

**Running head:**

“RESCEU: Understanding RSV”

**Title:**

REspiratory Syncytial virus Consortium in EUrope (RESCEU): Presumed risk factors and biomarkers for severe RSV disease and related sequelae. Protocol for an observational multi-centre, case-control study

**Authors:**

Kimberley Jefferies, Department of Paediatrics, Oxford Vaccine Group, Oxford, OX3 7LE, UK

Simon B Drysdale, Department of Paediatrics, Oxford Vaccine Group, Oxford, OX37LE, UK and  
Department of Paediatrics, St George’s University Hospital NHS Foundation Trust, London, SW17  
0QT

Hannah Robinson, Department of Paediatrics, Oxford Vaccine Group, Oxford, OX37LE, UK

Elizabeth Ann Clutterbuck, Department of Paediatrics, Oxford Vaccine Group, Oxford, OX37LE, UK

Luke Blackwell, Department of Paediatrics, Oxford Vaccine Group, Oxford, OX37LE, UK

Joseph McGinley, Department of Paediatrics, Oxford Vaccine Group, Oxford, OX37LE, UK

Gu-Lung Lin, Department of Paediatrics, Oxford Vaccine Group, Oxford, OX37LE, UK

Ushma Galal, Nuffield Department of Primary Care Health Sciences, Oxford, OX2 6GG

Harish Nair, Usher Institute, University of Edinburgh, Old Medical School, Teviot Place, Edinburgh,  
EH8 9AG

22 Jeroen Aerssens, Infectious Diseases, Janssen Pharmaceutica NV, 2340 Beerse, Belgium

23 Deniz Öner, Infectious Diseases, Janssen Pharmaceutica NV, 2340 Beerse, Belgium

24 Annefleur Langedijk, Department of Paediatrics, University Medical Center Utrecht, 3584 CX, The  
25 Netherlands

26 Louis Bont, Department of Paediatrics, University Medical Center Utrecht, 3584 CX, The  
27 Netherlands

28 Joanne G Wildenbeest, Department of Paediatrics, Immunology and Infectious Disease, Wilhelmina  
29 Children`s Hospitals, University Medical Center Utrecht, 3584 CX, The Netherlands

30 Federico Martinon-Torres, Translational Paediatrics and Infectious Diseases, Pediatrics Department,  
31 Hospital Clínico Universitario de Santiago, Spain and Genetics, Vaccines, Infections and Pediatrics  
32 research group, Instituto de Investigación Sanitaria de Santiago, University of Santiago de  
33 Compostela, Spain

34 Carmen Rodríguez-Tenreiro Sánchez, Translational Paediatrics and Infectious Diseases, Pediatrics  
35 Department, Hospital Clínico Universitario de Santiago, Spain and Genetics, Vaccines, Infections and  
36 Pediatrics research group, Instituto de Investigación Sanitaria de Santiago, University of Santiago de  
37 Compostela, Spain

38 Simon Nadel, Department of Paediatrics, Imperial College, London, SW7 2AZ, UK

39 Peter Openshaw, Faculty of Medicine, National Heart & Lung Institute, Imperial College, London,  
40 W2 1NY, UK

41 Ryan Thwaites, Faculty of Medicine, National Heart & Lung Institute, Imperial College, London, W2  
42 1NY, UK

43 Myra Widjojoatmodjo, Janssen Vaccines and Prevention B.V., 2333 CP Leiden, The Netherlands

44 Linong Zhang, Sanofi Pasteur, Cambridge MA 02139, USA

45 Thi Lien-Anh Nguyen, GSK Biologicals, 1330 Rixensart, Belgium

46 Carlo Giaquinto, Fondazione PENTA ONLUS, Torre di Ricerca Pediatrica, Corso Stati Uniti, 4,  
47 35127 Padova, Italy

48 Giuseppe Giordano, Fondazione PENTA ONLUS, Torre di Ricerca Pediatrica, Corso Stati Uniti, 4,  
49 35127 Padova, Italy

50 Eugenio Baraldi, Fondazione PENTA ONLUS, Torre di Ricerca Pediatrica, Corso Stati Uniti, 4,  
51 35127 Padova, Italy

52 Andrew J Pollard, Department of Paediatrics, Oxford Vaccine Group, Oxford, OX37LE, UK

53 The RESCEU investigators

54

55 **Corresponding author**

56 Dr Kimberley Jefferies

57 01865 611400

58 jefferiesk@hotmail.co.uk

59

60 Word count for abstract: 300

61 Word count for text: 3147

62

63

64

65

66

## 67 **ABSTRACT**

### 68 **Introduction:**

69 Respiratory syncytial virus (RSV) is the leading viral pathogen associated with acute lower  
70 respiratory tract infection (ALRI) and hospitalisation in children under five years of age worldwide.  
71 Whilst there are known clinical risk factors for severe RSV infection, the majority of those  
72 hospitalised are previously healthy infants. There is consequently an unmet need to identify  
73 biomarkers that predict host response, disease severity and sequelae.

### 74 **Objectives:**

75 The primary objective is to identify biomarkers of severe RSV acute respiratory tract infection  
76 (ARTI) in infants. Secondary objectives include establishing biomarkers associated with respiratory  
77 sequelae following RSV infection and characterising the viral load, RSV whole-genome sequencing,  
78 host immune response and transcriptomic, proteomic, metabolomic and epigenetic signatures  
79 associated with RSV disease severity.

### 80 **Study design:**

81 630 infants will be recruited across three European countries; the Netherlands, Spain and the United  
82 Kingdom. Participants will be recruited into two groups. Group 1 will consist of infants with  
83 confirmed RSV ARTI (includes upper and lower RTIs), 500 without and 50 with co-morbidities.  
84 Group 2 will consist of 80 healthy controls.

85 At baseline, participants will have nasopharyngeal, blood, buccal, stool and urine samples collected,  
86 plus complete a questionnaire and 14-day symptom diary. At convalescence (7 weeks  $\pm$  1 week post  
87 ARTI) specimen collection will be repeated.

88 Laboratory measures will be correlated with symptom severity scores to identify corresponding  
89 biomarkers of disease severity.

### 90 **Ethics and dissemination:**

UK study authorisation has been granted from the Health Research Authority and ethics approval from NRES South Central and Hampshire A. In Spain this has been granted by Comité de Ética de la Investigación de Santiago-Lugo and in the Netherlands by the Medical Ethical Committee, UMC, Utrecht. All study procedures adhere to International Conference on Harmonisation Good Clinical Practice guidelines.

ClinicalTrials.gov registration number: NCT03756766

*Key words: Respiratory syncytial virus, biomarkers, transcriptomics, proteomics, metabolomics, epigenetics*

## INTRODUCTION

Respiratory syncytial virus (RSV) is a major global health problem. It is the leading pathogen associated with acute lower respiratory tract infection (ALRI) and hospitalisation in infants [1] and contributes to about 13% of ALRI mortality in children younger than 5 years [2]. Premature birth, chronic lung disease, congenital heart disease, immunodeficiency and neuromuscular disease are among the known risk factors associated with severe RSV bronchiolitis [3–7]; however, the majority of hospitalised infants are previously healthy [8,9]. The clinical severity of bronchiolitis varies from mild forms manageable as an outpatient to severe cases requiring mechanical ventilation or extracorporeal membrane oxygenation (ECMO) in a paediatric intensive care unit (PICU) [10]. Two-thirds of infants have an RSV infection in their first year of life [11], 2-3% of these infants will require hospitalisation [6,11–13] and 2-6% of these admissions need management in a PICU [9,11,13,14]. Access to mechanical ventilation and intensive care support has helped maintain a low mortality in high-income countries (0%–1.5% in otherwise healthy infants with RSV) [15]. However, globally, RSV is estimated to be responsible for approximately 118,200 deaths each year in children under 5 years of age [2]. There is also evidence that early life RSV infection results in chronic airway dysfunction, increased risk of subsequent wheezing episodes and asthma [16,17].

There remains a lack of a safe and effective treatment or prophylactic agent for RSV infection. Currently, the only option available is passive protection provided by the monoclonal antibody, palivizumab (Synagis®; Swedish Orphan Biovitrum AB [Sobi™]) [18–22]; however, its high cost limits its use to those deemed high risk for severe disease. Therefore, there is a parallel need to assemble clinical resources to identify the correlates of severe RSV disease for clinical management, classification of disease severity in clinical trials and identification of biomarkers for severe disease to target safe and effective therapies [24].

## OBJECTIVES

The objectives of this study are to establish biomarkers correlated with and/or predictive of RSV ARTI (including upper and lower RTIs) disease severity among infants (primary objective); to determine which biomarkers are associated with subsequent respiratory sequelae following primary RSV infection and to quantify the health care costs and interruption to normal activities of daily living (secondary objectives). The primary and secondary endpoints are listed in table 1.

## **STUDY DESIGN**

This is a multi-national, multi-centre, case-controlled, observational study. 630 participants will be recruited across four sites including the University Medical Center Utrecht (UMCU), the Netherlands; Hospital Clínico Universitario de Santiago, Spain; Imperial College NHS Trust, London and participating hospitals in the Thames Valley and South Midlands Clinical Research Network (Oxford), UK. Infants will be recruited into two groups. Group 1 includes infants <12 months old with confirmed RSV ARTI, 500 without co-morbidities and 50 with co-morbidities (including congenital heart disease, bronchopulmonary dysplasia, prematurity defined as gestational age <37 weeks, Down's syndrome or any other significant medical condition) and Group 2 includes 80 healthy controls without RSV infection.

Infants in Group 1 will be further subdivided into four groups for data analysis. Regular review of participants recruited will ensure the target recruitment numbers for each sub-group are met:

- 1) Group 1a (n=250): healthy infants (without co-morbidities) with RSV infection requiring hospitalisation for at least 12 hours. Comparisons will be made between mechanically ventilated infants (severe phenotype) and those with less severe symptoms.
- 2) Group 1b (n=250): healthy infants with RSV infection not requiring hospitalisation (ie: participants who remain in the community or who are discharged home within 12 hours of registration if they present to the emergency department).

The analysis of Group 1a and 1b will answer the primary objective and the other groups will support the analysis of the secondary endpoints.

3) Group 1c (n=25): infants with RSV infection with any co-morbidity that would exclude them from group 1a and 1b requiring hospitalisation for at least 12 hours.

4) Group 1d (n=25): infants with RSV infection with any co-morbidity that would exclude them from Groups 1a and 1b, and not requiring hospitalisation

## RECRUITMENT

Potential participants for groups 1 and 2 will be recruited from both the hospital and community setting.

Methods of recruitment will include:

- Active surveillance: The clinical and research team working in the hospital setting (emergency department, paediatric ward, paediatric outpatient department or paediatric intensive care unit) identifying and approaching eligible participants.
- Outreach to increase voluntary participation:
  - Poster advertising throughout local hospitals and general practitioner (GP) surgeries
  - Mail-out to health visitors and GPs to inform them of the study and encourage them to identify potential participants.
  - Media advertising through social media and website advertisement placed in locations relevant for the target age group with brief details of the study and contact details for further information.
- For Group 1b, infants may also be recruited through the RESCEU birth cohort study in which children are actively followed in the first year of life.

Potential participants will have the opportunity to contact their respective research sites for additional information by telephone or email.



## ASSESSMENT OF ELIGIBILITY

Formal assessment of trial eligibility will follow informed written consent. Eligible participants for

Group 1 must fulfil the following criteria:

- Male or female infant <12 months of age at enrolment.
- Confirmed RSV positive ARTI. Confirmed using an RSV point of care test (either a rapid antigen detection test or rapid RSV PCR test) or a laboratory RSV PCR test.
- Hospitalised for <48 hours at enrolment or within 96 hours of illness onset (for those not admitted).
- Available for a 7 week ( $\pm 1$  week) convalescent visit.
- Has not received medication to treat RSV infection (e.g. ribavirin), had no prior exposure to an RSV investigational vaccine or medication, has not received immunoglobulins or monoclonal antibodies (including palivizumab) or used oral steroids or montelukast within 7 days of enrolment.

Group 2 (healthy controls) participants:

- Cannot have had a respiratory illness or received a vaccination in the past 7 days
- Must not have a history of a concurrent significant medical illness or have been born <37 weeks gestation.

(See supplementary table 1 for eligibility criteria for both groups)

## INFORMED CONSENT

For both groups, consent will be obtained by a member of the research team who is suitably qualified and experienced and has been authorised by the chief or principal investigator. Parents or guardians will be provided with written and verbal versions of the study information booklet. This details the exact nature and rationale for performing the study, what is involved and the risks and benefits. They

213 must sign the latest approved version of the informed consent form before any study specific  
214 procedures are performed.

215 For group 1, in some centres where RSV status is not determined as part of routine clinical care,  
216 initial consent will be obtained to perform a bedside RSV test. Only infants who have a confirmed  
217 RSV positive ARTI (either via a bedside test or diagnostic laboratory PCR test) are eligible for  
218 enrolment.

219 For both consent steps, it is clearly stated that participation is voluntary and that the parent, guardian  
220 or legally authorised representative is free to withdraw their infant from the study at any time, for any  
221 reason without prejudice to future care, without affecting their legal rights and with no obligation to  
222 give the reason for withdrawal. They will be allowed as much time as required to consider the  
223 information, and the opportunity to question the investigator, their GP or other independent parties  
224 before deciding whether to participate.

225 Additional informed written consent will be obtained for storage of participants samples in local  
226 biobanks for future studies.

227

## 228 **STUDY PROCEDURES AND SCHEDULES FOR DATA COLLECTION**

### 229 **GROUP 1- active RSV ARTI**

230 After informed written consent has been obtained and RSV positive ARTI confirmed, enrolled  
231 participants will undergo sampling. This will include two nasopharyngeal swabs, one synthetic  
232 absorption matrix (SAM) swab [25] or buccal swab, venepuncture and collection of a stool and urine  
233 sample. Participants' parents or guardians will also be asked to complete a baseline questionnaire  
234 (supplement 2) and a 14-day respiratory symptom diary (supplement 3). For those being treated as an  
235 inpatient, daily nasopharyngeal swabs will be collected until discharge to monitor the kinetics of RSV  
236 viral load.

Participants will subsequently have a convalescent visit at 7 weeks ( $\pm 1$  week) after discharge from hospital, or post onset of symptoms for participants recruited in the community and not admitted to hospital. This will be followed by annual questionnaires (supplement 4 and 5), sent via mail or email, to assess respiratory symptoms and sequelae and determine health care costs, resource use and Health Related Quality of Life (HRQoL) data (Group 1 study overview can be seen in figure 1).

### ***Baseline participant information***

For group 1 participants, baseline information will be collected either in the hospital or community setting. Information collected will include patient demographics, medical advice sought, medical history, clinical examination findings, laboratory or radiological investigations and treatment received. A standardised respiratory clinical severity score (ReSVinet score [26]) will also be calculated. This records details such as feeding intolerance, medical intervention required, respiratory difficulty, respiratory frequency, presence of apnoea, overall condition and presence of fever [26].

### ***Baseline questionnaire and 14-day respiratory symptom diary***

Group 1 participants will complete a baseline questionnaire to collect information about household demographics, attendance at nursery or child-minders, family history of atopy (asthma/eczema/hay fever), exposure to household smoke or pets and a baseline assessment of quality of life.

The 14-day respiratory symptom diary will also be initiated at baseline and completed either online or as a paper copy. If a paper source document is used, data will be entered into the electronic data capture system (REDCap) by the clinical research team.

Data captured in the daily diary includes whether the respiratory symptoms are ongoing, what symptoms are present (cough, coryza, wheeze, shortness of breath, feeding difficulties and

temperature), the infants overall health and the health and wellbeing of their parents, guardians or legal representatives.

At the convalescent visit (7 weeks  $\pm$  1 week post ARTI) a member of the clinical research team will ensure the baseline questionnaire and 14-day diary has been completed and entered into REDCap.

### ***Procedures & sampling***

At baseline, group 1 participants will have two nasopharyngeal swabs taken (one in M4RT transport medium and one in Amies transport medium) and a SAM swab [25] (site dependent) for RSV confirmation and viral load, PCR for other respiratory viruses and microbiome and transcriptomic analysis. These samples can also be obtained from a nasopharyngeal aspirate if taken as part of routine clinical care. If the child is intubated and ventilated and has a bronchoalveolar lavage done as part of their clinical care, a sample will also be taken for analysis.

Venous blood samples will be obtained at a maximum volume of 0.8ml/kg. Samples will be collected into Paxgene RNA (0.2-0.5ml) tubes for transcriptomic analysis, serum tubes ( $\geq$ 1.8ml) for proteomic, metabolomic, systems serology, RSV proteins F, Ga, Gb and N and pre-F and post-F neutralising antibody analysis; serum clot or EDTA and buccal samples for epigenetic and genome-wide association study (GWAS) analysis and lithium heparin tubes ( $\geq$  1-2ml) to obtain whole blood for cellular immunology (includes flow cytometric cell phenotyping and intracellular cytokine staining).

The stool specimen will be analysed for microbiome and the urine for metabolomics.

### ***Annual questionnaires***

Questionnaires will be sent to participants' parents or guardians at the first, second and third birthdays, for a maximum of 3 years depending on the recruitment date in relation to the study end

283 date. Contact will be made via telephone, text or email and the questionnaire will be distributed via  
284 email or mail each year.

285 The questionnaire determines whether the child has had any respiratory symptoms, including wheeze,  
286 since the original RSV illness, whether they sought advice from a healthcare professional, what  
287 treatment they received (e.g. antibiotics, inhaled bronchodilator or corticosteroids), duration of  
288 symptoms and frequency of recurrence. It also includes information on the presence of atopy,  
289 household smoke exposure and whether the child received their routine vaccinations.

290

## 291 **GROUP 2- Healthy controls**

292 For Group 2 participants, baseline samples will include up to two nasopharyngeal swabs, a SAM swab  
293 or buccal swab, venous bloods, a stool specimen and a urine specimen. These specimens will be tested  
294 as for the Group 1 participants. A week after enrolment the participants' parents or guardians will be  
295 contacted to ensure the participant has remained well and free from respiratory symptoms. If they  
296 have developed symptoms in this time period another participant will be recruited as a replacement.  
297 Participants will subsequently receive annual questionnaires as for group 1.

298 Details of sampling procedures and data collection for Groups 1 and 2 can be found in supplement 6.

299

## 300 **SPECIMEN PROCESSING**

301 Specimens will undergo initial processing in local laboratories to enable storage until being  
302 transferred to the appropriate sites for final processing and analysis. This includes industry partners of  
303 the European Federation of Pharmaceutical Industries and Associations (EFPIA)  
304 (e.g. GlaxoSmithKline, Sanofi Pasteur, Janssen Pharmaceuticals and AstraZeneca) who are members  
305 of the RESCEU project, Fondazione PENTA for the treatment and care of children with HIV-ONLUS

(PENTA) and academic sites which include the University of Oxford and the University Medical Centre Utrecht (UMCU). Further details of sample processing can be found in supplement 7.

## **ADVERSE EVENTS DEFINITION AND REPORTING**

The International Conference on Harmonisation (ICH) definition of a serious adverse event will be used. No medicinal products will be administered during this observational study. Participants will only have venepuncture and the collection of nasopharyngeal, buccal, urine and stool samples, hence reporting of serious adverse events (SAEs) will be limited to those relating directly to the collection of research samples or death regardless of cause.

Should an SAE occur to a participant, during or immediately after the visit, it will be reported to the local Principal Investigator (PI) and subsequently to the Chief Investigator (CI) within 24 hours of the study staff being aware of the SAE.

The local investigators opinion will be used to determine whether the event was ‘related’ to the research procedures. The CI will determine whether ‘related’ events are expected or unexpected in relation to those procedures. Where the CI deems an SAE to be ‘related’ and ‘unexpected’ the SAE will be reported to the Research Ethics Committee.

## **TRIAL MONITORING**

Site specific trial monitoring will be in place according to Good Clinical Practice (GCP) guidelines. Following written Standard Operating Procedures, the monitors verify that the clinical trial is conducted and data generated, documented and reported in compliance with the protocol, GCP and applicable regulatory requirements.

## **SAMPLE SIZE CALCULATON**

The consortium-wide, sample size estimation is based on a microarray analysis of the primary outcome comparing disease severity in group 1a and 1b participants, and assuming a binary biomarker (biomarker positive or biomarker negative). Biomarkers were deemed to be predictive if an odds ratio of at least 1.2 (log odds=0.18, mean difference=0.2) for the biomarker presence/absence between the severe RSV sample and the non-severe RSV controls was recorded. Using RSV PCR, 226 otherwise healthy infants with severe RSV disease requiring hospitalisation and 226 age- and sex-matched cases with RSV not requiring hospitalisation will be recruited to achieve 90% power (SD 0.4) to detect a log odds ratio of 0.18 (mean difference=0.2) with a true biomarker rate of 0.5% and a False Discovery Rate (FDR) of 5%. This is estimated using a two-sided Mann-Whitney U or Wilcoxon Rank-Sum test assuming that the actual data distribution is logistic. We plan to recruit 10% over these numbers to account for anticipated drop out for a sample size of 500. Groups 1c, 1d and 2 are exploratory cohorts.

## STATISTICS AND ANALYSIS PLAN

Individual datasets obtained from different endpoints (*e.g.* GWAS, metabolomics, proteomics, transcriptomics) will be analysed separately in association with the RSV disease severity. With the availability of nasal microbiome data, we will also analyse the interaction between bacterial species colonizing the nasopharynx and disease severity and the clinical outcome.

Additionally, large data sets will be investigated for integrative computational analyses in order to identify biomarker profiles associated with differing disease severity, in the context of biomarker discovery. Specificity of the results will be tested against 1) existing literature and publicly available data, 2) ongoing prospective and observational RESCEU studies designed to be used for biomarker validation (*i.e.* infant birth cohort, older-adults cohort, COPD cohort), 3) analysis of existing validation datasets that RESCEU has access to (*i.e.* Danish Neonatal Screening Biobank, existing data from a prospective study in Kilifi, Kenya, adult challenge studies, and the MOSAIC study) further details are available at <http://resc-eu.org/>. The biomarker validation will be performed with the consideration of

population groups (by age: infants, older-adults; by susceptibility: COPD subjects; by location) and disease severity measures (RSV severity, clinical variables).

For correlates of disease severity, various sources of biomarker data (*e.g.* transcriptomic, metabolomic and proteomic data) will be used for predictive modelling. The challenge of integrating different data types (*e.g.* virology, immunology, transcriptomic, metabolomic and proteomic) will be tackled by investigating and considering different methods for the integration of the multi-omics data (25). Feature selection will be examined with well-established algorithms including support vector machines, random forest or nearest shrunken centroid classifiers, as well as more recent methods such as elastic net regularization, and with extensive inner cross-validation approaches, such as x-fold or LOOCV.

Provided the sample size is sufficient for each of the nasal microbial endpoints and there is evidence of a relationship between nasal microbiota and RSV severity, increased depth analysis will be performed using relevant methods described above.

## **REGULATORY APPROVALS AND TRIAL REGISTRATION**

Study recruitment commenced in October 2017 and is currently ongoing. Within the UK, study authorisation has been granted from the Health Research Authority (IRAS project ID: 231136) and Research Ethics Committee (REC) approval has been granted by South Central and Hampshire A (NRES reference 17/SC/0522). In Spain this has been granted by Comité de Ética de la Investigación de Santiago-Lugo (2017/395) and in the Netherlands by the Medical Ethical Committee, UMC, Utrecht (reference 17/563).

## **ETHICS AND DISSEMINATION**

The investigator will ensure the study is conducted in accordance with the principles of the declaration of Helsinki, relevant regulations and with GCP. The study staff will ensure that the



participants' anonymity is maintained. The study will comply with the data protection act so participants are anonymised and only identified with a participant ID. All documents will be stored securely and only accessible by study staff and authorised personnel.

The samples obtained are intended to be used solely for research and not for diagnostic purposes. In the case of an incidental abnormal finding, the results will be discussed with the responsible clinical team who will discuss the implications with the participants. Venepuncture can be moderately painful but pain reducing methods will be offered prior to the procedure (eg: anaesthetic cream or sucrose) and bruising may occur. If the first attempt is unsuccessful, further attempts will be made if verbal consent is obtained from the participants' parent/carer or legal guardian. Nasopharyngeal swabs can cause brief discomfort but the procedure lasts less than 10 seconds and is performed by trained personnel. Nose bleeds are possible but uncommon. Obtaining the stool and urine sample will cause no discomfort.

## **PUBLICATION**

The investigators will be involved in reviewing drafts of the manuscripts, abstracts and any other publications arising from the study. Participants are not given individual results but will have access to the overall study results via publications and the RESCEU website.

## **Acknowledgements**

AJP acknowledges support from the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre (BRC) for the conduct of this study and is an NIHR senior Investigator. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

All authors are members of the REspiratory Syncytial virus Consortium in EUrope (RESCEU). RESCEU has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement N° 116019. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA. <http://www.imi.europa.eu>.

## **RESCEU Investigators**

Harish Nair, Harry Campbell (University of Edinburgh); Philippe Beutels (Universiteit Antwerpen); Louis Bont, Joanne Wildenbeest, Debby Bogaert (University Medical Centre Utrecht); Andrew Pollard, Paul Klenerman, Charles Sande, Matthew Snape, Simon Drysdale, Christopher Butler (University of Oxford), Carlos Diaz, Eva Molero (Synapse), Peter Openshaw, Simon Nadel, Jadwicha Wedzicha (Imperial College London), Federico Martín-Torres, Carmen Rodríguez-Tenreiro (Servicio Galego de Saude), Terho Heikkinen (Turku University Central Hospital), Adam Meijer, Elisabeth Sanders (National Institute for Public Health and the Environment), Thea Kølsten Fischer (Statens Serum Institut), Maarten van den Berge (Academisch Ziekenhuis Groningen), Carlo Giaquinto, Eugenio Baraldi, Giuseppe Giordano (Fondazione PENTA for the treatment and care of children with HIV-ONLUS, PENTA); Judy Hackett, Laura Dillon (AstraZeneca), Charles Knirsch (Pfizer), Antonio Gonzalez Lopez, Thi Lien-Anh Nguyen (GlaxoSmithKline), Scott Gallichan, Linong Zhang (Sanofi Pasteur), Jeroen Aerssens, Myra Widjoatmodjo (Janssen), Eric Hillson, Brian Rosen (Novovax)

## Footnote page

### Competing interests

AJP led research on RSV vaccines on behalf of the University of Oxford which ended in 2016, funded by Okairos. AJP chairs the UK Department of Health's (DH) Joint Committee on Vaccination and Immunisation (JCVI) and is a member of the World Health Organization's (WHO) Strategic Advisory Group of Experts.

FMT has received financial and non-financial support outside the submitted work as well as trials fees through his institution from GSK Pfizer, Novavax, Sanofi Pasteur MSD, Ablynx, Jansen, Regeneron, and Medimmune. FMT is a member of the European Technical Advisory Group on Vaccines for WHO-Europe. The views expressed in this manuscript are those of the authors and do not necessarily reflect the views of the JCVI, the DH, or the WHO.

JA and DÖ are employees of Janssen Pharmaceutica. MW is employee of Janssen R&D Vaccines. LZ is employee of Sanofi Pasteur. TLAN is employee of GSK Biologicals.

LB has regular interaction with pharmaceutical and other industrial partners. He has not received personal fees or other personal benefits. UMCU has received major funding (>€100,000 per industrial partner) for investigator initiated studies from AbbVie, MedImmune, Janssen, the Bill and Melinda Gates Foundation, Nutricia (Danone) and MeMed Diagnostics. 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 MedImmune. UMCU has received minor funding for participation in trials by Regeneron and Janssen from 2015-2017 (total annual estimate less than €20,000). UMCU received minor funding for consultation and invited lectures by AbbVie, MedImmune, Ablynx, Bavaria Nordic, MabXience, Novavax, Pfizer, Janssen (total annual estimate less than €20,000). LB is the founding chairman of the ReSViNET Foundation.

### Funding

RESCEU has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 116019. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

<http://www.imi.europa.eu>

**Correspondence and requests for reprints should be addressed to**

Kimberley L Jefferies

Department of Paediatrics, Oxford Vaccine Group, Oxford, OX3 7LE, UK

01865 611400

jefferiesk@hotmail.co.uk

## References

1. De Steenhuijsen P, WAA, Heinonen S, Hasrat R et al. Nasopharyngeal microbiota, host transcriptome, and disease severity in children with respiratory syncytial virus infection. *Am J Respir Crit Care Med* **2016**;194(9):1104–15.
2. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet* 2017; 390: 946-958
3. Hall CB, Powell KR, MacDonald NE et al. Respiratory syncytial viral infection in children with compromised immune function. *N Engl J Med* **1986**; 315(2):77–81.
4. Navas L, Wang E, de Carvalho V, Robinson J. Improved outcome of respiratory syncytial virus infection in a high-risk hospitalized population of Canadian children. *The Journal of Pediatrics* **1992**; 121:348–54.
5. Wang EEL, Law BJ, Boucher FD et al. Pediatric Investigators Collaborative Network on Infections in Canada (PICNIC) study of admission and management variation in patients hospitalized with respiratory syncytial viral lower respiratory tract infection. *J Pediatr* **1996**; 129(3):390–5.
6. Boyce TG, Mellen BG, Mitchel EF, Wright PF, Griffin MR. Rates of hospitalization for respiratory syncytial virus infection among children in Medicaid. *J Pediatr* **2000**; 137(6):865–70.
7. Walsh EE, Peterson DR, Falsey AR. Risk Factors for Severe Respiratory Syncytial Virus Infection in Elderly Persons. *J Infect Dis* **2004**; 189(2):233–8.
8. Sala KA, Moore A, Desai S, Welch K, Bhandari S, Carroll CL. Factors associated with disease severity in children with bronchiolitis. *J Asthma* **2015**; 52(3):268–72.
9. Hall CB, Weinberg GA, Iwane MK et al. The Burden of Respiratory Syncytial Virus Infection in Young Children. *N Engl J Med* **2009**; 360(6):588–98.
10. Wright M, Piedimonte G. Respiratory Syncytial Virus Prevention and Therapy: Past, Present, and Future. *Pediatr Pulmonol* **2011**; 46(4):324–47.
11. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of Primary Infection and Reinfection with Respiratory Syncytial Virus. *Am J Dis Child* **1986**; 140:543–6.
12. Stockman LJ, Curns AT, Anderson LJ, Fischer-Langley G. Respiratory Syncytial Virus-associated Hospitalizations Among Infants and Young Children in the United States, 1997-2006. *Pediatr Infect Dis J* **2012**; 31(1):5–9.
13. Deshpande SA, Northern V. The clinical and health economic burden of respiratory syncytial virus disease among children under 2 years of age in a defined geographical area. *Arch Dis Child* **2003**; 88(12):1065–9.
14. Hasegawa K, Tsugawa Y, Brown DFM, Mansbach JM, Camargo CA. Trends in Bronchiolitis Hospitalizations in the United States, 2000-2009. *Pediatrics* **2013**;132(1):28–36.
15. Szabo SM, Gooch KL, Bibby MM, et al. The risk of mortality among young children hospitalized for severe respiratory syncytial virus infection. *Paediatr Respir Rev* **2013**;13(S2):S1–8.
16. Blanken MO, Rovers MM, Molenaar JM, et al. Respiratory Syncytial Virus and Recurrent

- Wheeze in Healthy Preterm Infants. *N Engl J Med* **2013**; 368(19):1791–9.
17. Sigurs N, Aljassim F, Kjellman B, et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. *Thorax* **2010**; 65(12):1045–52.
18. Carbonell-Estrany X, Bont L, Doering G, Gouyon JB, Lanari M. Clinical relevance of prevention of respiratory syncytial virus lower respiratory tract infection in preterm infants born between 33 and 35 weeks gestational age. *Eur J Clin Microbiol Infect Dis* **2008**; 27(10):891–9.
19. Hussman JM, Lanctôt KL, Paes B. The cost effectiveness of palivizumab in congenital heart disease: a review of the current evidence. *J Med Econ* **2013**; 16(1):115–24.
20. Resch B, Resch E, Müller W. Should respiratory care in preterm infants include prophylaxis against respiratory syncytial virus infection? The case in favour. *Paediatr Respir Rev* **2013**; 14(2):130–6.
21. Resch B, Resch E, Müller W. Should respiratory care in preterm infants include prophylaxis against respiratory syncytial virus infection? The case against. *Paediatr Respir Rev*. **2013**; 14(2):128–129.
22. Frogel M, Stewart DL, Hoopes M, Fernandes AW, Mahadevia PJ. A Systematic Review of Compliance with Palivizumab Administration for RSV Immunoprophylaxis. *J Manag Care Pharm* **2010**; 16(1):46–58.
23. The Green Book 2013. Available at: <https://www.gov.uk/government/collections/immunisation-against-infectious-disease-the-green-book#the-green-book>. Accessed 30 January 2019
24. Brown PM, Schneeberger DL, Piedimonte G. Biomarkers of Respiratory Syncytial Virus (RSV) infection: specific neurotrophin and cytokine levels provide increased accuracy in predicting disease severity. *Paediatr Respir Rev* **2015**; 16(4):232–40.
25. Thwaites RS, Jarvis HC, Singh N, et al. Absorption of Nasal and Bronchial Fluids: Precision Sampling of the Human Respiratory Mucosa and Laboratory Processing of Samples. *J Vis Exp* **2018**; 131: e56413, doi:10.3791/56413
26. Justicia-Grande AJ, Pardo-Seco J, Cebey-López M, et al. Development and Validation of a New Clinical Scale for Infants with Acute Respiratory Infection: The ReSVinet scale. *PLoS ONE* **2016**; 11(6): e0157665. doi:10.1371/journal.pone.0157665

**Table 1: Primary and Secondary Endpoints**

<b>Sampling procedures and outcome measures</b>	
	<b>Group 1 (RSV positive ARTI cases, both hospitalised and non-hospitalised)</b>
	<b>Group 2 (healthy controls without RSV)</b>
<b>Primary Endpoints</b>	
<b>To establish biomarkers correlated with or predictive of RSV disease severity in infants (Primary analysis will compare healthy infants (without co-morbidities) with RSV infection requiring hospitalisation for at least 12 hours (Group 1a) with healthy infants with RSV infection not requiring hospitalisation/ discharged home within 12 hours of registration in the emergency department (Group 1b))</b>	<p>- <i>At enrolment</i>: nasopharyngeal swabs (microbiome, transcriptome, viral PCR), buccal swab, blood (transcriptomics, pre/post-F RSV antibodies, metabolomics, flow cytometry, cellular immunology), urine (metabolomics), stool (microbiome) sampling.</p> <p>- <i>At convalescence</i> (7 weeks <math>\pm</math> 1 week post ARTI) all of the above samples are repeated (group 1 only)</p> <p>-Severity of ARTI assessed using the ReSVinet score* and the need for hospitalisation.</p>
<b>Secondary Endpoints</b>	
<b>To establish biomarkers associated with sequelae following RSV infection in infants</b>	<p>- <i>At enrolment</i>: Analysis of nasopharyngeal swabs (microbiome, transcriptome, viral PCR), buccal swab, blood (transcriptomics, pre/post-F RSV antibodies, metabolomics, flow cytometry, cellular immunology), urine (metabolomics) and stool (microbiome) samples.</p> <p>-<i>At convalescence</i> (7 weeks <math>\pm</math> 1 week post ARTI) the above samples will be repeated (Group 1 only)</p> <p>- Sequelae determined from parental questionnaires completed at 1, 2 and 3 years of age</p>
<b>To characterise the viral load and genetic sequence of RSV associated with mild and severe disease</b>	<p>- <i>At enrolment</i>: nasopharyngeal sampling (viral load, multiplex PCR, RSV sequencing)</p> <p>-Daily nasopharyngeal swabs for hospitalised participants</p> <p>- <i>At convalescence</i> (7 weeks <math>\pm</math> 1 week post ARTI) samples repeated.</p> <p>-Illness severity assessed using the ReSVinet score* and the need for hospitalisation.</p>
<b>To characterise the immune response to mild and severe RSV disease</b>	<p>-<i>At enrolment</i>: blood samples for cellular immunology (flow cytometric cell phenotyping and intracellular cytokine staining), systems serology, presence and characteristics of pre and post-F neutralising antibodies</p> <p>-<i>At convalescence</i> (7 weeks <math>\pm</math> 1 week post ARTI) blood samples repeated</p>

	-Illness severity assessed using the ReSVinet score* and the need for hospitalisation.
<b>To determine health care costs, health care resource use, interruption of normal activities, and Health Related Quality of Life in RSV-associated ARTI patients and their families</b>	<ul style="list-style-type: none"> <li>- Baseline questionnaire** at enrolment</li> <li>- Annual questionnaires**, maximum 3 years (to quantify number of hospital admissions, number of days admitted and other healthcare advice sought and how often)</li> <li>- 14-day symptom diary** during illness</li> <li>(Group 1 only)- to quantify duration of hospital stay and symptoms.</li> </ul>

(\*\* baseline and annual questionnaires, as well as the 14-day symptom diary will elicit HR-QoL and the impact of RSV related ARTI with Group 2 responses acting as controls. These questionnaires will be completed by the parents or legal guardians and have been designed specifically for this study- see supplementary information for details)

570 (ARTI includes both upper and lower respiratory tract infections). \* The ReSVinet scale [26]. PLoS ONE **2016**; 11(6): e0157665.  
571 doi:10.1371/journal.pone.0157665