Global molecular diversity of RSV – the “INFORM RSV” study

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Abstract

Background: Respiratory syncytial virus (RSV) is a global cause of severe respiratory morbidity and mortality in infants. While preventive and therapeutic interventions are being developed, including antivirals, vaccines and monoclonal antibodies, little is known about the global molecular epidemiology of RSV. INFORM is a prospective, multicenter, global clinical study performed by ReSViNET to investigate the worldwide molecular diversity of RSV isolates collected from children less than 5 years of age.

Methods: The INFORM study is performed in 17 countries spanning all inhabited continents and will provide insight into the molecular epidemiology of circulating RSV strains worldwide. Sequencing of > 4000 RSV-positive respiratory samples is planned to detect temporal and geographical molecular patterns on a molecular level over five consecutive years. Additionally, RSV will be cultured from a subset of samples to study the functional implications of specific mutations in the viral genome including viral fitness and susceptibility to different monoclonal antibodies.

Discussion: The sequencing and functional results will be used to investigate susceptibility and resistance to novel RSV preventive or therapeutic interventions. Finally, a repository of globally collected RSV strains and a database of RSV sequences will be created.

Keywords: Respiratory syncytial virus, Next generation sequencing, Temporal and geographical diversity, Molecular epidemiology, Monoclonal antibodies, Vaccines

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**Article summary**

**Strengths**

- INFORM RSV is large enough to identify drivers of spatial and temporal distribution.
- Sequencing platform was selected based on a comparative pilot study.
- RSV is cultured to translate genotype to function.
- INFORM RSV is collaborating with others including researchers from the UEDIN, WHO and NIH.

**Limitations**

- Clinical information is limited, no follow-up data available.

**Background**

Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infections in children worldwide [1]. While most children infected with RSV suffer from runny noses, coughing and wheezing, RSV infection can escalate to bronchiolitis, pneumonia and even death [2]. Globally in 2015, 48,000–74,500 children under the age of 5 years died with RSV in-hospital, predominantly in low- and middle-income countries [2].

Although RSV is recognized as a global health problem, there is no licensed vaccine currently available anywhere in the world. Efforts to develop a vaccine initially failed in the 1960s when the first vaccine candidate, a formalin-inactivated vaccine, did not protect against RSV in children but instead induced exacerbated lung disease after subsequent RSV exposure requiring hospitalization and causing death [3, 4]. The potential risk of enhanced disease has hampered vaccine development such that, even after more than 50 years of effort, no vaccine is available yet. An alternative approach for prevention of RSV disease is passive immunization with monoclonal antibodies (mAbs). RSV-IGIV (RespiGam), an intravenous immunoglobulin containing high titers of RSV neutralizing antibodies, was initially approved in 1995 as a passive immunization strategy but was discontinued in 2003 after its replacement by the more potent mAb palivizumab (humanised mAb that targets the RSV fusion (F) protein) [5]. Palivizumab is the only currently approved prophylaxis and its use is limited to high-risk infants (premature, heart and lung disease, Down syndrome) in high-income countries [3]. These data demonstrate that neutralizing Abs are efficient in preventing RSV disease and that antibody levels correlate with RSV disease prevention. The development of suptavumab (REGN22222), another mAb targeting the RSV F protein as a preventive strategy for use in preterm infants was discontinued in 2017 as it failed to meet the primary endpoint of preventing medically-attended RSV infections [6, 7]. A promising mAb candidate currently in clinical development is nirsevimab (MEDI8897), which targets the prefusion form of RSV F protein [8]. With a higher potency and extended half-life as compared to palivizumab, nirsevimab holds promise for protecting from RSV-associated lower respiratory disease for all infants entering their first RSV season and high-risk infants entering their first and second RSV seasons [7, 8].

Future clinical use of therapeutics, vaccines and mAbs to prevent RSV raises concerns about the emergence of local resistant strains [9, 10]. Therefore, RSV global surveillance is required. The Observational US Targeted Surveillance of Monoclonal Antibody Resistance and Testing of RSV (OUTSMART-RSV) surveillance program characterized circulating RSV strains in the U.S. during the 2017–18 season [11]. RSV strains that are resistant to palivizumab were found to be rare [10]. The frequency of natural resistance-associated polymorphisms for nirsevimab was also low (in vitro < 1%). However, the degree to which the acquisition of resistance will impact the effectiveness of current and future RSV therapeutics on a global scale remains unclear. To date, mAb-resistant mutants (MARMs) have not been thoroughly studied worldwide and little is known about the prevalence of naturally occurring resistant RSV strains either. The International Network For Optimal Resistance Monitoring of RSV (INFORM RSV) study will therefore prospectively describe the molecular epidemiology of RSV by monitoring temporal and geographic distribution of whole viral genome sequences. In addition to monitoring, we will construct a large repository of RSV sequence derived from a diverse geographic location. In the present article, we describe the methodology of the INFORM RSV study.

**Study objectives**

**Primary objective**

To investigate the molecular diversity of RSV isolates recovered from a global population of children less than 5 years of age over a five-year period.

**Secondary objectives**

1. To evaluate the prevalence of strains with polymorphisms in the binding regions for RSV mAbs
2. To compile a repository for RSV sequences
3. To perform functional virology studies
4. To test for susceptibility of newly identified RSV strains to RSV mAbs
5. To establish natural molecular evolution of RSV genomes before the widespread use of RSV mAbs or vaccines
Methods

Study design

INFORM RSV is a global clinical study initiated in 2017 by AstraZeneca to prospectively analyze RSV strains collected from children < 5 years of age. Collaborators were identified via the Respiratory Syncytial Virus Network (ReSViNET; www.resvinet.org). The ReSViNET Foundation is the international leading non-profit organization committed to reducing global burden of RSV infection. In the INFORM RSV study, RSV positive nasal samples will be collected from subjects as part of routine clinical care at local clinical sites and shipped to the laboratory of the University Medical Centre Utrecht (UMCU), the Netherlands, for sequencing and culturing.

In the INFORM RSV study, the goal is to collect and sequence approximately 4000 RSV positive respiratory samples during a 5-year period (2017–2022), which correlates to 50 or 100 samples per site per year (Additional files, Table 1). At the time of writing, the INFORM RSV study has been ongoing for 2 years and is currently conducted in 17 countries at 18 sites (Fig. 1). We aim to expand to other countries where disease burden studies are ongoing. To ensure both seasonal and geographical diversity, we endeavor to collect 10–20 samples per site per month, over the ~ five-month RSV season, which is on average 5 months long. If the site is able to collect more than the required number of samples, a subset will be randomly selected. Viral genomic sequencing will be performed on all samples by NGS using RT-PCR amplified cDNAs at the UMCU laboratory. To study molecular resistance, a subset of strains (~10%) will be randomly selected and cultured to evaluate functional susceptibility to anti-viral drugs being developed, and viral fitness of RSV variant with drug binding site changes or dominant changes in non-drug binding site.

Study participants

Children are eligible to participate in the study if they meet all the inclusion criteria (Table 1). Children can participate in the study when they fulfill all the following criteria: (1) under the age of 5 years at time of sampling, (2) admitted to the hospital or visiting the outpatient clinic, (3) tested positive for RSV or suspected to have RSV infection when RSV testing is not standard practice. Suspicions of RSV is defined by respiratory tract infection (RTI) symptoms. In instances where testing for RSV is not a standard of care, the informed consent procedure is performed before sample collection for study purposes. Lower and upper RTIs are not differentiated. Signed and dated written informed consent is obtained from parent(s)/legal representative(s) in accordance with the INFORM RSV study protocol, the International Conference on Harmonization Guideline on Good Clinical Practice E6 (ICH-GCP) and applicable national and international regulatory requirements including the Declaration of Helsinki. Children who meet the exclusion criteria of using preventive or treatment medication for RSV e.g. palivizumab, ribavirin or an experimental RSV mAb or vaccine will be excluded from participation.

Fig. 1 Countries participating in the INFORM RSV study. Red – Start in 2017; Blue – Start in 2018; Yellow – Start in 2019. The figure was created by ACL using Maptive (https://www.maptive.com)
Table 1 Eligibility criteria for the INFORM RSV study

| Inclusion Criteria | Exclusion Criteria |
|--------------------|--------------------|
| Age < 5 years      | Use of palivizumab or experimental medication for RSV |
| Confirmed RSV positive diagnosis | |
| Written informed parental consent | |

Sample collection, storage and shipment

After informed consent is obtained, nasopharyngeal samples are collected using flocked swabs and placed in Copan Universal Transport Medium (UTM). When patients are ventilated, bronchial aspirates are collected in Copan UTM. Samples are stored locally at −80 °C. When −80 °C storage is unavailable, samples can be stored at −20 °C before shipment to the UMCU laboratory. Samples are preferably stored in the original container and labeled with unique barcode provided by the UMCU corresponding to the INFORM RSV code. Samples are shipped frozen on dry ice to the UMCU laboratory for sequencing and culturing after each season.

Nucleic acid extraction and RSV subtyping

Nucleic acids are extracted from 250 to 500 μL of RSV positive nasal specimens using the MagNA Pure 96 DNA and Viral NA Large Volume kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions. Nucleic acids are eluted in 50 μL elution buffer. RSV subtyping and quantification is performed by multiplexed TaqMan RT-PCR analysis of the RSV N gene using RSV-A and RSV-B specific primer/probe mixes. The TaqMan RT-PCR reactions are performed on a StepOnePlus System (Applied Biosystems) according to the manufacturer’s protocol (Thermo Fisher Scientific). The purified amplicons are then quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. Subsequently, the normalized PCR products are subjected to Next Generation Sequencing (NGS) library construction using the Nextera XT DNA Library Prep Kit according to the manufacturer’s protocol (Illumina). Illumina sequencing adapters and barcodes are added to the tagmented DNA via PCR amplification using unique custom oligo sequences (Integrated DNA Technologies). Subsequently, the DNA is purified and size-selected using 0.6 X volume of Ampure XP reagent (Beckman Coulter, Inc.) according to the manufacturer’s protocol. Next, the purified DNA is quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific) and mixed in equimolar amounts. Sequencing is performed on the Illumina NextSeq500 platform (Illumina, Inc), generating paired-end 150 bp reads.

Table 2 Primers used in this study to amplify overlapping RSV genome fragments

| Primer                    | Sequence (5’-3’) |
|---------------------------|------------------|
| RSVA-fragment 1-Fw        | AAAATGCGTACWACAAACCTTC |
| RSVA-fragment 1-Rev       | GTTGTCCTGTTGTTGAGGC |
| RSVA-fragment 2-Fw        | CACGACTGACACACAAGGGAG | |
| RSVA-fragment 2-Rev       | GCTGATGGAACACCATGC |
| RSVA-fragment 3-Fw        | CGAGGTCCATCTTGGAATTGG |
| RSVA-fragment 3-Rev       | CACCCACCACTACATG |
| RSVA-fragment 4-Fw        | AGGGTGTTGATCAAAACTATGG |
| RSVA-fragment 4-Rev       | ACAGGAAAAAAAGTGTCAAACATG |
| RSVB-fragment 1-Fw        | AAAATGCGTACTCAACAGGAGATC |
| RSVB-fragment 1-Rev       | TTTCCTCCACGGAGACAGCATGAGAAT |
| RSVB-fragment 2-Fw        | TTTCCTCCACGGAGACAGCATGAGAAT |
| RSVB-fragment 2-Rev       | TGCACTTTTCTTTCATAGG |
| RSVB-fragment 3-Fw        | GTGATGAAAGTGGATATTAGG |
| RSVB-fragment 3-Rev       | TTTCCTCCACAGGAGATC |
| RSVB-fragment 4-Fw        | AAGGGTGTGACCAACAGCAG |
| RSVB-fragment 4-Rev       | AAGGGTGTGACCAACAGCAG |

RT-PCR amplification of RSV genomes and next generation sequencing

Upon RSV subtyping, the appropriate primer pairs are used to reverse transcribe and PCR amplify the four overlapping RSV genome fragments by using the SuperScript IV One-Step RT-PCR System (Invitrogen, CA) in a 9800 Fast thermal cycler (Applied Biosystems). The four overlapping genome fragments together comprise of the full RSV genome encompassing all viral genes, yet lacking the far 3’ and 5’ genome termini. Degenerate bases are used in places of genetically variable bases across RSV-A and RSV-B strains when necessary (Table 2). Cycling conditions are 55 °C for 10 min and 98 °C for 2 min, followed by 40 cycles of 98 °C for 10 s, 61 °C for 10 s and 72 °C for 3 min. Amplicons are verified on 1% agarose gels, pooled in equimolar amounts, and purified from 1% agarose gel using the GeneJet PCR Purification Kit (Thermo Fisher Scientific). The purified amplicons are then quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. Subsequently, the normalized PCR products are subjected to Next Generation Sequencing (NGS) library construction using the Nextera XT DNA Library Prep Kit according to the manufacturer’s protocol (Illumina). Illumina sequencing adapters and barcodes are added to the tagmented DNA via PCR amplification using unique custom oligo sequences (Integrated DNA Technologies). Subsequently, the DNA is purified and size-selected using 0.6 X volume of Ampure XP reagent (Beckman Coulter, Inc.) according to the manufacturer’s protocol. Next, the purified DNA is quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific) and mixed in equimolar amounts. Sequencing is performed on the Illumina NextSeq500 platform (Illumina, Inc), generating paired-end 150 bp reads.
RSV genome assembly and annotation
Assembly of the sequencing reads into complete genomes is performed with AstraZeneca’s Next-Generation Sequencing Microbial Surveillance Toolbox (NGS-MSTB) – a fully automated distributed pipeline implemented with a Common Workflow Language (CWL), and with a user interface based on the Galaxy bioinformatics workbench [12]. The main processing step is a targeted de-novo assembly using Ariba [13] with AstraZeneca’s customized assembly protocol tuned for robustness in the presence of mixed viral subpopulations and very high coverage variability. This is followed by post-assembly filtering of the low-abundance poorly assembled quasi-species. The pipeline creates a Web report with quality control metrics and genome browser views at the contig and individual read levels. A manuscript with detailed description of the assembly pipeline and its open-source release is in preparation.

The assignment of RSV subtypes is performed during the assembly process and the assignment of RSV genotypes is performed by phylogenetic clustering using a reference database of previously described genotypes [14].

To determine the polymorphisms in the F protein binding regions of RSV mAbs, the gene sequences are translated into amino acid sequences, aligned against reference sequences (NL13 strains), and the amino acid changes are recorded.

RSV culture
Frozen respiratory samples stored in UTM are thawed, combined 1:1 with DMEM (Dulbecco’s Minimal Essential Medium; Lonza) supplemented with 5% FBS and 100 μg/ml Normocin (InvivoGen), and subsequently filtered through a 0.45 μm filter. The filtrate is used to infect HEp-2 cells (60% confluent) in T25 flasks for 1 h at 33 °C and 5% CO2. The supernatant is replaced with fresh DMEM supplemented with 5% FBS and 100 μg/ml Normocin and placed back into the 33 °C humidified, 5% CO2 incubator. The viral culture is harvested upon reaching approximately 70% cytopathic effect (CPE) by centrifugation at 247×g for 10 min and combining the supernatant with 50% sucrose in dPBS (sterile filtered). The viruses are stored in 1 ml aliquots at −80 °C.

Data collection and management
Data is recorded on an electronic sample reporting form (SRF) (Table 3). SFRs from all sites are uploaded to a central database (eCASTOR) by Julius Clinical after which the clinical data are merged with the sequencing data. To ensure subject anonymity only a unique subject number and the age in months will be entered. Data will be locked after each season.

| Variables | Description |
|-----------|-------------|
| Site ID   | Age in months |
| Study ID  | Male / Female |
| Country   | < 24 h / > 24 h / Outpatient |
| Visiting date | Paediatric Intensive Care Unit / General Paediatric Ward / Outpatient clinic (including Emergency Department) |
| Age      | RSV detection method / molecular point-of-care-test |
| Gender   | A / B |
| Length of stay | -20 °C / -80 °C |
| Referring department | Calculated duration of pregnancy in weeks |
| RSV subtype | Congenital heart disease / Hematological malignancies / Neurological disease / Bronchopulmonary dysplasia / Other (specified in provided space) |
| Storage temperature | Yes (exclusive) / No / Partial |
| Gestational age at birth | Yes / No |
| Severe comorbidity | Smoking in household |
| Breastfeeding | Yes / No |
| Day care attendance | Other children in household under the age of 6 |

Table 3 Patient variables in the electronic case record form
Outcomes

Primary endpoint
RSV sequences from a global population of hospitalized children.

Secondary endpoints
1. Total number of RSV A and B subtypes and related genomes and the association of these subtypes with patient characteristics (Table 3)
2. Homology of the F gene from wild-type circulating RSV to that of reference strains
3. The total number of RSV strains with polymorphisms in RSV mAbs binding regions or antigenic sites of RSV F protein

Sample size calculation
The minimal number of samples needed for this study is 2500. The sample size will result in precise frequency estimates of RSV A and B subtypes as well as polymorphisms. The width of the 95% confidence interval (CI) will be no larger than 4%. In extremely low or high prevalence (e.g. < 7.5% or > 92.5%) the width of the 95% CI will be less than 2%. This study is also well powered to detect differences in the prevalence of subtypes (RSV A vs B). An estimate of the mean prevalence of RSV A (two-thirds) was derived from the study by Zhu et al. [10]. The INFORM RSV study has at least 90% power to detect a difference in the prevalence of subtypes between groups of 7% at an alpha of 0.05 (e.g. 70% RSV A in males vs. 63% RSV A in females), and at least 90% power to detect an effect size of 0.08 using a 4 degrees of freedom chi-square test. This means, for example, that this study can detect a difference in the distribution of RSV subtypes if the prevalence of RSV A across the sites were approximately as follows: 58, 62, 66, 70, and 74%. These sample size calculations were conducted in PASS software, using the two-sided CIs for single proportions with the simple asymptotic method with continuity correction and a chi-square test power analysis. Although 2500 samples are sufficient to detect the desired effect, and based on the minimal invasiveness for INFORM participants, the study will expand and add more countries to maximize insight in geographic and temporal diversity.

Discussion
In the INFORM RSV study, RSV isolates are subject to RSV subtyping and viral genome analysis. The main purpose of the project is to secure RSV samples to monitor RSV strains for changes in key epitopes recognized by mAbs. RSV is a member of the human orthopneumoviridae family [15], which are RNA viruses and therefore prone to genomic mutations. The possibility of immunological escape or viral resistance from mAbs approved or under development is a potential concern. In fact, a previous study performed by Regeneron (NCT02325791) to evaluate the efficacy and safety of suptavumab for the prevention of medically attended RSV infection in pre-term infants failed to meet its predefined efficacy endpoint based on its reduced efficacy against RSV B strains [16]. The reduced RSV B efficacy was due to a two-amino acid change at positions 172 and 173 in the antigenic site V region of the F protein, the epitope of suptavumab, which reduced susceptibility to suptavumab neutralization in vitro. It is therefore important that clinical studies involving anti-RSV F mAbs monitor for amino acid substitutions in antigenic binding regions of RSV isolates from subjects experiencing virologic failure, and to assess the impact of these changes on phenotypic susceptibility and viral fitness.

A key challenge for the INFORM RSV study is temporal diversity, as the timing of RSV outbreaks differs by season and location around the world. Another challenge is how to best integrate and interpret whole genome sequences in relation to clinical variables. To overcome this challenge, bioinformaticians from AstraZeneca, UMCU and Julius Clinical are working closely together to develop an integrated database and a robust pipeline to characterize the thousands of RSV sequences that will be generated.

In summary, this global prospective study aims at monitoring the molecular epidemiology of RSV to ensure that already approved therapeutics and those in development will be effective against currently circulating strains worldwide. The study has the potential to provide valuable information for vaccines, monoclonal antibodies and therapeutic drugs in development and will contribute to creating an international RSV repository.
How were the research question(s) and outcome measures developed and informed by their priorities, experience, and preferences?

Answer: Research questions and outcomes were developed by the UMC Utrecht and approved by the patient advisory board.

How were patients/public involved in the design of this study?

Answer: See above.

How were they involved in the recruitment to and conduct of the study?

Answer: Patients have not been involved in recruitment other than optimizing the patient information.

Were they asked to assess the burden of the intervention and time required to participate in the research?

Answer: See above.

How were (or will) patients and the public be involved in choosing the methods and agreeing?

Answer: See above.

Plans for dissemination of the study results to participants and wider relevant communities?

Answer: See above.

Authors’ contributions

All named authors in this article participated in the INFORM study. All authors read and approved the final manuscript. ACL, AG, TH, RTS, PR, FMT, MN, MH, CK, MB, RC, JP1*, JP2*, MPH and JGW were involved in patient recruitment. RJL, AE, MCV and FEJC performed laboratory analyses. ACL, CN, HJ, DET, AT, AR, MEA, DW, LKT, MTE and LB contributed to the design of the work, analysis and/or interpretation of data. ACL wrote the manuscript. *JP1 is corresponding to Jesse Papenburg, chosen because this name is furthest up in the author list.

Funding

The INFORM study received funding from AstraZeneca. The protocol has been developed by the authors. AstraZeneca has access to all data except viral sequences outside the RSV F and G genes. Data analysis is done by the principle investigator (Louis J Bont).

Availability of data and materials

As the current manuscript describes the study protocol and no other data, we do not have any raw data to share at the moment.

Ethics approval and consent to participate

The INFORM study has been approved by the ethic committees of all 18 participating sites:

- The Netherlands: The Medical Research Ethics Committee of the UMC Utrecht (reference number WAG/mb/17/016170).
- Italy: Ethics Committee for Clinical Testing of the Province of Padova of the Padova Hospital (no. 345 of 27/10/2016).
- Russia: The Department for Science, Innovation Development and Management of Health and Biological Risks, Ministry of Health of the Russian Federation.
- Germany: Ethics Committee of the Medical Faculty of the Philippus University Marburg.
- France: Ethics Committee Southwest and Overseas of the Crèteil Intercommunal Hospital Centre (ID-RCB No.: 2018-A02360–5S (file 1–18:73).
- Spain: Ethics Committee for Research Santiago-Lugo of the Hospital Centre University of Santiago (registration code 2017/397).
- South Korea: Medical Research Committee of the Seoul National University Hospital.
- Finland: Ethics Committee of the Hospital District of Southwest Finland, Turku.
- Australia: Human Research Ethics Committee of the Perth Children's Hospital.
- Brazil: The Research Ethics Committee of the Centro INFANT at Pontificia Universidade Catolica de Rio Grande do Sul (opinion number 2,569,872).
- Canada: Hamilton Integrated Research Ethics Board of the McMaster University.
- Canada: Research Ethics Board of the McGill University Health Centre.
- South Africa: Human Research Ethics Committee of the University of the Witwatersrand Johannesburg (no. M170966).
- Japan: Research Ethics Committee of the Fukushima Medical University (no. 29212).
- The United Kingdom: Health Research Authority of the King's College Hospital (no. 17/06/0466).
- Taiwan: Mackay Memorial Hospital Institutional Review Board (no. 19WMHS171e).
- Chile: Ethics Committee for Research on Human Subjects of the Faculty of Medicine, University of Chile.
- Mexico: Ethics Committee of the University Autónoma De Nuevo Leon, Faculty of Medicine.

Written informed consent was obtained from parent(s)/legal representative(s) of all children participating in the study.

Consent for publication

Not applicable.

Competing interests

FMT and JP are members of the editorial board of BMC Infectious Diseases. MCN has received grant funding from AstraZeneca. JP has received consulting/speaker fees/honoraria from AbbVie, Seegene Canada and Cepheid, and research grant funding outside of the current work from AbbVie, BD Diagnostics, AstraZeneca, Sanofi Pasteur, Hoffmann-La Roche and Janssen Pharmaceutical. LB has not received personal fees or other personal benefits. UMCU has received funding from AbbVie, AstraZeneca, Janssen, the Bill and Melinda Gates Foundation, Nutricia (Danone) and MeDed Medicos. UMCU has received major cash or in kind funding as part of the public private partnership IMI-funded RESCEU project from GSK, Novavax, Janssen, AstraZeneca, Pfizer and Sanofi. UMCU has received major funding by Julius Clinical for participating in the INFORM study sponsored by AstraZeneca. UMCU has received minor funding for participation in trials by Regeneron and Janssen from 2015 to 2017. UMCU received minor funding for consultation and invited lectures by AbbVie, AstraZeneca, Abyline, Bavaria Nordic, MabXience, Novavax, Pfizer, Janssen. LB is the founding chairman of the ReSViNET Foundation. Nirsevimab development is jointly funded by AstraZeneca and Sanofi Pasteur.

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