

# Investigator Studies Program (MISP) Protocol Template

## Requirements for Submitting a Full Proposal

### Section #1 - MISP Protocol Identification

<b>Study Title:</b>	<b>Risk factors for recurrent wheezing and asthma in association with age and severity of primary RSV infection: A population-based cohort study.</b>
<b>Request Date:</b>	
<b>Institution Name</b>	<b>FISABIO</b>
<b>Investigator Contact Information:</b> - Full address - Phone No. - Fax No. - e-mail address	Principal investigator    Mónica López-Lacort <sup>1</sup> , Mathematician, MSc
	Co-investigators        Dr. Cintia Muñoz-Quiles <sup>1</sup> , Biologist, PhD
	Dr. Alejandro Orrico-Sánchez <sup>1</sup> , PharmD, PhD
	Dr. Javier Díez-Domingo <sup>1</sup> , Pediatrician, MD, PhD
	<p><sup>1</sup>Vaccine Research Unit. FISABIO-Public Health</p> <p><sup>2</sup> Health Inequalities Unit. FISABIO-Public Health</p> <p>Avda Cataluña, 21 46020 Valencia, Spain Telephone: +34 961 925 968</p> <p>lopez_monlac@gva.es</p>

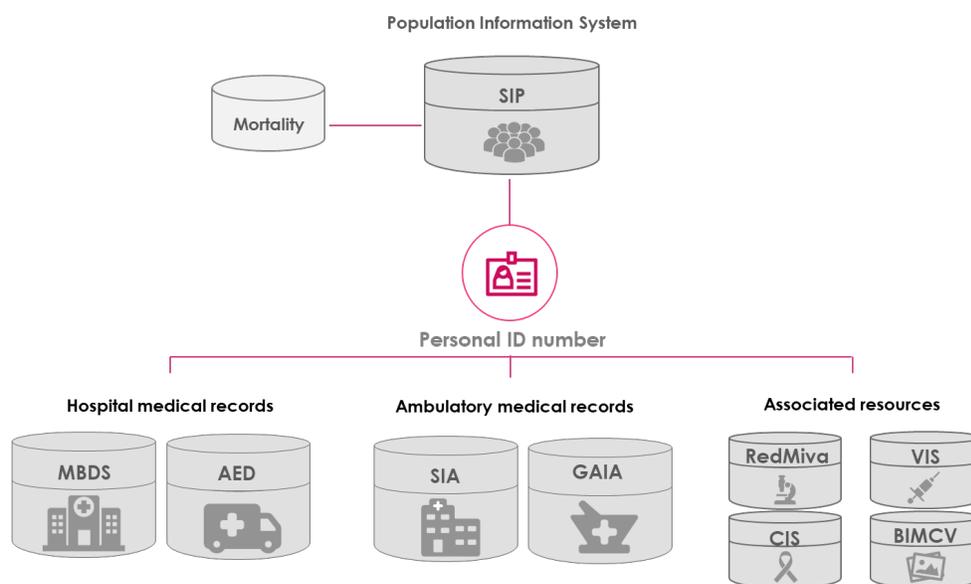
## Section #2- Core Protocol

<b>2.1 Objectives &amp; Hypotheses</b>	<p>2.1 List the objectives.</p> <p><b>Objectives</b></p> <p><b>Primary objective</b></p> <p>Assess the association between severe RSV-bronchiolitis and outpatient bronchiolitis during the first two years of life and their subsequent risk of recurrent wheezing and asthma in children older than two years.</p> <p><b>Secondary objectives</b></p> <p>Assess the risk of asthma and recurrent wheezing in children older than two years with an episode of severe RSV-bronchiolitis during their first two years of life respect to infants with an episode of severe non-RSV bronchiolitis and infants with no episode of bronchiolitis.</p> <p>Assess the association between the age at primary RSV-bronchiolitis and the subsequent risk of asthma and recurrent wheezing.</p> <p>Estimate the incidence rates of asthma among 6 year old subjects and older with RSV-bronchiolitis, non-RSV bronchiolitis and overall during their first two years of life.</p> <p><b>Exploratory objectives</b></p> <p>Describe the wheezing/asthma diagnosis before the bronchiolitis in children &lt; 2 years.</p> <p>2.1.1 List the clinical hypotheses.</p> <p>There are some evidences supporting that severe RSV infection in early childhood is associated with impaired lung function which persists beyond childhood and increases the risk of recurrent wheezing and asthma at a later age of life. Vaccination has the potential to be the most effective approach to reduce the global burden of diseases associated to RSV. The potential benefits of RSV interventions to reduce recurrent wheeze and asthma, if demonstrated, would significantly increase the cost-effectiveness of these RSV preventive interventions.</p> <p>The Valencia Region databases network allows collecting information of all primary care contacts, hospitalizations and the results of all microbiological tests performed in the Regional Health System (RHS).Data from these databases can be linked through a unique personal identification number. Using these databases we can differentiate between episodes of RSV-negative bronchiolitis and RSV-positive bronchiolitis in children less than 2 years old and can assess their risk of suffering future recurrent wheezing and asthma. Moreover, we can distinguish between severe RSV episodes (hospitalized) and non-severe (without hospitalization).</p> <p>Our initial hypothesis is that risk of asthma and recurrent wheezing might be higher among children suffering RSV-positive bronchiolitis (requiring hospitalization) than in children whose RSV episodes were managed at primary care centers, without a hospitalization required.</p>
--	--

<p><b>2.2 Background &amp; Rationale, Significance of Selected Topic &amp; Preliminary Data</b></p>	<p>Respiratory syncytial virus (RSV) is the most important cause of acute lower respiratory tract infection (ALRI) in infants and the major cause of hospitalizations, accounting for 66,000 – 199,000 deaths worldwide per year in children under 5 years of age (Mazur et al., 2015, Shi et al., 2017). In 2015 it was estimated that RSV was responsible of about 3 between 70% and 90% of children less than 1 and 2 years of age, respectively, have been infected with RSV ((CDC), 2016, Munoz-Quiles et al., 2016a, Regnier, 2013).</p> <p>We published a previous retrospective population-based study about the epidemiology of global bronchiolitis (following the McConnochie definition (McConnochie, 1983)) and RSV bronchiolitis (with RSV positive test) using primary care and hospitalization databases in children under 2 years of age (Munoz-Quiles et al., 2016b). The results showed that the incidence rate of bronchiolitis was estimated in 16.4 cases/100 children &lt;2 years per year, which means approximately 13,000-15,000 cases per year in the Valencia Region of Spain. Of them, 13% had 26% longer hospitalizations for RSV-bronchiolitis than the non-RSV diagnosed (the average length of stay (LOS) was 5 days) (Munoz-Quiles et al., 2016b). Moreover, infants between 2 and 10 weeks are considered a risk group when talking about severe bronchiolitis. The RSV-related costs in this Region could reach more than €10Mill considering indirect costs (Acedo et al., 2010).</p> <p>Epidemiological studies on bronchiolitis in Spain are scarce, and all of them, as the majority of European studies, are focused on hospitalizations. According to our results, 87% of the bronchiolitis cases on children &lt;2 years are managed as outpatients, what makes recommendable to include primary care visits in the evaluation of the bronchiolitis epidemiology and the burden of disease (Bueno Campana et al., 2008, Koehoorn et al., 2008, Ochoa Sangrador et al., 2013).</p> <p>Despite decades of RSV vaccine research, there is still no approved vaccine. Consequently, passive immunity with monoclonal antibodies is the only regulatory-approved option but, because of its high price, this antibody is only used in the highest-risk groups of individuals. Vaccination has the potential to be the most effective approach to reduce the global burden of diseases associated to RSV. Around 60 RSV vaccine candidates are under development, 16 of which are in clinical trials (Mazur et al., 2015, Murray et al., 2014). Pregnant women (in order to protect newborn babies through passive immunity) and infants are the two most promising candidates for RSV vaccines (Drysdale et al., 2016, Neuzil, 2016, RSV Vaccine Snapshot, 2016).</p> <p>There is growing evidence demonstrating that severe RSV infection in early childhood is associated with impaired lung function which persists beyond childhood and increases the risk of recurrent wheezing and asthma at a later age of life (Homaira et al., 2016, Sigurs et al., 2000, Sigurs et al., 2005). Asthma is the most common chronic reactive airway disease of childhood and is related with significant healthcare burden, especially emergency department and primary care visits (Homaira et al., 2018). The Strategic Advisory Group of Experts for Immunization (SAGE) suggested mobilizing resources to follow up subjects to evaluate the long term effects of RSV interventions in the context of uncertainties on whether RSV infection causes recurrent wheeze, which, if demonstrated, would substantially increase the cost-effectiveness of RSV preventive interventions (Giersing et al., 2017).</p> <p>In this study we propose to analyze, using “Real World Data”, the association between RSV-bronchiolitis during the first two years of life and the subsequent risk of recurrent wheezing and asthma in children older than two years. The association between the age at primary RSV-bronchiolitis and its severity (requiring or not hospitalization) will be also evaluated. The assessment of this potential association could inform future maternal and</p>
---	---

	infant RSV vaccination strategies.
<b>2.3 Study Design</b>	<p><b>Methods</b></p> <p><b>Study design</b></p> <p>A population-based, retrospective cohort study will be performed using the Valencia region's health care databases network.</p> <p><b>Study population</b></p> <p>The Valencia Region of Spain has a population of around 5 million inhabitants. The cohort will include all children who were born in Valencia Region between 2009 and 2015 and registered in the Regional Health System (RHS) (over 98%), with a follow-up ending on the date of data extraction (study period: 2009-date of data extraction).</p> <p><b>Inclusion criteria:</b></p> <ul style="list-style-type: none"> <li>- Subjects born in Valencia Region and covered by RHS and residing in the Valencia Region during study period.</li> </ul> <p><b>Exclusion criteria:</b></p> <ul style="list-style-type: none"> <li>- Subjects with less than 6 months of registration to the RHS.</li> <li>- Subjects with incorrect codification.</li> </ul> <p><b>Follow-up period:</b></p> <p>Date of inclusion in the study will be defined as date of birth and the follow-up will continue until the end of the study period (date of data extraction), when they leave Valencia (data of deletion from SIP) or in case of death, whichever came first.</p>
<b>2.4 Study Flowchart</b>	<p><b>Study setting and data sources</b></p> <p>The regional health system is divided into 24 health departments. Each of them includes at least 1 hospital (28 hospitals in total), 1 specialty center, and a variable number of ambulatory care centers. All primary care visits and hospitalizations are recorded in clinical databases.</p> <p>The databases of the Valencia Region have proven to be very reliable in the surveillance of vaccine preventable diseases (Morant-Talamante et al., 2013, Munoz-Quiles et al., 2016a, Munoz-Quiles et al., 2016b), as well as in the assessment of vaccine safety (Pérez-Vilar et al., 2015a) and effectiveness (Pérez-Vilar et al., 2015b). The positive predictive value (PPV) of RSV-bronchiolitis hospitalizations was 94% (López-Lacort et al., ESPID 2018) and over 90% for other diseases (Morant-Talamante et al., 2013, Pérez-Vilar et al., 2015a).</p>

Figure: Some databases of the Valencia Region



Data sources for the study will be:

Population-based administrative database

The regional population-based administrative database, SIP (Personal Information System), collects and updates identification data, geographic location, assignment of health services, and access to public health services for both residents of the Valencia Community and non-residents with access to public health services. It includes APSIG characteristic which is an identification code defined for each person at any time including: inhabitant's registration status, nationality (Spanish or not), sex, year of birth, health department assigned, health care insurance, residence status, migrations, work activity, geopolitical group, and social exclusion. Since 2005, SIP can be linked with the hospital discharge database.

Primary Care Database

Abucasis SIA-GAIA (Ambulatory Information System - Care provision management) is a primary care database used across the entire Valencian healthcare system. It was set up in 2006 and the percentage of the population included increased from 73.1% in 2007 to 88.8% in 2008 and to 95.7% in 2009. This database contains primary care diagnoses (physician coded using the International Classification of Diseases 9th Revision, Clinical Modification (ICD-9-CM)) and all drug prescriptions (using Anatomical Therapeutic Chemical (ATC) Classification System). In addition, the recorded text about each episode and patient by the paediatrician, other physician, and by the nurse responsible is included. The database is also used at Specialty care Centres and is considered reliable since 2007. GAIA includes MDIS (hospital dispensing module), which allows the registration of those prescriptions administered at the Primary Care or Specialty Centres.

Hospital Discharge Database

The Spanish hospital discharge database (CMBD, its acronym in English MBDS (minimum basic data set)), collects diagnosis and procedures as an assessment of medical activity. The coding system used is ICD-9-MC until 2016 and ICD-10-MC afterwards. The main discharge diagnosis is coded in first position, and diagnosis relevance decreases as the position number increases. Using MBDS is compulsory for all public hospitals, and over 95% of all discharges are included. According to the Spanish Ministry of Health, data are considered reliable since 2002.

RedMIVA

Database consisting of all the results (positive or negative) given by all the Microbiology laboratories of the Valencian Public System network. All data is transferred from the laboratories to the RedMIVA database on a daily basis. Data are considered reliable since 2008.

Vaccine Registry

All patient data can be linked to a vaccine registry (Registro de Vacunas Nominal, RVN), which is part of the population-based online registry (Sistema de Información Vacunal, SIV) put in place in 2000 that captures the immunization history of each individual. Data are registered from public and some private health centres. Available data includes vaccine by type, manufacturer, batch number, number of dose, place and administration date, and, if applicable, risk group. Data are considered reliable since 2005. Data from these databases can be linked through a unique personal identification number.

**2.5 Study Procedures**

**Case definition**

Our outcomes will be:

(a) Recurrent wheezing/asthma cases, will be defined as children  $\geq 2$  years with two or more wheezing/asthma ICD codes at least one month elapsed (table 1, ICD-9-CM until 2016 and ICD-10-CM afterwards) in hospital or primary care.

(b) Active asthma cases persisting at 6 years, will be defined as children  $\geq 5$  years with both at least one diagnostic code attributed to asthma (table 1) and the receipt of at least one anti-asthma drug prescription for obstructive airway diseases (table 2).

**Table 1**

	ICD-9	ICD-10	
<b>Asthma</b>	493	J45	<b>Asthma</b>
<b>Extrinsic asthma</b>	493.0x	J45.0	<b>Allergic asthma</b>
<b>Intrinsic asthma</b>	493.1x	J45.1	<b>Nonallergic asthma</b>
<b>Chronic obstructive asthma</b>	493.2x	J45.8	<b>Mixed asthma</b>
<b>Others forms of asthma</b>	493.8	J45.9	<b>Asthma unspecified</b>
<b>Exercise induced bronchospasm</b>	493.81	J46	<b>Status asthmaticus</b>
<b>Cough variant asthma</b>	493.82		
<b>Asthma unspecified</b>	493.9x		
<b>Wheeze</b>	786.07	R0.62	<b>Wheeze</b>
<b>Acute bronchospasm</b>	519.11	J98.01	<b>Acute bronchospasm</b>

*The following IDC-codes will be considered from the second encounter, the first appearance is related with a bronchiolitis case*

<b>Acute bronchitis and bronchiolitis</b>	466	J21	<b>Acute bronchiolitis</b>
<b>Acute bronchitis</b>	466.0	J20	<b>Acute bronchitis</b>
<b>Acute bronchiolitis</b>	466.1		
<b>Acute RSV bronchiolitis</b>	466.11	J21.0	<b>Acute RSV bronchiolitis</b>
<b>Acute bronchiolitis caused by other infectious organism</b>	466.19	J21.8, J21.9	<b>Unspecified acute bronchiolitis</b>

*ICD-codes related wheezing and asthma*

Table 2 (ATC codes)

<b>ATC-code</b>
<b>Oral corticoids</b>
H02AB02 dexamethasone
H02AB04 methylprednisolone
H02AB06 prednisolone
H02AB07 prednisone
<b>Ipratropium bromide</b>
R01AX03 ipratropium bromide
<b>R03BA inhaled corticosteroid</b>
R03BA01 beclometasone
R03BA02 budesonide
R03BA05 fluticasone
R03BA07 mometasone
<b>R03AC short- and long acting Beta2 agonist</b>
R03AC02 salbutamol
R03CC02 salbutamo
R03AC03 terbutaline
R03CC03 terbutalin
R03AC04 fenoterol
R03AC12 salmeterol
R03CC12 bambuterol
R03AC13 formoterol
<b>R03DC leukotrien receptor antagonist</b>
R03DC03 montelukast
<b>R03AK kombinationspræparater</b>
R03AK06 fluticasone, salmeterol
R03AK07 budesonide, formoterol
R03AK08 formoterol and beclometasone
R03AK13 salbutamol, beclometasone

*ATC-codes related anti-asthmatic drugs*

[https://www.whocc.no/atc\\_ddd\\_index/](https://www.whocc.no/atc_ddd_index/)

### Exposure

The exposure variable will be children under 2 years of age with an ICD-code related bronchiolitis in hospital (MBDS) or primary care (SIA).

The following categories will be used:

- (a) *Severe RSV-bronchiolitis*: The first hospitalization with an ICD-code related RSV-bronchiolitis in any diagnostic position or an ICD-code associated to bronchiolitis in any diagnostic position with a laboratory confirmed RSV result from RedMIVA (table 2).

- (b) Severe non RSV-bronchiolitis: The first hospitalization with an ICD-code related bronchiolitis (table 2 excluding 466.11/J21.0) in any diagnostic position with laboratory negative RSV result from RedMIVA.
- (c) Severe miscellaneous bronchiolitis: The first hospitalization with an ICD-code related bronchiolitis (table 2), excluding 466.11/J21.0, without laboratory test associated.
- (d) Outpatient bronchiolitis: The first diagnostic ICD-code associated with bronchiolitis (table 2) in primary care.
- (e) No-bronchiolitis case: A child from the cohort that had no registers of bronchiolitis-related diagnostic codes.

When a primary care visit is followed by a hospitalization in the following 30 days, it will be assumed to be the same bronchiolitis case and it will be considered a hospitalization case. A laboratory test will be considered associated to the admission if the sample is taken 5 days before the code registration to the discharge date (or 10 days after code registration).

**Table 3**

	ICD-9	ICD-10	
<b>Acute bronchitis and bronchiolitis</b>	466	J21	<b>Acute bronchiolitis</b>
<b>Acute bronchitis</b>	466.0	J20	<b>Acute bronchitis</b>
<b>Acute bronchiolitis</b>	466.1		
<b>Acute RSV bronchiolitis</b>	466.11	J21.0	<b>Acute RSV bronchiolitis</b>
<b>Acute bronchiolitis caused by other infectious organism</b>	466.19	J21.8, J21.9	<b>Unspecified acute bronchiolitis</b>

*ICD-codes related bronchiolitis*

**Explanatory variables**

Variables that are relevant to the diseases will be considered for the analysis: Birth date, age, sex, urban/rural residence, social exclusion risk, calendar year, month of birth, health department, zip code, municipality, health care district, having received any vaccination by a private provider, anti-pneumococcal vaccination, antibiotic consumption in the first year of life (ATC-code: J01). High-risk preterm babies (defined as infants who require hospitalization for prematurity) will be identified through the ICD-codes 765.XX or P07.XX (shortened gestation and low-birth-weight-related disorders) in MBDS.

**Data collection**

Data privacy will be protected by using anonymised data. The following variables will be requested to the different databases for the period from 1 January 2009 until the date of data extraction for subjects born from 1 January 2009 to 31 December 2015:

Data to be extracted from SIP:

- Identification block including SIP number, sex, date of birth and other geographical of birth, place and date of registration (excluding identifying data as surnames, ID card number, phone numbers, etc.).
- Regular location block that includes complete address, health map information as health department and census information among others (excluding exact data that allows the exact residence location of the subjects).
- Cessation block including cessation cause and description, cessation date and date of death (when applicable).

Data to be extracted from MBDS:

SIP number  
Health department  
Date of hospital admission  
Date of hospital discharge  
Diagnoses at discharge (main and secondary diagnoses)  
Procedures during the hospitalization  
Discharge destination (destination on discharge)

For any diagnosis code ICD-9-CM or ICD-10-CM related to bronchiolitis, wheezing Asthma (see table 1 and table 3), and prematurity (ICD-9-CM 765.XX , ICD-10-CM P07.XX).

Data to be requested from SIA-GAIA:

SIP number

*Morbidity data base*

Date of diagnosis activation (diagnosis of interest)  
Date of diagnosis deactivation (diagnosis of interest)  
Diagnosis description (diagnosis of interest)  
ICD-code (diagnosis of interest)

*Outpatient visits data base*

Date of the visit  
Diagnosis description  
ICD-code

*Specialist visits data base*

Date of the visit  
Diagnosis description  
ICD-code  
Professional attendance (GP/Specialist)  
Specialties Service

*Prescription*

Medical prescription number  
Dosage  
Number of units prescribed  
Treatment number  
Active ingredient code  
Active ingredient description  
ATC code

	<p>ATC description</p> <p>Pharmaceutical form</p> <p>Date of prescription (dd/mm/yyyy)</p> <p>ICD-code or prodedure</p> <p><i>Treatment</i></p> <p>Starting date of the treatment</p> <p>Closing date of the treatment</p> <p>Active ingredient code</p> <p>Active ingredient description</p> <p>ATC code</p> <p>ATC description</p> <p>ICD-code or prodedure</p> <p><i>Dispensation</i></p> <p>Number of units dispensed</p> <p>Active ingredient code</p> <p>Active ingredient description</p> <p>ATC code</p> <p>ATC description</p> <p>ICD-code or prodedure</p> <p>Date of dispensation</p> <p><i>MDIS</i></p> <p>Medication associated with wheezing /asthma ICD-codes</p> <p>MDIS- Active principles administered (ATC code)</p> <p>MDIS- Active principles administered</p> <p>MDIS- Active principles administered (description)</p> <p>MDIS- Date of administration</p> <p>MDIS- Number of units administered</p> <p>SIA: For any diagnosis code ICD-9-CM or ICD-10-CM related to bronchiolitis, wheezing and Asthma (see table 1 and 3).</p> <p>GAIA-MDIS: For any ATC-codes related anti-asthmatic drugs (table 2) and ATC-codes associated with antibiotic treatments (J01, only for children younger than 1 year (inclusive)).</p> <p><u>Data to be extracted from RedMIVA:</u></p> <p>All results for RSV detection in children younger than 2 years during the study period.</p>
--	--

	<p>SIP number</p> <p>Date of laboratory test (dd/mm/yyyy)</p> <p>Microorganism/antigen</p> <p>Date of test result (dd/mm/yyyy)</p> <p>Determination</p> <p>Laboratory test</p> <p>Result</p> <p><u>Data to be extracted from SIV:</u></p> <p>SIP number</p> <p>Administration date (anti-pneumococcal vaccination)</p> <p>Vaccination in a private or no private center (at least one dose of any vaccine administered in a private center)</p> <p><b>Limitations</b></p> <p>Using routinely collected data may introduce some bias because the data was not initially created for research purposes. Some cases of treated bronchiolitis or asthma may not have been codified, and therefore we may underestimate their incidence. However, no differences in time were anticipated in the previous study, and differences in local practices will be controlled by the health department.</p> <p>Possible biases related to changes in diagnostic procedures throughout the study period. The reliability of the bronchiolitis cases due to RSV recorded in the MBDS was estimated by calculating the PPV using the Valencia Microbiological Network (RedMiVa) as the reference standard and the PPV of bronchiolitis due to RSV diagnoses was 94% (López-Lacort, et al., ESPID, 2018). Moreover, the PPV of our databases' diagnostic codes was high for other diseases such as herpes zoster and intussusception.</p> <p>87% of cases of bronchiolitis in children under 2 years are managed as outpatients (Munoz-Quiles et al., 2016a, Munoz-Quiles et al., 2016b), where confirmation test are not usually required since the results do not modify the management of the disease. However, many of these cases have an associated laboratory RSV test requested in emergency department (without hospitalization). 80% of the bronchiolitis hospitalizations had an associated laboratory test and, as mentioned above, the PPV of bronchiolitis due to RSV diagnoses was 94% (López-Lacort, et al., ESPID, 2018).</p> <p><b>Regulatory and ethics considerations</b></p> <p>The study will be conducted in accordance with all applicable regulatory requirements, including all applicable subject privacy requirements, the Declaration of Helsinki, and the International Ethical Guidelines for Epidemiological Investigations.</p> <p>The study will be submitted for approval to the institutional review board. The Spanish Medicine Agency will be informed of the performance of the study (AEMPS) so it can be classified (as 'Estudio post-autorización otros diseños, EPA-OD') according to the existing legislation (Orden SAS/3470/2009). The Pharmacy Agency of the Valencia Government will also be informed about the study according to the existing legislation [Resolución de 16 de junio de 2009, de la Conselleria de Sanitat].</p>
--	--

<p><b>2.6 Study Duration</b></p>	<p>Two years</p> <table border="1"> <thead> <tr> <th colspan="2">TIMELINES</th> <th colspan="24">MONTHS</th> </tr> <tr> <th>Activities</th> <th>-1</th> <th>1</th> <th>2</th> <th>3</th> <th>4</th> <th>5</th> <th>6</th> <th>7</th> <th>8</th> <th>9</th> <th>10</th> <th>11</th> <th>12</th> <th>13</th> <th>14</th> <th>15</th> <th>16</th> <th>17</th> <th>18</th> <th>19</th> <th>20</th> <th>21</th> <th>22</th> <th>23</th> <th>24</th> </tr> </thead> <tbody> <tr> <td>Study design and protocol</td> <td>█</td> <td>█</td> <td></td> </tr> <tr> <td>IBR and AEMPS submission</td> <td></td> <td>█</td> <td>█</td> <td></td> </tr> <tr> <td>Data collection SIA, GAIA, CMBD, RedMiva</td> <td></td> <td></td> <td>█</td> <td></td> </tr> <tr> <td>Data management and analysis</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td></td> </tr> <tr> <td>Primary Objective</td> <td></td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>Secondary Objectives</td> <td></td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>Provisional Report Review and discusion</td> <td></td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>Secondary Objectives</td> <td></td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>Results interpretation and discusion</td> <td></td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td>█</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td>Final Report</td> <td></td> <td>█</td> <td></td> <td></td> </tr> <tr> <td>Manuscript Preparation and Submission</td> <td></td> <td>█</td> <td>█</td> </tr> </tbody> </table>	TIMELINES		MONTHS																								Activities	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Study design and protocol	█	█																									IBR and AEMPS submission		█	█																								Data collection SIA, GAIA, CMBD, RedMiva			█	█	█	█	█	█	█	█	█	█	█	█	█												Data management and analysis										█	█	█	█	█	█	█											Primary Objective													█	█	█	█	█										Secondary Objectives													█	█	█	█	█										Provisional Report Review and discusion													█	█	█	█	█	█									Secondary Objectives													█	█	█	█	█	█									Results interpretation and discusion														█	█	█	█	█	█	█	█						Final Report																								█			Manuscript Preparation and Submission																									█	█
TIMELINES		MONTHS																																																																																																																																																																																																																																																																																																																																																												
Activities	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24																																																																																																																																																																																																																																																																																																																																					
Study design and protocol	█	█																																																																																																																																																																																																																																																																																																																																																												
IBR and AEMPS submission		█	█																																																																																																																																																																																																																																																																																																																																																											
Data collection SIA, GAIA, CMBD, RedMiva			█	█	█	█	█	█	█	█	█	█	█	█	█																																																																																																																																																																																																																																																																																																																																															
Data management and analysis										█	█	█	█	█	█	█																																																																																																																																																																																																																																																																																																																																														
Primary Objective													█	█	█	█	█																																																																																																																																																																																																																																																																																																																																													
Secondary Objectives													█	█	█	█	█																																																																																																																																																																																																																																																																																																																																													
Provisional Report Review and discusion													█	█	█	█	█	█																																																																																																																																																																																																																																																																																																																																												
Secondary Objectives													█	█	█	█	█	█																																																																																																																																																																																																																																																																																																																																												
Results interpretation and discusion														█	█	█	█	█	█	█	█																																																																																																																																																																																																																																																																																																																																									
Final Report																								█																																																																																																																																																																																																																																																																																																																																						
Manuscript Preparation and Submission																									█	█																																																																																																																																																																																																																																																																																																																																				
<p><b>2.7 Statistical Analysis and Sample Size Justification</b></p>	<p><b>Estimation of sample size</b></p> <p>In our previous study a retrospective cohort of all children born between 2009 and 2012 was followed from birth to 2 years of age using the same population and health databases as the ones used for the present proposal (Munoz-Quiles et al., 2016b). The cohort consisted of 198,223 infants. Considering that annual birth cohort in the Valencia Region is about 48.000 infants we might expect that approximately 340.000 infants will be followed.</p> <p><b>Statistical analysis</b></p> <p>All analyses will be specified in more detail in a statistical analysis plan (SAP).</p> <p>Demographic characteristics will be summarized by descriptive statistics [proportions, frequencies, ranges, standard deviations and mean/median (depending on variable type)].</p> <p>A logistic regression model will be developed to analyze the risk of asthma and recurrent wheezing in children having suffered a bronchiolitis respect to children without bronchiolitis during their first two years of life. In the model adjustment we will consider the explanatory variables above-mentioned and their interactions to avoid the confusion.</p>																																																																																																																																																																																																																																																																																																																																																													

	<p>To estimate the effect of the age at primary bronchiolitis in the subsequent risk of asthma and recurrent wheezing only subjects with a bronchiolitis case will be selected. A logistic regression will be developed too considering age at bronchiolitis and the above-mentioned explanatory variables as covariates.</p> <p>Incidence rates of asthma among subjects 6 year old and older will be estimated as the number of incident events for each case definition divided by the total of population (and children-year). The incidence rates will be also calculated by bronchiolitis type. Results will be stratified by gender and age group. 95% confidence intervals will be calculated using the exact Poisson method for the different rates.</p> <p>Analyses of the anonymised data will be carried out using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria) and WinBUGS (BUGS "Bayesian Inference Using Gibbs Sampling").</p>																				
<b>2.8 Specific Drug Supply Requirements</b>	NA																				
<b>2.9 Adverse Experience Reporting</b>	<p>The study agreement outlines the requirement for adverse experience reporting. For clinical protocols, specific adverse experience reporting requirements must be identified in the protocol if the Model Study Agreement is not used (in general, this would apply to non-US. studies whose local requirements may prohibit the use of the agreement).</p> <p>Since the present proposal corresponds to a retrospective study using healthcare databases, adverse experience reporting is not expected. However, in case of any adverse experience detection we would proceed as described in the study agreement.</p>																				
<b>2.10 Itemized Study Budget</b>	<table border="1" data-bbox="370 1094 1341 1608"> <thead> <tr> <th data-bbox="370 1094 1182 1129">ACTIVITIES</th> <th data-bbox="1182 1094 1341 1129">COST</th> </tr> </thead> <tbody> <tr> <td data-bbox="370 1129 1182 1165">Study design</td> <td data-bbox="1182 1129 1341 1165">4.000,00 €</td> </tr> <tr> <td data-bbox="370 1165 1182 1201">Statistical analysis plan design</td> <td data-bbox="1182 1165 1341 1201">1.000,00 €</td> </tr> <tr> <td data-bbox="370 1201 1182 1291">Ethical and regulatory processes: IRB submission, IRB dispenses, Spanish Medicines Agency classification</td> <td data-bbox="1182 1201 1341 1291">1.000,00 €</td> </tr> <tr> <td data-bbox="370 1291 1182 1327">Study coordination</td> <td data-bbox="1182 1291 1341 1327">3.000,00 €</td> </tr> <tr> <td data-bbox="370 1327 1182 1430">Data management Data request, data extraction (4 databases), data cleaning, data merging and tabulation, data quality review.</td> <td data-bbox="1182 1327 1341 1430">29.000,00 €</td> </tr> <tr> <td data-bbox="370 1430 1182 1499">Data analysis and study results: statistical analysis plan implementation, preparation and presentation of study results.</td> <td data-bbox="1182 1430 1341 1499">5.000,00 €</td> </tr> <tr> <td data-bbox="370 1499 1182 1535">SUBTOTAL</td> <td data-bbox="1182 1499 1341 1535">43.000,00 €</td> </tr> <tr> <td data-bbox="370 1535 1182 1570">OVERHEAD 15%</td> <td data-bbox="1182 1535 1341 1570">6.450 €</td> </tr> <tr> <td data-bbox="370 1570 1182 1608">TOTAL BUDGET</td> <td data-bbox="1182 1570 1341 1608">49.450€</td> </tr> </tbody> </table>	ACTIVITIES	COST	Study design	4.000,00 €	Statistical analysis plan design	1.000,00 €	Ethical and regulatory processes: IRB submission, IRB dispenses, Spanish Medicines Agency classification	1.000,00 €	Study coordination	3.000,00 €	Data management Data request, data extraction (4 databases), data cleaning, data merging and tabulation, data quality review.	29.000,00 €	Data analysis and study results: statistical analysis plan implementation, preparation and presentation of study results.	5.000,00 €	SUBTOTAL	43.000,00 €	OVERHEAD 15%	6.450 €	TOTAL BUDGET	49.450€
ACTIVITIES	COST																				
Study design	4.000,00 €																				
Statistical analysis plan design	1.000,00 €																				
Ethical and regulatory processes: IRB submission, IRB dispenses, Spanish Medicines Agency classification	1.000,00 €																				
Study coordination	3.000,00 €																				
Data management Data request, data extraction (4 databases), data cleaning, data merging and tabulation, data quality review.	29.000,00 €																				
Data analysis and study results: statistical analysis plan implementation, preparation and presentation of study results.	5.000,00 €																				
SUBTOTAL	43.000,00 €																				
OVERHEAD 15%	6.450 €																				
TOTAL BUDGET	49.450€																				
<b>2.11 References</b>	<p><b>(CDC), C. f. D. C. a. P.</b> (2016). Respiratory Syncytial Virus Infection.</p> <p><b>Acedo, L., Diez-Domingo, J., Morano, J. A. &amp; Villanueva, R. J.</b> (2010). Mathematical modelling of respiratory syncytial virus (RSV): vaccination strategies and budget applications. <i>Epidemiol Infect</i> <b>138</b>, 853-60.</p> <p><b>Bueno Campana, M., Calvo Rey, C., Vazquez Alvarez, M. C., et al.</b> (2008). Viral respiratory tract infections in the first six months of life. <i>Anales de pediatria (Barcelona, Spain : 2003)</i> <b>69</b>, 400-5.</p>																				

**Drysdale, S. B., Sande, C. J., Green, C. A. & Pollard, A. J.** (2016). RSV vaccine use--the missing data. *Expert Rev Vaccines* **15**, 149-52.

**Giersing, B. K., Karron, R. A., Vekemans, J., Kaslow, D. C. & Moorthy, V. S.** (2017). Meeting report: WHO consultation on Respiratory Syncytial Virus (RSV) vaccine development, Geneva, 25-26 April 2016. *Vaccine*.

**Homaira, N., Briggs, N., Oei, J.-L., Hilder, L., Bajuk, B., Jaffe, A. & Omer, S. B.** (2018). Association of age at first severe RSV disease with subsequent risk of severe asthma: a population-based cohort study. *The Journal of infectious diseases*.

**Homaira, N., Luby, S. P., Hossain, K., Islam, K., et al.** (2016). Respiratory Viruses Associated Hospitalization among Children Aged <5 Years in Bangladesh: 2010-2014. *PloS one* **11**, e0147982-e0147982.

**Koehoorn, M., Karr, C. J., Demers, P. A., Lencar, C., Tamburic, L. & Brauer, M.** (2008). Descriptive Epidemiological Features of Bronchiolitis in a Population-Based Cohort. *Pediatrics* **122**, 1196-1203.

**López-Lacort, M., Muñoz-Quiles, C., Orrico-Sánchez, A., Díez-Domingo, J.** (2018). RSV bronchiolitis: validation of the diagnosis in hospitalizations (Poster). ESPID.

**Mazur, N., Martinon-Torres, F., Baraldi, E., Fauroux, B., et al.** (2015). Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. *Lancet Respiratory Medicine* **3**, 888-900.

**McConnochie, K. M.** (1983). BRONCHIOLITIS - WHATS IN THE NAME. *American Journal of Diseases of Children* **137**, 11-13.

**Morant-Talamante N, Díez-Domingo J, Martínez-Ubeda S, Puig-Barbera J, Aleman-Sanchez S, Perez-Breva L.** Herpes zoster surveillance using electronic databases in the Valencian Community (Spain). *BMC infectious diseases*. 2013;**13**:463.

**Munoz-Quiles, C., Lopez-Lacort, M., Domenech-Clar, R., Puig-Barbera, J. & Díez-Domingo, J.** (2016a). Respiratory syncytial virus infection in adults diagnosed in Valencian Region of Spain. *Revista Clínica Española* **216**, 508-510.

**Munoz-Quiles, C., Lopez-Lacort, M., Ubeda-Sansano, I., Aleman-Sanchez, S., et al.** (2016b). Population-based Analysis of Bronchiolitis Epidemiology in Valencia, Spain. *Pediatric Infectious Disease Journal* **35**, 275-280.

**Murray, J., Saxena, S. & Sharland, M.** (2014). Preventing severe respiratory syncytial virus disease: passive, active immunisation and new antivirals. *Archives of Disease in Childhood* **99**, 469-473.

**Neuzil, K. M.** (2016). Progress toward a Respiratory Syncytial Virus Vaccine. *Clin Vaccine Immunol* **23**, 186-8.

**Ochoa Sangrador, C., Gonzalez de Dios, J. & Grupo Investigador del Proyecto a, B.** (2013). Management of acute bronchiolitis in spanish emergency wards: variability and appropriateness analysis (aBREVIADo project). *Anales de pediatria (Barcelona, Spain : 2003)* **79**, 167-76.

**Pérez-Vilar S, Díez-Domingo J, Puig-Barberá J, Gil-Prieto R, Romio S.** (2015a) Intussusception following rotavirus vaccination in the valencia Region, Spain. *Hum Vaccin Immunother* **11**(7):1848-52.

**Perez-Vilar S, Díez-Domingo J, Lopez-Lacort M, Martínez-Ubeda S, Martínez-Beneito MA.** (2015b) Effectiveness of rotavirus vaccines, licensed but not funded, against rotavirus hospitalizations in the Valencia Region, Spain. *Bmc Infectious Diseases* **15**:92.

	<p><b>Regnier, S. A.</b> (2013). Respiratory syncytial virus immunization program for the United States: Impact of performance determinants of a theoretical vaccine. <i>Vaccine</i> <b>31</b>, 4347-4354.</p> <p><b>Shi, T., McAllister, D. A., O'Brien, K. L., et al.</b> (2017). Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. <i>Lancet</i> <b>390</b>, 946-958.</p> <p><b>Sigurs, N., Bjarnason, R., Sigurbergsson, F. &amp; Kjellman, B.</b> (2000). Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. <i>American Journal of Respiratory and Critical Care Medicine</i> <b>161</b>, 1501-1507.</p> <p><b>Sigurs, N., Gustafsson, P. M., Bjarnason, R., Lundberg, F., Schmidt, S., Sigurbergsson, F. &amp; Kjellman, B.</b> (2005). Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. <i>American Journal of Respiratory and Critical Care Medicine</i> <b>171</b>, 137-141.</p> <p><b>Stockman, L. J., Curns, A. T., Anderson, L. J. &amp; Fischer-Langley, G.</b> (2012). Respiratory Syncytial Virus-associated Hospitalizations Among Infants and Young Children in the United States, 1997-2006. <i>Pediatric Infectious Disease Journal</i> <b>31</b>, 5-9.</p> <p><b>RSV Vaccine Snapshot [Internet].</b> 2016. Available from:  <a href="http://www.path.org/vaccineresources/files/RSV-snapshot-December2016.pdf">http://www.path.org/vaccineresources/files/RSV-snapshot-December2016.pdf</a>.</p>
<p><b>2.12 Publication Plan</b></p>	<p>The dissemination actions will comprise at least:</p> <ul style="list-style-type: none"> <li>- Publication of a scientific paper in an indexed scientific journal</li> <li>- Participation in one national and in one international scientific meeting/congress</li> <li>- Publication on main findings in social media after publication on journal</li> </ul>
<p><b>2.13 Curriculum Vitae</b></p>	<p>Investigator should provide curriculum vitae in English and a listing of references to MSD.</p>
<p><b>2.13 Protocol Submission for Investigator -Initiated Studies</b></p>	<p>U.S. protocols should be submitted by US investigators directly or through the Global Research Specialist at <a href="http://www.merckisip.com">www.merckisip.com</a></p> <p>Non U.S. protocols should be submitted to the MSD office by the investigators.</p>