Clinical Study Protocol

Harnessing the beneficial non-specific effects of measles-mumps-rubella vaccine on infection with unrelated pathogens and allergic diseases in children – a single-centre phase IV randomised controlled trial with a factorial design comparing the current Swiss vaccination schedule with a modified schedule

Short title:	**N**on-specific **E**ffects of a modified **M**easles vaccination schedule to prevent **A**llergy and **U**nrelated infection in children
Trial acronym :	NEMAU
Translated title :	Bénéfices non-spécifique d'un schéma de vaccination modifié contre la rougeole
Study Type:	Randomised controlled trial
Study Categorisation:	A
Study Registration:	Clinicaltrial.gov NCT05758532
Study Identifier:	2022-00616
Sponsor-Investigator:	Dr Laure Pittet, MD-PhD Children's Hospital, University Hospitals of Geneva Rue Willy-Donzé 6, 1211 Geneva, Switzerland Tel: +41 79 55 38 277, laure.pittet@hcuge.ch
Investigational Product:	Measles-mumps-rubella vaccine, Priorix
Protocol Version and Date:	Version 1.2 of 08.03.2023

Signature Page

Study number	2022-00616 (Clinicaltrial.gov NCT05758532)
Study Title	Harnessing the beneficial non-specific effects of measles-mumps-rubella vaccine in children on infection with unrelated pathogens and allergic diseases – a single-centre phase IV randomised controlled trial with a factorial design comparing the current Swiss vaccination schedule with a modified schedule

The Sponsor-Investigator has approved the protocol version 1.2 (dated 08.03.2023), and confirms hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, the ICH-GCP guidelines and the local legally applicable requirements.

Sponsor-Investigator: Dr Laure Pittet

Geneva, 20.03.2023

Place/Date

Signature

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STUDY SYNOPSIS

Sponsor / Sponsor-Investigator	University Hospitals of Geneva / Laure Pittet
Study Title:	Harnessing the beneficial non-specific effects of measles-mumps-rubella vaccine on infection with unrelated pathogens and allergic diseases in children – a single-centre phase IV randomised controlled trial with a factorial design comparing the current Swiss vaccination schedule with a modified schedule
Short Title / Study ID:	**N**on-specific **E**ffects of a modified **M**easles vaccination schedule to prevent **A**llergy and **U**nrelated infection in children (NEMAU) 2022-00616
Protocol Version and Date:	1.2 08.03.2023
Trial registration:	Clinicaltrial.gov NCT05758532
Study category and Rationale	A (licenced vaccine, used in accordance with recommendation)
Clinical Phase:	Phase IV (licenced vaccine, within recommendation, evaluated for beneficial effect beyond the initial scope of approval)
Background and Rationale:	In addition to their specific effect against the target disease,[1] some vaccines induce broader changes to immune function, resulting in "**non-specific effects**".[2-16] Evidence suggests that measles vaccines can protect against unrelated infections and **reduce all-cause mortality by as much as half** in infants.[9 17 18] In two trials in Guinea-Bissau, an early dose of measles vaccine (before 9 months of age) was associated with reduced risk of hospital admission and of mild infections in the months following vaccination.[19 20] Importantly, the non-specific effects are mainly observed when the measles-containing vaccine (such as the measles-mumps-rubella (MMR) vaccine) is not given concomitantly with non-live vaccines.[21 22] Measles vaccination has also been associated with a reduced risk of allergy in children.[23] The immunological mechanisms underlying the non-specific effects of the measles vaccine have not been investigated, despite this vaccine being given almost universally to children. The overall objective of this project is to assess, in a randomised control trial (RCT), the effects of a "modified" MMR schedule in children, by an in-depth characterisation of both the clinical effects and the underlying immunomodulatory changes. The current Swiss administration schedule of giving MMR at 9 and 12 months of age ("current schedule") will be compared with a "modified schedule". This is expected to maximise the beneficial non-specific effects of MMR by giving it at 6 and 13 months of age, separately from other vaccines ("modified schedule"). Factorial analysis will enable assessment of the benefit of the intervention on each of the two doses of MMR separately or in combination.
Objective(s):	The clinical aims are to determine whether a modified schedule of MMR administration reduces both the risk and severity of: (i) infections with unrelated pathogens and (ii) atopic and allergic diseases. The laboratory aims are to: (i) quantify and characterise the immunological non-specific effects of MMR, and (ii) identify the biological pathways and molecular mechanisms that are altered by MMR vaccination.

<table>
<tr><td>Outcome(s):</td><td>Primary outcome: Number of parent-reported respiratory infections between 6 months and 9 months of age.
Main secondary outcomes:
Additional measures of infection
Eczema, food allergy, and wheezing diseases
Immune cell sub-populations and function over time
Transcriptional responses and epigenetic imprinting in immune cells
Vaccine responses and memory cells
Vaccine safety</td></tr>
<tr><td>Study design:</td><td>Phase IV, single-centre, 4-group, open-label, randomised controlled trial with a factorial design (primary outcome analysed as a 2-group trial)</td></tr>
<tr><td>Inclusion / Exclusion criteria:</td><td>6-month-old healthy children fully immunised with no contraindications to MMR and no indication for an early MMR or MMR-varicella vaccination</td></tr>
<tr><td>Measurements and procedures:</td><td>Parents will be asked to complete questionnaires once every 2 weeks to collect clinical data. Consent to blood tests is optional, and will determine whether a child participates in Part 1 only, or both Part 1 and Part 2.
Visits are planned at
6 months of age (randomisation, ± vaccination, ± blood collection),
9 months of age (± vaccination, ± blood collection),
11 to 13 months of age (vaccination), and
24 months of age (± blood collection).</td></tr>
<tr><td>Study Product / Intervention:</td><td>Measles-mumps-rubella (MMR) vaccine (Priorix): 0.5 ml injected intramuscularly in the deltoid region or in the anterolateral area of the thigh

Administration schedule will differ between the 4 groups, as follow:<table><tr><th>Age:</th><th>6 m</th><th>9 m</th><th>11-12 m</th><th>13 m</th></tr><tr><td>Group C.C.
(both MMR on Current schedule)</td><td>No vaccine</td><td>1st MMR</td><td>3rd DTP and PCV
2nd MMR</td><td>No vaccine</td></tr><tr><td>Group M.C.
(1st MMR Modified schedule, 2nd MMR Current schedule)</td><td>1st MMR</td><td>No vaccine</td><td>3rd DTP and PCV
2nd MMR</td><td>No vaccine</td></tr><tr><td>Group C.M.
(1st MMR Current schedule, 2nd MMR Modified schedule)</td><td>No vaccine</td><td>1st MMR</td><td>3rd DTP and PCV</td><td>2nd MMR</td></tr><tr><td>Group M.M.
(both MMR on Modified schedule)</td><td>1st MMR</td><td>No vaccine</td><td>3rd DTP and PCV</td><td>2nd MMR</td></tr></table>C: current schedule; DTP: diphtheria-tetanus-pertussis-containing vaccine; m: months of age; M: modified schedule; PCV: pneumococcal conjugate vaccine.</td></tr>
<tr><td>Control Intervention:</td><td>The interventional product given to the control group will be the same as for the intervention groups, but with a different schedule of administration (see table above).</td></tr>
</table>

Number of Participants with Rationale:	500 participants randomised into 4 groups (125 per group). Sample size is based on the primary outcome of respiratory infections. Based on the data from a previous trial, we expect a rate of respiratory infection of 0.29 episodes per month (SD 0.32) between 6 and 9 months of age in the control groups (Group C.M. or C.C.). By randomising 500 participants, we will have 80% power to detect a minimum of 30% reduction in this incidence with a 2-sided p-value of 0.05, assuming at least 85% completion rate of the 9 months questionnaire. The primary outcome will be analysed as a 2-group comparison: the 250 children receiving MMR at 6 months of age (Group M.C. or M.M.) will be compared with the 250 children receiving the MMR at 9 months of age (Group C.M. or C.C.). Among the 500 participants enrolled, 100 participants consenting to blood collection (Part 2) will be randomised in a different strata within the 4 groups (25 per group). Assuming that at least 60% of them will provide a sufficient blood sample at each time point, which will then be processed without any problem, these 100 participants in total are required for Part 2, to ensure that a minimum of 30 participants per group of 1st MMR timing (30 in Group M.C. or M.M., and 30 in Group C.M. or C.C.) are included in all analyses. This will provide >80% power to detect a biologically significant 1.5-fold-change in cytokine responses at the 5% significance level, based on the variance of previous infant cytokine and intracellular cytokine staining studies.
Study Duration:	3 years
Study Schedule:	Month Year of First-Participant-In (planned): March 2023 Month Year of Last-Participant-Out (planned): March 2026
Investigator(s):	Sponsor-investigator and Principal Investigator Dr Laure Pittet, MD-PhD Children's Hospital, University Hospitals of Geneva Rue Willy-Donzé 6, 1211 Geneva, Switzerland Tel: +41 79 55 38 277, laure.pittet@hcuge.ch Co-investigators Prof Arnaud Didierlaurent, PhD Center of Vaccinology, University of Geneva CMU, 1 rue Michel-Servet, 1211 Geneva, Switzerland Tel: +32 48 867 23 00, arnaud.didierlaurent@unige.ch Prof. Klara Pósfay Barbe, MD, MS Children's Hospital, University Hospitals of Geneva Rue Willy-Donzé 6, 1211 Geneva, Switzerland Tel: +41 22 372 5462, klara.posfaybarbe@hcuge.ch Prof Nigel Curtis, FRCPCH, PhD Infectious Diseases Unit, The University of Melbourne and Murdoch Children's Research Institute, Royal Children's Hospital Melbourne 50 Flemington Road, 3052 Parkville, Victoria, Australia Tel +61 3 9345 6366, nigel.curtis@rch.org.au Dr Nicole Messina, PhD Infectious Diseases Group, The Murdoch Children's Research Institute 50 Flemington Road, 3052 Parkville, Victoria, Australia Tel +61 3 9936 6461, nicole.messina@mcri.edu.au
Study Centre:	Single-centre, University Hospitals of Geneva

Statistical Considerations:	The full details of the analysis will be provided in the statistical analysis plan, which will be finalised prior to database lock. The primary outcome (number of respiratory infections between 6 and 9 months of age) will be compared between the two group of 1st MMR timing (Group M.C. and M.M. *vs.* Group C.M. and C.C.) using negative binomial regressions, adjusted for sex (male/female), day-care attendance (yes/no), presence of comorbidities (yes/no), older siblings (yes/no), and will be presented as adjusted incidence rate ratios (aIRRs) with 95% confidence intervals. Other comparative analyses at the 9-months and 2-years endpoints will be done using chi-square test (or Fisher's test when appropriate) for categorical variables, and paired t-test or Wilcoxon signed-rank test for continuous variables, depending on the distribution of the variable. Time-to-event outcomes will be assessed using Cox proportional hazards with adjustment for baseline risk factors. For the 2-years endpoints, factorial analysis will enable to measure the effect on all outcomes of the intervention made to the first and the second dose of MMR, separately or in combination. Regression will be used to identify independent factors associated with each of the outcomes. All significant predictors ($p<0.20$) will be then included in a stepwise backward multivariate analysis. Trauma and accident outcomes will also be compared between the two groups and will act as control events, as MMR is not believed to change their risk of occurrence. Subgroup analysis are planned (by sex, risk factor, underlying maternal immunity, etc.). The analyses will be done in collaboration with the Clinical Research Center at Geneva University Hospitals.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, ICH-GCP, as well as all national legal and regulatory requirements.

ABBREVIATIONS

AE	Adverse Event
BASEC	Business Administration System for Ethical Committees, (https://submissions.swissethics.ch/en/)
BCG	Bacille Calmette-Guérin
CA	Competent Authority (e.g. Swissmedic)
CCER	Geneva Cantonal Ethics Commission
CEC	Competent Ethics Committee
CI	Confidence interval
CRF	Case Report Form
ClinO	Ordinance on Clinical Trials in Human Research *(in German: KlinV, in French: Oclin, in Italian: OSRUm)*
eCRF	Electronic Case Report Form
CTCAE	Common terminology criteria for adverse events
DSMB	Data and Safety Monitoring Board
DSUR	Development safety update report

DTP	Diphtheria-tetanus-pertussis-containing vaccine
GCP	Good Clinical Practice
Group C.C.	Receive both MMR on Current schedule
Group C.M.	Receive 1st MMR on Current schedule and 2nd MMR on Modified schedule
Group M.C.	Receive 1st MMR on Modified schedule and 2nd MMR on Current schedule
Group M.M.	Receive both MMR on Modified schedule
Ho	Null hypothesis
HRA	Federal Act on Research involving Human Beings *(in German: HFG, in French: LRH, in Italian: LRUm)*
ICS	Intracellular cytokine staining
IFN-γ	Interferon-gamma
IMP	Investigational Medicinal Product
IIT	Investigator-initiated Trial
ITP	Idiopathic thrombocytopaenic purpura
ITT	Intention to treat
KOFAM	Coordination Office for Human Research *(in German: Koordinationsstelle Forschung am Menschen)*
MIS BAIR	*Melbourne Infant Study: BCG for Allergy and Infection Reduction*
MMR	Measles-mumps-rubella vaccine
MMRV	Measles-mumps-rubella-varicella vaccine
PCV	Pneumococcal conjugate vaccine
PI	Principal Investigator
PICF	Participant informed consent form
RCT	Randomised controlled trial
SDV	Source Data Verification
SNSF	Swiss National Science Foundation
SOP	Standard Operating Procedure
SPC	Summary of product characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
Th	T helper cell
TLR	Toll-like receptor
TMF	Trial Master File
WHO	World Health Organization

SUMMARY OF THE REVISION HISTORY IN CASE OF AMENDMENTS

Version Nr, Version Date	Chapter	Description of change	Reason for the change
1.0, 24.03.2022		Initial release	
1.1, 06.05.2022, following CCER review	Many	Terminology: “optimised” schedule changed to “modified” schedule	The neutral term “modified” is more suitable, as the benefit of the alternate schedule is not proven yet
	3.1	Added precision on investigators’ role	Requested by CCER
	7.4, 8.5, and 9.2.5	Added precision on participant follow-up If withdrawal is due to an AE	Requested by CCER
	9.2	Added precision on blood transport and analysis	Requested by CCER
1.2, 08.03.2023, protocol tidy up before the inclusion of the first participant	Title page, synopsis, 1.2, 2.1, 13, 14.1	Added the Clinicaltrial.gov and KOFAM registration numbers Updated PI phone number Updated planned start and completion dates Updated funding section	Update needed
	1.5	Change of monitoring institution Slight change in monitoring plan	Following advice from HUG’s Clinical Trials Unit’s monitoring unit
	2.7, 7.2, 9.3.1	Updated places for advertising the study	Following advice from the Communication Unit of HUG
	9.2	Added justification for collecting the participants’ date of birth	Following advice from the CCER

STUDY SCHEDULE

Visit number	V1		V2		V3[a]			V4
Time point	t_0	t_0 to t_{+3m}	t_{+3m}	t_{+3m} to t_{+6m}	t_{+6m}	t_{+7m}	t_{+6m} to t_{+18m}	t_{+18m}
Age of participant	6mo	6mo to 9mo	9mo	9mo to 12mo	12mo	13mo	12mo to 24mo	24mo
Allowed time frame	±2w	±1w	±2w	±1w	±2w	±2w	±1w	±4w
Recruitment								
Screening, eligibility check	X							
Inclusion and randomisation	X							
Baseline questionnaire [b]	X							
Intervention: MMR vaccination								
- Control Group C.C.			X		X			
- Intervention Group M.C.	X				X			
- Intervention Group C.M.			X			X		
- Intervention Group M.M.	X					X		
Assessments								
Clinical assessment [c]	X		X		X			X
Clinical questionnaires		X…X…X…X[d]		X…X…X…X[e]			X…X…X…X[e]	
Vaccine diary (S/AE)		X[f]		X[f]			X[f]	
Contact with treating paediatrician	X		X		X			X
Health record check	X		X					X
Blood collection (Part 2 only)	X		X					X

C: current schedule; MMR: measles-mumps-rubella vaccination; m: month; M: modified schedule; mo: month-old; S/AE: (serious) adverse event; V: visit; w: week.

[a] Timing of V3 differs between groups: 13 mo for Group M.M. and Group C.M., and 12 mo for Group M.C and Group C.C. If funding is insufficient, V3 might be simplified and delegated to the treating paediatrician.

[b] Includes demographics, medical history.

[c] Includes physical examination and eczema assessment using SCORAD.

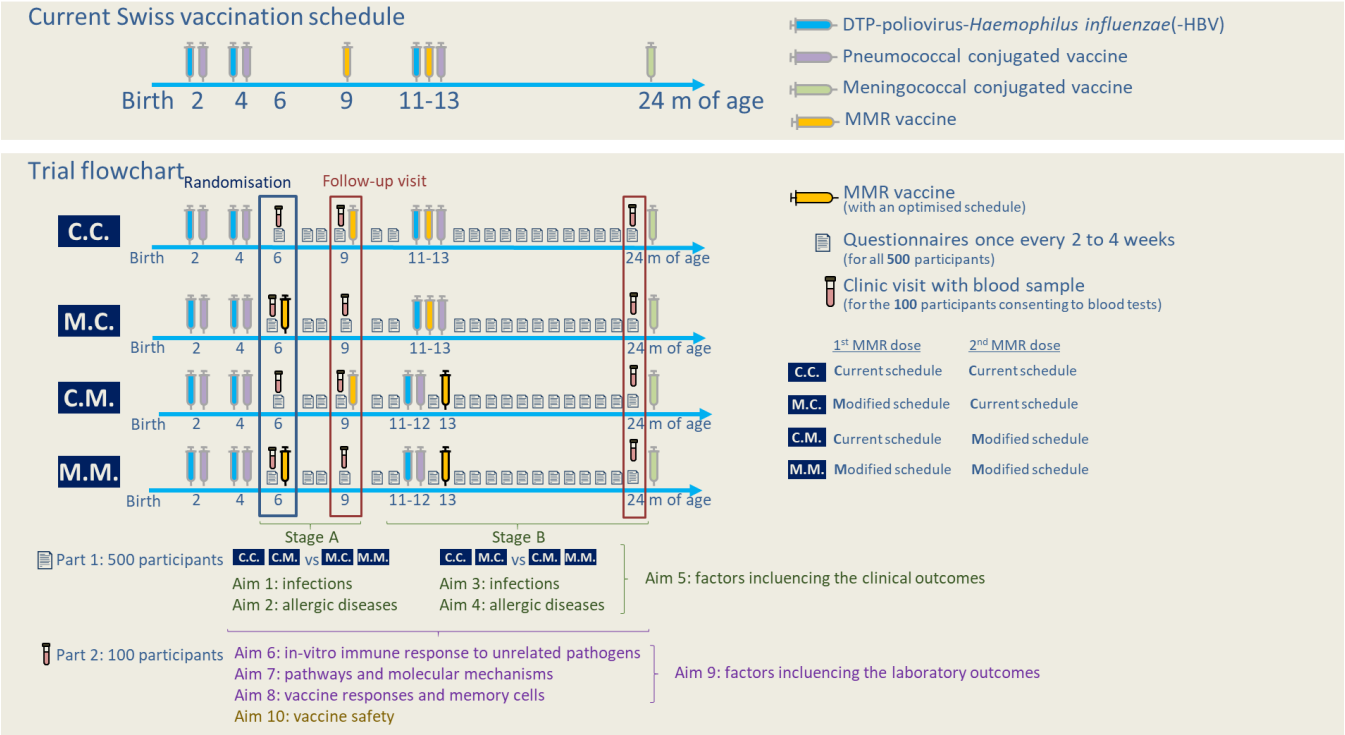
[d] every 2 weeks.

[e] every month.

[f] follow-up 42 days after vaccination.

		Stage B: comparison for the timing of the **2nd MMR**		
		2nd MMR 1m after the 12y DPT-PCV (**M**odified)	2nd MMR concomitant with the 12y DPT-PCV (**C**urrent)	
Stage A: comparison for the timing of the **1st MMR**	1st MMR at 6m (**M**odified)	**Group M.M.**, n=125 (including 25 in Part 2)	**Group M.C.**, n=125 (including 25 in Part 2)	N=250 (including 50 in Part 2)
	1st MMR at 9m (**C**urrent)	**Group C.M.**, n=125 (including 25 in Part 2)	**Group C.C.**, n=125 (including 25 in Part 2)	N=250 (including 50 in Part 2)
		N=250 (including 50 in Part 2)	N=250 (including 50 in Part 2)	

Participant allocation in 4 groups. C: current MMR schedule; DTP: diphtheria-tetanus-pertussis vaccine; m: month; M: Modified MMR schedule; PCV: pneumococcal conjugate vaccine.



Current Swiss administration schedule and Study flowchart

(see section 6.1.3)

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor, Sponsor-Investigator

Sponsor	University Hospitals of Geneva Rue Gabrielle-Perret-Gentil 4, 1211 Geneva, Switzerland
Sponsor representative	Dr Laure Pittet, MD-PhD Children's Hospital, University Hospitals of Geneva Rue Willy-Donzé 6, 1211 Geneva, Switzerland

This study is a single-centre investigator-initiated trial, with only one sponsor-investigator.

1.2 Principal Investigator(s)

Principal investigator	Dr Laure Pittet, MD-PhD Children's Hospital, University Hospitals of Geneva Rue Willy-Donzé 6, 1211 Geneva, Switzerland Tel: +41 79 55 38 277, laure.pittet@hcuge.ch
Other investigators	Prof Arnaud Didierlaurent, PhD Center of Vaccinology, University of Geneva CMU, 1 rue Michel-Servet, 1211 Geneva, Switzerland Tel: +32 48 867 23 00, arnaud.didierlaurent@unige.ch Prof Klara Pósfay Barbe, MD, MS Children's Hospital, University Hospitals of Geneva Rue Willy-Donzé 6, 1211 Geneva, Switzerland Tel: +41 22 372 5462, klara.posfaybarbe@hcuge.ch Prof Nigel Curtis, FRCPCH, PhD Infectious Diseases Unit, The Royal Children's Hospital Melbourne 50 Flemington Road, 3052 Parkville, Victoria, Australia Tel +61 3 9345 6366, nigel.curtis@rch.org.au Dr Nicole Messina, PhD Infectious Diseases Group, Murdoch Children's Research Institute 50 Flemington Road, 3052 Parkville, Victoria, Australia Tel +61 3 9936 6461, nicole.messina@mcri.edu.au
Clinical evaluators	Dr Laure Pittet, MD-PhD Prof. Klara Pósfay Barbe, MD, MS Prof Nigel Curtis, FRCPCH, PhD

1.3 Statistician ("Biostatistician")

Support in data collection, management, statistical analysis and interpretation will be provided by data managers and study statisticians from the Clinical Trials Unit (CTU) and the Methodologic Support Unit of the Clinical Research Center at Geneva University Hospitals.

Trial statistician	Prof Thomas Pernegger, MD, PhD, MS, MPH Methodologic Support Unit University Hospitals of Geneva Rue Gabrielle-Perret-Gentil 4, 1211 Geneva Tél. +41 22 372 90 37, thomas.perneger@hcuge.ch

1.4 Laboratory

Immunology laboratory	Center of Vaccinology, University of Geneva CMU, 1 rue Michel-Servet, 1211 Geneva, Switzerland

1.5 Monitoring institution

Monitoring will be performed according to ICH Good Clinical Practice (GCP) by the HUG's Platform for Clinical Research in Paediatrics, Gynaecology and Obstetrics.

1.6 Data Safety Monitoring Committee

An independent Data and Safety Monitoring Board (DSMB) will be convened three times during the study: before the first recruitment and at 6 and 18 months after the first recruitment.

The DSMB will monitor safety, data completeness, and the general study conduct. All the details of the DSMB analyses will be outlined in the DSMB charter.

The DSMB will be composed of individuals with the appropriate expertise, including at least three independent clinicians and/or biostatisticians who, collectively, have experience in the management of biostatistics and the conduct and monitoring of randomised controlled trials. Members of the DSMB will be independent of trial conduct. The DSMB will provide its input to the Principal Investigator.

1.7 Any other relevant Committee, Person, Organisation, Institution

Not applicable.

2. ETHICAL AND REGULATORY ASPECTS

Before the start of the study, the protocol, patient information and consent forms, and other study documents (e.g., case report form) will be submitted to the Geneva Cantonal Ethics Commission (CCER) for formal approval. Any substantial amendment to the protocol (see Swissethics definitions for amendments, www.swissethics.ch) will be submitted to the Geneva Ethics Committee for approval before it can take effect.

The clinical study will only begin once written approval from all required authorities has been received.

Any additional requirements imposed by the authorities will be implemented.

2.1 Study registration

This study is registered on www.clinicaltrials.org (NCT05758532) and on the website of the Coordination Office for Human Research (KOFAM), which is operated by the Federal Office of Public Health (SNCTP000004942).

2.2 Categorisation of study

This clinical trial is a Category A.

The measles-mumps-rubella vaccine is authorised in Switzerland to be used in infants, and can be given as of 6 months of age according to the Swiss Federal Commission for Vaccination,[24] the World Health Organization (WHO),[25] and most international guidelines. The use of the measles-mumps-rubella vaccine in the present trial is therefore in accordance with the prescribing information, within the same group of individuals, and at the same dosage.

2.3 Competent Ethics Committee (CEC)

The Competent Ethics Committee of this trial will be the “Commission cantonale d'éthique de la recherche” (CCER) of the Canton of Geneva. Dr Laure Pittet is responsible to ensure approval. As it is a single-centre study there are no other CCER involved.

The decision of the CCER will be communicated in writing to the Sponsor-Investigator before commencement of this study. As described above, no changes will be made to the protocol without prior CCER approval, except where necessary to eliminate apparent immediate hazards to study participants.

Premature study end or interruption of the study will be reported within 15 days. The regular end of the study will be reported to the CCER within 90 days, and the final study report within one year after the study’s end. Amendments will be reported as described in Section 2.10.

2.4 Competent Authorities

This clinical trial is a Category A and therefore there is no need to seek approval from Swissmedic.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, and Swiss Law and Swiss regulatory authority’s requirements. The CCER and regulatory authorities will receive Annual Safety and interim Reports and be informed about study stop/end in agreement with local requirements.

2.6 Declaration of interest

There is no conflict of interest to declare.

2.7 Patient Information and Informed Consent

The parents of potential subjects will be informed about the trial via pamphlets distributed in maternity services, hospitals, day-cares, pharmacies, community centres, and paediatricians’ offices, email, notice board and website/social media. The pamphlets include a summary of the study and a QR code with a link to a website where they can read further information and access the participant informed consent form (PICF), with contact details for further questions. Interested parents are given the opportunity to talk with a member of the research team if they have any questions, and to consult the treating paediatrician prior to enrolment.

Parents of potential subjects will then be screened by investigators (or designee) who will explain to the parent of each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. The parent of each subject is informed that the participation in the study is voluntary, that they may withdraw their children from the study at any time, and that withdrawal of consent will not affect subsequent medical assistance and treatment. The parent of each subject are informed that they can ask any questions, and consult with family members, friends, treating physicians or other experts before deciding about their participation in the study. Enough time will be given to the parents of the subjects to make an informed decision.

The parents of the subjects are also informed that

- the treating physician of their child will be contacted to inform about the trial, to corroborate their child’s medical history and to seek additional information;
- authorised individuals other than their treating physician may examine their child’s medical records;
- the blood samples taken as part of the study will be coded and stored for a period of maximum 15 years; this duration has been chosen given the likelihood that progress in the nascent field of non-specific effects of vaccines may benefit substantially from the reanalysis of study samples. Upon the approval of the Sponsor-investigator, samples may be sent outside of Switzerland to collaborating research laboratories for non-commercial research purposes.

The parent of each subject is given a subject information sheet and a consent form describing the study and providing sufficient information for the parent to make an informed decision about their child’s participation in the study.

The formal consent of the parent of each subject, using the approved consent form, is obtained before the subject is submitted to any investigation procedure.

The parent of each subject should read, understand, and voluntarily agree before signing and dating the informed consent form, and is given a copy of the signed document. The consent form is signed and dated by the subject and the PI (or designee). The signed consent form it is retained as part of the investigation records.

There is no compensation for participating in the trial.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that she shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited.

The assignment to each subject of a unique subject identification number through REDCap ensures subject confidentiality.

The subject's parent will be informed that for data verification purposes, authorised representatives of the Sponsor-Investigator, a competent authority, the DSMB, or the CCER may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.9 Early termination of the study

The Sponsor-Investigator or the CCER may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention.

2.10 Protocol amendments

Substantial amendments are only implemented after approval of the CCER.

Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the CCER. Such deviations shall be documented and reported to the sponsor and the CCER as soon as possible.

All non-substantial amendments are communicated to the CCER within the Annual Safety Report. Any deviations from the protocol and GCP will be documented in a protocol deviation form and filed in the trial master file (TMF), and will be reported to the CCER as soon as possible.

3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

Vaccines have non-specific effects

In addition to preventing a large number of deaths from their target disease,[1] some vaccines induce broader changes to immune function, resulting in "off-target" or "**non-specific effects**".[2-16] Evidence from epidemiological, clinical, immunological and animal studies suggest that some vaccines can provide "accidental benefits" against unrelated infections (Fig 1),[9] allergic diseases (Fig 2),[23 26 27] autoimmune diseases, and malignancies. Treatment of non-invasive bladder cancer with bacille Calmette-Guérin (BCG) vaccine is, for example, a licensed use of a non-specific effect.[28-31] The clinical impact of non-specific effects have been mainly studied for BCG, measles-containing vaccines and diphtheria-tetanus-pertussis (DTP) vaccines in low-income countries, in which mortality rates are high. The level of evidence for different vaccines and for different clinical manifestations varies considerably. The non-specific effects of vaccines appear to be influenced by several factors including sex, age at vaccination (e.g. birth, infancy, adulthood, elderly), sequence of administration (e.g. concomitant or separate to an/other(s) vaccine(s)), underlying risk (e.g. due to setting or predisposition), as well as other immune modulators (e.g. vitamin A).[7 32]

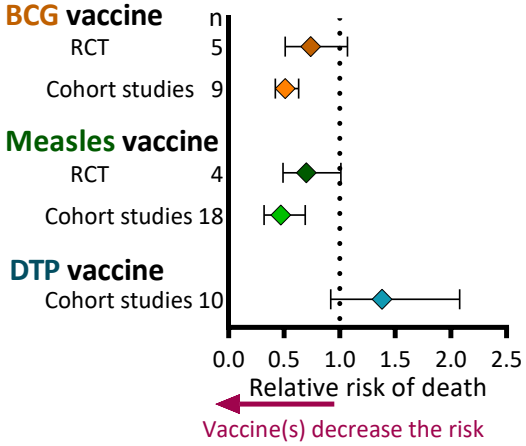


Fig 1. Summary of WHO meta-analysis results of relative risk for all-causes mortality (with 95% confidence interval) following vaccination with BCG, measles, or DTP in RCTs and cohort studies. Adapted from ref[2 9]
n = number of studies included in the meta-analysis.

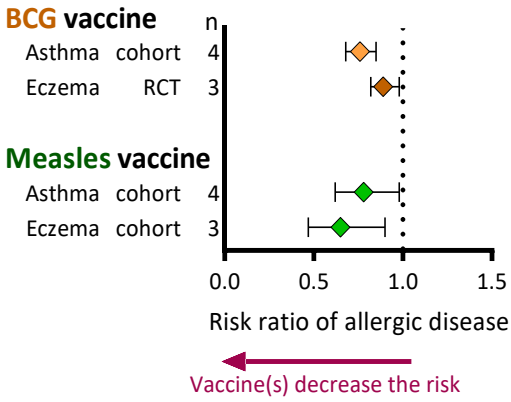


Fig 2. Summary of meta-analysis results of risk ratios for asthma or eczema (with 95% confidence interval) following vaccination with BCG or measles vaccine in RCTs and cohort studies. Adapted from ref[2 16 23 26]
n = number of studies included in the meta-analysis.

Briefly, the following summarises the current state of knowledge on the non-specific effects of vaccines:

1) Reduction of all-cause mortality following early-life BCG or measles vaccines in RCT

Data from observational studies and RCTs in high mortality settings suggest that **live-attenuated vaccines**, such as BCG and measles vaccines, **reduce all-cause mortality by as much as half** (Fig 1);[9] this protection is attributed to reduced deaths from respiratory tract infections and sepsis in infants, in settings with a low prevalence of tuberculosis or measles.[17 18] This is mainly observed following neonatal or early-life vaccination (first weeks of age).

2) Reduction of unrelated morbidity following measles vaccines in RCT

In a RCT in Guinea-Bissau, an early dose of measles vaccine was associated with reductions in **non-hospitalised infectious episodes** of diarrhea, vomiting, and fever.[20] An RCT in adults recently reported that a measles vaccine booster reduced the risk of COVID-19 symptoms and the need for COVID-19 treatment.[33]

3) Controversial finding of detrimental non-specific effects following non-live vaccines

Beneficial non-specific effects are **not reported for non-live vaccines** - such as the DTP vaccine.[9 34] Observational studies in low-income countries suggest that in certain situations and settings, the DTP vaccine might have detrimental non-specific effects associated with increased all-cause

mortality (Fig 1), a finding that has been highly controversial and that is not well characterised, given the ethical and practical challenge of designing an RCT evaluating DTP.[9 35-40]

4) Importance of the sequence of administration of measles vaccine in observational studies

In both low- and high-income countries, **observational** studies report that measles vaccine, when it is the **most recent vaccine received**, is associated with a lower rate of hospital admission for unrelated infectious diseases in children,[19 21 41-43] particularly **if given alone** (i.e. not concomitantly with DTP or other non-live vaccines).[21 22] This is mainly observed following infant or toddler vaccinations (first months to first years of age).

5) Potential benefit of live-vaccine-last schedule

In both low- and high-income countries, observational studies suggest that the non-specific effects of vaccines are determined by the **most recent vaccine administered** and might be affected by the concomitant and/or subsequent administration of other vaccines. The strategy of a "**live-vaccine-last schedule**" is to give live-attenuated vaccines as soon as possible after non-live vaccines, to counteract any potential detrimental non-specific effects of a non-live vaccine by beneficial non-specific effects induced by a subsequent live-attenuated vaccine.

6) Sex-differential non-specific effects of vaccines

Sex-differential effects have been observed for non-specific effects of vaccines,[7 11 14 15] including greater benefit of measles vaccine observed in girls,[9 18 19] and possibly increased all-cause mortality in girls following DTP,[44-48] compared with boys. The immune mechanisms underlying these findings are not yet fully understood.[11]

7) Reduction of allergic diseases following early-life BCG or measles vaccines

In relation to **allergic** diseases, RCTs suggest that early-life BCG vaccination reduces the prevalence of eczema, particularly in those at high risk (Fig 2).[16 49] In cohort studies, early-life BCG vaccination also reduces the prevalence of asthma. Measles vaccines are also associated with a reduced risk of eczema and asthma in cohort studies, **but no RCT has investigated this yet**.[23]

The immunological mechanisms underlying the non-specific effects of measles vaccine are unknown

Research investigating the immunological mechanisms that potentially underlie the non-specific effects of vaccines has focused on BCG vaccine, with most studies in adults (Fig 3).[2 7 50-55] Briefly, the non-specific effects of BCG is postulated to result from inducing:

(i) **heterologous lymphocyte responses**, mediated by both "**bystander activation**" (activation of unrelated B cells and/or T cells following infection- or vaccine-induced immune stimulation) and **cross-reactivity** (molecular mimicry between antigens of different pathogen species or strains);

(ii) **memory in innate immune cells** ("trained immunity") mediated by epigenetic and metabolic rewiring, resulting in an increase in the strength of the IFN-γ/Th1 response to unrelated pathogens,[32 50 56 57] and an overall decrease in Th2 responses − which is known to trigger allergies.[58-61]

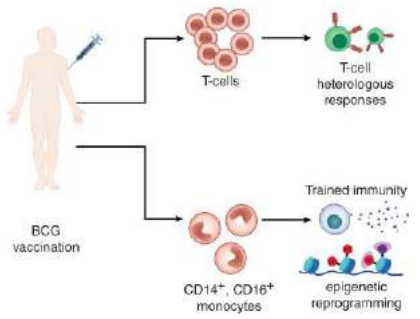


Fig 3. The immunological mechanisms proposed to mediate the non-specific effects of BCG vaccine: heterologous T-cell responses and trained immunity (innate immune memory). From ref[4]

These mechanisms result in an enhanced immune response and a more effective host defence when faced with a subsequent unrelated pathogen.

Immunomodulatory effects on in-vitro responses to unrelated pathogens have also been reported following measles vaccination.[62-68] **However, the underlying immunological mechanisms and pathway(s) have not yet been fully evaluated**. These might resemble those observed following BCG given the similarities in clinical observations.

Exploiting the non-specific effects of vaccines could have substantial public health benefits

Given the importance of the potential effects on infant health worldwide, the WHO recently emphasised the need for further RCTs to evaluate the non-specific effects of vaccines.[69 70] In particular, **there is a need to assess the measles vaccine-induced immunomodulation, both clinically and immunologically**, as:

(i) measles vaccines are widely used;
(ii) research on non-specific effects of measles vaccines is scarce, mainly based on observational studies;
(iii) vaccination schedule modification, as a result of a better understanding of the non-specific effects of measles vaccines, could have measurable effects.

Previous studies in Africa report that the administration of measles vaccine at an earlier age (before 9 months) is associated with reduced hospital admissions and general morbidity in the following months,[19 20] with conflicting effects on mortality.[71-73] Understanding the pathways involved in the non-specific effects of measles vaccine would enable them to be exploited further, in particular when evaluating the optimal timing and sequence of vaccinations, aiming to maximise both the direct effects of vaccination and the non-specific effects of live-attenuated vaccines, and to minimise any potentially harmful non-specific effects of non-live vaccines.[5 74-77 78 79] Scheduling a live-attenuated vaccine immediately after non-live vaccines has been postulated to **potentially save an extra one million of deaths per year** in children living in low-income countries.[80]

This trial will assess the non-specific effects of measles vaccine with a translational approach

The aim of the trial is to assess the non-specific effects of an early dose of a measles vaccine (the measles-mumps-rubella vaccine, MMR) in children, evaluating both the clinical impact (on unrelated infections and allergic diseases) and the underlying immunomodulatory mechanisms. This will be done through the most rigorous clinical approach (i.e., an RCT, as recommended by WHO), and will provide evidence on whether the beneficial effects reported in low-income settings are replicated in children living in a high-income country. Specifically, the trial will compare the non-specific effects of an earlier dose of MMR (at 6 months of age) with the regular scheduled dose (at age of 9 months), with the aim of optimising the timing of the administration of this vaccine. It will also assess the influence of giving MMR alone, compared with administering MMR concomitantly with non-live vaccines. The two interventions (bringing forward the 1st MMR dose and giving the 2nd MMR alone) have never been evaluated within the same trial, and this proposed trial will therefore be the first to measure the benefit of both interventions separately and in combination. The separate assessment of the two components will provide a better understanding of the mode of action of the modified schedule.

The study team has experience in studying the non-specific effects of vaccines

Thanks to grants from the SNSF (*Early Post-Doc.Mobility Grant* P2GEP3-178155) and from the HUG (*Fond de perfectionnement*), the PI, **Laure Pittet**, completed three years as a post-doc at the Murdoch Children's Research Institute in Melbourne, Australia, with Prof Nigel Curtis and Dr Nicole Messina, focusing on the non-specific effects of BCG. She had the opportunity, to work on the RCT "*Melbourne Infant Study: BCG for Allergy and Infection Reduction*" (**MIS BAIR**), in which 1272 newborns were randomised to receive BCG vaccine or no-BCG at birth, with follow-up for 5 years (NCT01906853).[16 81-83] They reported that neonatal BCG vaccination was able to reduce the incidence of eczema in high-risk infants (defined as having two atopic parents),[16] but that there was insufficient evidence to support a recommendation to give neonatal BCG vaccination to all newborns in high-income countries for the general prevention of eczema or to reduce lower respiratory tract infections (Fig. 4).[83]

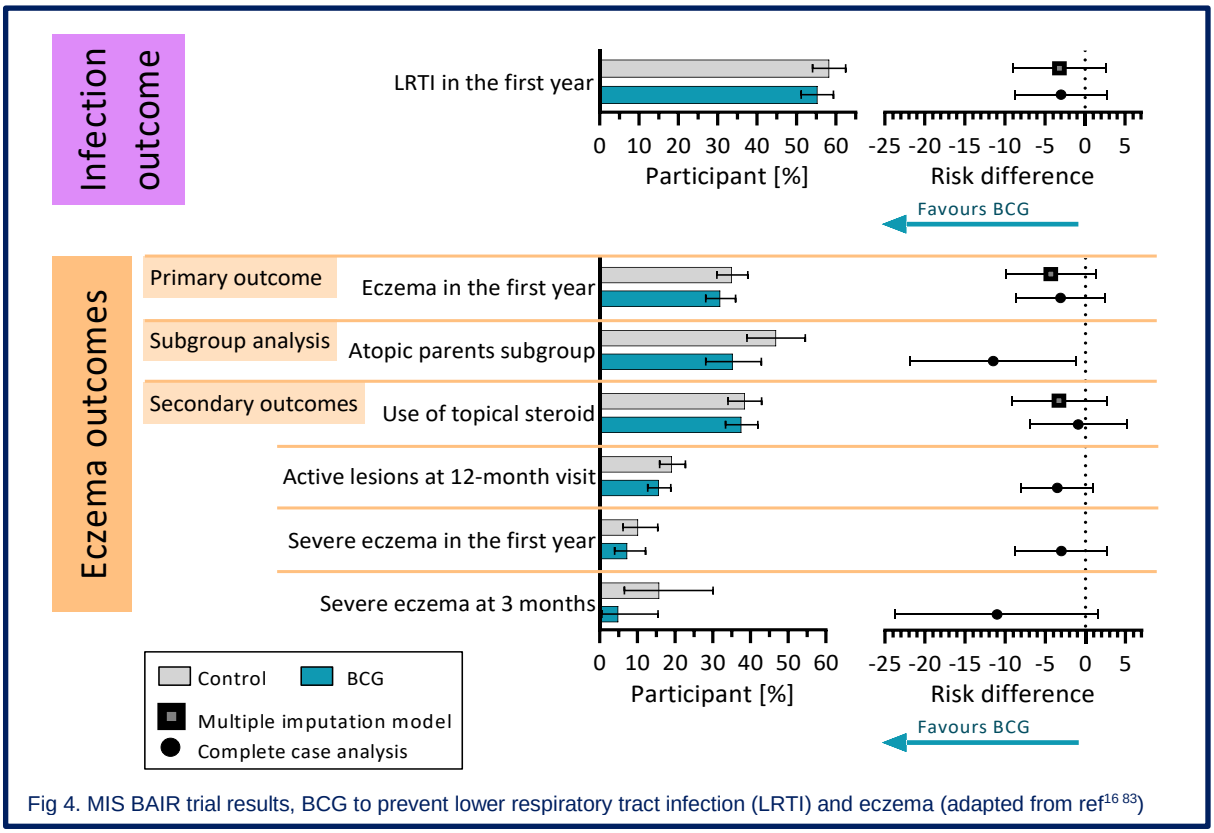


Fig 4. MIS BAIR trial results, BCG to prevent lower respiratory tract infection (LRTI) and eczema (adapted from ref[16 83])

In-vitro stimulation assays with heterologous stimulants were performed in a subset of the MIS BAIR participants at 7 days, 7 months and 13 months of age, and the study team reported on the immunological changes following neonatal BCG vaccination.[84 85] They also took the opportunity of the 13-month blood sample to compare participants who had already received MMR with those who had not yet received it, and observed that both IFN-γ and TNF-α secretions were higher in response to heterologous stimulation in MMR-vaccinated participants (Fig. 5).

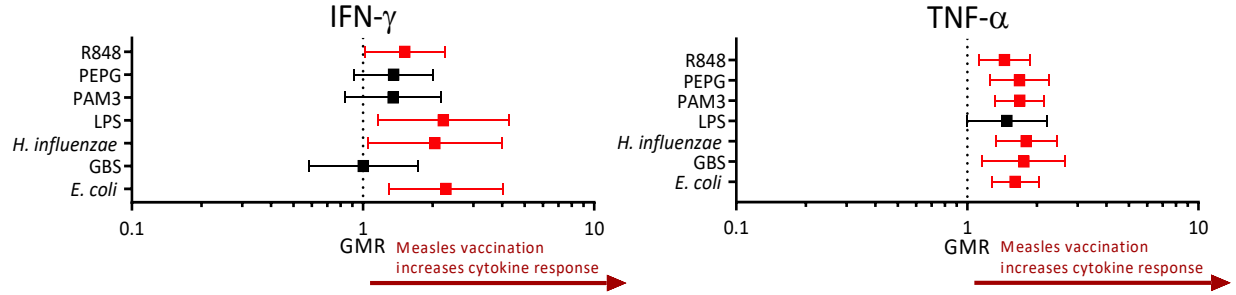


Fig 5. The effect of measles vaccination vs. no measles vaccination on cytokine responses to heterologous stimulants in 96 13-month-old participants of the MIS BAIR trial (including 70 measles vaccine-naïve participants and 26 who had received their first measles vaccine dose at a median of 23.5 (interquartile range 10) days) before the blood test). Geometric mean ratios (GMR) are shown with 95% CI. GMR > 1.0 indicates that cytokine levels were higher in measles-vaccinated participants compared with measles-naive participants. Significant results (p<.05) are depicted in red (manuscript in preparation). GBS: Group B *Streptococcus*; IFN: interferon; LPS: lipopolysaccharide, TLR4 agonist; PEPG: peptidoglycan, TLR2/4 agonist; R848: resiquimod, TLR7/8 agonist; PAM3: TLR1/2 agonist; TNF: tumor necrosis factor.

In another RCT, the *Early Life Vaccines and Immunity Study* (**ELVIS**) trial with 4 arms,[86 87] immunomodulation induced by neonatal BCG measured by in-vitro stimulation assays was found to be influenced by the co-administration of hepatitis B vaccine, a non-live vaccine (Fig. 6). These findings highlight the interaction between vaccines when they are co-administered.

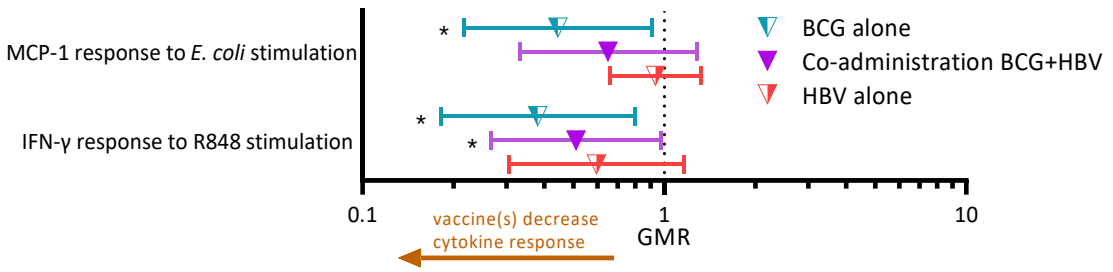


Fig 6. The effect of neonatal BCG and/or hepatitis B (HBV) on cytokine responses to heterologous stimulants in 128 10-day-old participants in the ELVIS trial. Geometric mean ratios (GMR) are shown with 95% confidence interval. The "No vaccine" group is defined as the reference group. GMR > 1.0 indicate that cytokine levels were higher in the vaccinated group (BCG alone, BCG+HBV, or HBV alone) compared with the "No vaccine" group. The figure illustrates that: (i) BCG vaccination modulated the neonatal innate immune response; (ii) HBV vaccine alone did not alter heterologous cytokine responses; but (iii) co-administration of HBV had influenced the immunomodulation induced by BCG. *: *p*<.05; IFN: interferon; MCP-1: monocyte chemoattractant protein-1; R848: resiquimod, a TLR7/8 agonist. (Adapted from [87])

During the SARS-CoV-2 pandemic, the group has set up the **BRACE** trial (*BCG vaccination to reduce the impact of COVID-19 in healthcare workers*) that is evaluating the non-specific effects of BCG to reduce the prevalence and severity COVID-19 in healthcare workers (NCT04327206).[88] [89] This international placebo-controlled RCT began in March 2020, with funding from the Gates Foundation and others. The group randomised a total of 6828 healthcare workers from 5 countries who are being followed up for one year with blood samples, questionnaires, and daily recording of symptoms. The trial includes many secondary outcome that evaluate whether BCG vaccine reduces the severity of other respiratory illnesses and allergic diseases, as well as the immunological mechanisms that underlie the non-specific effects of BCG.

In parallel with this, the group performed systematic reviews of the non-specific effects of vaccines,[2] [90] and have written the first chapter on this topic for the Plotkin's "Vaccines" textbook, which is recognised as the most authoritative vaccinology reference book.[2]

Prof Arnaud Didierlaurent is the head of the research group Translational Vaccine Immunology at the Department of Pathology and Immunology, Faculty of Medicine, University of Geneva. He has 20-years of experience in translational research in academia and industry, including 12-year experience at GSK in global roles, from research to product launch. His team works on understanding inflammatory response to infections and vaccines. Their main focus is the understanding of the role of innate immunity in vaccine responses (e.g. antigen-presenting cells), which are key players in conditioning long-lasting antibody and T-cell responses. Through a translational approach, combining clinical research and animal model studies, the team aims to characterise which component of the innate immune response to vaccines (e.g. changes in gene expression level, inflammatory mediators, etc.) translate into a change in the quality of the antigen specific response (e.g. antibodies, T- and B-cell response) when the former is altered by immunosuppression. Through a better characterisation of the hypo-responsiveness observed in immunocompromised individuals, the group aims to improve vaccination strategies for these populations that are at higher risk of infections. In addition, the team also explores the mechanism of action of various vaccines technologies in animal models, including adjuvants, focusing on the role of local innate immunity in vaccine response.

Prof Klara Posfay Barbe is the head of the General Paediatrics at the University Hospitals of Geneva. She is also the Director of the Clinical Research Platform for Paediatrics, Gynaecology and Obstetrics, a hub of SwissPedNet since 2012. Her research topics include infectious diseases in immune-compromised patients, and has substantial personal experience in setting up and running vaccination trials.

Prof Nigel Curtis has expertise in combining clinical and laboratory components in studies on non-specific effects of vaccines. He is Professor of Paediatric Infectious Diseases at The University of Melbourne, the head of Infectious Diseases at the Royal Children's Hospital Melbourne and the Group Leader of the Infectious Diseases research group at the Murdoch Children's Research Institute. He has hosted Laure Pittet during her post-doc and is the PI of the BRACE, MIS BAIR and ELVIS clinical trials. He will act as an advisor on the trial.

Dr Nicole Messina has a strong track record in defining immunological mechanisms that contribute to allergic and infectious diseases, and cancer. She has expertise in the immunological and transcriptomic aspects of the proposed project as well as co-ordinating the collection and processing of longitudinal

biological samples from neonates and infants. She is the lead of the immunological aspects of the BRACE, MIS BAIR and ELVIS clinical trials. She will act as an advisor for all the immunity aspect of the trial.

The group already has a substantial personal experience in setting up and running vaccination trials, managing teams, and studying the interaction between vaccines and the immune system. The topic of the PI's MD-PhD was the safety and immunogenicity of vaccines in immunocompromised patients, focusing on live-attenuated vaccines (e.g. MMR) after liver transplantation,[91-95] and the immune responses to pneumococcal conjugate vaccine,[96-98] under the supervision of Prof Klara Posfay-Barbe. In parallel, the PI's MD thesis (2016) described the deleterious impact of *Bordetella holmesii* on the pertussis re-vaccination strategy.[99-103] The PI's other publications focus on general paediatrics and infectious diseases.[1 87 104-127] The group also has a great network of collaborations, and is connected to the key international experts working on the non-specific effects of vaccines. This project would greatly complement and strengthen our country's expertise in vaccinology, studying an innovative new perspective: the effect of vaccines on the development and maturation of the immune system.

3.2 Investigational Product and Indication

Measles-mumps-rubella vaccine

Active substance	Measles virus, Schwarz strain* (min. $10^{3.0}$ TCID50) Mumps, RIT 4385 strain derived from Jeryl Lynn strain* (min. $10^{3.7}$ TCID50) Rubella, Wistar RA 27/3 strain** (min. $10^{3.0}$ TCID50) * produced in chick embryo cells ** produced in human diploid (MRC-5) cells
Excipients	Powder: amino acids, lactose (anhydrous), mannitol, sorbitol Solvent: water for injections
Trade name	Priorix
Marketing authorisation holder	GlaxoSmithKline AG GLN 7601001000674 Talstrasse 3-5, 3053 Münchenbuchsee Tel: +41318622111, Fax: +41318622200, swiss.info@gsk.com
Dosage form	Powder for Injection with solvent for resuspension
Dose and route of administration	0.5 ml injected intramuscularly in the deltoid region or in the anterolateral area of the thigh

TCID50: Median Tissue Culture Infectious Dose

3.3 Preclinical Evidence

Not applicable, the measles-mumps-rubella vaccine is authorised in Switzerland and strongly recommended since 1985 in infants. According to the summary of product characteristics, non-clinical data reveal no special hazard for humans based on general safety studies.[128]

3.4 Clinical Evidence to Date

3.4.1 Safety and immunogenicity of the MMR vaccine

The Cochrane review on the safety and immunogenicity of MMR vaccines has recently been updated.[129] It includes 138 studies with a total of 23,480,668 participants, with 51 studies (10,248,159 children) assessing vaccine effectiveness and 87 studies (13,232,509 children) assessing vaccine safety.

3.4.1.1 Effectiveness

According to the Cochrane review, vaccine effectiveness in preventing **measles** is 95% after one dose (95% CI, 87% to 98%) and 96% after two doses (95% CI 72% to 99%). The effectiveness of Jeryl Lynn containing MMR vaccine in preventing **mumps** is 72% after one dose (95% CI, 26% to 92%), 86% after two doses (95% CI, 65% to 96%). Vaccine effectiveness against **rubella**, using a vaccine with the BRD2 strain which is only used in China, is 89% (95% CI, 58% to 97%).

3.4.1.2 Safety

3.4.1.2.1 Data from the summary of product characteristics [128]

The safety profile presented below is available in the summary of product characteristics of the MMR vaccine PRIORIX, and is based on a total of approximately 12,000 subjects administered PRIORIX in clinical trials.

In controlled clinical studies, signs and symptoms were actively monitored during a 42-day follow-up period. The vaccinees were also requested to report any clinical events during the study period.

The most common adverse reactions following PRIORIX administration were injection site redness and fever ≥38°C (rectal) or ≥37.5°C (axillary/oral).

3.4.1.2.1.1 Tabulated list of adverse reactions

Adverse reactions reported are listed according to the following frequency:

- Very common: (≥ 1/10)
- Common: (≥ 1/100 to < 1/10)
- Uncommon: (≥ 1/1,000 to < 1/100)
- Rare: (≥ 1/10,000 to <1/1,000)

Clinical trial data

System Organ Class	Frequency	Adverse reactions
Infections and infestations	Common (1%-10%)	Upper respiratory tract infection
	Uncommon (0.1%-1%)	Otitis media
Blood and lymphatic system disorders	Uncommon (0.1%-1%)	Lymphadenopathy
Immune system disorders	Rare (0.001%-0.1%)	Allergic reactions
Metabolism and nutrition disorders	Uncommon (0.1%-1%)	Anorexia
Psychiatric disorders	Uncommon (0.1%-1%)	Nervousness, abnormal crying, insomnia
Nervous system disorders	Rare (0.001%-0.1%)	Febrile convulsions
Eye disorders	Uncommon (0.1%-1%)	Conjunctivitis
Respiratory, thoracic and mediastinal disorders	Uncommon (0.1%-1%)	Bronchitis, cough
Gastrointestinal disorders	Uncommon (0.1%-1%)	Parotid gland enlargement Diarrhoea Vomiting
Skin and subcutaneous tissue disorders	Common (1%-10%)	Rash
General disorders and administration site conditions	Very common (>10%)	Redness at the injection site Fever ≥38°c (rectal) or ≥37.5°c (axillary/oral)
	Common (1%-10%) to Very common (>10%)*	Pain and swelling at the injection site Fever >39.5°c (rectal) or >39°c (axillary/oral)

*In general, the frequency category for adverse reactions was similar for the first and second vaccine doses. The exception to this was pain at the injection site which was “Common” after the first vaccine dose and “Very common” after the second vaccine dose.

Post-marketing data

The following adverse reactions have been identified in rare occasions during post-marketing surveillance. Because they are reported voluntarily from a population of unknown size, a true estimate of frequency cannot be provided.

System Organ Class	Adverse reactions
Infections and infestations	Meningitis Measles-like syndrome Mumps-like syndrome (including orchitis, epididymitis and parotitis)
Blood and lymphatic system disorders	Thrombocytopenia Thrombocytopenic purpura
Immune system disorders	Anaphylactic reactions
Nervous system disorders	Encephalitis* Cerebellitis Cerebellitis-like symptoms (including transient gait disturbance and transient ataxia) Guillain-Barré syndrome Transverse myelitis Peripheral neuritis
Vascular disorders	Vasculitis
Skin and subcutaneous tissue disorders	Erythema multiforme
Musculoskeletal and connective tissue disorders	Arthralgia Arthritis

* **Encephalitis** has been reported with a frequency below 1 per 10 million doses. The risk of encephalitis following administration of the vaccine is far below the risk of encephalitis caused by natural diseases (measles: 1 in 1000 to 2000 cases; mumps: 2-4 in 1000 cases; rubella: approximately 1 in 6000 cases).

Accidental intravascular administration may give rise to severe reactions or even shock.

3.4.1.2.1.2 Overdose

Cases of overdose (up to 2 times the recommended dose) have been reported during post-marketing surveillance. No adverse events have been associated to the overdose.

3.4.1.2.2 Data from the Cochrane review [129]

3.4.1.2.2.1 Aseptic meningitis

There is evidence supporting an association between aseptic meningitis and MMR vaccines containing Urabe and Leningrad-Zagreb mumps strains, but **no evidence supporting this association for MMR vaccines containing Jeryl Lynn mumps strains** (rr 1.30, 95% CI 0.66 to 2.56; low certainty evidence).

3.4.1.2.2.2 Febrile seizure

The analyses provide evidence supporting an association between MMR/MMR+V/MMRV vaccines (Jeryl Lynn strain) and febrile seizures. Febrile seizures normally occur in 2% to 4% of healthy children at least once before the age of 5. The **attributable risk febrile seizures vaccine-induced is estimated to be from 1 per 1700 to 1 per 1150 administered doses**.

3.4.1.2.2.3 Idiopathic thrombocytopaenic purpura

The analyses provide evidence supporting an association between MMR vaccination and idiopathic thrombocytopaenic purpura (ITP). However, the risk of ITP after vaccination is smaller than after natural infection with these viruses. After natural infection, ITP occurs in 5 cases per 100,000 (1 case per 20,000) per year. **The attributable risk is estimated about 1 case of ITP per 40,000 administered MMR doses**.

3.4.1.2.2.4 Other

There is no evidence of an association between MMR immunisation and:

- **Encephalitis** or **encephalopathy** (rate ratio 0.90, 95% CI 0.50 to 1.61; 2 observational studies; 1,071,088 children; low certainty evidence)
- **Autistic spectrum disorders** (rate ratio 0.93, 95% CI 0.85 to 1.01; 2 observational studies; 1,194,764 children; moderate certainty).

There is insufficient evidence to determine the association between MMR immunisation and:

- **Inflammatory bowel disease** (odds ratio 1.42, 95% CI 0.93 to 2.16; 3 observational studies; 409 cases and 1416 controls; moderate certainty evidence).

Additionally, there is no evidence supporting an association between MMR immunisation and:

- Cognitive delay
- Type 1 diabetes
- Asthma
- Dermatitis/eczema
- Hay fever
- Leukaemia
- Multiple sclerosis
- Gait disturbance
- Bacterial or viral infections.

3.4.2 Safety and immunogenicity of the MMR vaccine before 9 months of age

Traditionally, the MMR vaccine was given as of 12 months of age, an age when passive transplacental transferred maternal antibody has disappeared and can no longer interfere with the vaccine response. Since then, the epidemiology of measles has changed, measles infection has become rarer and most infants are now born to uninfected, vaccinated mothers. Infants born to vaccinated mothers lose their passive immunity approximately 3 months earlier than infants born to mothers who have naturally acquired immunity (Fig 7).[130] **Infants born to vaccinated mothers therefore mount an adequate immune response to the measles vaccine at a younger age**.

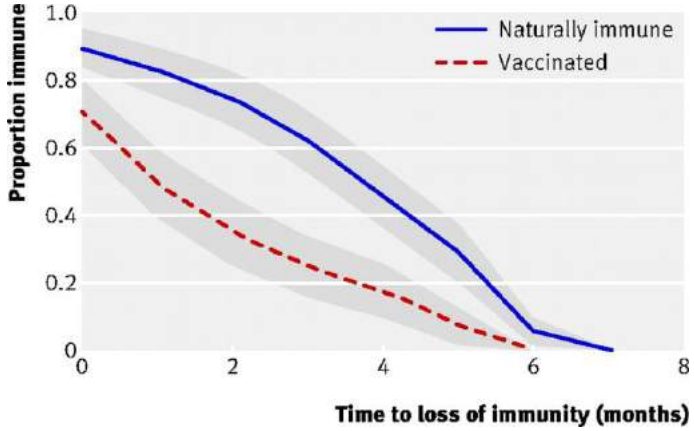


Fig 7. Proportion of infants born to vaccinated women (in red) or to naturally immune women (in blue) still immune as a function of time to loss of immunity. Shaded area is 95% confidence interval. From ref[131]

In the only systematic review available, the pooled estimated measles seroconversion rate was 76% (95% CI, 71 to 82) after vaccination at 6 months of age.[131] However, this estimation is based on observational studies that took place before the switch from mothers who had naturally acquired immunity to measles-vaccinated mothers, and is therefore unlikely to reflect today's reality.

Importantly, the systematic review did not identify any safety issue with similar profile of adverse events across the ages.

In Switzerland, MMR is already known to be safe and well tolerated in 6-month-old infants; it is already recommended by the Swiss Federal Commission for Vaccination in certain situations such as pre-travel and during outbreaks.[24]

3.5 Rationale for the dosage, route, regimen

The dose (0.5 ml) and route (intra-muscular injection) remains unchanged. The only change is the age of administration. With the administration of the first dose of MMR at 6 months of age instead of 9 months of age, it provides a unique 3-month long window during which we can study the differences between the two groups, until the control group receives the MMR vaccine at 9 months of age.

3.6 Explanation for choice of comparator (or placebo)

The control group will follow the usual vaccination schedule, with the first dose of MMR given at 9 months of age; they will not receive any intervention at 6 months of age (no placebo injection).

It has been deliberately chosen not to use a placebo to increase acceptability and overall feasibility.

3.7 Risks / Benefits

Safety of MMR vaccine at 6 months of age: MMR is already known to be safe and well tolerated in 6-month-old infants; it is already recommended by the Swiss Federal Commission for Vaccination in certain situations.[24] This study should therefore be considered as a low-risk study.

Risk of measles outbreak: In the eventuality of a measles outbreak, participants will be able to receive MMR vaccine if indicated, as recommended by the Swiss Federal Commission for Vaccination.[24] This is unlikely to happen given the low incidence of measles in this age group in Switzerland (1.16 per 100 000 inhabitants in 2020).

Benefit of MMR vaccine at 6 months of age: Infants born to unvaccinated mothers in an under-vaccinated population are at risk of contracting measles even right after birth, and therefore have the highest risk of complications, including subacute sclerosing panencephalitis. Transplacentally transmitted anti-measles antibodies can temporarily protect infants born to vaccinated mothers from measles infection, but their level and sustainability vary. This passive immunity is not sufficient to protect infants throughout their first year of life, and measles vaccination should start as early as possible, especially in areas with endemic measles virus transmission, such as Switzerland. In these regions, the first dose of measles-containing vaccine should be given as early as possible. Lowering the age of the administration of the first dose of measles vaccine is particularly important in the current post-vaccination era. Indeed, the peak concentration of vaccine-induce measles-specific antibody is typically lower than that induced by wild-strain infection. As a result, the antibody concentration in infants born to vaccinated mothers is at least three-fold lower than in infants born to mothers who have naturally acquired immunity.

Overall benefit of the trial: this translational trial would be the **first RCT to evaluate the non-specific effects and in-depth immunogenicity of early MMR in a high-income country**, with a comprehensive assessment of both clinical and immunological outcomes, including cell function and memory. It will also be among the first trials of the non-specific effects of vaccines to relate in-vitro findings to clinical outcomes. The robust setting of the study and immune read-outs are expected to provide high value data to confirm preliminary data from previous studies in low-income countries that are often limited by the high rate of missing data and the imprecision of many of the measures. A better understanding of non-specific effects has the potential to provide crucial progress in vaccine research, both for existing and future pathogens. Moreover, vaccination schedules could be easily adapted if early MMR vaccination is proven to have beneficial non-specific effects. **The outcomes of this study will directly inform and benefit vaccination strategies worldwide**.

The study of the non-specific effects of vaccination is an emerging branch in vaccinology and translational research. Improved understanding will have a high impact on immunisation schedules and infant health, as well as expected implications for alternative use of vaccines, for vaccination policies, and for the design of novel immunotherapeutic approaches. Infectious diseases remain a leading cause of morbidity and mortality in children under five years of age worldwide. While the development of pathogen-specific vaccines continues to be one of the pillars of infectious diseases prevention, understanding the beneficial and potential detrimental non-specific effects of vaccines will enable manipulation of these effects to optimise vaccine strategies and improve infant health. Rather than targeting a specific pathogen, “trained immunity-based vaccination” could be designed to provide broader protection by enhancing the innate immune response.[132] In addition, considering the rise in anti-vaccination movements and greater public interest in the effects of vaccines, it is important that both the specific and non-specific effects of vaccines are rigorously investigated and understood. A solid evidence based knowledge is urgently needed to counter potential future vaccine non-compliance resulting from concerns over the use of non-live vaccines.

3.8 Justification of choice of study population

The subjects chosen are the target population for the intervention, should the modified schedule show a benefit. As older subjects are unlikely to be MMR-naïve, 6-months-old infant are the population of choice to study the non-specific effects of MMR. Moreover, the clinical significance and duration of non-specific effects of vaccines, as well as the underlying mechanisms, probably differ with age. The immune system is more susceptible to immunomodulation in neonates than in older individuals; for example,

BCG-induced immunological changes differ between neonates, in whom pro-inflammatory cytokine responses to unrelated pathogens were found to be generally reduced,[84] infants,[85 133-136] and adults, in whom pro-inflammatory cytokine responses to unrelated pathogens are generally increased.[137 138] In one RCT lead by our group, the immunological changes measured following neonatal BCG vaccination differed at 7 days,[84] and 7 months of age,[85] within the same cohort of participants.

4. STUDY OBJECTIVES

4.1 Overall Objective

The aims of the proposed RCT are to determine whether optimising the MMR schedule reduces the burden of disease in childhood (infection with unrelated pathogens and allergic diseases), and to explore the underlying immunological mechanisms that could explain these clinical effects. This study will compare the current Swiss vaccine administration schedule with a modified schedule, designed to maximise the beneficial non-specific effects of MMR.

Two interventions will be assessed in the modified schedule:

Stage A: To administer the first dose of MMR at an earlier age
Stage B: To administer the second dose of MMR subsequently to (and not concomitantly with) other the non-live vaccines that are planned to be administered at this age (namely diphtheria-tetanus-pertussis-containing vaccine and the pneumococcal vaccine)

The impact of these two interventions will be measured separately and in combination using a factorial design (see Section 6).

4.2 Primary Objective

Stage A

AIM1: Determine whether early MMR vaccination (6 months of age) reduces the number of **respiratory infections** with unrelated pathogens between 6 months and 9 months of age.

Hypothesis: Children receiving early MMR are less likely to develop infections between 6 months and 9 months of age because of trained immunity induced by the live-attenuated MMR vaccine, compared to controls who received a non-live vaccine (DTP) as their last vaccination. The difference between the two groups is most likely to be seen for respiratory infections,[19 43 139-141] justifying it to be the primary objective.

4.3 Secondary Objectives

Clinical objectives

Stage A

AIM1: Determine whether early MMR vaccination (6 months of age) reduces the number and severity of **infections** with unrelated pathogens between 6 months and 9 months of age.

Hypothesis: Children receiving early MMR are less likely to develop infections between 6 months and 9 months of age because of trained immunity induced by the live-attenuated MMR vaccine, compared to controls who received a non-live vaccine (DTP) as their last vaccination. The difference between the two groups is most likely to be seen for respiratory infections,[19 43 139-141] but might as well be observed for other infections, justify them to be included as secondary objectives.

AIM2: Determine whether early MMR vaccination (6 months of age) reduces the risk and severity of **atopic and allergic diseases** between 6 months and 9 months of age.

Hypothesis: MMR acts as an early-life stimulus and prevents allergy by skewing the developing immune system toward a Th1 response, with corresponding down-regulation of Th2 responses.[58-61]

Stage B

AIM3: Determine whether the administration of the second MMR vaccine after the 12-month DTP and

PCV (not simultaneously with) reduces the number and severity of **infections** with unrelated pathogens between 13 months and 24 months of age.

Hypothesis: Children receiving MMR on its own (whose last vaccine received is therefore a live-attenuated vaccine) are less likely to develop infection because of trained immunity induced by the MMR vaccine, compared to controls who received non-live vaccine (DTP and/or PCV) simultaneously with MMR as the last vaccine.

AIM4: Determine whether administration of the second MMR vaccine after the 12-month DTP and PCV (not simultaneously with) reduces the risk and severity of **atopic and allergic diseases** between 13 months and 24 months of age.

Hypothesis: MMR given on its own might be more capable to prevent allergy by modelling the developing immune system toward a Th1 response, and corresponding down-regulation of Th2 responses.

Stages A and B

AIM5: Determine whether one of the two interventions has more impact, or if they are **synergistic**, and evaluate how children, maternal and environmental **factors influence the clinical outcomes**.

Hypothesis: The two intervention are synergistic and the non-specific effects observed are modulated by host factors (e.g. sex, ethnicity), exposure (e.g. day-care, siblings, pets, smoking), underlying immunity (e.g. previous infections), and familial history (e.g. allergy).

Laboratory objectives

Stages A and B

AIM6: Determine the **immunological non-specific** effects of MMR using in-vitro stimulation assays.

Hypothesis: MMR induces persistent changes in innate immune cells that modify their responsiveness to unrelated pathogens. The in-vitro immune response to various pathogen-derived triggers differs between the 2 groups, due to vaccine-induced modulation of the immune system.

AIM7: Identify the **biological pathways** and molecular mechanisms that underpin non-specific effects of MMR.

Hypothesis: Similar to the effects of BCG vaccination in adults, MMR alters infants' innate immunity by inducing epigenetic reprogramming of immune cells (e.g. monocytes) in a process called "trained immunity", associated with altered gene expression and clinical outcome.

AIM8: Determine the **immunogenicity** of early MMR vaccination.

Hypothesis: MMR vaccine is immunogenic at 6 months of age, inducing high seroprotection rate and memory cells; there is no indication to administer a third dose. As infants born to vaccinated mothers the subjects are more likely to have lost their transplacentally transferred maternal antibodies and are therefore more likely to mount an adequate immune response to the measles vaccine at a younger age.

AIM9: Determine whether one of the two interventions has more impact, or if they are **synergistic**, and evaluate how children, maternal and environmental **factors influence the laboratory outcomes**.

Hypothesis: The two intervention are synergistic and the non-specific effects observed in blood analysis are also modulated by host factors (e.g. sex, ethnicity), exposure (e.g. day-care, siblings, pets, smoking), underlying immunity (e.g. previous infections), and familial history (e.g. allergy).

4.4 Safety Objectives

Stage A

AIM10: Determine the safety of early MMR vaccination.

Hypothesis: MMR vaccine is safe and well-tolerated at 6 months of age, inducing similar safety profile than when given later on.

5. STUDY OUTCOMES

5.1 Primary Outcome

Stage A

AIM1: Number of parent-reported respiratory infections between 6 months and 9 months of age.

Justification: As shown in previous studies on the non-specific effect of vaccines, the difference between the two groups is most likely to be seen for respiratory infections,[19 43 139-141] justifying it to be the primary outcome measure. This outcome measure is also clinically relevant given the high burden of disease attributable to respiratory infections in infants.

5.2 Secondary Outcomes

Clinical outcomes

Stages A and B

AIM1&3: **Infections**: Additional measures of infection, with measurement of the following events: any infection, otitis, upper/lower respiratory tract infection, gastroenteritis, urinary tract infection, osteoarticular infection, and trauma/accident (the latter being used as 'negative control' events), including (i) time to first event, (ii) prevalence, (iii) incidence, (iv) days free of event, (v) severity (duration, antibiotic use, hospitalisation, and outcome) of each event, measured between 6 months and 24 months of life.

Justification: The trained immunity induced by MMR vaccination will not only reduce the risk of infection overall, but also decrease their severity

AIMS2&4: **Allergic and atopic diseases**: Eczema, food allergy, and wheezing diseases measured as (i) time to first event/flare, (ii) prevalence, (iii) incidence of event/flaring, (iv) days free of event/flare, (v) severity (scores (see below), treatment, hospitalisation, impact on growth and quality of life) of each event/flare, measured between 6 months and 24 months of life.

Justification: The trained immunity induced by MMR vaccination will reduce the risk of developing allergic disease and decrease their severity

AIM5: **Influence of interventions, children, maternal and environmental factors on study outcomes**: Factorial and multivariate analyses will assess the impact of interventions (early MMR, non-concomitant MMR), host factors (e.g. sex, ethnicity), exposure (e.g. day-care, siblings, pets, smoking), underlying immunity (e.g. previous infections), and familial history (e.g. allergy) on clinical outcomes defined above.

Justification: Both interventions are expected to have non-specific effects but it is unclear to whether they are synergistic. The other factors listed above are known to possibly influence the non-specific effect of vaccines. Understanding to what extent they influence the non-specific effect of MMR would help identifying subgroup that would beneficiate the most from a modified vaccination schedule.

Laboratory outcomes

AIM6: **Immune cell sub-populations and responsiveness over time**, measured by immuno-phenotyping, cell activation and cytokine production following in-vitro stimulation with unrelated pathogens, Tool-like receptor (TLR) agonist or molecules derived from pathogens (as described in [84 85 142]), in bloods collected at 6 months, 9 months, and 24 months of age.

AIM7: **Transcriptional responses and epigenetic imprinting in immune cells** (e.g. monocytes) in bloods collected at 6 months, 9 months, and 24 months of age, identifying functional pathways that are impacted by MMR and that might contribute to the clinical non-specific effects observed.

AIM8: **Vaccine responses and memory cells** in bloods collected at 6 months, 9 months, and 24 months of age.

AIM9: **Influence of interventions, children, maternal and environmental factors on laboratory outcomes**: Factorial and multivariate analyses will assess the impact of interventions (early MMR, non-concomitant MMR), host factors (e.g. sex, ethnicity), exposure (e.g. day-care, siblings, pets, smoking), underlying immunity (e.g. previous infections), and familial history (e.g. allergy) on laboratory outcomes defined above.

5.3 Other Outcomes of Interest

Exploratory outcome measures may be examined, pending additional funding. These may include measures of metabolomic and lipidomic profile, in particular the quantification of glycoprotein acetyls, a marker of cumulative acute and chronic inflammation, commonly used as a surrogate of infection burden.[143]

5.4 Safety Outcomes

AIM10: **Vaccine safety**: occurrence and severity of local and systemic adverse events, as well as any serious adverse events, by systemic organ class, preferred term, severity using toxicity grading scale,[144] and relationship to the intervention from day 0 to day 42 post MMR.

6. STUDY DESIGN

6.1 General study design and justification of design

6.1.1 Study design and framework

This is a phase IV, single-center, open-label, randomised controlled trial with a factorial design, aiming to demonstrate the superiority of a modified vaccination schedule, compared with the current Swiss vaccination schedule for MMR.

6.1.2 Participants

500 children healthy children followed-up in Geneva, including a subset of 100 children with blood tests (see section 7 *Study population*, and section 11.2 *Sample size*).

6.1.3 Intervention and comparator

This trial will compare the current Swiss vaccine administration schedule (control group) with a modified schedule (intervention groups), designed to maximise the beneficial non-specific effects of MMR (Fig 8 and Table 1). The intervention will be the same (0.5 ml of the MMR vaccine injected intramuscularly), the only change is the timing of administration. **The factorial design will enable to measure the benefit of the intervention on each of the two doses of MMR separately or in combination** (Table 2). The intervention on the administration of the first dose of MMR is to give it at 6 months of age instead of 9 months of age; the intervention on the administration of the second dose of MMR is to give it 1 month after the 1-year non-live vaccines instead of concomitantly to them.

Whereas this will be a classic 2-arm trial with regard to the first intervention (early 1st MMR dose) and the primary outcome (number of respiratory infections between 6 and 9 months), the second phase will be a factorial trial, crossing the first component (early 1st MMR dose) with the second component (separated 2nd MMR dose). This design will allow a separate assessment of the impact of the 2 components on secondary outcomes measured until 24 months of age. The utility of the separate assessment of the 2 components is both scientific (better understanding of the mode of action of the modified schedule) and pragmatic (the modified schedule may imply that an additional medical visits is required (i.e. at 13 months of age to administer the 2nd MMR not concomitantly to the other 12-months vaccines) and if one was unnecessary (i.e. if the first component has the greatest impact, the additional impact from the second component is negligible) the intervention with only one of the two components would be more practical and cheaper).

	Current schedule	Modified schedule	Comment – Rational for change
1st DTP and PCV	2 m	2 m	No change
2nd DTP and PCV	4 m	4 m	No change
1st MMR	9 m	**6 m**	As soon as possible after 2nd DTP-PCV (both non-live vaccines)
3rd DTP and PCV	11-13 m	11-12 m	Before and not concomitantly to 2nd MMR
2nd MMR	11-13 m	**13 m**	Given on its own, after 3rd DTP-PCV (➔most recent vaccine live)

Table 1: Current Swiss vaccine administration schedule compared to the proposed modified schedule, with rational for change. m: months of age; PCV: pneumococcal conjugate vaccine (a non-live vaccine). See also Fig 8.

		Stage B: comparison for the timing of the **2nd MMR**		
		2nd MMR 1m after the 12y DPT-PCV (**M**odified)	2nd MMR concomitant with the 12y DPT-PCV (**C**urrent)	
Stage A: comparison for the timing of the **1st MMR**	1st MMR at 6m (**M**odified)	**Group M.M.**, n=125 (including 25 in Part 2)	**Group M.C.**, n=125 (including 25 in Part 2)	N=250 (including 50 in Part 2)
	1st MMR at 9m (**C**urrent)	**Group C.M.**, n=125 (including 25 in Part 2)	**Group C.C.**, n=125 (including 25 in Part 2)	N=250 (including 50 in Part 2)
		N=250 (including 50 in Part 2)	N=250 (including 50 in Part 2)	

Table 2. Participant allocation in 4 groups; see also Table 3. C: current MMR schedule; DTP: diphtheria-tetanus-pertussis vaccine; m: month; M: Modified MMR schedule; PCV: pneumococcal conjugate vaccine.

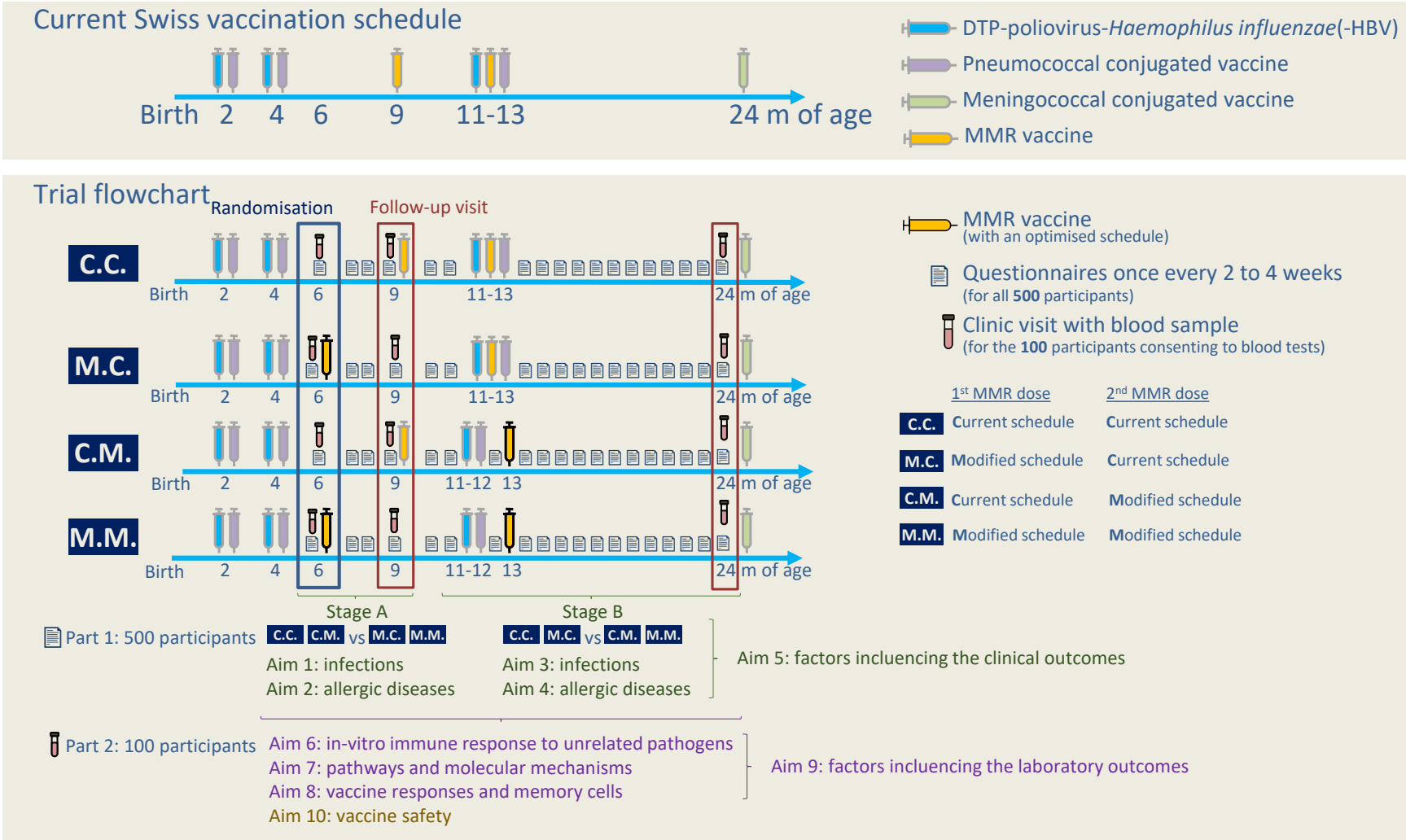


Fig 8: Current Swiss administration schedule and Study flowchart.

6.1.4 Duration of volunteers' participation

Participants will be followed-up for 18 months after randomisation, namely from 6 months to 24 months of age.

6.1.5 Study procedure and measurement

A minimum of three visits are planned, at 6 months of age (for inclusion, randomisation, ± vaccination, ± blood collection), 9 months of age (for ± vaccination, ± blood collection), and 24 months of age (for ± blood collection). Providing sufficient funding, an additional visit at 12-13 months of age (for vaccination) is planned at the Platform of clinical research in Paediatrics; if funding is insufficient, tasks will be delegated to the treating paediatrician who would do it during their routine follow-up visit. Parents will be asked to complete questionnaires once every 2 weeks for the first 3 months, then once every month.

6.2 Methods of minimising bias

6.2.1 Randomisation

Consent to blood tests at 6, 9 and 24 months of age will be sought, and will determine whether a child participates in Part 1 only, or both Part 1 and Part 2. Participant will be randomly allocated at inclusion to four equal groups, using a web-based randomisation procedure, stratified by consent to blood tests (yes/no), in randomly permuted blocks of variable length (4 or 8), enabling to prevent predictability of intervention assignments. The randomisation lists will be generated prior to the initiation of the study by someone not involved in the study analysis and will be integrated into REDCap.

6.2.2 Blinding procedures

Investigators involved in data cleaning and analyses will be blinded to allocation in order to avoid the introduction of bias between study arms, until statistical analyses have been completed.

6.2.3 Other methods of minimising bias

Validated questionnaire will be used when available (see section 9.2)

6.3 Unblinding Procedures (Code break)

Not applicable.

6.4 Risk mitigation of the trial design

Variability in infectious risk: Individual risk of infection depends on host (predisposition) and environmental (exposure) factors. Randomisation should ensure that risk factors are equally distributed between the two groups. The risk of those factors influencing the outcomes is also addressed by adjusting the analyses by the presence of comorbidities (host predisposition), siblings and day-care attendance (environmental exposure).

Recruitment: Recruitment will be through a network of private paediatricians highly motivated in contributing to clinical research. Pamphlets will be distributed in maternity services and paediatricians' offices; infants will be recruited during the first few months of age and parents will have opportunity to ask questions before the randomisation visit at 6 months of age. With over 5000 live births per year in Geneva, a minimum acceptation rate of 5% for Part 1 (questionnaires only) and of 1% for blood testing will enable recruitment to be completed in 18 months.

Study burden / acceptance of the study: The online questionnaire is being designed in partnership with parents in order to make it as user-friendly as possible. It will be completed every 2 weeks by participants' parents on a smartphone, computer or tablet following a participant-specific link sent by text or email, and will take less than 10 minutes to complete. The questionnaire includes many fields per participant but, thanks to branching algorithms, only items relevant to each participant will be displayed (based on the answers to previous questions). This design reduces redundancy and reduces the risk of missing or duplicate data.

Accuracy of parent-reported data: The questionnaire uses pre-filled fields, drop-down menus, and other measures to reduce the time taken for completion, and to increase the accuracy of the data. The data will be reviewed with the treating paediatrician, and the parents during the clinic visit, to retrieve any missing information. The child's medical records will also be checked. Clinical assessment will take place during the study visit to confirm some of the outcomes (e.g. presence of eczema using validated scores).

Blood collection in infants: Blood volume collected will be kept to the strict minimal and within the range proven to be safe in children.[145] The study visits will be held at the Platform of Clinical Research in Paediatric, Gynaecology & Obstetrics, and the blood collection will be done in collaboration with research nurses who are experienced in collecting blood from infants. The use of analgesia cream, correct positioning and distraction of the child will minimise pain. The minimum of a 3-months gap between the visits and blood collection will increase acceptability.

Extensive laboratory analysis: The teams of Prof Didierlaurent (Center of Vaccinology, Geneva), and Prof Curtis (Murdoch Children's Research Institute, Melbourne) have long experience in performing all the experiments described, including the in-vitro assays on whole blood and downstream sample analysis, as well as whole genome transcriptomic analysis, in infectious disease and vaccine studies.[146-148] We will also have access to the support of a bioinformatician who is currently working in the laboratory of Prof Didierlaurent and supporting in transcriptomic and epigenetic analyses. The genomic platform of the University of Geneva have internationally recognised expertise for such experiments (https://ige3.genomics.unige.ch/).

7. STUDY POPULATION

7.1 Eligibility criteria

7.1.1 Inclusion criteria

Participants fulfilling all of the following inclusion criteria are eligible for the study:

1) Informed Consent as documented by signature
2) 6-month-old children
3) In overall good health, without any clinically significant concomitant disease states (e.g., renal failure, hepatic dysfunction, cardiovascular disease, etc.) and no clinically significant abnormal finding on history and/or physical examination
4) Fully immunised for age according to the Swiss vaccination schedule
   a) with at least 2 doses of DTP-containing vaccine
   b) the last dose of vaccine received at least 2 weeks prior to enrolment

7.1.2 Exclusion criteria

The presence of any one of the following exclusion criteria will lead to exclusion of the participant:

1) Contra-indications to MMR, including
   a) immunosuppression (i.e. proven, suspected, or planned)
   b) allergy to a component of the vaccine
   c) receipt of a live-attenuated vaccine in the four weeks prior to inclusion
2) Vaccine refusal
3) Indication for an early MMR vaccination, including
   a) Measles outbreak
   b) Planned immunosuppression (indication to an accelerated schedule to be completed before starting an immunosuppressive treatment)
   c) Travel to a region with a high risk of measles outbreak
4) Indication for vaccination with MMR-varicella (MMRV) instead of MMR, including
   a) severe eczema
   b) parental will
5) Parental inability to follow the procedures of the study, e.g. due to language problems, psychological disorders, known/suspected non-compliance, substance abuse, etc.
6) Plan to move out of the country or have prolong absence during the trial
7) Other sibling included in the trial
   a) In the case of multiple pregnancy, only one child can be randomised.
8) Any temporary contra-indication to MMR, including child being sick (active significant illness)
   a) Inclusion can be delayed a few days until the illness resolves

7.2 Recruitment and screening

As mentioned in section 2.7, the parents of potential subjects are informed about the trial via pamphlets, email, notice board and/or website/social media in Geneva, Switzerland. The pamphlets include a pitch about the study and a QR code with a link to a website where they can read further information and access the participant informed consent form (PICF), with contact details for further questions. Interested parents are given the opportunity to talk with a member of the research team if they have any questions, and to consult the treating paediatrician prior to enrolment.

Parents of potential subject are then screened by investigators (or designee). If the subject is deemed to be eligible (see section 7.1), the investigators (or designee) explain to the parent the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. The parent of each subject is informed that the participation in the study is voluntary, that they may withdraw their children from the study at any time, and that withdrawal of consent will not affect subsequent medical assistance and treatment. The parent of each subject are informed that they can ask any question, and consult with family members, friends, treating physicians or other experts before deciding about their participation in the study. The parent of each subject is given a subject information sheet and a consent form describing the study and providing sufficient information for the parent to make an informed decision about their child's participation in the study. Enough time is given to the parents of the subjects. The formal consent of the parent of each subject, using the approved consent form, is obtained before the subject is submitted to any investigation procedure.

The parent of each subject should read, understand, and voluntarily agree before signing and dating the informed consent form, and is given a copy of the signed document. The consent form is signed and dated by the subject and the PI (or designee). The signed consent form it is retained as part of the investigation records.

There is no compensation for participating in the trial.

There are no further screening procedures for eligibility, including no test are required.

If enrolment goal are not met, measures will be taken to increase recruitment, such as extension within Geneva or to other location, or hiring supplementary staff.

7.3 Assignment to study groups

Once informed consent is signed, consent to blood tests at 6, 9 and 24 months of age will be sought, and will determine whether a child participates in Part 1 only, or both Part 1 and Part 2. For subjects consenting to blood tests, the baseline blood will be collected prior to randomisation. In the eventuality that the blood collection fails, the subject will be considered as not consenting to blood tests (participation to Part 1 only), given that the laboratory analysis in Part 2 cannot be made in the absence of a baseline blood sample.

As described in section 6.2.1, randomisation will be in 4 groups of similar size, using a web-based randomisation procedure, stratified by consent to blood tests (yes/no), in randomly permuted blocks of variable length (4 or 8), enabling to prevent predictability of intervention assignments. The randomisation lists will be generated prior to the initiation of the study by someone not involved in the study analysis and will be integrated into REDCap.

Subjects will be randomised at 6 months of age by an investigator (or designee) using the REDCap platform. Subjects will receive the two MMR vaccines according to their randomisation group (current schedule or modified schedule for the first and the second MMR dose, see section 6).

7.4 Criteria for withdrawal / discontinuation of participants

In accordance with the principles of the current revision of the Declaration of Helsinki, a volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give his/her reason(s) for doing so. In the present study this right is extended to the subject's parents. The Investigator may also withdraw the subject at any time in the interests of the subject's health and well-being. The DSMB may also recommend withdrawal of volunteers.

In addition, the subject may withdraw/be withdrawn for any of the following reasons:

- Ineligibility, jeopardising the health of the participant
    - either arising during the study or retrospectively, having been overlooked at screening
    - if the ineligibility is discovered after study inclusion, the participant will be withdrawn

from the study only in the event that the factor leading to ineligibility could directly jeopardise the health of the participant if the participant continues in the trial.

- Significant protocol deviation
  - failure to follow protocol procedures that specifically relate to the primary outcome of the study
- Volunteer non-compliance with study requirements
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures

The reason for withdrawal will be recorded in a CRF. If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the subject's parent, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

It is not planned to replace subject who have withdrawn from the study, should it be less than the maximum 15% allowed in the sample size calculation (see section 11.2).

Any subject who do not complete the questionnaires and fails to attend scheduled follow-up visits with neither communication with the study team nor a clear rationale will be deemed to have withdrawn from the study. If a subject withdraws from the study, clinical data and blood samples collected before his/her withdrawal from the trial will be used/stored unless the subject's parent specifically requests otherwise.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products

All participants will receive all vaccines planned by the Swiss vaccinations schedule, the intervention being only a change in the administration scheme (Table 3).

Age:	6 m	9 m	11-12 m	13 m
Group C.C. (both MMR on **C**urrent schd.)	No vaccine	1st MMR	3rd DTP and PCV 2nd MMR	No vaccine
Group M.C. (1st MMR **M**odified schd., 2nd MMR **C**urrent schd.)	1st MMR	No vaccine	3rd DTP and PCV 2nd MMR	No vaccine
Group C.M. (1st MMR **C**urrent schd., 2nd MMR **M**odified schd.)	No vaccine	1st MMR	3rd DTP and PCV	2nd MMR
Group M.M. (both MMR on **M**odified schd.)	1st MMR	No vaccine	3rd DTP and PCV	2nd MMR

Table 3: MMR schedule in the 4 study groups. C: current schedule; DTP: diphtheria-tetanus-pertussis-containing vaccine; m: months of age; M: modified schedule; PCV: pneumococcal conjugate vaccine; schd: schedule. See also Table 2 and Fig 8.

As recommended by the Swiss vaccinations schedule, the dose of the intervention is the following:

- **MMR**: 0.5 ml of MMR vaccine injected intramuscularly in the deltoid region or in the anterolateral area of the thigh.

The following vaccines are expected to be administered by the treating paediatrician at 11-12 months of age, as planned and recommended by the Swiss vaccinations schedule:

- **DTP**: 0.5 ml of a diphtheria, tetanus, pertussis, poliovirus, ± *Haemophilus influenzae* type b ± hepatitis B vaccine injected intramuscularly in the deltoid region or the anterolateral area of the thigh.
- **PCV**: 0.5 ml of pneumococcal conjugate vaccine injected intramuscularly in the deltoid region or the anterolateral area of the thigh.

8.1.1 Experimental Intervention

The interventional product is the measles-mumps-rubella vaccine Priorix from GlaxoSmithKline (see section 3.2). It comes as a powder (that is the lyophilised MMR component) and a solvent for solution for injection in a pre-filled syringe.

The lyophilised measles-mumps-rubella component is a white to slightly pink powder.

The solvent is a clear and colourless solution.

The vaccine must be reconstituted by adding the entire contents of the pre-filled syringe of solvent to the vial containing the powder. The mixture should be well shaken until the powder is completely dissolved in the solvent. Due to minor variation of its pH, the reconstituted vaccine may vary in colour from clear peach to fuchsia pink without deterioration of the vaccine potency.[128]

There will be no deviation from the commercial product and the usual administration mode. The vaccine (0.5 ml) will be injected intramuscularly in the deltoid region or in the anterolateral area of the thigh.

8.1.2 Control Intervention (standard/routine/comparator treatment)

The interventional product given to the control group will be the same as for the intervention group (see section 8.1.1), but with a different schedule of administration (see section 8.1).

8.1.3 Packaging, Labelling and Supply (re-supply)

Not applicable, we will used the same vaccine as used routinely.

8.1.4 Storage Conditions

The MMR vaccines will be stored in their original package in order to protect them from light, and kept in the refrigerator (2°C-8°C). It should not be freeze.

After reconstitution, the vaccine should be injected promptly after reconstitution, or stored at 2°C-8°C and used within 8 hours of reconstitution.

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

See section 8.1 and 8.1.1.

8.2.2 Control Intervention

See section 8.1 and 8.1.2.

8.3 Dose modifications

Not applicable.

8.4 Compliance with study intervention

Not applicable.

8.5 Data Collection and Follow-up for withdrawn participants

As stated in section 7.4, If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the subject's parent, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

8.6 Trial specific preventive measures

Not applicable.

8.7 Concomitant Interventions (treatments)

Not applicable.

8.8 Study Drug Accountability

Not applicable, we will used the same vaccine as used routinely.

8.9 Return or Destruction of Study Drug

Not applicable, we will used the same vaccine as used routinely.

9. STUDY ASSESSMENTS

9.1 Study flow chart(s) / table of study procedures and assessments

Visit number	V1		V2		V3[a]			V4
Time point	t_0	t_0 to t_{+3m}	t_{+3m}	t_{+3m} to t_{+6m}	t_{+6m}	t_{+7m}	t_{+6m} to t_{+18m}	t_{+18m}
Age of participant	6mo	6mo to 9mo	9mo	9mo to 12mo	12mo	13mo	12mo to 24mo	24mo
Allowed time frame	±2w	±1w	±2w	±1w	±2w	±2w	±1w	±4w
Recruitment								
Screening, eligibility check	X							
Inclusion and randomisation	X							
Baseline questionnaire [b]	X							
Intervention: MMR vaccination								
- Group C.C.			X		X			
- Group M.C.	X				X			
- Group C.M.			X			X		
- Group M.M.	X					X		
Assessments								
Clinical assessment [c]	X		X		X			X
Fortnightly questionnaire		X…X…X…X[d]		X…X…X…X[e]			X…X…X…X[e]	
Vaccine diary (S/AE)		X[f]		X[f]			X[f]	
Contact with treating paediatrician	X		X		X			X
Health record check	X		X					X
Blood collection (Part 2 only)	X		X					X

C: current schedule; MMR: measles-mumps-rubella vaccination; M: modified schedule; m: month; mo: month-old; S/AE: (serious) adverse event; V: visit; w: week.

[a] Timing of V3 differs between groups: 13 mo for Group M.M. and Group C.M., and 12 mo for Group M.C and Group C.C. If funding is insufficient, V3 might be simplified and delegated to the treating paediatrician.

[b] Includes demographics, medical history.

[c] Includes physical examination and eczema assessment using SCORAD.

[d] every 2 weeks.

[e] every month.

[f] follow-up 42 days after vaccination.

9.2 Assessments of outcomes

Clinical outcomes

Source of information:

1) Online parent questionnaires sent every two weeks for the first 3 months, then monthly.
2) Contact with the treating paediatrician at 9 and 24 months for completion of any missing illness data.
3) Access to medical records.

The data collected (Table 4) will enable the extraction of the following measurement for each participant for both infectious and allergic episodes: number of episodes between 6 months and 2 years of life, interval between inclusion and first episode, characteristics of each episode (e.g. diagnosis, duration) and treatment (e.g. type, route, duration), complications, hospitalisation (e.g. reason, length of stay, outcomes), number of days free of episode. Episodes of trauma/accidents will be used as control events.

Data collected at inclusion, confirmed with treating paediatrician and hospital records
Demographics: parental age, education, ethnicity, country of birth; household composition, age of siblings; location of home (countryside, urban); socioeconomic group; date of birth*.
Potential confounding and or mediating factors pre-randomisation: maternal pregnancy factors (mode of delivery, infection, antibiotics, probiotics, smoking, supplements, vaccines), infant factors (sex, gestational age, season of birth, sepsis, antibiotics, probiotics, alimentation, supplements and medications); family history of allergic disease (eczema, hay fever, asthma, food and non-food allergies); household smoking; pet exposure.
Data collected with online questionnaires, confirmed with treating paediatrician and hospital records
Infection outcome data: episodes of: upper/lower respiratory tract infection (including croup, pharyngitis, otitis media, bronchiolitis, bronchitis, pneumonia), cold sores/mouth ulcers, gastroenteritis, urinary tract infection, bone-joint infection, other infection. *For all infections*: diagnosis, pathogen(s), site of infection, date, symptoms, duration, severity, outcome, health care use, hospital admission (more than 1 day), treatment.
Trauma/accident data (used as control events): medically attended accidents, burns or trauma. *For all occurrences*: date, severity, health care use, hospital admission (more than 1 day).
Allergic disease outcome data: presence of eczema, food allergy, wheezing disease. *Eczema* - diagnosis using adapted version of the UK diagnostic tool,[149 150] parent-reported doctor-diagnosed, and clinical evaluation during study visits; symptoms; severity and impact on quality of life using the POEM[151] and the SCORAD[152] scores. *Food-allergy* – parent-reported doctor-diagnosed food allergy or sensitisation; diagnostic test used: participants with suspicion of food allergy will be seen by a specialist, as routinely done in Geneva. As recommended,[153] diagnosis will be made using oral food challenge, basophil activation test, or mast cell activation test; sensitisation defined using skin prick tests results and serum-specific IgE concentration (including allergen component-resolved diagnostics). *Wheezing diseases* – parent-reported doctor-diagnosed wheezing episode (bronchiolitis or infant asthma).[154] *For each allergic disease*: age at diagnosis, date, duration and severity of episodes/flares, outcome, treatment.
Potential confounding factors post randomisation: breastfeeding (age at cessation); formula (type); solid feeding (age at introduction of different foods); smoking exposure; childcare attendance (index & siblings); travel; pet exposure; vitamin D supplementation; probiotic intake; paracetamol/ibuprofen use; antibiotics; other vaccinations (which and when).

Table 4: Summary of clinical data that will be collected.

* Collection of participants' date of birth is needed to determine the season of birth (see table 4 above), as well as the time range for the visits (see section 9.3).

Laboratory outcomes

Source of information:

In the voluntary subgroup of participants whose parent consented to blood tests, blood will be collected at 6 months, 9 months, and 24 months of age to perform in-vitro stimulation assays, immune cell evaluation and vaccine responses. Just after collection, the blood will be transported to Prof Didierlaurent's laboratory at the Centre for Vaccinology of the University of Geneva, which is geographically very close. and in which the assays are established. The time points will enable us to evaluate how the immune system matures and, thanks to the control group, distinguish age-induced effects from the vaccine-induced early effects.

9.2.1 Assessment of primary outcome

Stage A

AIM1: **Respiratory infections between 6 months and 9 months of age**.

Outcome measure: Incidence of parent-reported respiratory infections between 6 months and 9 months of age using fortnightly REDCap questionnaires, with validation of data by confirmation with treating paediatrician and medical records.

9.2.2 Assessment of secondary outcomes

Clinical outcomes

Stages A and B

AIM1&3: **Infections**

Outcome measure: Additional measures of infection, with measurement of the following events: any infection, otitis, upper respiratory tract infection, lower respiratory tract infection, gastroenteritis, urinary tract infection, osteoarticular infection, and trauma/accident (the latter being used as control event), including:

(i) **Time to first event** – Calculated as:
For participants who have an event:
Date of event onset - date of randomisation
For participants who did not have an event:
Earliest censoring date - date of randomisation

(ii) **Prevalence** - Calculated as:
number of participants who have an event / total number of participants

(iii) **Incidence** - Calculated as:
number of events / total time of follow-up

(iv) **Days free of event** - Calculated as:
number of days free of event / total days of follow-up of participants

(v) **Severity of event** – measured as:
1. Duration – Per event, calculated as: *date of recovery - date of onset*
2. Antibiotic use - Per event, including: *yes/no, antibiotic name, dose, route, duration*
3. Hospitalisation - Per event, including: *yes/no, ward/critical care, duration, treatment received*
4. Outcome - Per event, including: *uneventful/complication/sequel/death*

of each category of event, measured between 6 months and 24 months of life, using fortnightly REDCap questionnaires, with validation of data by confirmation with treating paediatrician and medical records.

AIMS2&4: **Allergic and atopic diseases**

Outcome measure: Occurrence and flare of eczema, food allergy, and wheezing diseases (defined as mentioned in table 4) measured as:

(i) **Time to first event** – Calculated as:
For participants who have a flare:
Date of event onset - date of randomisation
For participants who did not have a flare:
Earliest censoring date - date of randomisation

(ii) **Prevalence** - Calculated as:
number of participants who have a flare / total number of participants

(iii) **Incidence** - Calculated as:
number of flares / total time of follow-up

(iv) **Days free of flare** - Calculated as:
number of days free of flare / total days of follow-up of participants

(v) **Severity of flare** – measured using:
1. Scores – e.g. *POEM*[151] and *SCORAD*[152] (see table 4)
2. Treatment use - Per flare, including: *yes/no, treatment name, dose, route, duration*
3. Hospitalisation - Per flare, including: *yes/no, ward/critical care, duration*
4. Outcome - Per flare, including: *uneventful/complication/sequel/death*
5. Impact on growth – Calculated using: *evolution of biometric percentile*
6. Impact on quality of life – Calculated using scores, e.g. items from *POEM*[151] and *SCORAD*[152]

of each event/flare, measured between 6 months and 24 months of life, using fortnightly REDCap questionnaires, with validation of data by confirmation with treating paediatrician and medical records.

AIMS5: **Influence of interventions, children, maternal and environmental factors on study outcomes**

Outcome measure: as defined above. Factorial and multivariate analyses will assess the impact of interventions (early MMR, non-concomitant MMR), host factors (e.g. sex, ethnicity), exposure (e.g. day-care, siblings, pets, smoking), underlying immunity (e.g. previous infections), and familial history (e.g. allergy) on the clinical outcomes defined above.

Laboratory outcomes

AIM6: **Immune cell sub-populations and responsiveness over time**

Outcome measure: Bloods will be collected at 6 months, 9 months, and 24 months of age and in-vitro whole blood stimulation will be done with unrelated pathogens and Toll-like receptor (TLR) agonists, such as:

Unrelated pathogens
Staphylococcal aureus
Streptococcus pneumoniae
Escherichia coli
Candida albicans
Respiratory syncytial virus (RSV)

TLR-agonists		
TLR2/4 agonist	PEPG	Peptidoglycan
TLR3 agonist	Poly(I:C)	Polyinosinic-polycytidylic acid
TLR4 agonist	LPS	Lipopolysaccharide
TLR7/8 agonist	R848	Resiquimod

Table 5: Example of unrelated pathogens and Toll-like receptor (TLR) agonists used for stimulations.

The assay strip for stimulation will be made in batches, to maximise standardisation of the stimulant volume, and stored at -80°C until the addition of blood. Cytokine secretion and immune cell activation will be measured in stimulated samples using the following procedures:

Cytokine secretion: Whole blood diluted 1:1 in RPMI will be incubated together with either a heat-killed pathogen, a TLR-agonist or media alone. Cytokine expression will be quantified in supernatants by multiplex assays through measurement of cytokines concentrations (e.g. IFN-γ, IFN-α/β, IP-10, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p40, MCP-1, MIG, MIP-1β, and TNF-α) in response to the in-vitro stimulation.

Intracellular cytokine staining (ICS) assays: Whole blood diluted 1:1 in RPMI will be cultured in the stimulation strips described in the presence of cytokine secretion inhibitors for the last 6 hours. Innate and adaptive immune cell (e.g. monocytes subsets, dendritic cell subsets, neutrophils, NK cells, NK T cells, T cells and B cells) activation and cytokine production (e.g. IFN-γ, IP-10, IL-1β, IL-4, IL-6, IL-10, IL-12p40, IL-17, IL-22 and TNF-α) will be assessed by ICS and flow/spectral cytometry as previously described.[114 142 155]

AIM7: **Transcriptional responses and epigenetic imprinting in immune cells**

Outcome measure: (e.g. monocytes) in bloods collected at 6 months, 9 months, and 24 months of age, identifying functional pathways that are impacted by MMR and that might contribute to the clinical non-specific effects observed.

In brief, PBMC and granulocytes will be isolated from peripheral blood samples collect at 6 months, 9 months, and 24 months of age using a density gradient. Monocytes (or other relevant immune cell subsets identified in AIM 6) will be isolated by fluorescence activated cell sorting (FACS, cell sorter) and

analysed by transcriptomic and epigenetic analysis, to identify immune and cellular pathways that have been altered by MMR vaccination.

Transcriptomic signatures*:* Purified immune cell populations from a minimum of 20 paired samples (i.e. sample taken at 6 months, 9 months, and 24 months of age of 20 individuals) per group of primary outcome analysis (20 in Group M.C. or M.M., and 20 in Group C.M. or C.C.; see section 11.2) will be stimulated with an unrelated pathogen or media alone (Table 5). The choice of stimulation will be informed by the findings from AIM 6 (i.e. those inducing the most differences between the two groups). RNA will be isolated and RNA sequencing (RNA-Seq) will be done and mapped. Differentially expressed genes (DEGs) will be compared between groups and time points.

Epigenetic reprograming: DNA will be isolated from a minimum of 20 paired purified immune cell populations samples per group (as defined above), and measure DNA methylation will be measured. Analysis of differential methylation will be done and methylation sites will be allocated to nearby genes. This will be done in similar cells and samples as for the transcriptomic analysis so both results could be correlated.

Pathway and integration analysis*:* Pathway analysis of transcriptomic and epigenomic data will be done using standard ontological analysis software.[156] Dynamic gene promoters and distal regions with DNA methylation changes will be scanned for transcription factor motif enrichment.

AIM8: **Vaccine responses and memory cells**

Outcome measure: quantification of vaccine-specific immunoglobulin and immune cells, including memory cells at 24 months of age, compared to pre-vaccination (at 6 months or 9 months of age, depending on the group) and between groups. In order to increase the numbers for the 24-months-old between groups comparison, the 24 months blood sample with be proposed to all 500 participants, regardless of the Part, but is of course optional.

AIM9: **Influence of interventions, children, maternal and environmental factors on laboratory outcomes**

Outcome measure: as defined above. Factorial and multivariate analyses will assess the impact of interventions (early MMR, non-concomitant MMR), host factors (e.g. sex, ethnicity), exposure (e.g. day-care, siblings, pets, smoking), underlying immunity (e.g. previous infections), and familial history (e.g. allergy) on the laboratory outcomes defined above.

9.2.3 Assessment of other outcomes of interest

Exploratory outcome measures may be examined, pending additional funding.

9.2.4 Assessment of safety outcomes

AIM10: **Vaccine safety**: occurrence and severity of local and systemic adverse events, as well as any serious adverse events, by systemic organ class, preferred term, severity using standard grading scales (Table 6),[144 158] and relationship to the intervention from day 0 to day 42 post MMR (see section 10)

Parents will be asked to fill down a 42-day vaccine diary to record adverse events including local reaction (pain, redness, tenderness, and swelling) and systemic reaction (fever, fatigue, irritability, vomiting, diarrhoea, myalgia, arthralgia, conjunctivitis, skin rash, and parotitis). Investigators will also record solicited and unsolicited events at study visits and will check with the treating paediatrician and the medical record.

	Grade 0 None	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life threatening
Local reaction					
Pain	None	Does not interfere with activity	Repeated use of nonnarcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room visit or hospitalisation
Redness	None	1a: < 2.5 cm 1b: 2.5 - 5 cm	5.1 - 10 cm	>10 cm	Necrosis or exfoliative dermatitis
Tenderness	None	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Emergency room visit or hospitalisation
Swelling / induration	None	1a: < 2.5 cm 1b: 2.5 - 5 cm and does not interfere with activity	5.1 - 10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis
Systemic (general) reaction					
Fever	None	38.0°C to 38.4°C	38.5°C to 38.9°C	39.0°C to 40.0°C	>40.0°C
Fatigue	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalisation
Irritability	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalisation
Nausea / vomiting	None	No interference with activity, or 1-2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	Emergency room visit or hospitalisation for hypotensive shock
Diarrhoea	None	2-3 loose stools or < 400 g/24 hours	4-5 stools or 400-800 g/24 hours	≥6 watery stools or >800 g/24 hours or requires outpatient IV hydration	Emergency room visit or hospitalisation
Myalgia / arthralgia	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalisation
Conjunctivitis	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalisation
Skin rash	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalisation
Parotitis	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalisation

Table 6: Toxicity grading of adverse event following vaccination, according to the Food and Drug Administration. (2007). "Guidance for Industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical" Retrieved 20.12.2021, from

https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf

9.2.4.1 Adverse events

All adverse events, both solicited and unsolicited, will be recorded through day 42 after vaccination. For each adverse event, date and time of onset, duration, resolution, action taken, assessment of intensity/ severity (Table 6) and relationship with study treatment will be recorded in REDCap.

Solicited adverse events, will be collected through day 42. They include the following:

9.2.4.1.1 Solicited local adverse event

- Pain
- Redness
- Tenderness
- Swelling / induration

9.2.4.1.2 Solicited systemic (general) adverse event

- Fever
- Fatigue
- Irritability
- Nausea / vomitting
- Diarrhea
- Myalgia / arthralgia
- Conjunctivitis
- Skin rash
- Parotitis

9.2.4.1.3 Unsolicited adverse event

Unsolicited adverse events will be recorded during study visits, and through consulting with the treating paediatrician and the medical record, or if the subject's parent spontaneously contact the investigator.

9.2.4.2 Laboratory parameters

Not applicable, it is not planned to assess laboratory parameters.

9.2.4.3 Vital signs

Not applicable, it is not planned to assess clinical parameters.

9.2.5 Assessments in participants who prematurely stop the study

As stated in sections 7.4 and 8.5, If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the subject's parent, until the AE has resolved, stabilised or a non-trial related causality has been assigned.

9.3 Procedures at each visit

Subjects will be asked to attend 4 visits at the Platform for clinical research in paediatrics over an 18-months study period (from 6 months to 24 months of age). The screening and pre-enrolment process will vary between participants and is not considered as a study visit. Each study visit is described in detail below. An overview is found in section 9.1.

9.3.1 Screening: 0 to 6 months of age (between t_{-6m} and t_0)

Information about the trial: The parents of potential subjects are informed about the trial via pamphlets, email, notice board and/or website/social media. The pamphlets include a pitch about the study and a QR code with a link to a website where they can read further information and access the participant informed consent form (PICF), with contact details for further questions. Interested parents are given the opportunity to talk with a member of the research team if they have any questions, and to consult the treating paediatrician prior to enrolment.

Screening by investigators: Parents of potential subject are then screened by investigators (or designee) that explains to the parent of each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. The parent of each subject is informed that the participation in the study is voluntary, that they may withdraw their children from the study at any time, and that withdrawal of consent will not affect subsequent medical assistance and treatment. The parents of each subject are informed that they can ask any question, and consult with family members, friends, treating physicians or other experts before deciding about their participation in the study. Enough time is given to the parents of the subjects.

9.3.2 Visit 1: Baseline and randomisation, 6 months of age ($t_0 \pm 2$ weeks)

Eligibility check by investigators, with review of past medical history and of vaccination records, re-evaluation of inclusion and exclusion criteria.

Target clinical examination will be performed if needed, to ensure that there is no temporary exclusion criteria (e.g. if the child is sick, the inclusion can be delayed a few days until the illness resolves).

Overview of the trial by investigators, using the study flow chart and the participant informed consent form. Correct comprehension of the trial will be checked, with the possibility for parents to ask any remaining question.

Blood collection will be discussed in further details for interested parents.

Further information will be given to all parents, including that

- The treating physician of their child will be contacted to inform about the trial, corroborate their child's medical history and seek additional information.
- Authorised individuals other than their treating physician may examine their child's medical records.
- The blood samples taken as part of the study will be coded and stored for a period of maximum 15 years; this duration has been chosen given the likelihood that progress in the nascent field of non-specific effects of vaccines may benefit substantially from the reanalysis of study samples. Upon the approval of the Sponsor, samples may be sent outside of Switzerland to collaborating research laboratories for non-commercial research purposes.
- There is no compensation for participating in the trial.
- They are free to withdraw at any time. Unless they specifically request complete consent withdrawal, the long-term data collection, including access to medical records, will continue, in order to avoid bias as much as possible.

Informed consent will be sought, once the subject's parent confirm understanding and voluntarily agreeing in participating; the parent and the investigator will both date and sign the consent form.

Inclusion in the trial, the parent is given a copy of the signed document; the original copy is securely filed in the study documentation.

Baseline questionnaire will be filled down by the subject parents and reviewed with the investigator. It includes questions on demographics, medical history, and exposure, as detailed in Table 4 in section 9.2.

Eczema assessment will be performed, using *SCORAD*[152] score.

Part 2 only: **blood will be collected** (maximum 15 ml), in subjects whose parent accepted blood collection, for the laboratory tests listed in section 9.2.2. This is done after informed consent is obtained but prior to randomisation. In the eventuality that the blood collection fails, the subject will be considered as not consenting to blood tests (participation to Part 1 only), given that the laboratory analysis in Part 2 cannot be made in the absence of a baseline blood sample.

Randomisation in the trial, using a web-based randomisation procedure integrated into REDCap, stratified by consent to blood tests (yes/no), in randomly permuted blocks of variable length (4 or 8), enabling to prevent predictability of intervention assignments.

Distribution of the vaccination plan according to the group the subject has been randomised to.

Group M.C. or M.M. only: **MMR vaccination (first dose)**; Subjects randomised to the Group M.C. or M.M. (modified schedule for the 1st MMR dose) will receive one dose of MMR vaccine. After reconstitution of the vaccine by adding the entire content of the pre-filled syringe of solvent to the vial containing the powder, the vaccine (0.5 ml) will be injected intramuscularly in the deltoid region or in the anterolateral area of the thigh. The subject will be distracted during the injection, in order to minimise the pain. The subject will remain under close observation for 20 minutes after the injection.

Group C.M. or C.C. only: **No vaccination**; Subject randomised to the Group C.M. or C.C. (current schedule for the 1st MMR dose) will not receive any vaccine at 6 months.

Vaccine diary will be presented to the parents, who will be instructed to fill it for the next 42 days.

Fortnightly questionnaire will be presented to the parents, who will be instructed to fill them every 2 weeks for the next 18 months.

Contact details will be given to the parent, consisting in an emergency card with a phone number to call in case of questions about the trial or upon occurrence of any adverse event.

Scheduling next visits.

Once the subject and their parent have left:

Data entry will be checked by the investigator to ensure that no information is missing. Parents could be contacted if any data is missing.

Contact with treating paediatrician will be made, to inform about the inclusion and the randomisation, confirm the understanding of the trial procedures and the vaccination plan, and answer any questions.

9.3.3 Visit 2: Follow-up, 9 months of age (t_{+3m} ± 2 weeks)

Review of the fortnightly questionnaires by investigators, together with the parent, to ensure concordance and the absence of missing data; instruction to continue to fill them every 2 weeks until the subject is 24 months old.

Review of vaccine diary, by investigators, together with the parent, to ensure concordance and the absence of missing data; instruction to fill a second vaccine diary the next 42 days.

Review of vaccination records, by investigators, to ensure that all is in order. Review with the parent the vaccination plan and ensure it is well understood.

Overview of the next steps of the trial by investigators, using the study flow chart to ensure correct comprehension of the parent, with the possibility for parents to ask any question.

Target clinical examination will be performed if needed, to ensure that there is no temporary contra-indication for the vaccination (e.g. if the child is sick, the vaccination can be delayed a few days until the illness resolves) or for the blood test (e.g. if the child is sick or recovering, the visit can be delayed a few days until the illness resolves, as it might influence the laboratory test).

Eczema assessment will be performed, using *SCORAD*[152] score.

Part 2 only: **blood will be collected** (maximum 15 ml), in subjects whose parent accept blood collection, for the laboratory tests listed in section 9.2.2. In the eventuality that the blood collection fails or if parents does not accept blood collection at this visit, the subject will remain in Part 2, as they have already provided a baseline blood and further blood collection might succeed.

Group C.M. or C.C. only: **MMR vaccination (first dose)**; Subject randomised to the Group C.M. or C.C. (current schedule for the 1st MMR dose) will receive one dose of MMR vaccine. After reconstitution of the vaccine by adding the entire content of the pre-filled syringe of solvent to the vial containing the powder, the vaccine (0.5 ml) will be injected intramuscularly in the deltoid region or in the anterolateral area of the thigh. The subject will be distracted during the injection, in order to minimise the pain. The subject will remain under close observation for 20 minutes after the injection.

Group M.C. or M.M. only: **No vaccination**; Subject randomised to the Group M.C. or M.M. (modified schedule for the 1st MMR dose) will not receive any vaccine at 9 months.

Scheduling next visits.

Once the subject and their parent have left:

Data entry will be checked by the investigator to ensure that no information is missing. Parents could be contacted if any data is missing.

Contact with treating paediatrician will be made, to ensure concordance of the data collected with the medical record and the absence of missing data, as well as to confirm the understanding of the next step of the trial and answer any questions.

9.3.4 Visit 3: Follow-up, 12 or 13 months of age ($t_{+6\text{-}7m}$ ± 2 weeks)

Note: if funding is insufficient, this visit might be simplified and delegated to the treating paediatrician.

Review of the fortnightly questionnaires by investigators, together with the parent, to ensure concordance and the absence of missing data; instruction to continue to fill them every 2 weeks until the subject is 24 months old.

Review of vaccine diary, by investigators, together with the parent, to ensure concordance and the absence of missing data; instruction to fill a third vaccine diary the next 42 days.

Review of vaccination records, by investigators, to ensure that all is in order:
- The Group M.C. and C.C. (12 months old) should not have received the 12-months vaccination yet.
- The Group C.M. and M.M. (13 months old) should have received the third doses of DTP and PCV13 a month ago.

Review with the parent the vaccination plan and ensure it is well understood.

Overview of the next steps of the trial by investigators, using the study flow chart to ensure correct comprehension of the parent, with the possibility for parents to ask any question.

Target clinical examination will be performed if needed, to ensure that there is no temporary contra-

indication for the vaccination (e.g. if the child is sick, the vaccination can be delayed a few days until the illness resolves).

Eczema assessment will be performed, using *SCORAD*[152] score.

MMR vaccination (second dose); all subject, regardless of the group, will receive one dose of MMR vaccine. After reconstitution of the vaccine by adding the entire content of the pre-filled syringe of solvent to the vial containing the powder, the vaccine (0.5 ml) will be injected intramuscularly in the deltoid region or in the anterolateral area of the thigh. The subject will be distracted during the injection, in order to minimise the pain. The subject will remain under close observation for 20 minutes after the injection.

***Group M.C. and C.C. only:* concomitant vaccination with DTP and PCV**; Subject randomised to the Group M.C. and C.C. (current schedule for the second MMR dose) will receive their third dose of DTP and PCV concomitantly to the second dose of MMR, as planned by the Swiss vaccination schedule.

Scheduling next visits.

Once the subject and their parent have left:

Data entry will be checked by the investigator to ensure that no information is missing. Parents could be contacted if any data is missing.

Contact with treating paediatrician will be made, to ensure concordance of the data collected with the medical record and the absence of missing data, as well as to confirm the understanding of the next step of the trial and answer any questions.

9.3.5 Visit 4: Follow-up, 24 months of age (t_{+18m} ± 4 weeks)

Review of the fortnightly questionnaires by investigators, together with the parent, to ensure concordance and the absence of missing data.

Review of vaccine diary, by investigators, together with the parent, to ensure concordance and the absence of missing data.

Review of vaccination records, by investigators, to ensure that all is in order and make a copy to be stored as a source document.

Target clinical examination will be performed if needed, to ensure that there is no temporary contra-indication for the blood test (e.g. if the child is sick or recovering, the visit can be delayed a few days until the illness resolves, as it might influence the laboratory test).

Eczema assessment will be performed, using *SCORAD*[152] score.

***Part 2 only:* blood will be collected** (maximum 15 to 20 ml), in subjects whose parent accept blood collection, for the laboratory tests listed in section 9.2.2, and possibly other exploratory outcomes (depending on funding).

***Part 1 only:* optional blood collection** (maximum 15 to 20 ml), for the laboratory tests assessing vaccine responses and long-term immunity, as listed in AIM 8 in section 9.2.2, and possibly other exploratory outcomes (depending on funding).

End of the trial for the subject.

Once the subject and their parent have left:

Data entry will be checked by the investigator to ensure that no information is missing. Parents could be contacted if any data is missing.

Contact with treating paediatrician will be made, to ensure concordance of the data collected with the medical record and the absence of missing data.

10. SAFETY

10.1 Drug studies

The *Safety Reporting SOP* provide more detail on safety reporting.

For this Category A trial, only certain adverse events (AE) are recorded: specifically serious adverse events as defined below, and non-serious adverse events of interest specified in section 10.1.1.1.

During the entire duration of the study, all serious adverse events (SAEs) are collected, fully investigated and documented in source documents and case report forms (CRF). Study duration encompassed the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period.

10.1.1 Definition and assessment of (serious) adverse events and other safety related events

10.1.1.1 Definition of adverse event

An **Adverse Event (AE)** is any untoward medical occurrence in a patient or a clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with the study procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. [ICH E6 1.2]

For the period of vaccination to 42 days post vaccination only, the following solicited adverse events are non-serious adverse events of interest for this study; they will be collected using vaccine diary.

10.1.1.1.1 Solicited local adverse event

- Pain
- Redness
- Tenderness
- Swelling / induration

10.1.1.1.2 Solicited systemic (general) adverse event

- Fever
- Fatigue
- Irritability
- Nausea / vomitting
- Diarrhea
- Myalgia / arthralgia
- Conjunctivitis
- Skin rash
- Parotitis

10.1.1.1.3 Unsolicited adverse events

Unsolicited adverse events will be recorded during study visits, and through consulting with the treating paediatrician and the medical record, or if the subject's parent spontaneously contact the investigator. They are defined as an adverse event of interest and will be reported as such only if they began within the first 42 days following the intervention and if the causality assessment deems the event to be possibly, probably or definitely associated to the intervention.

10.1.1.2 Definition of serious adverse event

A **Serious Adverse Event (SAE)** is classified as any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires in-patient hospitalisation or prolongation of existing hospitalisation, or
- results in persistent or significant disability/incapacity.

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalisation, but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. [ICH E2A]

SAEs should be followed until resolution or stabilisation. Participants with ongoing SAEs at study termination (including safety visit) will be further followed up until recovery or until stabilisation of the disease after termination.

10.1.1.3 Assessment of Causality

Both Investigator and Sponsor-investigator make a causality assessment of the event to the study drug, based on the criteria listed in the ICH E2A guidelines:

Relationship	Description
Definitely	The AE is **clearly related** to the intervention, there is a • Temporal relationship • Recurrence after rechallenge • (or other proof of drug cause)
Probably	The AE is **likely related** to the intervention, there is a • Temporal relationship • No other cause evident
Possibly	The AE **may be related** to the intervention, there is a • Temporal relationship • Other cause possible
Unlikely	The AE **is doubtfully related** to the intervention, as it does not fulfil the above conditions
Not related	The AE **is clearly not related** to the intervention, as a causal relationship can be ruled out

10.1.1.4 Unexpected Adverse Drug Reaction

An "unexpected" adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the product information or the summary of product characteristics (section 3.4.1.2). [ICH E2A]

10.1.1.5 Suspected Unexpected Serious Adverse Reactions (SUSARs)

The Sponsor-Investigator evaluates any SAE that has been reported regarding seriousness, causality and expectedness. If the event is related to the investigational product and is both serious and unexpected, it is classified as a SUSAR.

10.1.1.6 Assessment of Severity

Severity of AE will be assessed using standard grading scales, namely the Food and Drug Administration toxicity grading scale for all solicited adverse events (Table 6),[144] and the Common Terminology Criteria for Adverse Events CTCAE Version 5.0 for any unsolicited AE (CTCAE)[158] https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_8.5x11.pdf

10.1.2 Reporting of serious adverse events (SAE) and other safety related events

10.1.2.1 Reporting of SAEs

All SAEs must be reported immediately and within a maximum of 24 hours to the Sponsor-Investigator of the study. The Sponsor-Investigator will re-evaluate the SAE and return the form to the site.

SAEs resulting in death are reported to the Ethics Committee via BASEC within 7 days.

10.1.2.2 Reporting of SUSARs

A SUSAR needs to be reported to the Ethics Committee (local event via local Investigator) via BASEC within 7 days, if the event is fatal, or within 15 days (all other events).

10.1.2.3 Reporting of immediate safety and protective measures

All suspected new risks and relevant new aspects of known adverse reactions that require immediate safety-related measures, must be reported to the Sponsor-Investigator within 24 hours. The Sponsor-Investigator must report these measures within 7 days to the Ethics Committee (local event via local Investigator) via BASEC.

10.1.2.4 Reporting and Handling of Pregnancies

Not applicable.

10.1.2.5 Periodic reporting of safety

An Annual Safety Report is submitted once a year to the local Ethics Committee via Sponsor-Investigator.

10.1.3 Follow up of (Serious) Adverse Events

All AEs or SAEs that result in a subject withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the volunteer consents to this).

10.2 Assessment, notification and reporting on the use of radiation sources

Not applicable.

11. STATISTICAL METHODS

11.1 Hypothesis

Null Hypothesis (H_0): Children receiving early MMR at 6 months of age (Group M.C. or M.M.) will have the same number of respiratory infections between 6 months and 9 months of age than children receiving the regular MMR at 9 months of age (Group C.M. or C.C.).

Alternative hypothesis (H_1): Children receiving early MMR at 6 months of age (Group M.C. or M.M.) will have less respiratory infections between 6 months and 9 months of age compared with children receiving the regular MMR at 9 months of age (Group C.M. or C.C.).

11.2 Determination of Sample Size

The sample size calculation for the clinical outcome (500 participants) is based on the primary outcome of respiratory infections. Based on the data from a previous trial, we expect a rate of respiratory infection of 0.29 episodes per month (SD 0.32) between 6 and 9 months of age in the control groups (Group C.M. or C.C.). By randomising 500 participants, we will have an 80% power to detect a minimum of 30% reduction in this incidence with a 2-sided p-value of 0.05, assuming at least 85% completion rate of the 9 months questionnaire. The 500 participants will be randomised into 4 groups of similar size (see Section 6 and table 3 in Section 8.1): for the primary outcome analysis, the 250 children receiving early MMR at 6 months of age (Group M.C. or M.M.) will be compared with the 250 children receiving the regular MMR at 9 months of age (Group C.M. or C.C.).

		Timing of the **2nd MMR**		
		2nd MMR 1m after the 12y DPT-PCV (**M**odified)	2nd MMR concomitant to the 12y DPT-PCV (**C**urrent)	
Primary outcome comparing the timing of the **1st MMR**	1st MMR at 6m (**M**odified)	**Group M.M.**, n=125	**Group M.C.**, n=125	N=250
	1st MMR at 9m (**C**urrent)	**Group C.M.**, n=125	**Group C.C.**, n=125	N=250
		N=250	N=250	

Table 7. Participant allocation in 4 groups; for the primary outcome analysis, the Groups M.C. and M.M. will be compared with the Groups C.M. and C.C. C: current MMR schedule; DTP: diphtheria-tetanus-pertussis vaccine; m: month; M: Modified MMR schedule; PCV: pneumococcal conjugate vaccine.

The sample size calculation for the laboratory outcomes (100 participants) is more conservative as it depends on the success of the blood collection and of the laboratory processing for each of the time points. The use of paired samples (taken from the same infant at different ages) reduces the effect of inter-individual heterogeneity and provides greater statistical power with lower participant numbers.

Among the 500 participants enrolled, 100 participants consenting to blood collection (Part 2) will be randomised into 4 groups of similar size (table 2 in Section 6.1): for the laboratory outcome analysis, the 50 children receiving early MMR at 6 months of age (Group M.C. or M.M.) will be compared with the 50 children receiving the regular MMR at 9 months of age (Group C.M. or C.C.). Assuming that at least 60% of participants from Part 2 will provide a sufficient blood sample at each time point, which will then be processed without any problem, these 100 participants in total are required for Part 2, to ensure that a minimum of 30 participants per group of 1st MMR timing (30 in Group M.C. or M.M., and 30 in Group C.M. or C.C.) could be included in all analyses.

		Timing of the 2nd MMR		
		2nd MMR 1m after the 12y DPT-PCV (**M**odified)	2nd MMR concomitant to the 12y DPT-PCV (**C**urrent)	
Primary outcome comparing the timing of the 1st MMR	1st MMR at 6m (**M**odified)	**Group M.M.**, n=25	**Group M.C.**, n=25	N=50
	1st MMR at 9m (**C**urrent)	**Group C.M.**, n=25	**Group C.C.**, n=25	N=50
		N=50	N=50	

Table 8. Participant allocation in 4 groups; for the laboratory outcome analysis, the Groups M.C. and M.M. will be compared with the Groups C.M. and C.C. C: current MMR schedule; DTP: diphtheria-tetanus-pertussis vaccine; m: month; M: Modified MMR schedule; PCV: pneumococcal conjugate vaccine.

Cytokine secretion & ICS studies: with a minimum of 30 participants per group of 1st MMR timing (30 in Group M.C. or M.M., and 30 in Group C.M. or C.C.) who could be included in all analyses (minimum of 60 in total) we would have >80% power to detect a biologically significant 1.5-fold-change in cytokine responses at the 5% significance level, based on the variance of previous infant cytokine and ICS studies. [84 85 137 142 155 156 159-162]

Transcriptomic analysis: a minimum of 20 participants per group of 1st MMR timing (20 in Group M.C. or M.M., and 20 in Group C.M. or C.C.; 40 participant in total) provides 80% power to detect a 1.25 fold-change in RNA expression counts for RNASeq.[163]

Epigenetic analyses: a minimum of 20 participants per group of 1st MMR timing (20 in Group M.C. or M.M., and 20 in Group C.M. or C.C.; 40 participant in total) provides 80% power to detect a mean difference of 9% at p<0.05 and a difference of 30% at the genome wide level.[164]

11.3 Statistical criteria of termination of trial

Not applicable.

11.4 Planned Analyses

The full details of the analysis will be provided in the statistical analysis plan, which will be finalised prior to database lock. Briefly, all tests will be 2-tailed and a P<.05 will be considered statistically significant for the primary outcome. Non-parametric tests will be used when variables are not normally distributed.

11.4.1 Datasets to be analysed, analysis populations

The groups will be compared by intention-to-treat analysis. Sensitivity analysis will be done using an as-treated (per-protocol) analysis and will only include participants with available data who received the interventions as planned.

Subgroup analysis are planned (by sex, risk factor, underlying maternal immunity, etc.) and will be detailed in the statistical analysis plan.

11.4.2 Primary Analysis

This will be a classic 2-arm trial with regard to the first intervention (early 1st dose of MMR) and the primary outcome (number of respiratory infections between 6 and 9 months of age). The analysis of the

primary outcome will be performed by means of a regression model (either logistic, if the primary outcome is dichotomised as 0 vs >=1, or negative binomial, if numbers of infection episodes are left uncategorized) and will compare the two group of 1st MMR timing (Group M.C. or M.M. *vs.* Group C.M. or C.C.). The model will be adjusted for sex (male/female), day-care attendance (yes/no), presence of comorbidities (yes/no), older siblings (yes/no), and will be presented as adjusted odd ratios, hazard ratio, or incidence rate ratios (aIRRs) with 95% confidence interval, according to the model chosen.

It will be done in collaboration with the Clinical Research Center at Geneva University Hospitals, once all participants have attended the 9 months visit.

11.4.3 Secondary Analyses

The socio-demographic and clinical characteristics of the participants will be described using standard descriptive statistics (frequencies, medians and interquartile ranges, or means and standard deviation). Other comparative analyses at the 9-months and 2-years endpoints will be performed using chi-square test (or Fisher's test when appropriate) for categorical variables, and paired t-test or Wilcoxon signed-rank test for continuous variables, depending on the distribution of the variable. Time-to-event outcomes will be assessed using Cox proportional hazards with adjustment for baseline risk factors. Factorial analysis will enable to measure the effect on all outcomes of the intervention made to the first and the second dose of MMR, separately or in combination.

The infection outcomes of the 2-year endpoint will be analysed in two stages:
1) first we will examine the infection count distributions in the 4 experimental conditions: standard schedule (Group C.C.), early first dose (Group M.C.), separated 2nd dose (Group C.M.), both intervention (Group M.M.). We will check if the effects of one intervention appear similar across strata of the other intervention. This will be done to identify strong qualitative interactions (e.g., absence of effect in one stratum versus presence in the other), and to guide the regression models.
2) then we will use negative binomial regression models, starting with a model that includes an interaction term:
log(infections) = b0 + b1*early + b2*separate + b3*early*separate where "early" and "separate" designate the 2 components of the intervention, both coded as 0 or 1. We will consider the coefficient b3 to decide on further analysis. If b3 is of small magnitude (in clinical and scientific terms) we will conclude to the absence of a statistical interaction (on a multiplicative risk scale), and we will drop the interaction term from further analysis. If b3 is of substantial size, further analysis will proceed as though the trial was a 4-arm trial, with 3 interventions (Group M.C, Group C.M. and Group M.M.) compared to the standard schedule arm (Group C.C.). The assessment of b3 will be based primarily on scientific and clinical relevance, not on statistical significance.

Model in absence of interaction:
log(infections) = b0 + b1*early + b2*separate (+ any other adjustment variables)

The interpretation of the coefficients is
b0: log of number of infection events under standard schedule (Group C.C.)
b1: log of relative risk of infection under early first dose, averaged over strata of separate 2nd dose
b2: log of relative risk of infection under separate 2nd dose, averaged over strata of early first dose

Model if a substantial interaction is detected:
log(infections) = b0' + b1'*early + b2'*separate + b3'*combined (+ any other adjustment variables)

The variable "combined" will be set to 1 in the combined intervention arm, and 0 in the other arms

The interpretation of the coefficients will be
b0': log of number of infection events under standard schedule (Group C.C.)
b1': log of relative risk of infection under early first dose (Group M.C.), versus standard
b2': log of relative risk of infection under separate 2nd dose (Group C.M.), versus standard
b3': log of relative risk of infection with both components (Group M.M.), versus standard

The other adjustment variables will be defined before closure of the trial, and specified in the statistical analysis plan. They will be variables associated with the risk of infection, such as immune status, having siblings, exposure to day care, etc.

Note on power in presence of interaction:
The power and sample size calculations assumed the absence of an interaction, so that all participants would be used for the estimation of each intervention effect. If an interaction is present

and the second model is used, the effective sample size available for the evaluation of each of the 3 interventions will be halved. This will cause a loss of precision and of power. The confidence intervals for each intervention effect will be widened by a factor 1.4 (square root of 2). We believe that even in such a situation the trial would produce useful knowledge; even if the results were non-significant (statistically) for one intervention or the other, they would facilitate the planning of further trials, and would be available for meta-analysis.

Subgroup analysis are planned (by sex, risk factor, underlying maternal immunity, etc.) and will be detailed in the statistical analysis plan. The null hypothesis is that there is no difference between subgroups, whereas the alternate hypothesis is that the intervention is more beneficial in certain subgroups.

The analyses will be done in collaboration with the Clinical Research Center at Geneva University Hospitals, once all participants have attended the 24 months visit.

11.4.4 Interim analyses

An interim safety analysis will be performed after the first 250 subjects have been randomized and reached the 9-months-old visit; a safety report detailing the results of the analysis will be compiled for the DSMB and the CCER.

11.4.5 Safety analysis

AE and SAE will be reported by group, using standard descriptive statistics (frequencies, medians and interquartile ranges, or means and standard deviation) for each AE of interest (see section 10)

- prevalence
- day of onset
- size (e.g. for redness or induration)
- duration
- severity (using standardised grading scales)
- causality with the intervention
- treatment
- resolution

The analyses will be done thrice, in collaboration with the Clinical Research Center at Geneva University Hospitals, at the same time of the interim analysis, the same time of the primary analysis, the same time of the final analysis.

11.4.6 Deviation(s) from the original statistical plan

A detailed statistical analysis plan will be written as a separate document. It will be further updated as needed. All deviations from the planned analyses will be recorded in the trial master file, and reported and justified at the end of the trial.

11.5 Handling of missing data and drop-outs

Efforts will be made to minimise missing data, with regular check for data completeness during study visit and through contacting the treating paediatrician. Moreover, our team is both flexible and mobile (with experience in performing home visit or phone call when needed), as most data can be collected through self-reporting using telephone and email communication, even if not ideal.

Missing data, including those due to study dropouts, will be marked as such in interim analyses, the clinical study report, and any published manuscripts reporting trial data. Missing data will be taken into consideration using several methods, among them responder analysis (worst-case/best-case scenarios), complete-case analysis (modified ITT) and potentially multiple imputation. These sensitivity analyses will be used if required to validate study findings and will be detailed in the statistical analysis plan.

12. QUALITY ASSURANCE AND CONTROL

Approved SOPs will be used for all procedures, including clinic visit, phone calls, and laboratory procedures. The PI is responsible for proper training of all involved study personnel.

12.1 Data handling and record keeping / archiving

Data will be collected in electronic Case Reports Form (eCRF) in REDCap. All study related documents will be archived electronically on the secured server of the HUG. The full details of the data management will be provided in a data management plan.

12.1.1 Case Report Forms

All questionnaires answered by parents will be directly entered in REDCap through eCRF; these include baseline questionnaires, fortnightly questionnaires and vaccine diaries.

Investigators will also use eCRF during study visits or when calling the treating paediatrician.

REDCap is suitable for direct data entry as it a secure, web based software platform designed to support data capture for research studies, as it provide an intuitive interface for validated data capture and have audit trails for tracking data manipulation and export procedures; access to the database is restricted by unique password and username.

On project-specific documents, participants are only identified by a unique participant number. The participant identification list will be stored by the principal investigator and will be protected from unauthorised or accidental disclosure by password. Only de-identified data will be used. Published data will summarise group outcomes only, individual study participants will not be identified.

12.1.2 Specification of source documents

For this trial source documents are original documents, data, and records that can help for the completion of the eCRF, keeping in mind that most CRF will be completed directly by the parent or the investigator without any source document. In this particular setting, source documents may include, but are not limited to: PICF; clinical notes (medical history, vital signs, physical examination, AE data, and concomitant medication and vaccination details) from investigators, treating paediatrician, or the subject's medical record; laboratory records; paper CRF vaccine diaries (e.g. rescue CRF used when completion of eCRF is impossible); vaccination record; and any correspondence. All source data and paper CRFs will be stored securely.

12.1.3 Record keeping / archiving

All study documents must be archived for a minimum of 10 years after study termination or premature termination of the clinical trial. The Investigator will maintain appropriate medical and research records for this trial, in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The Principal Investigator, co-investigators and clinical research nurses will have access to records. Investigators will permit monitors, ethical and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of monitoring, quality assurance reviews, audits and evaluation of the study safety and progress.

Data will be archived at HUG's Platform for clinical research in paediatrics, gynaecology and obstetrics. At the end of the project, the entire database will be archived in a reusable format. Archives encompass all raw data, meta-data, transformed data, transformation operations, deviations, version history, and audit trails. Redeployment of the entire database is therefore possible whenever needed. The comprehensive archive will remain property of the Sponsor-Investigator.

12.2 Data management

The Principal Investigator will have the responsibility for overseeing the reception, entry, cleaning, querying, analysis and storage of all data that accrue from the study by designated persons. Data management will be performed by the Platform of clinical research in Paediatric, Obstetrics and Gynaecology, in collaboration with the UIC (Unité d'Investigation Clinique), a unit which is part of CRC (Centre de Recherche Clinique / CTU) at the HUG (Hôpitaux Universitaires de Genève) and the Faculty of Medicine UniGE (Geneva University).

For each set of data, quality control and triggers to computerised logic and/or consistency checks will be systematically applied in order to detect errors or omissions. Standard operating procedure (SOP) will be used to standardise data collection, to ensure reliability and consistency. All study personnel will be instructed, trained and supervised on a regular basis to correctly collect and enter data into the electronic database. Subjects' questionnaires (baseline questionnaires, fortnightly questionnaires and vaccine diaries) will be directly filled up by the participants' parents through computer-assisted questionnaires (eCRF). Answers from parent's questionnaires are directly linked to the REDCap database which will minimise errors in data collection. After integration of all corrections in the complete set of data, the database will be locked and saved before being released for statistical analysis.

12.2.1 Data Management System

The UIC is certified ISO 9001/2008, and the unit guarantees best practices in the field of clinical data management.

All data and applications are physically stored in dedicated data centre on HUG premises. The physical hardware consists of enterprise-grade servers, networking, and storage solutions from tier 1 vendors and trustworthy and stable GNU/GPL solutions.

Data are physically stored in a mySQL ver.14 RDBMS (Relational Database Management System) using a dedicated CDMS software (Clinical Database Management System). This CDMS is a central Web-based system consolidating all CRF related data, whether the project is mono or multi centric.

All applications are hosted on certified virtual servers running Red Hat Linux Enterprise in a VMWare ESX environment. There are several dedicated servers for each system, ensuring separation between the testing / pre-production environment and the production environment. System updates are applied only after validation in the pre-production environment.

HUG infrastructure is under the responsibility of DSI (Direction des Systèmes d'Information at HUG). All exploitation, monitoring and backups operations are performed by DSI, in accordance with UIC policies.

All systems and applications are continuously monitored. Appropriate measures are automatically taken whenever an alert is issued.

Backups operations are performed by the DSI service at HUG, in accordance with UIC policies. Frequent backups are performed using the best enterprise backup solutions at HUG and are physically stored in a fire-proof safe. Backup strategy compromises an optimised hourly, daily, monthly, and yearly retention plan:

- Server backups (DSI): 1x differential per day (14 days retention, with mirroring), 1x full every month (retention 12 months), annual (preserved infinitely)
- Database backups (UIC): hourly, (retained 24 hours), daily (retained 2 months), monthly (preserved infinitely)

12.2.2 Data security, access and back-up

Physical access to the data centre is logged and limited to authorised personnel using badge authentication. On a regular basis, vulnerability testing is performed to reduce potential exposure. Remote access to servers is limited to authorised personnel. Connections to servers are encrypted using SSH. System logs are stored in a dedicated centralised system for audit purposes. The internal HUG network is protected by multiple firewalls, proxy, reverse-proxy and anti-virus solutions. Web servers operate under SSL (HTTPS) certifications, ensuring Web connections are encrypted and secure.

All study information collected by study personnel will be entered into an electronic database (REDCap). Access to the database will be restricted by unique password and username and back-up copies made and stored separately. Only people part of the investigation team, the sponsor team, the affiliated reviewers or auditors, as well as inspection authorities are given access to data.

Personal accounts are granted individually for each person. Identification is made by a personnel ID and a password. Failure to provide the correct password after a limited number of attempts automatically deactivates the faulty account (protection against non-authorised attacks).

Only institutional e-mail addresses will be accepted for any communication of sensitive data regarding account creation and management. Pen-Tests (simulation of malware attacks) are regularly performed, and measures taken whenever necessary.

12.2.3 Analysis and archiving

Data will be collected directly by the subjects' parents (through REDCap questionnaire) or via study nurses and investigators. A dedicated CDMS project will be created for the eCRF (using RedCAP®). This eCRF will reflect the visit plan and all subject-related data to be collected, as described by the study protocol. CRF data are of different data-types, mainly numeric values, encoded values, text descriptions, etc. Coding, catalogues and multi-choice values will be preferably used as opposed to free-text values. Automatic score calculations will be performed whenever appropriate (e.g. POEM and SCORAD severity scores). The eCRF will ensure high data quality control by applying logical and managerial controls over data capture in order to ensure consistency, completeness and coherence of data.

A more exhaustive and detailed Data Management Plan will be generated and applied during the whole data management process (until data are archived), after the sponsor-investigator has obtained the grant and contracted UIC.

The investigation team will enter the data interactively in the CDMS by means of its interactive graphical

interface. Data will be edited and viewed by the investigation personnel based on the role of each participant.

All modifications to the eCRF during the study conduct will be tracked and dealt with consequently (version numbering). Data will be electronically and systematically revalidated after each major change, and corrections/completeness applied whenever necessary.

All system log-ins, interventions, modifications and form status changes will be thoroughly recorded in an Audit Trail log system. Deviations to the protocol will be also tracked and resolution status indicated.

Data and metadata will be exported anonymously in due time in CSV – where required – read into Stata to produce Stata dataset (.dta) files along with associated data dictionary information. Data checking and cleaning will be performed using Stata, and cleaned datasets for use in analyses will be stored in Stata format (Stata version 16). Each step of this process will be monitored through the implementation of individual passwords and regular backups in order to maintain appropriate database access and to guarantee database integrity.

12.2.4 Electronic and central data validation

Validation/review will be performed in parallel by appropriate people designated by the sponsor-investigator (monitors, data mangers, etc.). Up-to-date graphical and text reports, as well as descriptive statistics, will be made available to monitor data in real time. An elaborated electronic process to comment, query and claim correction actions on data via reviewing will reinforce data quality throughout the study conduct and termination. Whenever appropriate, use of controlled vocabulary and common international catalogues (ATC/DCI, MedDRA, NCBI Taxonomy, etc.) will be used.

12.3 Monitoring

The monitoring strategy has been defined as low-risk, according to the SCTO guidelines and the Risk-Based Monitoring (RBM) Score Calculator published on https://www.sctoplatforms.ch/en/scto-platforms/monitoring-17.html

A site-initiation visit will be conducted before inclusion of the first participant.

During routine monitoring visits, the verifications will concern:

• Existence and completion of informed-consent forms (100% of the participants)

• Source-data verification for key data (IMP) for 20% of the participants, including first participant

• Trial master file

A close-out visit will be conducted at the end on the study.

12.4 Audits and Inspections

Study documentation, source data/documents and staff will be accessible to auditors/inspectors from the CCER, or any authorised authority as needed; all questions will be answered during inspections. All involved parties will keep participant data strictly confidential.

12.5 Confidentiality, Data Protection

See section 12.2.2 for more detail on data protection. Direct access to source documents will be permitted for the purposes of monitoring (section 12.3), audits and inspections (section 12.4) (ICHE6, 6.10). Sponsor-investigator, investigators, and the data management team will have access to protocol, dataset, statistical code, etc. during and after the study (publication, dissemination).

12.6 Storage of biological material and related health data

Coded samples will be stored independent from the study only with the participant's express consent. All other unused samples will be destroyed at the end of the study.

13. PUBLICATION AND DISSEMINATION POLICY

The trial protocol is registered on ClinicalTrials.gov (NCT05758532) and will be published in a journal once recruitment has started. Dissemination of the findings is planned, regardless of the results, through the WHO, in peer-reviewed journals and at scientific conferences. There will not be any publication restriction. Rule for authorship will follow the ICMJE recommendation.

Sex-subgroup analyses are planned and will be published regardless of the results.

Once the database is cleaned and locked, it will be deposited in a data sharing repository archiving system. Access to the data will follow the rules of the repository system.

14. FUNDING AND SUPPORT

14.1 Funding

The salary of the PI is first being paid by the University of Geneva, through a position of *Cheffe de Clinique scientifique*, granted by the HUG for the set-up of the proposed project, and then by an *Ambizione* grant from the Swiss National Science Foundation. The study will be funded by the *Ambizione* grant, the grant *Projet de Recherche et Developpement* from the HUG and the *STARTER-MD* grant from the Foundation Louis-Jeantet and the Private Foundation of the HUG. Funders have no role in the design of the study, the analysis of its data, or the decision to publish its results. A copy of the agreements are filed in the TMF.

The study can be simplified in case of insufficient funding. Examples of simplifications include:

- Stopping follow-up after the 9-months visit: the primary outcome analysis would still be possible as it focuses on the period between 6 and 9 months of age.
- Delegation of visits and vaccine administration to treating paediatricians, especially those with no blood sample.
- Simplifying the laboratory experiments, with a reduced number of participants (the minimal required by the sample size calculation).
- Starting with a pilot study (e.g. 60 to 100 participants for clinical outcomes, 20 participants for laboratory outcome) to assess feasibility and verify the assumptions made in the sample size calculation.

14.2 Other Support

Should this be needed, some of the laboratory expenses will be covered by research funds from the Center of Vaccinology, Department of Pathology and Immunology, Faculty of Medicine of Geneva (Prof Arnaud Didierlaurent), and the Infectious Diseases Group at the Murdoch Children's Research Institute, Melbourne, Australia (Prof Nigel Curtis).

15. INSURANCE

Insurance for this investigator-initiated trial will be provided by the Sponsor (HUG). A copy of the certificate is filed in the TMF.

16. PATIENT AND PUBLIC INVOLVEMENT

The platform "PP + 3P" (Patients partenaires + Proches, Professionnels et Public) of the University Hospital of Geneva has been consulted during the design of this proposal and will be actively involved throughout the study.

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18. APPENDICES

PRIORIX subst sèche c solv (GlaxoSmithKline AG) Compendium suisse des médicaments. Information professionnelle. Mise à jour mai 2019

Priorix powder and solvent for solution for injection in a pre-filled syringe. Summary of Product Characteristics. GlaxoSmithKline UK. Updated 20-May-2020.

Case Report Form, draf v1.0 of 25.03.2022

Participant Information and informed consent form, v1.2 of 08.03.2023

Rougeole, oreillons, rubéole, fact sheet of the Federal Office of Public health (distributed to participants)

Pamphlets template, v1.0 of 13.05.2022