under evaluation for RSV prevention is mAb immunoprophylaxis for infants and active vaccination for paediatric (two to 12 years), adult (18 to 65 years), and older adult (> 65 years) populations. Overall, the data revealed that higher SNA titre was associated with lower RSV incidence rates across all disease severity levels and populations (Fig. 1a). Consistent with the pathophysiology of RSV, within each population the incidence rates of higher severity RSV were lower than those of lower severity disease levels. This is likely because not all participants with mild disease progress to severe disease (e.g., LRTI or hospitalization) that is usually indicative of virus spreading to the lower airway tract.

A similar relationship was observed in data from published human challenge clinical trials. Consistent with field clinical trials, increasing mean SNA titre resulted in a lower incidence of asymptomatic and symptomatic RSV infection following challenge with RSV (Fig. 1b). Also in agreement with field trials, the incidence rates for symptomatic infection were lower than those for all (including asymptomatic) infections. Based on the literature, the incidence of symptomatic and asymptomatic RSV infection in adults was much

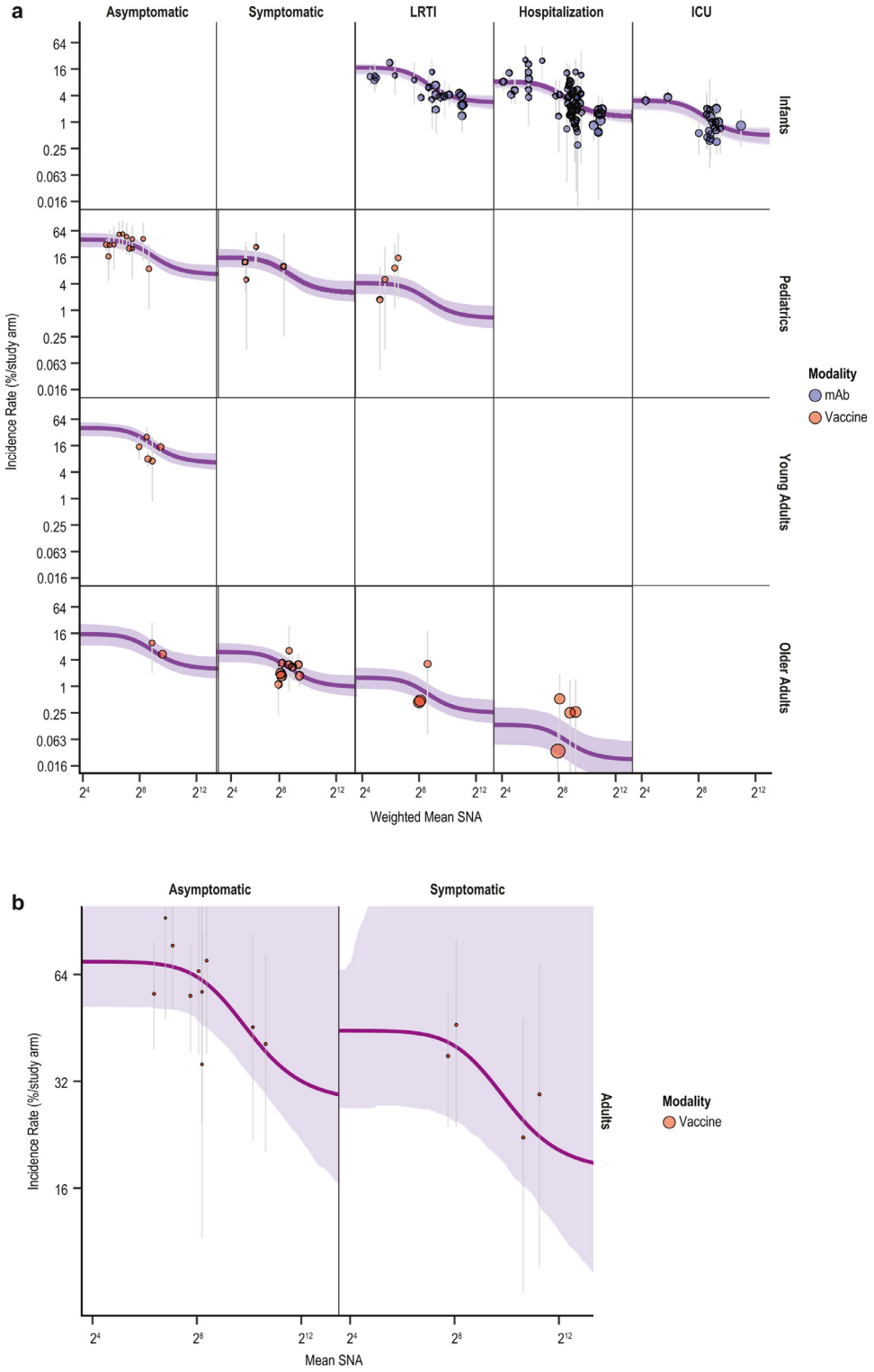


Fig. 1. Published field and challenge study data indicate higher SNA results in lower incidence rates of RSV. (a, b) Curves for incidence rate versus FOI-weighted mean SNA titre. The mean of the SNA titre time-course (weighted by the FOI for field studies) was plotted as the independent variable for visualization. Each point represents a paired reported incidence rate and weighted mean SNA titre for a study arm in the literature. Error bars indicate the 95% confidence interval (CI) for the reported incidence rate. The size of a data point corresponds to its relative contribution to the model. Solid lines represent the model-fitted relationship between incidence rate and SNA titre for a typical trial. The shaded purple area represents the 95% CI of the model-fitted relationship. Higher mean SNA titre results in (a) lower seasonal RSV incidence rates across populations and disease severity in clinical field trials and (b) lower RSV incidence rates for adults in human challenge trials. In panels a and b, 40 and 1 data points, respectively, with a reported incidence rate of zero were included in the model fit but are not shown to increase visibility of key features. Similarly, the y-axis has been scaled to enable the visibility of salient data properties and results in some vertical bars representing CI to be truncated. The y-axis of panel b has been truncated at an incidence rate of 100%. Disease severity level definitions are provided (**Supplementary Fig. S2**). FOI, force-of-infection; SNA, serum neutralizing antibody; LRTI, lower respiratory tract infection; ICU, intensive care unit.

higher in challenge trials relative to field clinical trials because all participants in challenge trials are directly exposed to a high inoculum of RSV and tested daily, whereas only a portion of field study participants may come into contact with RSV and a fraction of those are tested for the virus. All human challenge clinical trials from the literature evaluated active vaccines (not mAbs) for prevention.

Data extracted from both field and challenge clinical trials were modelled simultaneously based on a sigmoidal relationship between the probability of acquiring RSV infection (or RSV disease) and the SNA titre. The independent variable, SNA titre, was modelled as a function of time over the duration of the study and was weighted by the RSV force of infection (FOI). The model-fitted relationship—or the protection curve—for each disease severity, population, and study type is shown (Fig. 1a and b); in general, the observed data are well described by the model. The maximum incidence rate (Rmax) of each disease severity level was modelled as a fraction of the Rmax for the previous disease severity level using an inverse logit function (e.g., Rmax for symptomatic infection was modelled as a fraction of asymptomatic infection). Within a disease severity level, maximum incidence rates of asymptomatic infection were similar between paediatric and adult populations. Separate parameters were used to account for maximum incidence rates that were observed to be lower in older adult and higher in infant populations. The minimum incidence rate for each disease level (i.e., Rmin, maximum protective effect at high SNA titres) was modelled as a fraction of the maximum incidence rate (MinMaxRatio). This fraction was the same across all populations in the model. The IT50 (half maximal inhibition titre) parameter, or the $\log_2$ SNA titre needed to achieve a 50% reduction between the log minimum and log maximum incidence rate, was not significantly different between mAbs and vaccines for any populations or disease severity levels in clinical field trials. Other factors, such as the impact of underlying disease (e.g., chronic lung disease, congenital heart disease) and addition of vaccine adjuvant were also evaluated during model development. The inclusion of these factors as covariates on model parameters did not significantly improve the overall model fit and were not included in the final model.

Due to inherent differences between challenge trials and field trials (e.g., direct inoculation with high titre virus versus natural infection), additional model parameters were estimated to account for differences in the protection curve for challenge trials. The IT50 was estimated to be higher for human challenge trials, as a higher SNA titre was needed to achieve protection. A separate parameter ($RMax_{chall}$) was included to account for higher maximum incidence rates of asymptomatic and symptomatic RSV infection observed in human challenge clinical trials due to direct viral inoculation of study participants and frequent testing for infection. The maximum protective

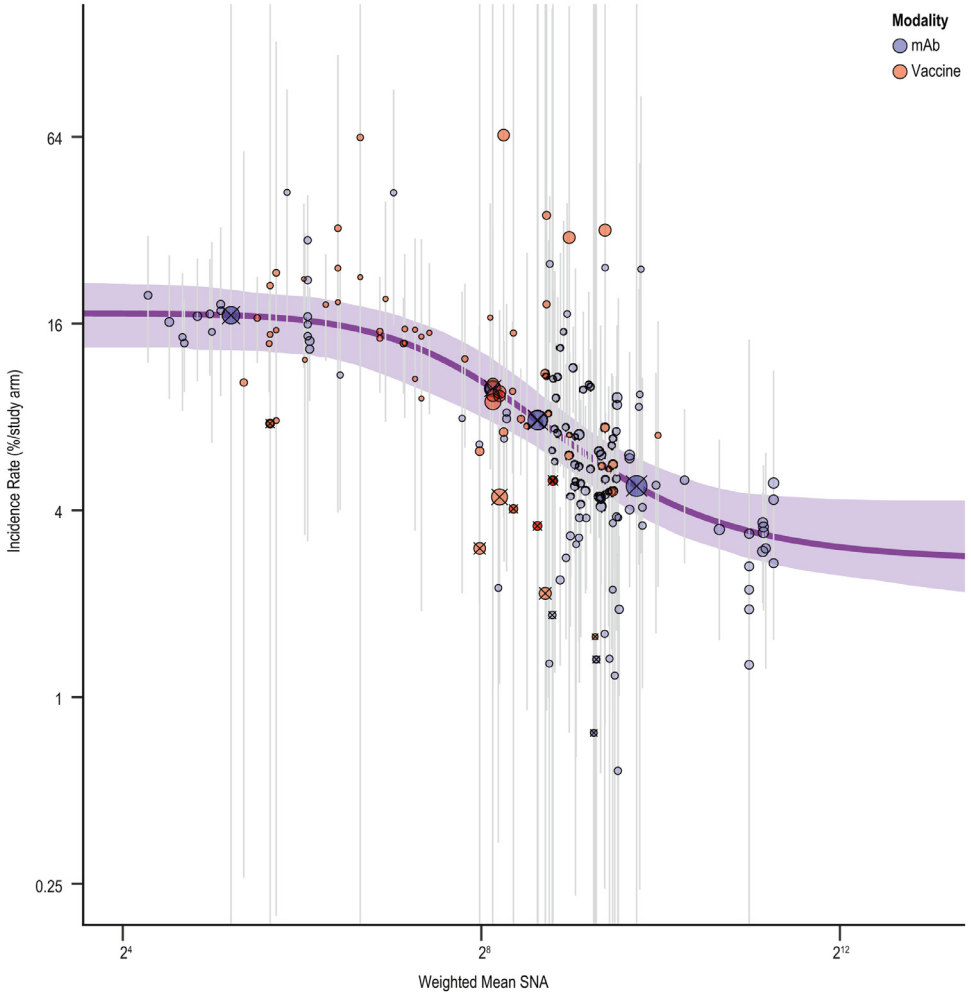


Fig. 2. Integrated MBMA scaled by V^2ACHER reveals a strong relationship between increasing SNA titre and decreasing incidence of RSV LRTI in infants.

All data points and model predictions have been scaled to RSV LRTI in infants using the V^2ACHER method. The shaded purple area represents the 95% CI of the model-fitted relationship. Nine data points with an incidence rate of zero were included in the model but are not shown to improve visibility of key features. Similarly, the y-axis has been scaled to enable the visibility of salient data properties and some vertical bars representing CI are truncated. The points with crosses represent study arms with reported zero incidence rates; these points may appear as non-zero incidence rates after V^2ACHER scaling. In one study (**Supplementary Table S1**, PMID: 29373476) the reported incidence rates were zero for all study arms but the model fit this finding well; the V^2ACHER visualization method represents this by showing as points (shown as crosses) very close to the curve.

effect [as defined by the value of one minus the ratio of the minimum to the maximum incidence rate ($MinMaxRatio_{chall}$) within a clinical level] was also lower in challenge trials, likely due to the extremely sensitive methods [e.g., daily collection of nasal specimens for quantitative polymerase chain reaction (PCR) and symptom scores] used to assess all subjects for infection, and was accounted for in the model. Also, the ratio between the maximum incidence rate for asymptomatic and symptomatic infection was different in challenge trials versus field trials, as expected, and this was accounted for. The hill coefficient (γ)–a model shape parameter related to potency that determines the steepness of the protection curve–was found to be similar across field and challenge trials. Additional details on the covariate structure of the model are provided (Materials and Methods, **Supplementary Table S4**).

In the absence of rich data for each level of disease severity, population, and study design, visualization of the MBMA in a trellised manner (Fig. 1a and b) can make it difficult to understand and evaluate relationships between exposure and response variables across covariate values. To overcome this limitation, the V^2ACHER method for visualizing data and predictions using non-linear mixed-effects analyses with covariates was applied [23]. V^2ACHER allows data underlying complex models with multiple covariates and between-trial variability to be visualized on top of the model predictions in an unbiased manner, providing a transparent view of how the model fits the data. The result is that all observations, regardless of population, disease severity level, or study type (i.e., field or challenge), can be viewed over a single prediction on the same set of axes. Using V^2ACHER, all data points included in the model were scaled to RSV LRTI disease severity for an infant population in a field study. Using this method, a strong relationship between SNA titre and RSV incidence is apparent (Fig. 2), with observations from vaccines and mAb studies equally distributed above and below the predicted protection curve, reinforcing that SNA titre strongly correlates with protection, regardless of modality, population or disease severity level.

3.2. Model validation

3.2.1. Backwards translation using viral challenge experiments in cotton rat

Viral load reduction in the lung following prophylaxis with D25 (an RSV neutralizing antibody that binds to site Ø) and RB1 (a non-YTE parental antibody to MK-1654) was evaluated in a cotton rat challenge model using RSV (see Materials and Methods). Separately, lung viral load and serum antibody concentration data were extracted from an experiment with a similar design evaluating MEDI8897* (a non-YTE containing version of MEDI8897) [18] in cotton rats and plotted together with in-house generated data for D25 and RB-1. For all mAbs, higher serum concentrations at the time of PK sampling resulted in reduced viral load in the lung (**Supplementary Fig. S5**). A sigmoidal model was fit to serum mAb concentration versus lung viral load data. MK-1654 exhibited a slightly lower EC50 (concentration to achieve a 50% logarithmic reduction of the $\log_2$ viral load between the maximum and minimum $\log_2$ viral loads) than D25 and MEDI8897*; however, the result with MK-1654 and MEDI8897* may not be directly comparable due to differences in assay methodologies and sensitivities. The mAb concentrations were

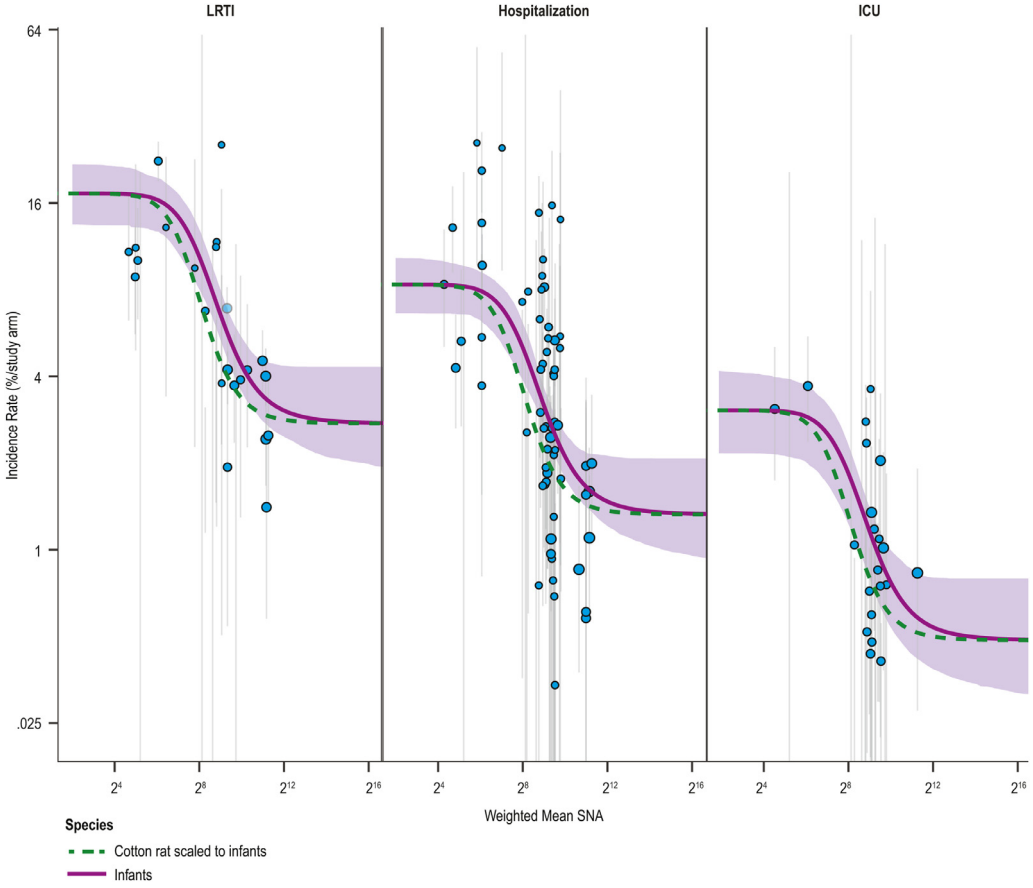


Fig. 3. Overlay of cotton rat challenge model with RSV incidence rate model in infants shows consistency. Solid points represent the reported incidence rate and weighted mean SNA titre for a stratified study arm in infants from the literature. Error bars indicate the 95% CI for the reported incidence rate. The size of each point corresponds to its relative model weight. Lines represent the model-fitted relationship between incidence rate and SNA titre for Tier-1 (i.e., rigorous, randomised controlled trials) infant studies lasting 151 days (solid purple) and the scaled viral load vs SNA titre relationship from cotton rat (dashed green). The shaded purple area represents the 95% CI of the model-fitted relationship. Seventeen data points with an incidence rate of zero were included in the model but are not shown to improve visibility of key features.

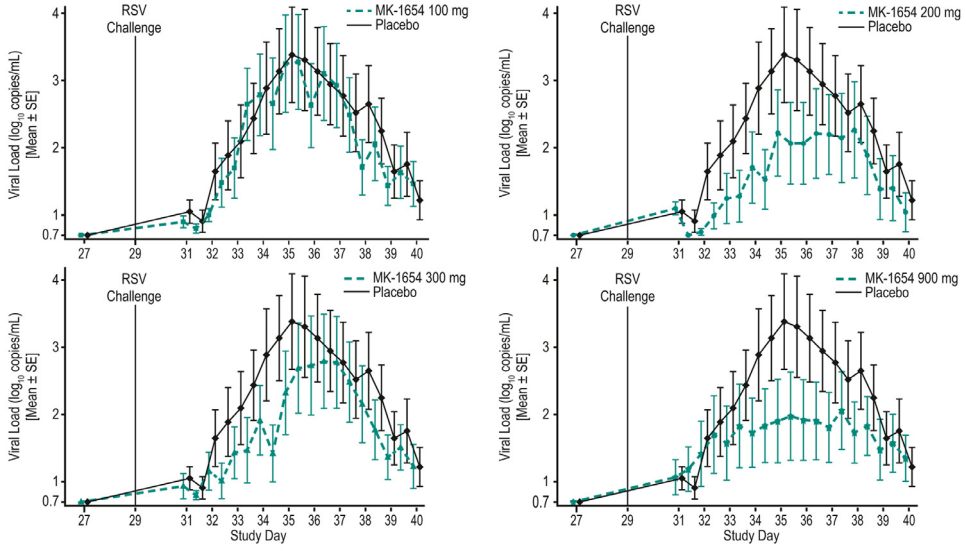
subsequently converted to SNA equivalents based on the PK/SNA relationship from healthy adults [13] and $\log_2$ minimum/maximum viral load was scaled to minimum/maximum incidence rates of RSV infection in infants for the purposes of comparison to the clinical MBMA, as has been previously described [24].

Consistent with the clinical MBMA, the cotton rat lung protection curve scaled to infants predicted a strong decrease in expected RSV incidence rates as SNA titres increase.. Importantly, the relationship between SNA titre and RSV incidence rate predicted from scaling the cotton rat challenge data approximated the relationship identified for infants from the clinical MBMA across disease severity levels (Fig. 3).

3.2.2. Forward translation using phase 3 results from REGN2222

A second validation exercise evaluated whether the RSV MBMA could reliably predict phase 3 efficacy results from a published trial

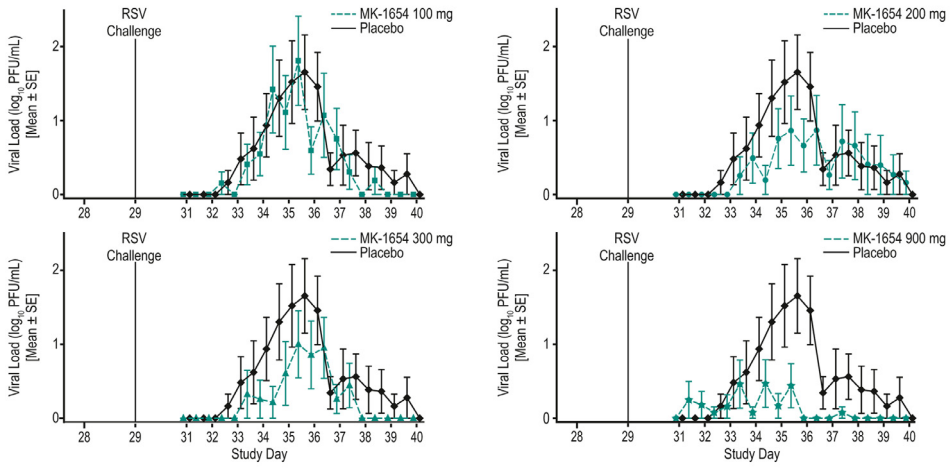
a. Nasal RSV viral load by RT-qPCR



b. Analysis of area under the viral-load curve by RT-qPCR, study days 31-40

Treatment	n	Least-square mean	(95% CI)	Treatment vs placebo difference in LS mean (90% CI)	*P* value (2-sided)
Placebo	15	21.25	(13.96, 28.55)		
MK-1654 100 mg	13	19.94	(12.11, 27.78)	-1.31 (-10.25, 7.64)	0.808
MK-1654 200 mg	13	14.74	(6.90, 22.58)	-6.51 (-15.46, 2.43)	0.229
MK-1654 300 mg	14	16.44	(8.89, 23.99)	-4.81 (-13.58, 3.96)	0.363
MK-1654 900 mg	13	15.33	(7.50, 23.17)	-5.92 (-14.87, 3.02)	0.273

c. Nasal RSV viral load by quantitative culture



d. Analysis of area under the viral-load curve by quantitative culture, study days 31-40

Treatment	n	Mean	SD	Median	Range
Placebo	15	5.36	6.41	5.34	(0.00, 20.13)
MK-1654 100 mg	13	4.37	5.32	0.00	(0.00, 12.08)
MK-1654 200 mg	13	3.51	6.35	0.00	(0.00, 20.29)
MK-1654 300 mg	14	2.45	4.32	0.00	(0.00, 13.64)
MK-1654 900 mg	13	1.18	3.05	0.00	(0.00, 10.13)

Fig. 4. Viral load as determined by RT-qPCR and quantitative culture following intranasal challenge with RSVA after single intravenous doses of MK-1654 or placebo (a to d). Mean nasal RSV viral load titres with corresponding standard are shown as measured by RT-qPCR at study Days 27 to 40 (a) and by quantitative culture at study Days 31 to 40 (c). The analysis of the VL-AUC is displayed for RT-qPCR (b) and quantitative culture (d). In (b), the least square mean is based on an analysis of variance (ANOVA) model with fixed effects for treatment. P values [2-sided t-test] and the CIs were constructed assuming a normal distribution for the VL-AUC. In each of the tables, n denotes the number of participants who were randomised, dosed with MK-1654 or placebo, received RSVA inoculation and contributed to the analysis. SD = standard deviation. RT-qPCR = reverse transcription quantitative real-time PCR.

evaluating REGN2222 (also known as suptavumab, an anti-RSV mAb previously under development) for RSV prevention in infants [15]. In this randomised trial, preterm infants received either placebo, a single dose of 30 mg/kg REGN2222 or two doses of 30 mg/kg separated by 57 days, and were followed for 150 days for the development of medically attended RSV infection, including hospitalization or outpatient LRTI. For the purposes of this validation, only efficacy for the prevention of RSVA was used, as an RSVB variant resistant to REGN2222 was highly prevalent during the trial rendering the molecule ineffective against RSVB [15]. The predictions from the MBMA-based *in silico* trial simulations were generally consistent with the trial (**Supplementary Fig. S6**). The predicted efficacy (95% CI) against RSV A LRTI or hospitalization for REGN2222 in the phase 3 trial was 0·70 (0·65–0·75) for the single-dose group and 0·81 (0·75–0·85) for the two-dose group, suggesting a dose response. The observed efficacy (95% CI) of REGN2222 vs. placebo for the prevention of RSV A LRTI or hospitalization was 0·62 (-0·05–0·86) for the one-dose group and 0·61 (-0·07–0·86) for the two-dose group. This study was not powered to detect a statistically significant effect for the prevention of RSVA only, and thus confidence intervals around the reported efficacy estimates for the clinical trial were wide.

3.3. Human challenge study and model predictions

3.3.1. Human challenge trial results

Next, a double-blind, randomised RSVA human challenge study was conducted to inform on the RSV MBMA and validate its application for the prediction of efficacy. In this study, 80 adults were administered one of four single dose levels of MK-1654 or placebo in a 1:1:1:1:1 ratio on Day 1 (**Supplementary Tables S5 and S6**). The four different dose levels (100, 200, 300, and 900 mg) of MK-1654 were selected so that the resultant SNA titres would be distributed across a range of potentially minimally- to highly-protective titres. After administration of MK-1654, RSV SNA titres increased in a dose-dependent manner. The PK profile and SNA titres were consistent with results observed in a previous phase 1 study in adults [13] (**Supplementary Figs. S7 and S8**). On Day 29, seventy participants were challenged intranasally with RSVA (14 participants each in the 100 mg, 200 mg, and 300 mg groups, 13 in the 900 mg group, and 15 in the placebo group). RSV nasal viral load was measured using RT-qPCR from study Days 31–40 (corresponding to Days 2 to 11 after challenge). One participant from the MK-1654 100 mg group and one participant from the MK-1654 200 mg group were missing RT-qPCR results on four (Day 37 through 40) and five (Day 36 through 40) days, respectively. These two participants were excluded from the analyses. RSV nasal viral load (VL) and area under the curve (AUC) decreased as the MK-1654 dose increased from 100 to 200 mg, but above 200 mg, no further decrease in viral load was observed (Fig. 4a and b). Based on a prespecified success criterion that the upper limit of the 90% CI of difference in mean VL-AUC by RT-qPCR between the MK-1654 900 mg group and placebo had to be less than 0 the primary hypothesis was not met (p=0.273, 2-sided). Viral load was also enumerated using a quantitative RSV culture, which enumerates only live/replication-competent virus (Fig. 4**c**). Analysis of the quantitative culture data demonstrated a clear trend of mean VL-AUC reduction in a dose-dependent manner across the dose range of 100 to 900 mg of MK-1654 (Fig. 4d). Participants were also followed for the incidence of symptomatic RSV infection from Days 31–40 (Table 2). In addition to symptomatic subjects, there were two participants in the study with asymptomatic RSV infection, one in the 100 mg group and one in the 300 mg group. As with VL-AUC by RT-qPCR, the incidence of symptomatic RSV infection decreased as the dose of MK-1654 increased from 100 mg to 200 mg, but no further decrease was observed at the two higher doses. There were no serious adverse events (AEs), dose-dependent pattern of AEs or discontinuations due to AEs in the trial (**Supplementary Table S7**). The safety profile of MK-1654 appeared similar to placebo and was generally consistent with results of the phase 1 study in adults [13].

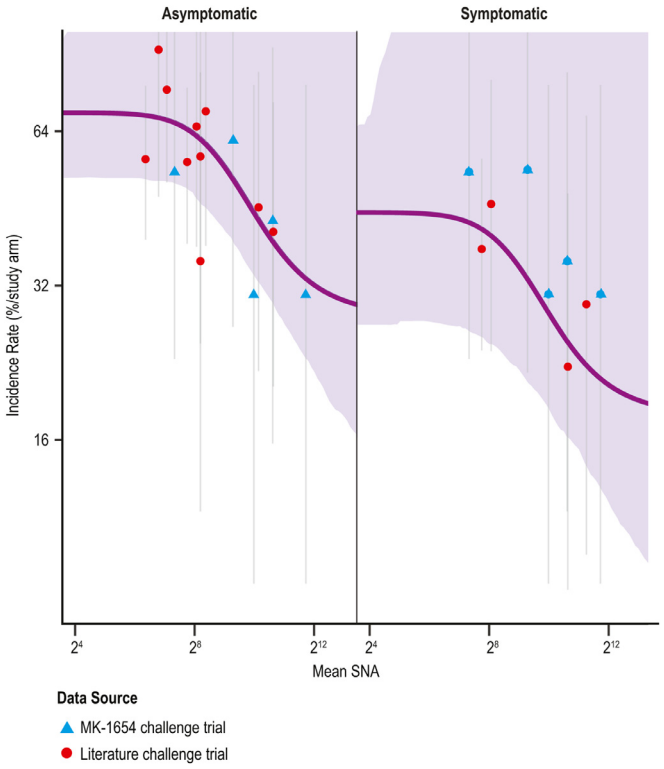


Fig. 5. MK-1654 human challenge trial efficacy results are consistent with MBMA predictions. Each point represents a paired reported incidence rate of either asymptomatic or symptomatic infection and mean SNA titre at the time of challenge from the MK-1654 challenge trial (blue triangles) or the literature challenge trials (red circles). Error bars indicate the 95% CI for the incidence rate. Purple lines represent the model-fitted relationship between incidence rate and weighted mean SNA titre based on all literature data (both field and challenge data but excluding data from this challenge trial). The shaded purple area represents the 95% CI of the model fitted relationship. One datapoint with an incidence rate of zero was included in the model but is not shown for readability. The y-axis has been truncated at an incidence rate of 100%.

Table 2
Proportion of participants with protocol defined symptomatic RSV infection on Days 2 to 11 after RSVA intranasal challenge.

Treatment	n	Symptomatic RSV Infection Participants with Symptomatic Infection (%)	95% CI
Placebo	15	8 (53·33)	(26·59, 78·73)
MK-1654 100 mg	13	7 (53·85)	(25·13, 80·78)
MK-1654 200 mg	13	4 (30·77)	(9·09, 61·43)
MK-1654 300 mg	14	5 (35·71)	(12·76, 64·86)
MK-1654 900 mg	13	4 (30·77)	(9·09, 61·43)

3.4. Translational comparison of human challenge trial results with the MBMA

Incidence rates from the MK-1654 human challenge study were evaluated for agreement with RSV MBMA predictions. As was planned in the design of the challenge study, SNA titres from placebo and MK-1654 treatment groups spanned the range of SNA titres that were included in the model (Fig. 5). Overall, the model was predictive of MK-1654 efficacy following viral challenge. Specifically, observed incidence rates of clinical level 1 disease (i.e., asymptomatic RSV infection) at a given mean SNA titre in the human challenge study agreed with the model, as the data points from the clinical trial were

a

MK-1654 Dose (mg)	Median RSV LRTI Predicted Incidence Rate (%/study)	Mean (95% CI) Predicted LRTI Efficacy
125	3.46	78.5 (73.2 – 82.8)
100	3.62	77.5 (72.2 – 81.8)
75	3.89	75.7 (70.3 – 80.2)
50	4.47	72.1 (66.1 – 77.1)
30	5.62	64.9 (58.0 – 70.7)
10	9.73	39.7 (28.9 – 48.9)
Placebo	16.0	–

b

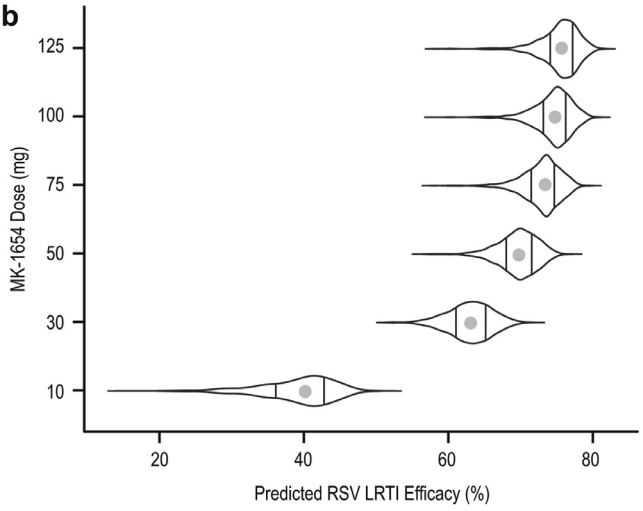


Fig. 6. In silico clinical trial simulations predict high efficacy against RSV LRTI for preterm and full-term infants. Simulation-based median predicted incidence rates and mean (95% CIs) for efficacy shown across dose levels (a) and distributions of predicted efficacy (b). Efficacy refers to the relative risk reduction of RSV LRTI between treatment and placebo groups following observation for 150 days. Each "violin" in b represents the distribution of predicted efficacy following simulation of 1000 clinical trials (3300 virtual infants enrolled to MK-1654 or placebo in a 2:1 ratio). Solid points represent the mean predicted efficacy for that dose level. Solid, vertical lines represent the upper and lower quartiles for the predicted distribution. LRTI, lower respiratory tract infection; CI, confidence interval.

distributed around the model predictions. In contrast, the incidence rates observed in the trial for symptomatic infection were systematically higher than that predicted by the model; however, model predictions (which are broad, due to large parameter uncertainty) fell within the confidence intervals for the observed incidence rates (which are also broad due to the small study size, see also Discussion).

3.5. Prediction of MK-1654 efficacy

The RSV MBMA model was used in a clinical trial simulation to predict the efficacy of MK-1654 for the prevention of RSV LRTI in a virtual population of healthy preterm and term infants representative of those expected to be enrolled in a late-stage trial. To simulate PK, a population PK model and PK/SNA relationship was developed using MK-1654 data in adults (Materials and Methods) and allometrically scaled to infants to account for differences in MK-1654 clearance and volume of distribution between these two populations. In the simulation, MK-1654 was administered either prior to or during (but before the peak of) the RSV season, and trends in RSV seasonality based on historical epidemiological data were taken into account. The RSV MBMA model was then used to predict incidence rates of LRTI through 150 days of follow-up (the duration of a typical RSV season in a temperate climate) for various dose-levels of MK-1654, which were compared to predicted incidence rates of LRTI for placebo groups to calculate efficacies. Clinical trial simulation results are provided in Fig. 6. A clear dose-response relationship, particularly between 10 and 50 mg, was observed with increasing dose levels of MK-1654 providing higher efficacy. For the target population, the mean efficacy began to plateau at approximately 76% with doses of MK-1654 of 75 mg or greater.

4. Discussion

In recent years, mAbs have emerged as major interventions for infectious diseases [25,26]. The dose selection for new mAb clinical candidates against RSV has traditionally relied on viral challenge studies in cotton rats to identify a therapeutic PK threshold [27]. This approach has generally shown translatability in the clinic, particularly for infants; however, it is based on a dichotomous PK target, which makes an unlikely assumption that infants with concentrations above or below this target are provided either complete or no protection, respectively, against infection. Key questions such as the duration for which humans should maintain concentrations above this protective target (particularly for a seasonal pathogen like RSV where exposure to the virus varies with time) and the application of the target across different patient populations, are not adequately addressed and may result in imprecise dose estimation for clinical populations.

The use of a validated model for efficacy prediction is important to guide decision-making in clinical development. Phase 3 trials require large investments, both financially and in terms of the time, risks, and effort of participants [27]. Therefore, calculating the probability of success for these trials is critical. Antibody titres from humoral immune responses have, traditionally, been used to predict immunogenicity, efficacy, and durability of prophylactic vaccines. Additionally, antibodies are easily measured from blood samples, making them an attractive biomarker to predict efficacy.

Numerous studies have demonstrated the relationship between levels of RSV SNA titre and protection from disease [28], including studies on mAb efficacy [3,6]. The MBMA described herein transforms the understanding of this relationship by using the totality of qualified published clinical RSV prevention data to further establish SNA as a correlate of prophylactic protection across both mAb and vaccine modalities.

The MBMA was validated using complimentary translational approaches. First, the protection curve for RSV infection in infants predicted by the MBMA was generally consistent with the protection curve established using the cotton rat challenge model for RSV. This backward translation, from clinical data represented by the MBMA to an animal model, supports the cotton rat as a reasonably predictive animal model for RSV prevention in infants. It also demonstrates that it is possible to continuously quantify the partial protection provided over a range of concentrations (as opposed to using a target PK threshold to predict complete versus absent protection). However, the curve in cotton rats was slightly left shifted compared to the MBMA. One possible explanation is that the cotton rat challenge study used in this validation exercise provided a direct measurement of viral replication in the lung, which may require lower SNA titers for protection due to higher penetration of antibody into the lung compared to the upper airway. Viral load in the lung is better aligned with the clinical disease of greatest concern (i.e., LRTI). These findings suggest that simple reliance on the viral load replication in the lung from an animal model (i.e., its direct use without appropriate quantitative considerations) might overestimate clinical efficacy across a range of concentrations and lead, ultimately, to underdosing in trials.

In an example of forward translation, using clinical trial simulation the developed MBMA reproduced efficacy results against RSV A from a phase 3 clinical trial of the mAb, REGN2222. Efficacy for the prevention of LRTI predicted by the model was generally in agreement with the efficacy reported in the publication for both dose groups, with the predicted mean efficacy falling within the observed

95% CI. However, for the clinical trial, the small study size and low number of observed RSV A cases resulted in wide confidence intervals, which may limit the interpretability (in isolation) of this component of the validation. Though predicted by the model, no apparent dose-response relationship was reported in the clinical trial data. This may be due to the wide confidence intervals and, therefore, an imprecise point estimate of efficacy in the two treatment arms. For example, one less case in the two-dose group would have increased the efficacy estimate by ~20%, resulting in a more apparent dose response. Alternatively, the timing of dosing or of the RSV A seasonality concurrent with trial conduct may have limited the ability to observe additional efficacy for the second dose administered on Day 57 (i.e., if less RSV A was circulating after the second dose than the first, then additional benefit of the second dose would be challenging to measure). When new larger trials with more clinical cases are reported with next generation RSV neutralizing mAbs, the validation exercise can be repeated with additional datasets.

As a second example of forward translation, a human RSV challenge study was conducted to confirm that MK-1654 provided protection against RSV infection consistent with MBMA predictions. To our knowledge, this is the first RSV challenge study conducted in humans using a mAb. To evaluate agreement between MBMA predictions and efficacy of MK-1654 in the clinic, the doses in the trial were selected to produce a range of SNA titres, from minimally- to highly-protective. Accordingly, the study dose-level group sizes were limited, and generally selected to show trends (enabling modelling) across dose-levels rather than statistically significant pairwise reductions in viral load or RSV infection rates at each dose. The results of the human challenge trial using MK-1654 were consistent with MBMA at the asymptomatic disease severity level, providing proof of concept that SNA titre produced by MK-1654 is a correlate of protection for efficacy in the clinic. Although the observed incidence of symptomatic RSV infection in the challenge study was higher than expected based on the model, the difference is well within the variability expected for a trial of this size, and the trend of the MK-1654 datapoints was consistent with the MBMA. Higher rates of symptomatic infection observed may be due to the liberal definition of RSV infection (Materials and Methods) used in the MK-1654 human challenge trial and the inoculation of a large quantity of virus, which could overcome inhibition by the mAb. Furthermore, there is a higher degree of uncertainty in the protection curve for symptomatic disease in a human challenge setting as all literature data used to fit the curve for this clinical level came from one published human challenge study. General agreement between efficacy observed in the MK-1654 challenge trial and model predictions further establishes proof of concept for MK-1654 and increases confidence in model predictions for an infant population. The results of our study support the use of the MBMA for efficacy predictions against various levels of disease in differing populations.

Clinical development of MK-1654 for single-dose prevention of RSV in infants entering their first RSV season remains ongoing. Trial simulations described herein predict that a single dose of the mAb will provide $\geq$ 75% efficacy for the prevention of RSV LRTI through at least 150 days. Efficacy in the target population is predicted to plateau at doses $\geq$ 75 mg, suggesting doses above this value produce SNA titres on or near the plateau of the exposure-response relationship, as defined by the MBMA. These efficacy predictions helped support initiation of a large phase 2b/3 outcome trial of MK-1654 in infants (NCT04767373).

The approach for RSV mAb efficacy prediction described here advances current approaches; nonetheless, there are inherent assumptions and limitations to the model and its applications to predicting clinical efficacy of MK-1654. First, it is assumed that the imputation of SNA titres for mAbs is sufficiently close to true titres. This is a reasonable assumption because mechanistically, RSV neutralizing mAbs work independently from host immune responses (i.e., innate, or B and T cell responses); protection is derived from specific antibodies that partition to mucosal sites to neutralize pathogen infection of respiratory epithelial cells [29]. This approach relies on fixed estimates of mAb potency which were based on both *in vitro* and *in vivo* data and further corroborated with in-house *ex vivo* experiments. The current model generally does not account for potential differences in partitioning to the site of action between mAbs or potential non-neutralizing efficacy mediated by Fc effector functions; therefore, clinical efficacy is strictly a function of neutralization titres in the serum. A refined understanding of this partitioning behaviour in humans across mAbs might improve model fidelity further. In addition, MBMA-predicted efficacy for mAbs does not account for the potential circulation or emergence of variants that confer resistance to the mAb, as was observed in the phase 3 trial of REGN2222 [15]. However, this potential could be built into future model iterations. The risk of mutations that confer resistance to MK-1654 is low given the conserved nature of its binding site on the F protein (site IV) [30–32] and the lack of circulating strains of RSV with resistance to MK-1654 [12]. Lastly, five decades of clinical data were available for model development. Several differences between study design (e.g., the method used for viral detection, amount of viral inoculum used in challenge trials) were not incorporated into the model and were instead accounted for using inter-trial variability. While many of these studies were small with concomitant high variability in their results, the MBMA technique enables combining the information across studies, thus giving summary results with lower variability, as clearly seen in Fig. 2. Such copious amounts of literature for model building may not be available for all pathogens; however, the impact of having fewer clinical data sets can be overcome, as demonstrated by models built on nonclinical data and more limited clinical data for newly emerging pathogens, such as SARS-CoV-2 [33,34]

The MBMA described here advances our current capability to predict the efficacy of RSV mAb and vaccine prophylaxis over current approaches. Furthermore, the model is generalizable beyond RSV. For example, the framework and methodology could aid the clinical design of vaccine and mAb trials for SARS-CoV-2 or other pathogens by predicting, with improved fidelity, the doses and frequencies of immunization needed to achieve protection. Further development of similar models has the potential to improve predictions of efficacy and trial design for any antibody-based passive or active immunotherapy.

Data sharing statement

Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA's data sharing policy, including restrictions, is available at http://engagezone.msd.com/ds_documentation.php through the EngageZone site or via email to dataaccess@merck.com.

Funding

This study was funded by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.

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Declaration of Competing Interest

BM, RR, LC, JC, WL, YZ, QH, WG, DS, BR, AE, SS, EL, and KV are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA (or were at the time of the study), and may hold stock in Merck & Co., Inc., Kenilworth, NJ, USA. JRS is an employee of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and may hold stock in Merck & Co., Inc., Kenilworth, NJ, USA and reports other investments that are less than 1% ownership for any company. JL, NP, LQ, HW, ES, BG, and FB are employed by Certara, Princeton, NJ, USA (or were employed at the time of the study) and may hold shares in Certara, Princeton, NJ, USA. Certara received funding from Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, for modelling work. ASYC is employed by Certara, Princeton, NJ, USA and holds stock in Certara, Princeton, NJ, USA and AstraZeneca, Cambridge, UK and is a chair of IQ consortium TALG and CLPG Pediatric PBPK group. JM, MK, AP, and JFK : nothing to disclose.

Acknowledgments

We would like to express our gratitude for the participants and study staff in the MK-1654 Protocol 005 challenge study. We thank Maria Luisa Sardu and Anna Largajolli of Certara, Princeton, NJ, USA for important Quality Control support of the modelling data. The authors would also like to thank members from Certara Data Science Group at Hyderabad and Menlo Park led by Raj Thatavarti and Nancy Zhang. We thank Andrew W. Lee of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA for careful reading of the manuscripts/abstracts from the literature searches performed for this study and for critically reviewing the manuscript. We also acknowledge Kara S. Cox for medical writing support, and Karyn Davis for editorial support, both are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA. Thanks also to Jesse Nussbaum, Michael Citron, Colleen Winstead (employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA at the time of this study), and Michelle Green (employed by Certara, Princeton, NJ, USA during the time of this study) for careful reading of the manuscripts and abstracts from the literature searches performed for this study. Thank you to Lyn Finelli for epidemiological data support and Qi Shen for clinical data assembly/quality control (both are employees of Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2021.103651.

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