RESEARCH PROTOCOL

PUFFIN trial

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PROTOCOL TITLE 'Pharmacogenetics Use For Further treatment Improvement in childreN'

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
ADRB2	**gene encoding beta-2-adrenergic receptor**
AE	**Adverse Event**
AR	**Adverse Reaction**
(c-)ACT	**(childhood-) Asthma Control Test**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
FDA	**Food and Drug Administration**
FeNO	**Fraction exhaled Nitric Oxide**
FEV1	**Forced Expiratory: Volume per second**
GCP	**Good Clinical Practice**
IC	**Informed Consent**
ICS	**Inhaled corticosteroids**
ICU	**Intensive Care Unit**
IgE	**Immunoglobulin E**
LABA	**Long acting beta2 agonists**
LTRA	**Leukotriene Receptor Antagonists**
NVK	**Nederlandse Vereniging voor Kindergeneeskunde (Dutch Society for Paediatrics)**
MARS	**Medication Adherence Reporting Scale**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**

OCS	**Oral corticosteroids**
PAQLQ	**Paediatric Asthma-related Quality of Life Questionnaire**
PACQLQ	**Paediatric Asthma-related Caregiver Quality of Life Questionnaire**
PedSQL	**Paediatric Quality of Life InventoryTM**
PCQ	**Productivity Cost Questionnaire**
QALY	**Quality Adjusted Life Year**
QoL	**Quality of Life**
RAST	**Radioallergosorbent-test (blood test to measure allergy)**
SABA	**Short acting beta2 agonists**
(S)AE	**(Serious) Adverse Event**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Asthma is the most common chronic disease in children. There is a large variability in treatment response to asthma medication and a one-size fits all approach might not be optimal for all paediatric patients. In children who are not well controlled on inhaled corticosteroids (ICS), guidelines suggest to double the dose of ICS or add a long acting beta-agonist (LABA). In children with asthma variation in the gene encoding the beta-2 adrenergic receptor *(ADRB2*), has been associated with poor response to long-acting beta-2 agonists (LABA). Children with asthma carrying a risk variant might therefore benefit more from doubling inhaled corticosteroids (ICS) than from adding LABAs.

Objective: To assess whether *ADRB2* genotype-guided asthma treatment in children with persistent asthma symptoms despite ICS treatment leads to better asthma control compared to non-genotype-guided asthma treatment.

Study design: National, multi-centre randomized controlled double blind trial

Study population: 310 children (6-17 years of age) with a doctor's diagnosis of asthma, who require a step-up in asthma treatment because of uncontrolled asthma symptoms despite adherent and adequate use of low dose ICS.

Intervention (if applicable): Participants will be randomized to 1) a genotype-guided treatment arm or 2) a usual care (non-genotype guided) control arm. In the genotype-guided arm, children will be treated based on their genotype of *ADRB2* (single nucleotide polymorphism rs1042713). Children homozygous for the risk variant (Arg16Arg) and heterozygotes (Arg16Gly) will be treated with doubling dosages of their ICS. Children homozygous for the wild type allele (Gly16Gly) will receive LABA as add-on to low dose ICS. In the control arm, children will be randomized between doubling ICS dosage or adding LABA, the two most common chosen options among respiratory paediatricians in the Netherlands when children are uncontrolled despite low dosages of ICS.

Main study parameter: Improvement of asthma control based on repeated measurement analysis of (childhood)-Asthma Control Test scores after 3 months.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The burden of the children and parents is moderate. The trial duration is 6 months, with 3 visits in the hospital and 4 additional online measurements (online diary). Biosamples will be taken (feces, saliva, nasal swabs) with limited burden to the children. There are no additional risks for participation in this study as only registered medication will be used. In our opinion a delay of 7-10 days before stepping up treatment in children with uncontrolled or poorly controlled symptoms is acceptable. In the future, the results of this study could lead to improved treatment for paediatric patients.

1. INTRODUCTION AND RATIONALE

The burden of asthma

Asthma is a chronic respiratory disease that affects approximately 300 million people worldwide [1]. It is characterized by recurrent respiratory symptoms and variable airflow limitation due to bronchial obstruction. Asthma is associated with a substantial health impact for the patient and large healthcare expenditures for society. In 2005, the total costs of childhood asthma for the 25 countries of the European Union were estimated at three billion euros, with the Netherlands contributing approximately 106 million euros and the UK 359 million euros [2]. These costs include medical and non-medical direct costs (e.g. GP visits, hospitalization and medication, diagnostics) as well as indirect costs (e.g. school days loss, caregiver productivity loss). One of the main drivers of direct medical costs is urgent asthma care [3]. Severe attacks of asthma symptoms (exacerbations) may lead to hospitalizations and admissions to the Intensive Care Unit. In the Netherlands, approximately 11% of the asthma patients are hospitalized each year [4]. Almost half of the costs associated with asthma management arise from hospital admission and unscheduled health care visits [5]. Despite progress in understanding this chronic disease, the burden of asthma due to hospitalizations and unscheduled visits is not decreasing.

Stepwise asthma treatment

Childhood asthma is treated in a stepwise approach. The first step of asthma treatment consists of short acting beta-2 agonists (SABA) as needed to relieve asthma symptoms. These drugs act as bronchodilators. Inhaled corticosteroids (ICS) are added to the treatment regime if asthma symptoms persist despite SABA use (step 2). ICS suppress the airway inflammation and are considered to be the cornerstone of maintenance asthma treatment. If a child's asthma remains uncontrolled, ICS dosage may be increased, or long-acting beta-2 agonists (LABA) or leukotriene receptor agonists (LTRA) can be added (step 3) [1]. However, the addition of a LTRA is not a very common chosen treatment option among respiratory paediatricians in the Netherlands.

The influence of genetic variation on asthma treatment response

There is large variability between patients in the level of symptom control or lung function improvement upon asthma maintenance treatment. In patients with poorly controlled asthma, improving adherence and inhaler technique are the first steps to improve asthma. However, even in clinical trials in which adherence to treatment is closely monitored, subgroups of patients remain symptomatic despite maintenance treatment [5]. Already in 2000, Drazen *et al.* suggested that up to 80% of the interindividual variance in lung function response upon

treatment in asthmatic patients could be due to genetic variations [6]. Since then, several candidate gene studies and a handful of genome-wide association studies (GWAS) have described genetic variants associated with response to asthma treatment.

One of these variants in a gene encoding for the beta-2 adrenergic receptor (*ADRB2*), has been positively associated poor response to long-acting beta-2 agonists [7-9]. This variant (rs1042713) is known as Arg16Gly since the 16th amino acid of the receptor is changed from glycine into arginine and the homozygous Arg16 variant is present in approximately 1 in 6 children [7]. A recent meta-analysis in the Pharmacogenomics in Childhood Asthma Consortium (PiCA) of 5 populations with 4,226 children and young adults of white Northern European and Latino origin showed that this variant was associated with an increased risk of asthma exacerbations when treated with LABA as add on treatment [7]. Per copy of the risk allele patients exposed to LABA had an increased risk of 52% for a severe asthma exacerbation. There was no increased risk for severe asthma exacerbations if patients were not exposed to LABA. An important observation from this meta-analysis is that the adverse effects of LABA were also observed in heterozygote carriers of the Arg16 variant, thereby providing evidence that over 60 percent of the population (17% homozygotes and 50% heterozygotes) is potentially at risk. This observational study indicated that a large group of paediatric asthma patients might not benefit from LABA and even suffer from more exacerbations, which may be preventable if they would have been treated differently. Besides that this leads to the research question whether genotyping is effective in children that are not under control on step 2 of asthma treatment, there is another question that remains unanswered. It is unclear how to treat the patients with the heterozygous variant. This is why it is very important to include heterozygous patients in our study.

Other clinical trials

One previous trial has been performed to address the effects of LABA in relation to *ADRB2* genotype, but only with children homozygous for the risk variant [10]. Sixty-two asthmatic children with the Arg16Arg genotype were randomized to treatment with ICS plus LABA or ICS plus LTRA and followed for 1 year. The trial showed that children treated with LTRA had fewer school absences, used less rescue medication, had less symptoms and a better quality of life compared to the group treated with LABA, with no effect on lung function scores between both study arms. The difference between both treatment groups could already be observed after 3 months (figure 1).

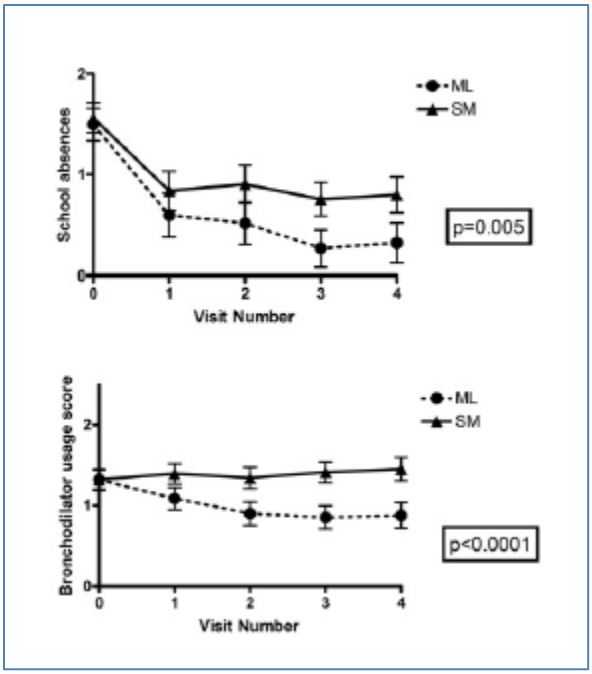

Figure 1. Change in asthma-related school absences and use of salbutamol reliever use in 62 asthmatic children with Arg16Arg genotype treated with LABA or alternative drug. Change in asthma-related school absences and the use of salbutamol reliever over a 1-year study period with 3-monthly visits between groups treated with ICS plus oral montelukast (ML) or ICS+LABA plus placebo montelukast (SM). The differences in school absences and bronchodilator usage can already be observed early in the trial (after 3 months, visit 1) [10].

Why should children be studied?

It is important to study children with asthma instead of adults since ample data indicate that this genetic risk effect is mainly observed in the paediatric population. The adverse effects of LABA might be more prominent in children than in adults, which was clearly shown in a meta-analysis on the risk of LABA of 110 controlled clinical trials with 60.954 patients performed by the Food and Drug Administration (FDA) [11]. It could be that adults with asthma are less vulnerable to the negative effects of LABA due to the influence of other modifying factors such as increased airway wall rigidity (caused by airway remodeling over time), long-term inflammation or a different affinity of the beta-2 adrenergic receptors to their agonists [12]. However, a subsequent safety trial mandated by the FDA found no significant difference between the risk of serious asthma events in children receiving a combination of LABA and ICS compared to children who only received an ICS [13].

Therefore, it is important to study the effect of this genetic variant on the treatment outcome in children. This study will include children with asthma to test whether ADRB2-genotype guided treatment will lead to better and faster asthma control.

Why should other –omics markers also be included in this study?

There are some first results pointing in the direction that –omics markers are predictive in responsiveness to asthma medication, for example in microbiomics, metabolomics, transcriptomics and epigenomics. A genomic susceptibility alone does not seem to be enough to drive asthma phenotypes. The interaction with the environment might play a major role in driving a predisposed genetic background towards the breakage of immune tolerance

and the development of asthma, likely by influencing microbiotic, bacterial metabolomics and/or the epigenome and the transcriptome. Obtaining more information about the whole phenotype of the asthmatic children via nose swabs and feces sampling is one of our secondary outcomes.

Recent data has shown that the nasal epithelium is an excellent proxy tissue for –omics' studies of the lower airways [14]. In addition to the primary analyses, we therefore aim to perform an in-depth integrative -omics analysis of children unresponsive to therapy with double dosage of ICS.

Supporting this concept, several studies have shown associations between epigenetic markers and asthma or asthma-related outcomes [15]. A new pool of biomarkers may be found in the gut (faeces) of asthmatic patients. It has been shown that the microbial composition changes in the airways and gut of asthmatic patients compared to healthy individuals. Levels of short chain fatty acids (SCFA) and branched chain fatty acids (BCFA) are an indication of changed carbohydrate and protein fermentation, respectively, by commensal bacteria, such as *bifidobacteria* and *bacteriodes* [16]. SCFA and BCFA have been demonstrated to be important for regulation of asthma-associated inflammatory responses [17]. The human microbiome is acquired during life, and it is a very important changeable property. Several studies have identified molecules and mechanisms that connect diet, the gut microbiota and immune-related diseases such as asthma [18].

Methylation of inflammatory genes changes over time in asthmatics starting at the early stages of disease development. Differences in methylation patterns between asthmatics and healthy individuals may be detectable as early as in cord blood [9]. In asthmatics methylation patterns may determine the dynamics of disease from progression to remission. On the other hand treatment and disease control may also affect methylation in a feedback loop. Identification of key epigenetic markers can lead to a change in treatment strategies from palliative to preventive [19]. Furthermore, recent literature suggests that patients with severe asthma carry methylation changes in specific genes compared to non-severe patients [20].

Taken together, -omics dimensions (such as microbiomics & bacterial metabolomics, breathomics, transcriptomics and epigenomics) may constitute the final bridge between genetic-predisposition and actual disease development. Single-dimension biomarker approaches to phenotype asthma are increasingly regarded to be inaccurate and outdated, especially since different dimensions can interact (Figure 1).

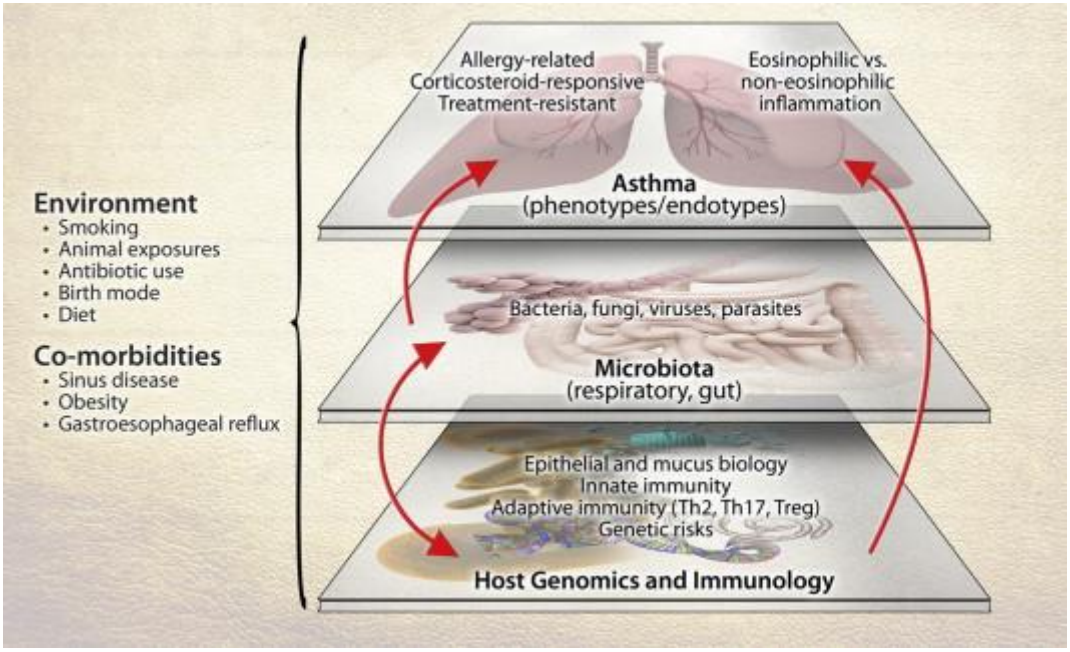

Figure 1. Host (epi)genomics, immunology and microbiota likely collectively influence asthma treatment response (Huang et al, JACI 135:25-30, 2015).

Precision Medicine (also known as Personalized Medicine) is the field that aims to optimize effectiveness of treatment for individual patients or patient groups by taking into account individual patient characteristics. Many different factors can play a role in determining the best treatment for a particular patient. Paediatric asthma patients differ considerably from adult patients in the nature of the disease, its triggers, the course of disease and therefore the response to therapy. In paediatric asthma the collection of airway biopsy specimens and invasive procedures in general are difficult. Therefore we consider it of utmost importance to target tissues and materials for biomonitoring that can be easily and non-invasively gathered in routine clinical practice, such as saliva, exhaled breath, and faeces. Rather than using specimens that could never be part of a clinical strategy for optimising drug therapy. Biomarkers that will be studied in this proposal are (epi)genetic factors (nose swabs), microbiome (feces) and metabolomic markers in exhaled breath.

Assessing the costs and benefits of ADRB2-genotyping

Alongside this RCT, a cost-utility analysis will be conducted in order to quantify the incremental costs and benefits of introducing a genetic-based diagnostic tool into clinical asthma practice from a societal perspective. A key research question that we will consider is if ADRB2-prospective genotyping is found to be clinically effective, is whether this translates into economically important differences in patients' health, their quality of life, healthcare resource utilization and costs.

2. OBJECTIVES

Primary Objective:

- To assess whether *ADRB2* genotype-guided treatment leads to better asthma control after 3 months compared to usual care in children who are uncontrolled despite adherent and adequate use of ICS

Secondary Objectives:

- To assess whether *ADRB2* genotype-guided treatment leads to better asthma control at 6 months.
- To assess whether *ADRB2* genotype-guided treatment leads to improved quality of life (QoL), fewer school absences, fewer exacerbations, and better lung function compared to usual care in children at 3 and 6 months
- To assess whether *ADRB2* genotype-guided treatment leads to fewer changes in asthma therapy at 3 months, compared to usual care.
- To assess whether *ADRB2* genotype-guided treatment leads to a shorter time to reach asthma control, compared to usual care
- To assess the cost-utility of *ADRB2*-genotype guided treatment
- To identify –omics-biomarkers for non-response to ICS treatment

3. STUDY DESIGN

Study design: national, multi-centre, double-blind randomized controlled trial

Duration: 6 months, with 3 visits in the hospitals (at t=0, t=3 months and t=6 months)

Setting: Patients are recruited at out-patient asthma clinics in secondary and tertiary care hospitals in the Netherlands.

Description: Three hundred ten children (6 to 17 years of age) with a doctor's diagnosis of asthma and uncontrolled asthma symptoms despite adherent and adequate use of ICS for at least three months (step 2 asthma treatment) will be recruited by secondary and tertiary care centers in the Netherlands. All participants are eligible for step-up asthma treatment (from step 2 to step 3) as assessed by the treating paediatrician/paediatric pulmonologist Participants will be randomized to a genotype-guided treatment arm (n=155) or to a usual care, non-genotype guided, arm (n=155) (Figure 2) and followed for 6 months.

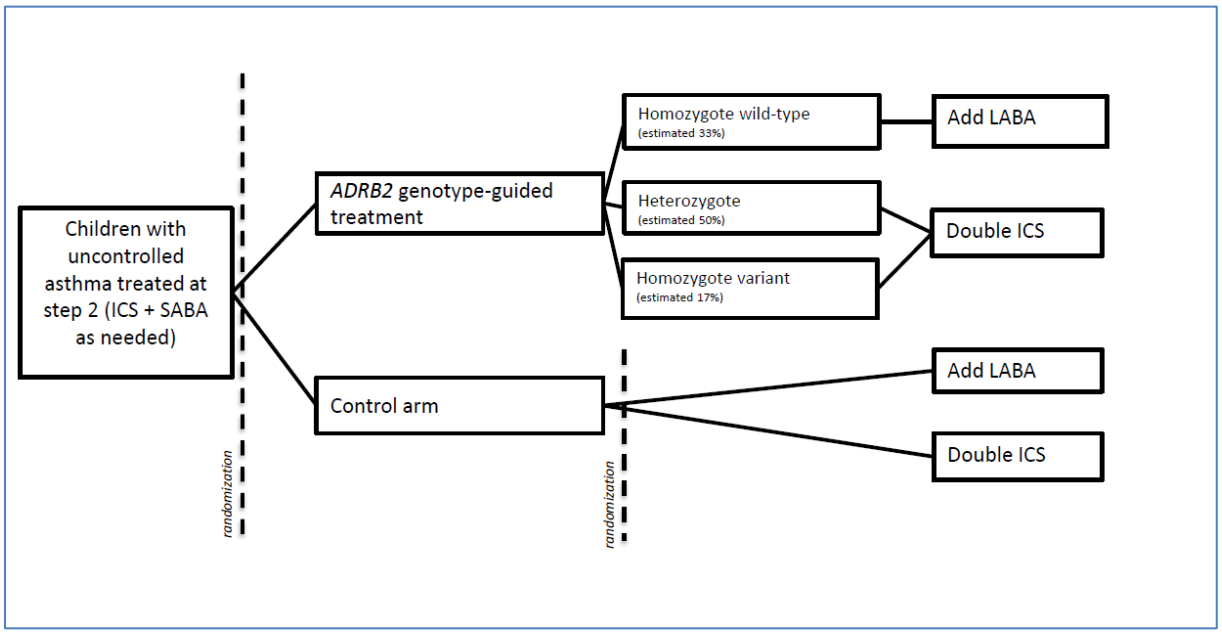

Figure 2. PUFFIN study design

Genotyping before start treatment

During the baseline visit in the hospital, clinical data and biological samples (including a DNA sample) will be collected. Upon this visit, the DNA sample will be send to the Clinical Chemistry department of the Erasmus MC (Head: Prof. R. van Schaik) to perform genotyping of the *ADRB2* gene within one week. The treating physician will adapt the treatment regime

of the participant based on the treatment advice of the study coordinator (Table 1). For the children in the genotype-arm, this will be based on the genotype. The treating physician will not know (be blinded) whether the treatment advice was based on the genotype (intervention arm) or based on randomization (control arm). The participant will be followed for 6 months. If the participant is still uncontrolled at t=3 months, treatment will be adapted according to Table 1. All children will be genotyped, in order to assess the influence of the genotype on treatment outcome in the usual arm group retrospectively. The children should use the same inhalation device during the study to avoid confusion on how they should inhale their medication.

Table 1. Treatment regimes

	Arg16Arg or Arg16Gly	**Gly16Gly**	
Therapy: month 0- 3	Double ICS	Double ICS	ICS+LABA
Therapy for month 4-6 if still uncontrolled at 3 months	Normal dosage ICS and LTRA	Normal dosage of ICS and LABA	Double ICS

Intervention arm: ADRB2 genotype-guided treatment arm

In the genotype-stratified arm, children will be treated based on their *ADRB2* genotype. Children homozygous for the risk variant Arg16 and heterozygotes (Arg16Gly) will be treated with doubling dosages of their ICS. Children homozygous for the wild type allele (Gly16Gly) will receive LABA.

Control arm: Non genotype-guided treatment arm

In the control arm, genotyping will be performed for retrospective analysis, but the genotype information will not be used to guide treatment. Children in this study arm will be randomized again between doubling ICS dosage (n=75) or LABA treatment (n=75), the two most commonly preferred add-on options among paediatric pulmonologists in the Netherlands. We choose to randomize between both treatments options, since international guidelines do not agree on the preferred treatment option [1,15].

Furthermore, to test our hypothesis it is necessary to have enough children in the control group with Arg16Arg or Arg16Gly to be treated with LABA. The amount of children treated with LABA and ICS should be equal in the control group. Therefore we decided to randomise children in the control group over doubling ICS (n=77) and adding LABA (n=77). This will lead to an estimated number of children with Arg16Arg or Arg16Gly of 51 who will get LABA add on. In this way the power is high enough to determine the

effectivity of both treatment options in the three genotypes. We find it important to define effectivity next to the question whether genotyping benefits children with asthma. In the control group DNA samples will be obtained for retrospective analysis.
It is safe to randomise the children again who are randomised within our control arm, because treatment with a double dose of ICS and adding a LABA are both standard of care. A Cochrane review from 2009 has shown that both treatments have proven to be equally effective in both children and adults
Randomisation in the control arm is important because it would be futile if the children in this arm would be treated with the same therapy by accident. Randomisation is necessary to make the trial as small and effective as possible. At this moment physicians do not have the tools to determine which therapy is the best for every child. This is why we think it is correct to randomise in the control arm.

Based on the previous studies of Lipworth *et al.* [10], and Turner *et al.* [7], we hypothesize that children with one or two Arg16 alleles in *ADRB2* will experience less asthma control, thus randomization in the control arm will ensure sufficient children with these *ADRB2* genotypes to be exposed to LABA in order to test our hypothesis. We will perform interim genetic analysis to verify that we include sufficient children with these risk genotypes in our study. Our trial is designed to reflect clinical practice as closely as possible, therefore the choice of the type of ICS or LABA will be done by the treating physician.

4. STUDY POPULATION

4.1 Population (base)

Children will be selected from the asthma clinic of at least 15 participating Dutch hospitals. In general, children (6-18 years) who are well-controlled on step 2 treatment will be treated by general practitioners (GPs). GP guidelines suggest to refer children with asthma not well controlled on step 2 treatment. Therefore, children treated in the hospitals will be at least on step 2 treatment. We expect that approximately 25% of these children are poorly controlled or uncontrolled on step 2 treatment. The hospitals that expressed their intent to participate in the study were confident that they were able to recruit enough patients in the study.

Plan B

The contact with other Dutch hospitals is good, and in case the current sites fail to include enough patients, we will extent the inclusion sites to other hospitals. The novel initiated Dutch consortium Paediatric Pulmonology will also play an active role in stimulating hospitals to participate.

Another option is to let general practitioners play a role in our patient inclusion.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Between 6-18 years of age
- Doctor's diagnosis of asthma (ever) based on FEV1 reversibility ≥ 12 % and/or bronchial hyperresponsiveness.
- Current asthma symptoms (based on ACT (≥12 years) or C-ACT (<12 years) score ≤ 19
- ICS use ≥ 3 months before inclusion (start dosage ICS, treatment step 2 according to childhood asthma guideline NVK, Table 3)
- Adequate inhalation technique (based on validated checklist score [21])
- Self-assessed good adherence to maintenance asthma treatment
- Understanding of Dutch language
- Internet access a home, willing to fill in internet questionnaires

Table 3. ICS dosing step 2

ICS	Dosage (µg)
Beclomethason	2 dd 200

Beclomethason (extra fine)	2 dd 100
Budesonide	2 dd 200
Fluticason	2 dd 100-125
Ciclesonide	1 dd 160

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Active smoking
- Congenital heart disease
- Serious lung disease other than asthma (Cystic Fibrosis, Primary Ciliary Dyskinesia, congenital lung disorders, severe immune disorders)
- LABA use in past 6 months
- Omalizumab use
- ICU admission in the previous year

4.4 Sample size calculation

A sample size analysis was performed for Arg16 homozygotes double dose ICS vs. LABA treatment (our main research question), with power 80%, α=0.05 2-sided, and based on the ACT scores and correlation between repeated measurements in a previous performed RCT in children with asthma (the BATMAN study) (SD=3.6, r=0.58) [22]. Taking into account a minimal clinically relevant significant difference of 3 points on the ACT scores, that 16% of the children in the PACMAN study were carrying Arg16Arg, and that ACT will be measured 4 times (baseline t=0, t=1, t=2 and t=3) before the primary endpoint (at t=3 months), a minimum of 153 children need to be included in each study arm. When the analysis will be adjusted for the baseline value, this will reduce the variance of the estimate of difference between the group by a factor 1-r^2, and thereby increase the power. Because not all children might be willing to complete the trial we will include a total of 310 children. The power is based on the inclusion of 16% of children carrying Arg16Arg. We will count the amount of children with this genotype when 310 children have been included. If this amount is not reached after 310 children we will continue the trial until we have included the expected 49 children with the Arg16Arg genotype.

5. TREATMENT OF SUBJECTS

Intervention: ADRB2 genotype-guided treatment
The intervention consists of genotype-guided treatment. All children in the trial require additional asthma treatment (step 3). There are two preferred treatment options among paediatric pulmonologists in the Netherlands: 1) doubling ICS dosage or 2) adding LABA to the standard dose of ICS. In the intervention group, the choice of treatment will be based on the child's ADRB2 genotype (rs1042713). This variant is known as Arg16Gly since the 16th amino acid of the receptor is changed from glycine into arginine. Children homozygous for the risk variant Arg16 and heterozygotes (Arg16Gly) will be treated with doubling dosages of their ICS. Children homozygous for the wild type allele (Gly16Gly) will receive LABA.

Comparator: Non genotype-guided treatment
In the comparator group choice of treatment will be based on randomization (1:1) to the two treatment options (doubling ICS dosage or adding LABA treatment) and will not be based on ADRB2 genotype. This arm reflects usual care. Genotyping will be performed for retrospective analysis, but the genotype information will not be used to guide treatment.

This study meets the definition of a "clinical study with limited intervention" as is defined in the new EU regulation Article 2, Clause 3. Children with asthma are treated in this study with drugs that are approved by the European Medicines Agency and are used according to the approved Summary of Product Characteristics. Children with asthma who are uncontrolled asthma on treatment step 2 will be treated according to step 3, which is double dose ICS or adding a LABA. It is not clear which of the two treatment options in step 3 is most effective in the different phenotypes of children. In this study the choice for the drugs will be determined by a genetical test or by randomisation. The procedures in this study are minimal invasive. They will consist of three visits in the hospital, monthly questionnaires and obtaining samples (saliva, feces, nose swabs).

INVESTIGATIONAL PRODUCT

5.1 Name and description of investigational product(s)

Product and dose in mcg	Active substance
AirFluSal aerosol 25/125, 25/250 Sandoz	Salmeterol/fluticason
AirFluSal Forspiro 50/500 Sandoz	Salmeterol/fluticason
Salmeterol 25 aerosol Fisher	Salmeterol
Salmeterol 25 aerosol GSK	salmeterol
Salmeterol/Fluticason 25/125, 25/250 aerosol Vincion	Salmeterol/fluticason
Salmeterol/Fluticason 25/125, 25/250, 50/500 aerosol GSK	Salmeterol/fluticason
Seretide 25/50, 25/125, 25/250 aerosol GSK	Salmeterol/fluticason

Seretide Diskus 50/100, 50/250, 50/500 inhalatiepoeder GSK	Salmeterol/fluticason
Serevent 25 aerosol GSK	Salmeterol
Serevent Diskus 50 GSK	Salmeterol
Aerivio Spiromax 50/50	Salmeterol/fluticason
Fluticasonproprionaat 125, 250 aerosol Vincion	fluticason
Fluticasonproprionaat 50, 125, 250 aerosol GSK	Fluticason
Budesonide 200 aerosol Allgen	Budesonide
Budesonide 200 aerosol Mylan	Budesonide
Budesonide Novolizer 200, 400	budesonide
Budesonide Easyhaler 100, 200, 400	budesonide
Formoterol Novolizer 6, 12	Formoterol
Formoterol Easyhaler 12 Sandoz	Formoterol
Atimos 12 Chiesi	Formoterol
Oxis turbuhaler 12	Formoterol
Oxis turbuhaler 6	Formoterol
Alvesco dosisaerosol 160	ciclesonide
Beclometason 100 aerosol TEVA	Beclometason
Beclometason 250 aerosol TEVA	Beclometason
Beclometason 50 aerosol TEVA	beclometason
Beclometason 50 aerosol Allgen	beclometason
Beclometason 100, 250, 500 aerosol Mylan	Beclometason
Beclometason 100, 250, 500 aerosol Sandoz	beclometason
Qvar 50, 100 aerosol	beclometason
Foster 100/6, 200/6 aerosol Chiesi	Beclometason/formoterol
Qvar 50, 100 redihaler	beclometason
Bufoler Easyhaler 320/9, 160/4.5	Budesonide/formoterol
Duoresp Spiromax 160/4.5	Budesonide/formoterol
Flutiform 50/5, 125/5, 250/10 aerosol	Fluticason/formoterol
Foster NEXThaler 100/6, 200/6 Chiesi	Beclometason/formoterol
Relvar Ellipta 92/22, 184/22 GSK	Fluticasonfuroaat/vilanterol
Symbicort 200/6 aerosol	Budesonide/formoterol
Symbicort Turbuhaler 100/6, 200/6, 400/12	Budesonide/formoterol

A meta-analysis of 25 clinical trials with a total of 5572 children has shown that on average there was no difference in efficacy between the ICS+LABA combination and the double dose ICS combination [23].

5.2 Summary of findings from non-clinical studies

A summary of findings from non-clinical studies can be found in the Summary of Product Characteristics (SPC) on the pages defined below:

Product and dose in mcg	**Location of findings from non-clinical studies:**
AirFluSal aerosol 25/125, 25/250 Sandoz	Section 5.3, page 18
AirFluSal Forspiro 50/500 Sandoz	Section 5.3, page 20
Salmeterol 25 aerosol Fisher	Section 5.3, page 11
Salmeterol 25 aerosol GSK	Section 5.3, page 12
Salmeterol/Fluticason 25/125, 25/250 aerosol Vincion	Section 5.3, page 13
Salmeterol/Fluticason 50/100, 50/250, 50/500 aerosol GSK	Section 5.3, page 18
Seretide 25/50, 25/125, 25/250 aerosol GSK	Section 5.3, page 17
Seretide Diskus 50/100, 50/250, 50/500 inhalatiepoeder GSK	Section 5.3, page 17
Serevent 25 aerosol GSK	Section 5.3, page 11
Serevent Diskus 50 GSK	Section 5.3, page 9
Aerivio Spiromax 50/50	Section 5.3, page 19
Fluticasonproprionaat 125, 250 aerosol Vincion	Section 5.3, page 12
Fluticasonproprionaat 50, 125, 250 aerosol GSK	Section 5.3, page 12
Budesonide 200 aerosol Allgen	Section 5.3, page 11
Budesonide 200 aerosol Mylan	Section 5.3, page 8
Budesonide Novolizer 200, 400	Section 5.3, page 11
Budesonide Easyhaler 100, 200, 400	Section 5.3, page 11
Formoterol Novolizer 6	Section 5.3, page 8
Formoterol Novolizer 12	Section 5.3, page 8
Formoterol Easyhaler 12 Sandoz	Section 5.3, page 7
Atimos 12 Chiesi	Section 5.3, page 7
Oxis turbuhaler 12	Section 5.3, page 7
Oxis turbuhaler 6	Section 5.3, page 7
Alvesco dosisaerosol 160	Section 5.3, page 8
Beclometason 100 aerosol TEVA	Section 5.3, page 6
Beclometason 250 aerosol TEVA	Section 5.3, page 6
Beclometason 50 aerosol TEVA	Section 5.3, page 6
Beclometason 50 aerosol Allgen	Section 5.3, page 7
Beclometason 100, 250, 500 aerosol Mylan	Section 5.3, page 7
Beclometason 100, 250, 500 aerosol	Section 5.3, page 7

Sandoz	
Qvar 50, 100 aerosol	Section 5.3, page 6
Foster 100/6, 200/6 aerosol Chiesi	Section 5.3, page 15
Qvar 50, 100 redihaler	Section 5.3, page 8
Bufoler Easyhaler 320/9, 160/4.5	Section 5.3, page 14
Duoresp Spiromax 160/4.5	Section 5.3, page 30
Flutiform 50/5, 125/5, 250/10 aerosol	Section 5.3, page 17
Foster NEXThaler 100/6, 200/6 Chiesi	Section 5.3, page 16
Relvar Ellipta 92/22, 184/22 GSK	Section 5.3, page 41
Symbicort 200/6 aerosol	Section 5.3, page 10
Symbicort Turbuhaler 100/6, 200/6, 400/12	Section 5.3, page 13

5.3 Summary of findings from clinical studies

A summary of findings from clinical studies can be found in the Summary of Product Characteristics (SPC) on the pages defined below:

Product and dose in mcg	**Location of findings from clinical studies:**
AirFluSal aerosol 25/125, 25/250 Sandoz	Section 5.1, page 14
AirFluSal Forspiro 50/500 Sandoz	Section 5.1, page 12
Salmeterol 25 aerosol Fisher	Section 5.1, page 9
Salmeterol 25 aerosol GSK	Section 5.1, page 9
Salmeterol/Fluticason 25/125, 25/250 aerosol Vincion	Section 5.1, page 10
Salmeterol/Fluticason 50/100, 50/250, 50/500 aerosol GSK	Section 5.1, page 12
Seretide 25/50, 25/125, 25/250 aerosol GSK	Section 5.1, page 13
Seretide Diskus 50/100, 50/250, 50/500 inhalatiepoeder GSK	Section 5.1, page 11
Serevent 25 aerosol GSK	Section 5.1, page 9
Serevent Diskus 50 GSK	Section 5.1, page 6
Aerivio Spiromax 50/50	Section 5.1, page 13
Fluticasonproprionaat 125, 250 aerosol Vincion	Section 5.1, page 10
Fluticasonproprionaat 50, 125, 250 aerosol GSK	Section 5.1, page 9
Budesonide 200 aerosol Allgen	Section 5.1, page 10
Budesonide 200 aerosol Mylan	Section 5.1, page 7
Budesonide Novolizer 200, 400	Section 5.1, page 10
Budesonide Easyhaler 100, 200, 400	Section 5.1, page 10
Formoterol Novolizer 6	Section 5.1, page 8
Formoterol Novolizer 12	Section 5.1, page 8
Formoterol Easyhaler 12 Sandoz	Section 5.1, page 7
Atimos 12 Chiesi	Section 5.1, page 6

Oxis turbuhaler 12	Section 5.1, page 6
Oxis turbuhaler 6	Section 5.1, page 6
Striverdi respimat 2,5	Section 5.1, page 10
Alvesco dosisaerosol 160	Section 5.1, page 6
Beclometason 100 aerosol TEVA	Section 5.1, page 6
Beclometason 250 aerosol TEVA	Section 5.1, page 6
Beclometason 50 aerosol TEVA	Section 5.1, page 6
Beclometason 50 aerosol Allgen	Section 5.1, page 6
Beclometason 100, 250, 500 aerosol Mylan	Section 5.1, page 6
Beclometason 100, 250, 500 aerosol Sandoz	Section 5.1, page 6
Qvar 50, 100 aerosol TEVA	Section 5.1, page 5
Foster 100/6, 200/6 aerosol Chiesi	Section 5.1, page 10
Qvar 50, 100 redihaler	Section 5.1, page 7
Bufoler Easyhaler 320/9, 160/4.5	Section 5.1, page 11
Duoresp Spiromax 160/4.5	Section 5.1, page 28
Flutiform 50/5, 125/5, 250/10 aerosol	Section 5.1, page 13
Foster NEXThaler 100/6, 200/6 Chiesi	Section 5.1, page 11
Relvar Ellipta 92/22, 184/22 GSK	Section 5.1, page 36
Symbicort 200/6 aerosol	Section 5.1, page 8
Symbicort Turbuhaler 100/6, 200/6, 400/12	Section 5.1, page 10

5.4 Summary of known and potential risks and benefits

Chapter 10 describes our risk analysis.

5.5 Description and justification of route of administration and dosage

Dosage will be according to the SPC's of the products.

5.6 Dosages, dosage modifications and method of administration

There will be no dosage modifications or different methods of administration other than described in the SPC's of the products.

5.7 Preparation and labelling of Investigational Medicinal Product

All investigational medicinal products in this study are used according to the Nederlandse Vereninging van Kindergeneeskunde (NVK) and according to registration as described in the SPC of each product. The products will be selected and prescribed as standard care. At the moment of inclusion patients are already being treated with other inhalation products (SABA and single dose ICS), which will be continued once in the study.

5.8 Drug accountability

At inclusion the investigators will document the name of the medication (ICS or LABA), the manufacturer, batch-number and expiry date of the product in the case report form.

6. METHODS

6.1 Study parameters/endpoints

6.1.1 Main study parameter/endpoint

Change in asthma control based on repeated measurement analysis of ACT or C-ACT scores at t=3 months. We will choose t=3 months as a primary outcome, since the effect of genotype-guided treatment on asthma control should already be visible within this period (based on the Scottish trial in Arg16Arg children [10]). Furthermore, in case a child is not controlled within this period, we do not find it ethical to continue the same medication. This reflects normal clinical practise at the outpatient clinic. However, in order to study the effect on a longer term, children will be followed for 6 months in total.

6.1.2 Secondary study parameters/endpoints (if applicable)

- change in asthma control at t=6 months (repeated measurement analysis)
- time to ACT ≥ 20
- change in asthma-related quality of life scores
- change in fatigue score
- school absences
- exacerbations (oral corticosteroids use, ER visits, hospital admissions)
- time to first exacerbation
- amount of changes in therapy at t=3 months
- change in lung function (FEV1 pre- and postbronchodilator) at t=3 and t=6
- change in FeNO at t=3 and t=6 (in centers where FeNO analysers are available)
- change in nasal gene expression and nasal gene methylation in relation to the treatment effect at t=3 and t=6
- Incremental cost per Quality Adjusted Life Year (QALY)
- Incremental costs per avoided exacerbation

6.2 Randomisation, blinding and treatment allocation

Randomisation between intervention and control arm

Participants will be randomized 1:1 to the intervention arm or the control arm. Block randomization with randomly chosen block sizes and stratified per center will be applied. Randomization software will be used to generate randomization codes.

Randomisation within control arm

Children in the control arm will be randomized 1:1 to a) doubling ICS dosage or b) adding LABA to the treatment regime. Block randomization with randomly chosen block sizes and stratified per center will be applied. Randomization software will be used to generate randomization codes.

Blinding

The study will be double blinded, both patients and paediatricians will be blinded for the study arm and will not know whether the treatment advice was based on the *ADRB2* genotype of the child or not. The researchers of the Clinical Trial Unit of the department of Respiratory Medicine at the AMC will have access to the randomization arm and according to the algorithm (Table 1) will provide the treating physician and research nurses at the inclusion sites with a treatment advice for step 3.

Breaking the randomization code

The randomization code will be broken in case of severe asthma exacerbations requiring ICU admission.

6.1 Study procedures

The study consists of 3 clinical visits (t=0, t=3 months (± 2 weeks) , t=6 months (± 2 weeks)) and monthly online questionnaires (Figure 3).

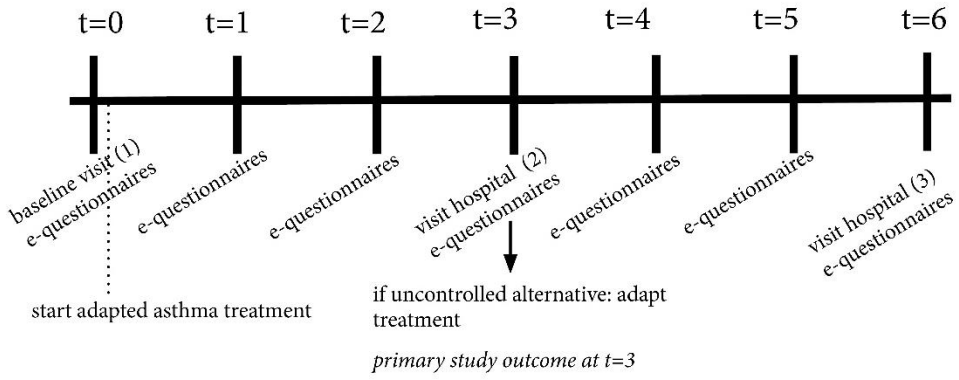

Figure 3: Timeline PUFFIN trial

Informing participants

During a regular care visit, the treating physician will inform the parents and the patient about the study. The physician will contact the PUFFIN investigators. Patients and parents will get at least 24 hours to consider participation. A research assistant of the hospital of the child will

contact the patients and parents by phone to inform whether they would like to participate. In case they are willing to participate, a study visit will be planned within 2 weeks.

Measurements during clinical visit 1

Screening
Upon informed consent, patient will be screened to check whether he/she fulfill the inclusion criteria (e.g. current asthma symptoms, adequate inhalation technique (based on validated checklist (15), adherence to treatment). In case he/she fulfills these criteria, the following study measurements will be performed:

Lung function testing (in case this has not been performed during the last clinical routine visit, < 2 weeks preceding the study visit):

- Spirometry: Forced expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC) and FEV_1/FVC ratio, Forced Expiratory Flow after 75% of the exhaled volume (FEF75) before and after inhalation of salbutamol will be measured.
- Fraction of exhaled nitric oxide (FeNO) will be measured before spirometry.

Children will be instructed to stop their short acting bronchodilators at least 8 hours prior to lung function testing.

NB: A lung function measurement could have been assessed during the screening visit. In this case, this measurement is eligible to count for visit 1.

Clinical review
Measure height/weight.

Questionnaires:
Children and parents are asked to complete several questionnaires (if possible online):

- *Asthma control test*

  The validated Dutch version of the Asthma Control Test (ACT, for children of ≥ 12 years) or Childhood Asthma Control Test (C-ACT, for children < 12 years) inquires on asthma symptoms in the past 4 weeks.

- *Exacerbations*

  Severe exacerbations (asthma-related unscheduled health care visits, use of OCS, admissions), as well as mild-to-moderate exacerbations (sudden increase of

symptoms, asthma attack requiring additional rescue medication, unplanned visits to general practitioner for asthma) and school absences (in days) due to asthma symptoms will also be recorded.

- *Asthma medication use and adherence to maintenance treatment*

Questions on current asthma medication will be included. Furthermore, patients will be asked to complete the medication adherence reporting scale (MARS). Parents and children are asked for consent to extract medication dispensing data of the child from the local pharmacies

- *Asthma-Quality of Life*

In children aged 12 years and older, asthma-related quality of life will be measured with the 13-item self-reported Dutch validated version of the Paediatric Asthma-Related Quality of Life Questionnaire (PAQLQ) [24] for children and expressed as overall asthma-related quality of life. In children aged below 12 years, we use the Paediatric Asthma-Related Caregiver Quality of Life Questionnaire (PACQLQ)[25].

- *Productivity loss parents*

Modules of the Productivity Cost Questionnaire (PCQ) [26] to assess the loss of productivity of the caregivers will be included in the questionnaire.

- *Fatigue*

The PedsQL Multidimensional Fatigue Score [27] will be included to assess symptoms of fatigue in the past month.

- Control of Allergic Rhinitis

In case, children suffer from allergic rhinitis in addition to asthma, The Control of Allergic Rhinitis and Asthma Test (CARAT) [28] will be used to assess the level of control of allergic rhinitis.

- *Other questions*

Furthermore we will include questions on allergy and rhinitis complaints, environmental factors, pre- and postnatal factors, demographics and diet (related to the microbiome).

Noninvasive procedures

Two nasal epithelial swab (Copan Flocked Swabs), nr 56380CS01 per nostril will be taken for DNA and RNA isolation. This sample will be taken by gently rotating the flocked swab at the site of the lower inferior turbinate. Furthermore, a saliva sample will be taken for genomic DNA isolation. We will also ask the children to send a feces sample to the researchers after the study visit for microbiomics/metabolomics analysis. Within the AMC inclusion site, breath

will be analyzed using the SpiroNose. For this measurement, children just have to breathe into the SpiroNose.

Laboratory testing:
Nasal epithelial swabs: DNA and RNA isolation will be performed. Whole genome gene-expression and epigenomics analyses will be performed.
Saliva: DNA will be isolated and genotyped for the *ADRB2* gene.
Feces: short-chain fatty acids will be measured, 16s RNA sequencing will be performed to analyze the microbiome.

Clinical visit 2 (t=3 months (± 2 weeks))
During this visit asthma control is assessed. In case children are still not controlled with current treatment, they will receive alternative treatment according to Table 1.
The following measurements will be performed during this visit:

- clinical review (similar to clinical visit 1)
- lung function measurements (similar to clinical visit 1), FeNO measurement
- online asthma questionnaire (less extensive as clinical visit 1, but including questions on current asthma medication use, symptoms, asthma-related quality of life, fatigue and exacerbations)
- Nose swabs for DNA and RNA extraction.

Clinical visit 3 (t=6 months (± 2 weeks))
Same measurements as clinical visit 2.

Online monitoring / e-diary
A web-based patient filewill be developed to obtain monthly data and allow patients and their caregivers to upload data and complete questionnaires. An external partner (Patient 1) will develop this research database that meets all safety and privacy legislations. The web-based application will be accessible from every device with an internet connection (such as computer, tablet, smartphone). Data entered through the electronic questionnaire will be directly sent to a secured database, will be time and date logged and can be accessed by staff with sufficient level of access rights. Pop-up messages and emails will be used to alert the patients to uncompleted questionnaires. When participants do not fill in their questionnaire monthly, research staff will contact the parents/patients and try to motivate compliance.

The questionnaires include questions on current asthma medication use, symptoms, asthma-related quality of life, fatigue and exacerbations. Furthermore, children and parents can note changes in medication use and symptoms to discuss this with their physician during the clinical visit..

6.2 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. These subjects will then be treated according to standardized care. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

6.2.1 Specific criteria for withdrawal (if applicable)

Children who will be admitted to the ICU during the study will be withdrawn from the study.

6.3 Replacement of individual subjects after withdrawal

A dropout rate of 5% has been foreseen for this study, based on previous experiences with a similar study population in the BATMAN study [ZonMW project number: 171002101] in which patients were followed for 1 year. Therefore, subjects will not be replaced if they drop out of the study.

6.4 Follow-up of subjects withdrawn from treatment

Subjects will be followed up by their own paediatrician and treated according to national guidelines.

6.5 Premature termination of the study

Since all the medication used in this trial is already used in clinical practise, we do not foresee safety reasons for premature termination of this study.

7. SAFETY REPORTING

7.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

7.2 AEs, SAEs and SUSARs

7.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to trial procedure. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

7.2.2 Serious adverse events (SAEs)

A serious adverse event occurring during the trial is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event. The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events, except for the following SAEs: SAEs related to asthma diagnosis including exacerbations and hospitalisation for asthma exacerbations but (with the exception of asthma-related ICU admissions).

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events. The following SAEs will be reported in line listings every 6 months: SAEs related to asthma diagnosis.

7.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met during the trial:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

7.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

7.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

7.5 Data Safety Monitoring Board (DSMB)

We consider the risk associated with participating in this study moderate:

- The study includes a vulnerable participant group (children),
- However, the medication in this study is already used in clinical care,
- And, there are no high risk measurements or interventions associated with significant safety issues, outside what is considered normal practice for this patient population

Therefore, we do not consider it is necessary to establish a DSMB for this study.

8. STATISTICAL ANALYSIS

8.1 Primary study parameter(s)

The trial results will be analysed according to the intention to treat principle. The primary study parameter is improvement of asthma control after 3 months. Asthma control is measured each month and is represented in a score obtained from 5-item asthma control questionnaires (ACT or CACT). The score ranges on a scale from 5 to 25 (continuous variable).

To assess whether children with a variant genotype have a poorer improvement in asthma control we will assess:

1. The improvement in asthma control is different between patients with Arg16Arg treated with LABA and treated with double dose ICS
2. The improvement in asthma control is different between patients with Arg16Gly treated with LABA and treated with double dose ICS
3. The improvement in asthma control is different between patients with Gly16Gly treated with LABA and treated with double dose ICS

To assess the clinical impact of a genotype-guided strategy on improvement in asthma control we will assess whether:

4. The improvement in asthma control is different between patients in the genotype-guided treatment arm compared to the control arm

Univariate repeated measurement analysis will be used, as well as multivariate analysis correcting for the following covariates: age, gender. In case the outcome variable follows a normal distribution, parametric tests are applied, in case the outcome variable does not follow a normal distribution, appropriate non-parametric testing will be applied. In addition, descriptive statistics will be used to describe the characteristics of the study population.

8.2 Secondary study parameter(s)

The following secondary study parameters will be assessed in this trial. In case a continuous outcome variable does not follow a normal distribution, appropriate non-parametric testing will be applied.

1. <u>change in asthma control score at t=6 months</u>

Changes in asthma control scores at t=6 months between patients with different genotypes treated with LABA or double dose ICS, and between patients in the intervention arm and in the control arm will be compared.

2. time to ACT ≥ 20 (time-to-event variable)

A log-rank or similar statistical test will be used to compare time to reach well controlled asthma between patients with different genotypes treated with LABA or double dose ICS, and between patients in the intervention arm and in the control arm.

3. change in asthma-related quality of life scores

Asthma-related quality of life is measured using the questions of the PaQLQ, and is measured at various time intervals during the trial. The questions of the PaQLQ are divided into three domains (activity limitations, symptoms, emotional function) and consist of 23 questions (with a 7 point scale). All questions are equally weighted and the overall score is the mean of the responses to all questions. Differences in mean/median of asthma-related quality of life scores from the beginning to the end of trial (t=6 months) between patients with different genotypes treated with LABA or double dose ICS, and between patients in the intervention arm and in the control arm are assessed using appropriate testing.

4. change in fatigue score

Fatigue score will be measured at the beginning and the end of the trial using the PedSQL. This 18-item questionnaire measures fatigue in three domains (general fatigue; sleep fatigue, cognitive fatigue). Each question can lead to 0-4 points. The total fatigue score ranges between 0-72. The differences in median score will be compared between patients with different genotypes treated with LABA or double dose ICS, and between patients in the intervention arm and in the control arm using appropriate testing.

5. school absences

The proportion of patients with school absences, as well as the median/mean amount of school absences, will be compared between patients with different genotypes treated with LABA or double dose ICS, and between patients in the intervention arm and in the control arm using Chi-squared and appropriate testing.

6. exacerbations (oral corticosteroids use, ER visits, hospital admissions)

Exacerbations will be defined as the use of short courses of oral corticosteroids, asthma-related ER visits and asthma-related hospital admissions. The proportion of patients with exacerbations, as well as the median/mean amount of exacerbations per group, will be compared between patients with different genotypes treated with LABA or double dose

ICS, and between patients in the intervention arm and in the control arm using appropriate testing.

7. time to first exacerbation

A log-rank test will be used to compare time to a new exacerbation between patients with different genotypes treated with LABA or double dose ICS, and between patients in the intervention arm and in the control arm. For the definition of exacerbation see secondary outcome #6.

8. amount of changes in therapy at t=3 months

At t=3 months physicians can adapt the treatment of participants in case they are still not well controlled (see table 1). We will assess whether patients in the intervention arm have fewer changes in therapy (continuous variable) at t=3 compared to patients in the control arm, using appropriate statistical testing.

9. change in lung function (FEV1 pre- and postbronchodilator) at t=3 and t=6

Differences in change in lung function at t=3 and t=6 will be assessed between patients with different genotypes treated with LABA or double dose ICS, and between patients in the intervention arm and in the control arm using Kruskall-Wallis tests or one-way ANOVA as appropriate.

10. change in FeNO at t=3 and t=6

FeNO is measured as a continuous variable in parts per billion. We will assess whether the median/mean level of FeNO is different between patients with different *ADRB2* genotypes treated with LABA or double dose ICS, and between patients in the intervention arm and in the control arm using appropriate testing (logFeNO).

8.3 Other study parameters

The following –omics analysis will be performed:

1. change in nasal gene expression and nasal gene methylation in relation to the treatment effect at t=3

   For each sample, DNA methylation level at each CpG site will be calculated in percentage by B= (M/M+U) * 100%. Where M is the signal strength of methylated CpG given by Illumina HumanMethylation450 BeadChip array, and U is the signal

strength of unmethylated CpG. For each marker a T-test will be used to assess methylation variation between responders and non-responders to treatment at t=3 months. Multiple comparison adjustment will be performed adopting a Bonferroni correction for the number of probes that will be successfully analysed after quality control

2. change in microbiome profile and treatment effect at t=3
   Using 16S RNA sequencing, each sample sequence set will be sub-sampled to 8,700 sequences. Differences in abundance will be detected using a Kruskal-Wallis generating a Benjamini-Hochberg false-discovery rate corrected p-value, whereby responders and non-responders to treatment at t=3 months are compared.

3. Exhaled air at t=0, t=3 and t=6 only for the AMC inclusion site
   Exhaled air will be sampled using the SpiroNose (AMC, Amsterdam & Comon Invent, Delft, NL). In its current form, the SpiroNose is a spirometry coupled electronic nose based on metal-oxide semiconductor (MOS) sensors with high between-sensor reproducibility. Patients will perform 5 tidal breaths, then, after a single deep inspiratory vital capacity manoeuvre and a 5 second breath hold, the patient exhales a vital capacity volume into the measurement setup. The exhaled air is directly measured by the SpiroNose, which is connected to an Ethernet cable for immediate secure data transmission to the online BreathCloud server. Data can be downloaded for further processing and analysis with offline pattern recognition software.

ETHICAL CONSIDERATIONS

8.4 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version 2013, 19-10-2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts. We will comply with the principles enshrined in the Council of Europe Convention on human rights and biomedicine – known as the Bioethics Convention (Oviedo). Its main purpose is to protect individuals against exploitation

8.5 Recruitment and consent

Eligible asthmatic children will be selected in the participating hospitals. The parents and children will be informed orally about the study by the treating physicians who will contact the investigators. The treating physician will provide the patient and parents with the

appropriate patient information forms. They will be given at least 24 hours to consider their decision and are free to reconsider their decision at any moment during the study. A research assistant will contact the participants and parents to ask whether they would be willing to participate. If they are willing to participate a study visit is scheduled in their own hospital within 1 week. Before start of the study visit written consent is obtained.

8.6 Objection by minors or incapacitated subjects (if applicable)

We will act according to the code of conduct of objection by minors from the Netherlands Association for Paediatric Medicine: Code of conduct relating to expressions of objection by minors participating in medical research - Netherlands Association for Paediatric Medicine:

"Code of conduct"

1. *Individual children respond differently to diagnostic and treatment procedures and to participation in medical research. Various factors help to determine the nature of the response: the way the child is prepared for what is going to happen, the parent-child relationship, the doctor-patient relationship, the child-friendliness of the environment in which the procedure takes place and so on. One child will not be unduly disturbed by having an injection (even if he or she winces or makes some other display of pain), while another will find the experience distressing. Although responses vary considerably from child to child, there is a general correlation between the degree of 'invasiveness' of a procedure and the strength of the response. In some cases, fear regarding participation or a particular procedure will prompt a child to object. Patient and understanding explanation and reassurance will generally be sufficient to enable the research or the procedure to proceed without problems. Where a newborn child or infant is concerned, it is much harder to ascertain whether objection has been expressed. As a general rule, however, it is reasonable to suggest that a child may be deemed to object if its behaviour clearly differs in nature or degree from that normally displayed by the child when confronted with situations not encountered in everyday life. In this context, situations not encountered in everyday life may be considered to include diagnostic or therapeutic procedures.*

2. *Before seeking consent for a child's participation in medical research, an investigator must fully inform the child's custodial parent(s) or guardian about what is proposed. Information should be provided orally and in writing. The nature of the procedures involved in the research should be discussed with the parents and their views sought*

on the child's likely response. The possibility of the child objecting to participation and the type of behaviour that should be regarded as an expression of objection should also be discussed. The investigator should also explain what is to happen in the event of the child objecting. The consent obtained from the parents should include agreement to the proposed procedure for dealing with expressions of objection by the child.

3. *The consent statement signed by parents should stipulate that, if the child should object to participation in the research, consent for its further participation will be invalidated.*

4. *If prior to the research there is doubt as to whether a child should participate, consideration may be given to involving the patient in the research for an agreed pilot period.*

5. *While the research is in progress, the behaviour of the child should be continually assessed at the research location to determine whether the child's behaviour is within the bounds normally associated with the child when confronted with situations not encountered in everyday life. If a child's behaviour is not within these bounds, he or she should be deemed to have expressed an objection in the sense of the WMO.*

6. *The parents, the investigator(s) and possibly a behavioural scientist should be involved in assessment of a child subject's behaviour. Assessment of a child subject's behaviour should not be a one-off exercise, but should continue through all phases of the research.*

7. *The parents of a child subject should be able to withdraw their consent at any point during the research. If a child subject expresses an objection, the child's participation should be discontinued.*

8. *In all medical research involving child subjects, the burden associated with participation should be minimised; where non-therapeutic research is concerned, the law stipulates that it must be negligible. Medical studies often involve the combination of research procedures with diagnostic procedures necessary in connection with the subject's treatment. Where research involves an invasive procedure, such as a finger prick or venapunction, this should if possible be combined with a procedure necessary for diagnostic or treatment purposes, such as blood sampling. If possible, a*

needle or line that has already been inserted should be utilised, so that the number of 'jabs' is kept to the minimum. The burden can also be reduced by the use of plasters with local anaesthetic. The various steps to be taken with a view to minimising the burden should be detailed in the research protocol and in the information given to the parents and subjects.

9. *The following should be noted in the research file or the medical (status) report, as appropriate:*
   (a) the outcome of any trial participation;
   (b) the consent of the custodial parent(s) or guardian, including the procedure to be followed in the event of a possible expression of objection;
   (c) an account of the subject's participation in the research, stating whether objection was expressed;
   (d) an assessment as to whether the subject's behaviour constitutes objection, as referred to above;
   (e) the names of the people responsible for assessing the subject's behaviour, as described above;
   (f) an assessment as to whether the subject's behaviour in the course of the study constitutes objection;
   (g) the steps taken to minimise the burden associated with participation.
   The protocol for a medical research project in which minors are to be used as subjects should state that the NVK's code of conduct for dealing with subjects' expressions of objection in the course of the research will be adhered to.

10. *This code of conduct will be evaluated in consultation with the research community two years after its initial publication and amended as necessary."*

This code of conduct was approved by the Board of the Netherlands Association for Paediatric Medicine (NVK) on 21 May 2001 and published in NVK Newsletter no. 3, June 2001.

8.7 Benefits and risks assessment, group relatedness

Risks: Nasal swabbing (soft tips swabs, Copan Flocked Swabs), nr 56380CS01 provides a minimal burden to the children. We have performed the same method in the MAKI3 trial in 6 year old children, and found that all children were able to donate the nasal swabs with minimal burden. This soft-flocked method has been developed since nasal brushing with

cytobrushes is painful and provides a significant burden to the children. Children have reported an itchy feeling, and in rare circumstances tearing eyes. We regard the collection of saliva and faces as having a minimal burden.
Repeated lung function measurements can be experienced as uncomfortable in case of spirometer induced bronchoconstriction, which happens < 3 % in asthmatic children. However, this is part of usual asthma care and we will administer salbutamol 400 mcg (pMDI with spacer). The risks associated with participation can be considered negligible and the burden minimal.
This study includes only minors, since the effect of the *ADRB2* genotype on response to asthma medication seems to be restricted to the paediatric population. This patient group is expected to have the most benefit from prospective *ADRB2* genotyping before starting treatment.
Benefits: in the intervention arm, children may suffer from fewer asthma symptoms compared to usual care.

8.8 Compensation for injury

The sponsor has a liability insurance which is in accordance with article 7 of the WMO.

8.9 Incentives (if applicable)

Patients and parents will receive compensation in travel costs based on travelled kilometres and parking costs for the hospital visits. Children will receive a small present for participation.

9. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

9.1 Handling and storage of data and documents

Collected data and samples will be coded complying with the Dutch Personal Data Protection Act. The code will not include patient initials and birth-date, but will include a code of the hospital were inclusion took place. The key to link patients and their genotypes or biomarkers will be securely stored, and accessible only to the principal investigators of each participant centre.

Information gathered by the study will be used only for aggregate analysis, and will not be released with any information that identifies research participants. DNA Genotyping of the saliva samples will be performed in one centre (ErasmusMC), and the DNA samples will be coded and unlinked to individual respondent identifiers. Microbiome/metabolome feces samples will be analysed and stored at the AMC. Transcriptomic and epigenomic samples will be stored in Groningen and analysis will be performed at the UMC Groningen.

We will conduct a genome wide association study (GWAS) in a later stadium to identify possibly other involved SNPs in asthma disease in children. This data may be also useful to compare with (and later include in) worldwide databases with children with asthma.

9.2 Monitoring and Quality Assurance

The study will be monitored by an external monitoring (CRU). For more details see the monitoring plan (K).

9.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

9.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed

the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

9.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

9.6 Public disclosure and publication policy

We will follow the basic principles of the CCMO statement on publication policy. The protocol will be published at clinicaltrials.gov and trialregister.nl before the first patient is included in the study. The results of research will be submitted for publication to peer-reviewed scientific journals, and will also be updated at clinicaltrials.gov and trialregister.nl.

10. STRUCTURED RISK ANALYSIS

10.1 Potential issues of concern

Not applicable.

10.2 Synthesis

All products described in chapter 5 are registered in The Netherlands. In this study the products are used according the registration and there is a wide experience with these products in daily care of children.

On basis of these considerations chapter 10.2 was not further completed.

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