# The effect of FP-025, a MMP-12 inhibitor, on allergen-induced airway responses, airway inflammation and aspects of airway remodeling in subjects with mild eosinophilic house dust mite (HDM)-allergic asthma

(HDM)-allergic asthma

**Protocol Number: FP02C-18-001**

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| Protocol title | The effect of FP-025, a MMP-12 inhibitor, on allergen-induced airway responses, airway inflammation and aspects of airway remodeling in subjects with mild eosinophilic house dust mite (HDM)-allergic asthma |
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# 1 SIGNATURE PAGE FOR SPONSOR

Hereinafter called Foresee Pharmaceuticals Co., Ltd.

Investigational drug name: FP-025

Protocol number: FP02C-18-001

[redacted]
Senior Vice-President

DocuSigned by:
[redacted]
08 September 2021 | 06:23 PDT

Signature Date

Signer Name: [redacted]
Signing Reason: I approve this document
Signing Time: 08 September 2021 | 06:23 PD
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# 2 SIGNATURE PAGE FOR INVESTIGATOR

Investigational drug name: FP-025

Protocol number: FP02C-18-001

I agree to the terms and conditions relating to this study as defined in this Protocol, electronic Case Report Form (eCRF), and any other protocol-related documents. I fully understand that any changes instituted by the investigator(s) without previous agreement with the sponsor would constitute a violation of the protocol, including any ancillary studies or procedures performed on study subjects (other than those procedures necessary for the well-being of the subjects).

I agree to conduct this study in accordance with the Declaration of Helsinki and its amendments, International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines and applicable regulations and laws. In particular, I will obtain approval by an Ethics Committee or Institutional Review Board (EC/IRB) prior to study start and signed informed consent from all subjects included in this study. In addition, I will allow direct access to source documents and agree to inspection by auditors from the sponsor and Health Authorities. I will ensure that the study drug(s) supplied by the sponsor are being used only as described in this protocol. Furthermore, I confirm herewith that the sponsor is allowed to enter and utilize my professional contact details and function in an electronic database for internal purposes and for submission to worldwide Health Authorities.

[redacted]
Principal Investigator

DocuSigned by:
[redacted] 06 September 2021 | 08:56 EDT
Signature Date
Signer Name: [redacted]
Signing Reason: I approve this document
Signing Time: 06 September 2021 | 08:56 EDT
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Principal Investigator

Signature Date
Signer Name: [redacted]
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# PROTOCOL CHANGES LOG

**Amendment 3, dated 06 September 2021**, amendment 2 issued on 22 April 2021, amendment 1 issued on 09 August 2018, original protocol issued 05 March 2018.

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20EC of the European Parliament and the Council of the European Union.

With this amendment, an interim analysis is added to compute the conditional power of a significant difference between FP-025 and placebo treatment at the planned end of the study, using unblinded data of at least 11 completed subjects.

| Section | Update |
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| **Header** | ~~22 April~~**06 September 2021**, Amendment ~~2~~**3**<br>QPS Logo has been updated. |
| **Title page** | Document status version Amendment ~~2~~**3**<br>Date ~~22 April~~ **06 September 2021** |
| **Section 6.2.3 Methacholine/Histamine Challenge** | Although interacting with different receptors, both histamine and methacholine are regarded as 'a direct agent' ~~by agonist' producing bronchoconstriction~~ as outlined in the international guidelines by leading societies (ATS/ERS)), **and** both have been (interchangeably) used to assess nonspecific airway hyperresponsiveness according to the same protocol in standard clinical practice as well as in the context of the allergen bronchoprovocation test [15, 16]. |
| **Section 8.3 Inhaled allergen (HDM) challenge** | Subjects with a~~n~~ LAR will be contacted for a phone call check up by research staff usually within 24-48 hours after the challenge day, or otherwise at the discretion of the investigator. |
| **Section 8.4.2.1 Baseline Characteristics** | • Hematology and clinical chemistry and urinalysis (refer to Section 8.4.4.~~7~~**8**) |
| **Section 8.4.4.7 Overall Asthma Symptoms** | Overall asthma symptoms will be assessed using a visual analogue scale (VAS). **A standard VAS includes a scale of 100 mm. When a scale of another size is used, the values will be adjusted to match the standard VAS size of 100 mm.** |
| **Section 8.9.2 Treatment Assignment** | ~~The~~**For the purpose of an interim analysis (see Section 10.9),** the randomization code **of the subjects who have completed the study** will be unblinded and made available for data analysis ~~only~~**. Unblinding of the randomization code will also apply** after study closure, i.e., when the study has been **fully** completed, the protocol deviations determined and the clinical database declared complete, accurate, and locked. |
| **Section 8.9.3 Double-blinding** | The study is performed in a double-blinded fashion. ~~The investigator and study staff, the subjects, the monitors and the sponsor's staff will remain blinded to the treatment until study closure.~~ The investigational drug and its matching placebo are indistinguishable and medication containers will be packaged in the same way. The randomization code will be kept strictly confidential. It is accessible only to the pharmacist |

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| | on site, who is not involved in the conduct and analysis of the study, and will keep the randomization **code**/scheme strictly confidential.<br><br>**The investigator and research staff, the subjects, the monitors and the sponsor's staff will remain blinded to the treatment until study closure. For the interim analysis (see Section 10.9), only the independent statistician will be unblinded to the study treatment of those subjects who have completed the study and have been included in the interim analysis.** |
| **Section 10.1 Statistical Analysis Plan** | A statistical analysis plan (SAP) will be written and finalized before the **final** study closure, i.e., database closure and unblinding of the randomization code of the study. The SAP will provide full details of the individual analyses, the data displays and the algorithms to be used for data derivations. A SAP for safety, tolerability and PD will be written by QPS Qualitix Taiwan. A PKAP for the PK analyses will be written by QPS LLC. **All analyses on efficacy outcomes including their relationships or relationships between PK and PD/clinical/biomarker outcomes will be overseen by the independent statistician (Prof A.H. Zwinderman).**<br><br>The SAP **will include** the link of major protocol deviations/violations to the analysis sets. |
| **Section 10.2.1 Sample Size** | The sample sizes for this study are based on previous allergen challenge studies [9] and previous local experiences including several outcomes (airway physiology and several (diluted) biomarkers), taking into account additional variability due to a 2 ~~center~~**centre** setup. |
| **Section 10.2.2 Procedure for accounting for missing, unused, and spurious data** | In **the** calculation of percentages, subjects with missing data if not included in an analysis will not be considered in numerator or denominator unless otherwise specified. |
| **Section 10.3 Pharmacodynamic Parameters** | Analyses of all ~~other~~**(including additional) PD parameters and any relationships between** parameters will be detailed in the SAP. |
| **Section 10.8 Exploratory Analyses** | Exploratory data-driven analyses can be performed at a later timepoint, i.e. after finalizing the CSR and can be ~~later on~~ added as an addendum **later on**. |
| **Section 10.9 Interim Analysis** | **After at least 11 subjects have successfully completed both treatment periods (with fully analyzable data sets of airway responses and sputum cytospins), an interim analysis will be conducted in order to compute the conditional power of a significant difference between FP-025 and placebo treatment on the LAR as well as on the sputum eosinophil counts (reflecting target engagement), at the planned end of the trial in the presence of sufficiently high PK levels and at the original expectation of the effect of FP-025 on the LAR. If the conditional power is less than 25%, the trial may be stopped for futility. However, such a decision** |

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| | **will depend on the size of the FP-025 treatment-effect on secondary and exploratory outcomes.**<br><br>**In addition, the size of a potential carry-over effect of active treatment in the first treatment period into effects of treatment in the second treatment period will be investigated. If the size of the carry-over is larger than expected, the statistical analysis for the remaining subjects may be amended.**<br><br>**No adjustment of the sample size is made because with the intended sample size of 32 evaluable subjects, the power of the original effect is over 95% and this remains the case with the addition of the interim analysis.**<br><br>**In the absence of any clinically relevant AEs, recruitment and study conduct will continue during (preparation of) the interim analysis.** |
| **Section 10.10 Clinical Study Report** | The CSR will include the data and the analysis of both the primary and secondary parameters; exploratory parameters and analyses may be added ~~later on~~ in an addendum to the CSR **later on**. |
| **Section 12.1.3.2 Database Management and Quality Control** | Any changes to the eCRF after that time **require** a database unlock and can only be made after receipt of written approval of the Sponsor. |

**Amendment 2, dated 22 April 2021**, amendment 1 issued on 09 August 2018, original protocol issued 05 March 2018.

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20EC of the European Parliament and the Council of the European Union.

Currently, there is a ubiquitous shortage of the raw materials to produce methacholine, making it difficult to obtain the methacholine needed for the methacholine challenges in this study. Methacholine will therefore be replaced by histamine, whenever there is an insufficient supply of methacholine. Both agents produce similar effects within the airways and in clinical practice are used interchangeably to document airway hyperresponsiveness. Importantly, both are well-documented in connection to bronchial allergen challenge. Within the study, subjects will receive either methacholine or histamine.

| Section | Update |
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| **Header** | ~~09August 2018~~**22 April 2021**, Amendment ~~1~~**2** |
| **Title page** | Document status version Amendment ~~1~~**2**<br>Date ~~09August 2018~~**22 April 2021** |
| **Key Study Personnel** | The order of the collaborating parties/sub-investigators was updated: Khalid Adb-Elaziz was placed before Els Weersink. |
| **Abbreviations** | Added:<br>**PC20FEV1(Hist): Provocative concentration of histamine causing a 20% fall in FEV1** |
| **Section 5 Protocol Synopsis** | Secondary objectives:<br>• To determine the treatment effect (i.e. Day 1 versus Day 10) of multiple oral doses of FP-025 versus placebo on baseline parameters (such as blood eosinophils, FeNO and ~~PC20methacholine).~~ **PC20FEV$_1$(Meth)/PC20FEV$_1$(Hist))**. |
| **Section 5 Protocol Synopsis** | Secondary endpoints:<br>o Airway hyperresponsiveness expressed as PC20FEV$_1$(Meth) **or PC20FEV$_1$(Hist)** (Day 10-Day 12);<br>• Potential treatment effect (FP-025 versus placebo) on baseline parameters (i.e. Day 1 versus Day 10), including:<br>o Blood eosinophils;<br>o PC20FEV$_1$(Meth) **or PC20FEV$_1$(Hist)**;<br>o FeNO.<br>Exporatory endpoints:<br>• Potentially longer lasting treatment effects 14 days following Period 2 (FP-025 versus placebo) (spirometry (baseline FEV1), PC20FEV$_1$(Meth) **or PC20FEV$_1$(Hist)** and airway and systemic biomarkers (i.e. blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum). |
| **Section 5 Protocol Synopsis** | Summary of Study Design |

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| | On Screening Day 2, spirometry with subsequent methacholine/**histamine** challenge (for calculation of the $PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)**) will be performed, followed by sputum induction (SI).<br><br>Following a washout period of at least 3 weeks and up to approximately 7 weeks, eligible subjects will be enrolled into the study and randomized on Day 1 if their asthma is within continuation criteria of screening (as assessed by history, (rescue) medication use, baseline $FEV_1$ and $PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)**).<br><br>On Day 1, after a short checklist, the following tests will be performed for baseline purposes, i.e. blood and urine sampling (both for routine safety and biomarkers), FeNO, IOS (if logistics allow), spirometry, EBC, NAL, NAB, methacholine/**histamine** challenge (with calculation of $PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)** for stability/eligibility check) and SI.<br><br>On Day 1 of Period 1, subjects should still have stable asthma (based on continuation criteria as applicable in the allergen challenge: i.e. history/medication use/spirometry and ~~PC20Methacholine~~ **$PC20FEV_1$(Meth)/$PC20FEV_1$(Hist)**) in order to be randomized by a blinded pharmacist and start with the study drug administration.<br><br>On Day 10 (i.e. approximately 24 hours pre-allergen), approximately 30-60 minutes after first daily dosing, subjects undergo the same procedures as on Day 1 (i.e. safety/stability check, blood and urine collection (biomarkers), FeNO, EBC, NAL, NAB, IOS (if logistics allow), spirometry, methacholine/**histamine** challenge and SI.<br><br>Fourteen (14) days (± 2 days) after final administration of FP-025 or placebo on Day 12 of Period 2, a follow-up visit will be scheduled. This visit will not only serve as a follow-up visit (e.g. checking weight and AEs, performing physical examination, ECG and pregnancy test, checking vital signs and collecting blood for clinical safety laboratory assessments), the 'duration of efficacy' will also be explored by performing the same procedures as performed on Day 1 and Day 10 (i.e. blood and urine samplings (safety/biomarkers), FeNO, EBC, NAL, NAB, IOS (if logistics allow), spirometry, methacholine/**histamine** challenge and SI). |
| **Section 5 Protocol Synopsis** | Inclusion criteria<br><br>8 On Screening Day 2, $PC20FEV_1$(Meth) should be <16 mg/mL if methacholine chloride is used (or adjusted by a factor of 1.2 if methacholine bromide is used). **If histamine is used, $PC20FEV_1$(Hist) should be <16 mg/mL.** |

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| **Section 5 Protocol Synopsis** | Pharmacodynamic parameters<br>Secondary parameters<br>Methacholine/**histamine** challenge:<br>• Provocative concentration of methacholine **or histamine** causing 20% fall of the $FEV_1$ from baseline ($PC20FEV_1$(Meth) **or PC20FEV$_1$(Hist)**)<br>Exploratory parameters<br>Impulse-oscillometry (IOS) post-~~metacholine~~**methacholine/histamine challenge** |
| **Section 5 Protocol Synopsis** | Statistical methodology<br>Pharmacodynamics<br>• Changes in allergen-induced AHR: i.e: $PC20FEV_1$(Meth) **or PC20FEV$_1$(Hist)** pre-post allergen (Day 10 versus Day12)<br>• Changes in blood eosinophils, FeNO and PC20FEV1(Meth) **or PC20FEV$_1$(Hist)** Day 1 versus Day 10 (potential treatment effect)<br>Exploratory<br>• Treatment effects: differences in changes in the following parameters (Day 1 versus Day 10): spirometry (baseline $FEV_1$), $PC20FEV_1$(Meth)**/PC20FEV$_1$(Hist)** and airway and systemic biomarkers (i.e. in blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum).<br>• Potentially longer lasting treatment effects 14 days following Period 2 (spirometry (baseline $FEV_1$), $PC20FEV_1$(Meth)**/PC20FEV$_1$(Hist)** and airway and systemic biomarkers (i.e. in blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum).<br>• IOS measurements following inhaled allergen and eventual methacholine/**histamine** challenges to assess the effect of study medication on the peripheral airways and for additional safety. |
| **Table 5-1: Visit and Assessment Schedule** | Methacholine/**histamine** challenge<br>Footnote b: FeNO, EBC, NAL, NAB, spirometry, methacholine/**histamine** challenge and SI to be performed in the morning (pre-dose). On Day 1, spirometry will be performed immediately after FeNO (since eligibility criterion).<br>Footnote e: By Medical history/Medication use/$FEV_1$ and ~~PC20methacholine~~ **PC20FEV$_1$(Meth)/PC20FEV$_1$(Hist)**.<br>Footnote l: To be performed before and after inhaled allergen challenge and methacholine/**histamine** challenge and after administration of ventolin.<br>Footnote m: To be performed after eligibility check (including $FEV_1$) and methacholine/**histamine** challenge with subsequent |

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| | PC20FEV$_1$(Meth) **or PC20FEV$_1$(Hist)** calculation on Day 1 of Treatment Period 1. |
| **Section 6.2 Study Rationale** | Added:<br>**Section 6.2.3 Methacholine/Histamine Challenge**<br>**Currently, there is a ubiquitous shortage of the raw materials to produce methacholine, making it difficult to obtain the methacholine needed for the methacholine challenges in this study. In case of insufficient supply of methacholine, histamine will be used instead in this study.**<br>**Although interacting with different receptors, both histamine and methacholine are regarded as 'a direct agent' by agonist' producing bronchoconstriction as outlined in the international guidelines by leading societies (ATS/ERS)), both have been (interchangeably) used to assess nonspecific airway hyperresponsiveness according to the same protocol in standard clinical practice as well as in the context of the allergen bronchoprovocation test [15, 16]. Furthermore, both histamine (which was historically the first agent) and methacholine have been used to predict the allergen PC20 in a similar way [9, 16]. Therefore, switching to histamine will not harm the subject safety nor the study objectives, particularly as the allergen-induced airway response on several (bio)markers will be compared within subjects. To allow for scientifically sound within-subject comparisons, whenever possible, subjects will receive the same challenge agent (i.e. methacholine or histamine) throughout the study. Both methacholine and histamine challenges will be performed according to the QPS standard operating procedures/work instructions in line with the above-mentioned international guidelines.** |
| **Section 7.2 Secondary Objectives** | To determine the treatment effect (i.e. Day 1 versus Day 10) of multiple oral doses of FP-025 versus placebo on baseline parameters (such as blood eosinophils, FeNO and ~~PC20methacholine).~~ **PC20FEV$_1$(Meth)/PC20FEV$_1$(Hist))**. |
| **Section 8.1.2 Secondary Endpoints** | ○ Airway hyperresponsiveness expressed as PC20FEV$_1$(Meth) **or PC20FEV$_1$(Hist)** (Day 10-Day 12);<br>• Potential treatment effect (FP-025 versus placebo) on baseline parameters (i.e. Day 1 versus Day 10), including:<br>○ Blood eosinophils;<br>○ PC20FEV$_1$(Meth) **or PC20FEV$_1$(Hist)**;<br>○ FeNO. |
| **Section 8.1.3 Exploratory Endpoints** | • Potentially longer lasting treatment effects 14 days following Period 2 (FP-025 versus placebo) (spirometry (baseline FEV1), PC20FEV1(Meth) **or PC20FEV1(Hist)** and airway and systemic biomarkers (i.e. blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum). |

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| **Section 8.2 Overall Study Design** | On Screening Day 2, spirometry with subsequent methacholine/**histamine** challenge (for calculation of the $PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)**) will be performed, followed by sputum induction (SI).<br><br>Following a washout period of at least 3 weeks and up to approximately 7 weeks, eligible subjects will be enrolled into the study and randomized on Day 1 if their asthma is within continuation criteria of screening (as assessed by history, (rescue) medication use, baseline $FEV_1$ and $PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)**).<br><br>On Day 1, after a short checklist, the following tests will be performed for baseline purposes, i.e. blood and urine sampling (both for routine safety and biomarkers), FeNO, IOS (if logistics allow), spirometry, EBC, NAL, NAB, methacholine/**histamine** challenge (with calculation of $PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)** for stability/eligibility check) and SI.<br><br>On Day 1 of Period 1, subjects should still have stable asthma (based on continuation criteria as applicable in the allergen challenge: i.e. history/medication use/spirometry and ~~PC20Methacholine~~ **$PC20FEV_1$(Meth)/$PC20FEV_1$(Hist)**) in order to be randomized by a blinded pharmacist and start with the study drug administration.<br><br>On Day 10 (i.e. approximately 24 hours pre-allergen), approximately 30-60 minutes after first daily dosing, subjects undergo the same procedures as on Day 1 (i.e. safety/stability check, blood and urine collection (biomarkers), FeNO, EBC, NAL, NAB, IOS (if logistics allow), spirometry, methacholine/**histamine** challenge and SI.<br><br>Fourteen (14) days (± 2 days) after final administration of FP-025 or placebo on Day 12 of Period 2, a follow-up visit will be scheduled. This visit will not only serve as a follow-up visit (e.g. checking weight and AEs, performing physical examination, ECG and pregnancy test, checking vital signs and collecting blood for clinical safety laboratory assessments), the 'duration of efficacy' will also be explored by performing the same procedures as performed on Day 1 and Day 10 (i.e. blood and urine samplings (safety/biomarkers), FeNO, EBC, NAL, NAB, IOS (if logistics allow), spirometry, methacholine/**histamine** challenge and SI). |
| **Section 8.3 Inhaled allergen (HDM) challenge** | <u>Continuation criteria allergen challenge:</u><br><br>Furthermore, $PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)** on Day 1 of Period 1 should be within 1.5 doubling doses of Screening and on Day 1 of Period 2 within 1 doubling dose of Day 1 of Period 1. Should the subject's values on Day 1 of either study period fall outside these ranges, subject may be rescheduled depending on the cause and at the discretion of the investigator. |

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| **Section 8.4.2.1 Baseline Characteristics** | Eligible subjects will furthermore be screened for the following baseline assessments/inclusion criteria:<br>• Spirometry (refer to Section 8.4.3.5)<br>• Methacholine/**histamine** challenge (refer to Section 8.4.3.6) |
| **Section 8.4.3.5 IOS and Spirometry** | IOS:<br>If logistics allow, IOS measurements will also be performed approximately 4-5 minutes before spirometric measurements scheduled on days without allergen challenge, or, in case of methacholine/**histamine** challenge, as defined in the respective SOPs/WIs. |
| **Section 8.3.4.6 Methacholine Challenge** | Changed section title to: Methacholine/**Histamine** Challenge<br><br>Methacholine/**histamine** challenge will be performed according to the SOP in line with current guidelines. **When methacholine bromide is not available, histamine should be used for the challenge. Whenever possible, subjects should receive the same challenge agent (i.e. methacholine or histamine) throughout the study.**<br><br>Shortly, incremental doses of methacholine bromide/**histamine** will be inhaled for 2 minutes by tidal breathing at approximately 5 minutes intervals. The challenge will be discontinued if a fall in $FEV_1$ of ≥20% from post-diluent baseline has been reached, or until the highest concentration has been administered. If a fall in $FEV_1$ of ≥20% from baseline has been reached, a $PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)** will be calculated by extrapolation as defined in the manual/SOP/WI. After the challenge, subjects will be given salbutamol to aid recovery and lung function will be measured after approximately 15-30 minutes (as per manual/SOP/WI). |
| **Section 8.4.6 Order of Assessments** | During the study periods, all protocol related-assessments will be conducted at the same time of the day +/- 1 hour. This means that, e.g. if **the** methacholine/**histamine challenge** has been conducted on Day 1 at 10:00, then on Day 10 and 12 this should be done between 9-11 am (starting time); applicable for both study periods. |
| **Section 8.4.7 Allowed Time Windows for PD, Safety and PK Assessments** | Scheduled time for exhaled air, EBC, NAL, NAB, IOS, spirometry, methacholine/**histamine** challenge and SI |
| **Section 8.5.1 Inclusion Criteria** | 8 On Screening Day 2, $PC20FEV_1$(Meth) should be <16 mg/mL if methacholine chloride is used (or adjusted by a factor of 1.2 if methacholine bromide is used). **If histamine is used, $PC20FEV_1$(Hist) should be <16 mg/mL.** |

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| **Section 8.10.1 Pharmacodynamic Parameters** | Secondary parameters<br><br>Methacholine/**histamine** challenge:<br>• Provocative concentration of methacholine **or histamine** causing 20% fall of the $FEV_1$ from baseline ($PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)**)<br><br>Exploratory parameters<br><br>**Impulse-oscillometry (IOS) post-methacholine/histamine challenge** |
| **Section 10.3 Pharmacodynamic Parameters** | All $FEV_1$ values post-allergen will be expressed as %fall from post-diluent baseline ($FEV_1$) and the highest, technically satisfactory $FEV_1$ value per pre-defined time point will be included into analysis. This excludes additional time points from analysis (e.g. if between a time interval additional spirometry has been performed for safety reasons – these values do not need to be included into analysis). $PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)** will be calculated as defined in the SOP. Analyses of all other parameters will be detailed in the SAP.<br><br>Secondary:<br>• Changes in allergen-induced AHR: i.e: $PC20FEV_1$(Meth) **or $PC20FEV_1$(Hist)** pre-post allergen (Day 10 versus Day12)<br>• Changes in blood eosinophils, FeNO and PC20FEV1(Meth) **or $PC20FEV_1$(Hist)** Day 1 versus Day 10 (potential treatment effect)<br><br>Exploratory:<br>• Treatment effects: differences in changes in the following parameters (Day 1 versus Day 10): spirometry (baseline $FEV_1$), $PC20FEV_1$(Meth)/**$PC20FEV_1$(Hist)** and airway and systemic biomarkers (i.e. in blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum).<br>• Potentially longer lasting treatment effects 14 days following Period 2 (spirometry (baseline $FEV_1$), $PC20FEV_1$(Meth)/**$PC20FEV_1$(Hist)** and airway and systemic biomarkers (i.e. in blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum).<br>• IOS measurements following inhaled allergen and eventual methacholine/**histamine** challenges to assess the effect of study medication on the peripheral airways and for additional safety. |
| **Section 11 References** | **15. Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. (1978) Reproducibility and comparison of responses to inhaled histamine and methacholine. Thorax. 33(6): 705–710.**<br><br>**16 Ravensberg AJ, Van Rensen ELJ, Grootendorst DC, De Kluijver J, Diamant Z, Ricciardolo FLM, Sterk PJ. (2007) Validated safety predictions of airway responses to house dust mite in asthma. Clin Exp Allergy 37(1):100-7.** |

**Amendment 1, dated 09 August 2018**, original protocol issued 05 March 2018.

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20EC of the European Parliament and the Council of the European Union.

The address information was updated, additional IOS measurements were added, optional asthma symptoms evaluations using VAS were added, duration of the screening period was extended, the number and timing of phone calls was updated, prior and concomitant medications were further specified, an additional blood biomarker sampling was added, and a section on pregnancy was added.

| Section | Update |
|---|---|
| **Header** | QPS: ~~CS0290 – 160508~~**160509**<br>~~05 March 2018~~**09August 2018**, ~~Version 1~~**Amendment 1** |
| **Title page** | Document status version ~~Version 1~~**Amendment 1**<br>Date ~~05 March 2018~~**09August 2018** |
| **Key study personnel** | Prof. Zuzana Diamant, MD PhD, pulmonologist – clinical pharmacologist<br>QPS Netherlands B.V.<br>~~Petrus Campersingel 123~~<br>**Hanzeplein 1, entrance 53**<br>9713 ~~AG~~**GZ** Groningen<br>The Netherlands<br><br>Rene Lutter, PhD<br>~~Academic Medical Centre,~~**Amsterdam UMC, University of** Amsterdam<br><br>Collaborating parties / **Sub-investigators**:<br><br>Els Weersink<br>~~Academic Medical Centre,~~**Amsterdam UMC, University of** Amsterdam<br><br>Khalid Abd-Elaziz, MBBS MD, clinical pharmacologist<br>QPS Netherlands B.V.<br>~~Petrus Campersingel 123~~<br>**Hanzeplein 1, entrance 53**<br>9713 ~~AG~~**GZ** Groningen<br>The Netherlands<br><br>Maarten van den Berge, MD PhD**, pulmonologist**<br><br>Clinical Facility:<br>QPS Netherlands B.V.<br>~~Petrus Campersingel 123~~<br>**Hanzeplein 1, entrance 53**<br>9713 ~~AG~~**GZ** Groningen<br><br>~~Academic Medical Centre,~~**Amsterdam UMC, University of** Amsterdam<br>C3, Lung Function Laboratory<br><br>Prof. A.H. Zwinderman, PhD<br>~~Academic Medical Centre,~~**Amsterdam UMC, University of** Amsterdam<br><br>~~Depts. Respiratory Medicine and Experimental Immunology~~<br>**Department of Clinical Epidemiology, Bioinformatics, and Biostatistics** |

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| | QPS ~~PV~~**Safety** Unit<br>~~Email: safetypv@qps.com~~<br>**Email safetyFP025@qps.com** |
| **Section 4, List of abbreviations** | ~~PV Pharmacovigilance~~<br>**VAS Visual Analogue Scale** |
| **Section 5, Protocol synopsis**<br>**Section 8.1.2, Secondary endpoints** | Secondary endpoints:<br>• Safety parameters include physical examination, clinical signs/symptoms reporting (MedDRA), (S)AEs, vital signs, lung function measurements**, overall asthma symptoms,** ECG and clinical safety laboratory outcomes (blood/urine). |
| **Section 5, Summary of study design**<br>**Section 8.2, Overall study design** | Prior to the study, there will be a Screening period consisting of 3 clinic visits (within ~~9 days~~**approximately 6 weeks**) to test subject eligibility.<br>On Screening Day 1, the following procedures will take place: signing of the informed consent, a check of the inclusion/exclusion criteria, medical history and demographics, physical examination, measurements of vital signs, height and weight, calculation of body mass index (BMI), blood sampling for blood eosinophils and clinical safety laboratory assessments, ECG, allergy testing and screening for drug and alcohol use, smoking (i.e. cotinine), pregnancy and HIV, Hepatitis B and Hepatitis C (i.e. serology). **At the discretion of the Principal Investigator (PI) or sub-investigators, some Screening Day 1 assessments (e.g. safety lab/pregnancy test/drug screen/cotinine screen/physical examination) may be moved to and/or repeated on Screening Day 2 and/or Screening Day 3.**<br>~~After~~**Within approximately 40 days after** Screening Day 1, potentially eligible subjects will start with more specific screening procedures (on 2 separate days ~~within 5 days~~**).(Screening Day 2 and Screening Day 3), preferably within 5 days, but maximally within 8 days). Subjects will be called by a physician or research staff within approximately 1 week prior to Screening Day 2 to check their asthma stability/medication intake/general health status.** On Screening Day 2, spirometry with subsequent methacholine challenge (for calculation of the $PC20FEV_1$(Meth)) will be performed, followed by sputum induction (SI). **If logistics allow, IOS will be added to spirometric measurements.** On Screening Day 3, there will be an inhaled HDM challenge with airway response measurements (**including** IOS and spirometry) **during and** up to approximately 8 hours post-allergen. **On completion of all response measurements following both inhalational challenges on Screening Day 2 and 3, subjects will receive rescue bronchodilator medication and lung function will be measured.** Upon leaving the clinic, all subjects will be provided with rescue medication (short acting beta2-agonist) to be used on an 'as needed basis' throughout the study. ~~After every HDM challenge the patient will receive patient emergency instructions~~**In addition, after each inhalational challenge, subjects will receive emergency instructions, and will be provided rescue medication (Ventolin). Within approximately 48 hours after Screening Day 3, subjects will be called** |

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| | **by a physician or research staff to check their asthma status/rescue medication use and general health.**<br><br>Following a washout period of at least 3 weeks and up to **approximately** 7 weeks, eligible subjects will be ~~randomized~~**enrolled** into the study~~.~~ **and randomized on Day 1 if their asthma is within continuation criteria of screening (as assessed by history, (rescue) medication use, baseline $FEV_1$ and $PC20FEV_1$(Meth)).** The study will consist of two identical study periods of 12 treatment days each, separated by a washout period of at least 3 weeks (and ~~no more than~~**up to approximately** 7 weeks).<br><br>**Subjects will be called by a physician or research staff within approximately 1 week prior to Day -1 of each study period to check their asthma stability/ medication intake/ general health status.** On Day -1, the checks performed on Screening Day 1 (i.e. medical history (includes asthma medication use, Adverse Events and asthma stability check)), physical examination, vital signs, weight, ECG, blood collection for clinical safety laboratory assessments and drug, alcohol, cotinine and pregnancy testing) will be repeated to reconfirm eligibility. **Subjects stay overnight in the unit at the discretion of the PI or subinvestigator.**<br><br>On Day 1, after a short checklist, the following tests will be performed for baseline purposes, i.e. blood and urine sampling (both for routine safety and biomarkers), FeNO, **IOS (if logistics allow),** spirometry, EBC, NAL, NAB, methacholine challenge (with calculation of $PC20FEV_1$(Meth) for stability/eligibility check) and SI.<br><br>On Day 1 of Period 1, subjects should still have stable asthma (based on ~~history/AEs~~**continuation criteria as applicable in the allergen challenge: i.e. history**/medication use/spirometry and PC20Methacholine) in order to be randomized by a blinded pharmacist and start with the study drug administration. **Should a subject not comply to the stability criteria, (s)he may be rescheduled at the discretion of the (co)PI or co-Investigator, depending on the underlying cause.**<br><br>In the event study medication will be taken at home (between 6-10 am/pm), the subject will be contacted **twice a day** on dosing days to receive confirmation of study medication intake.<br><br>On Day 10 (i.e. approximately 24 hours pre-allergen), approximately 30-60 minutes after first daily dosing, subjects undergo the same procedures as on Day 1 (i.e. safety/stability check, blood and urine collection (biomarkers), FeNO, EBC, NAL, NAB, **IOS (if logistics allow),** spirometry, methacholine challenge and SI. Subjects stay overnight in the unit. On Day 11, approx. 30-60 minutes after the morning dose, an inhaled allergen (HDM) challenge will be performed with airway response measurements (IOS and spirometry). During this study day, repeated PK sampling and blood biomarker sampling will be performed. ~~IOS measurements,~~ EBC, NAL and NAB will be performed at approximately 6.5 hours post allergen challenge. Subjects stay overnight in the unit.<br><br>~~There will be a check on the subjects within 1 week after each study period~~ ~~or~~**Within approximately 72 hours after Day 12 of each study period,** |

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| | **subjects will be called by a physician to check their asthma/medication intake/AEs and general health. If deemed necessary by the (co)PI, there may be another check on the subjects before and/or after each study period and in any case** following drop-out (either by phone or if needed and in case of drop-out, onsite the clinic).<br><br>Fourteen (14) days (± 2 days) after final administration of FP-025 or placebo on Day 12 of Period 2, a follow-up visit will be scheduled. This visit will not only serve as a follow-up visit (e.g. checking weight and AEs, performing physical examination, ECG and pregnancy test, checking vital signs and collecting blood for clinical safety laboratory assessments), the 'duration of efficacy' will also be explored by performing the same procedures as performed on Day 1 and Day 10 (i.e. blood and urine samplings (safety/biomarkers), FeNO, EBC, NAL, NAB, **IOS (if logistics allow),** spirometry, methacholine challenge and SI). |
| **Section 5, Pharmacodynamic parameters** | Impulse-oscillometry (IOS)**, post-allergen challenge,** mainly:<br><br>Exploratory parameters:<br>**Impulse-oscillometry (IOS) post-methacholine challenge** |
| **Section 5, Safety parameters**<br><br>**Section 8.10.2, Safety and tolerability parameters** | • Physical examination<br>• **(Optional) asthma symptoms evaluation (a VAS scale may be used for this purpose)**<br>• Clinical signs/symptoms reporting (MedDRA)<br>• (S)AEs<br>• Vital signs (HR, BP, temperature +/- $SpO_2$)<br>• Lung function measurements ($FEV_1$, $FEV_1$/FVC, PEF**, IOS**) |
| **Section 5, Statistical methodology**<br><br>**Section 10.3, pharmacodynamic parameters** | • To explore the effect of study medications on gene-transcriptomics from NAB pre- versus post-allergen.<br>• **IOS measurements following inhaled allergen and eventual methacholine challenges to assess the effect of study medication on the peripheral airways and for additional safety.**<br>• Only the MedDRA (safety) and PK parameters will be reported descriptively; the others will be analyzed either by paired t-testings or by ANOVA. |
| **Section 5, Table 5-1** | Screening within **~~9 days~~6 weeks**<br>(washout period of 3-**~~7weeks~~approximately 7 weeks** between periods)<br>S3[a]<br>In-/exclusion criteria[d]<br>Reconfirmation of Eligibility[~~d~~e]<br><br>Footnotes were updated.<br><br>~~Patient~~**Subject** instructions and/or diary review[er]<br><br>Urine drug screen, alcohol breath test, cotinine screen moved from Day 10 to Day 9.<br>**(Optional) overall asthma symptoms on Day 1, Day 10, Day 11, and Day 12.**<br>**Randomization on Day 1**<br>Optional ambulant visit <7 days after dosing on Day 12. |

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| | **Check health status optional on Day S2, S3, and Day 9. On Day -1, 1, 10, 11, and 12.**<br>**Phone call on Day S2, S3, -1, 2-4 5, and 6-8 days after dosing. Optional on Day 9.**<br>Footnotes:<br>~~a. Screening Day S3 should start within 5 days after Day S2.~~<br>**a.** **At the discretion of the PI or sub-investigator, Screening Day S1 some assessments (e.g. safety lab//pregnancy test/drug screen/cotinine screen/physical examination) can be moved to and/or repeated on Screening Day S2 and/or Screening Day S3. Screening Day S2 should start within approximately 40 days after Screening Day S1. Screening Day S3 preferably should start within 5 days after Day S2 but maximally within 8 days after Day S2.**<br>**b.** FeNO, EBC, NAL, NAB, spirometry, methacholine challenge and SI to be performed in the morning (pre-dose)~~; order of assessments depends on the day: on~~**. On Day** 1, spirometry will be performed immediately after FeNO (since eligibility criterion).<br>**d.** **Only on Screening Day S1, Screening Day S2 and Day-1 of Treatment Period 1.**<br>**e.** By Medical history~~/AEs~~/Medication use/FEV1 and PC20methacholine.<br>**j.** IOS to be performed **approximately 4-5 mins** before **all spirometric measurements during** inhaled allergen challenge, **in any case at baseline,** after diluent, and t=0.5, 1, 2, **3,** 4, 5, 6, 7 and 8 hours after inhaled allergen challenge~~.~~**, and after rescue medication. If logistics allow, IOS will also be performed approximately 4-5 minutes before spirometric measurements on days without inhaled allergen challenge.**<br>**k.** Screening Day S3 and Day 11: spirometry to be performed t=0~~,~~ **(i.e. pre-allergen and 10 mins post-diluent)** and 10, 20, 30, 45 minutes and t=1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hours after inhaled allergen challenge. If required for safety, additional spirometry to be performed e.g. at t=2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 hours after inhaled allergen challenge. If at Screening, there is a fall of 10-15% from baseline at 8 hours post-allergen, $FEV_1$ can be measured up to 9 h post-allergen.<br>**l.** **To be performed before and after inhaled allergen challenge and methacholine challenge and after administration of ventolin.**<br>**m.** **To be performed after eligibility check (including $FEV_1$) and methacholine challenge with subsequent $PC20FEV_1$(Meth) calculation on Day 1 of Treatment Period 1.**<br>**n.** **On Day 12 and at Follow-up, collected together with blood collection for clinical safety. On Day 11 together with pre-dose PK blood collection and approximately 6-7 hours post-allergen challenge**. Additional PD blood sampling for PBMC collection only on Day 1 and Day 10.<br>**p.** Collection times PK Day 11: (before first daily dose (t=0 hours), 1, 2, 4, 6, 8 and 9 hours post first daily dose). If clashing with other measurements then the efficacy measurements will prevail. **At Follow-up, collected together with blood collection for clinical safety and biomarkers.**<br>q. **Subjects have to stay overnight from Day -1 to Day 1 and from Day 10 to Day 12 for each period. An overnight stay from S2 to S3 is optional as is an overnight stay from Day 9 to Day 10.** Residence is preferred, but depending on the personal situation of the subject (at the discretion of the investigator, including living far or close from the clinic) and his/her medical condition a subject may or may not stay overnight. Subjects can only be dismissed from the unit at the discretion of the investigator and with rescue medication, personalized written instructions on how and when to use the rescue medication and emergency phone numbers.<br>**s.** **Phone call by a physician or research staff will be performed within approximately 1 week prior to Screening Day S2, within approximately 48 hours after Screening Day S3, within approximately 1 week prior to Day -1 of each treatment period, twice a day during the treatment periods when study medication is taken at home, within approximately 72 hours after Day 12 of each treatment period and within approximately 1 week after dosing on Day 12 of each period. If deemed necessary by the (co)PI more frequent phone calls or check-up visits may be scheduled during Screening/study.** |
| **Section 6.2.2, Allergen challenge model** | Inhaled allergen challenge can be safely conducted in dedicated research settings with ample expertise and experience with this disease model and the management of acute bronchoconstriction. Safety aspects and rescue medication will be managed and customized per subject by the investigator [9], and may include ~~any~~**combinations** of the following: administration of rescue medication (i.e. short-acting beta2-agonist) following allergen challenge, written instructions, emergency phone numbers, a follow-up |

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| | phone call and rescue medication; or any other safety measures as deemed necessary by the investigator. |
| **Section 8.3, Inhaled allergen (HDM) challenge** | The allergen challenge will be conducted using a standardized technique with incremental doses of inhaled house dust mite (HDM) extract as described in the literature [9] and as further detailed in the standard operating procedure (SOP) **and work instruction (WI)**.<br><br>During the study, IOS recordings (see Section 8.4.3.5) will precede spirometric measurements, and hence, will be performed within approximately **4-**5 min before actual spirometry time points at pre-, **during** and post-allergen **challenge**.<br><br>~~Patients~~**Subjects** with an LAR will be contacted for a phone call check up by research staff usually within 24-**48** hours after the challenge day, or otherwise at the discretion of the investigator.<br><br>During both study periods, generally, the 3 highest incremental doses of HDM extract will be used that provoked **(an EAR and subsequently)** a LAR during Screening. If during screening the EAR **(and subsequent LAR)** was reached following inhalation of **1 or** 2 allergen doses, an additional ~~lowest~~**lower** dose ~~will~~ **(to complete in total 3 doses) may** be added **at the discretion of the investigator** to ensure consistency of the procedure across subjects. ~~All other~~ **However, safety will always prevail, meaning that in some cases, subjects will receive fewer allergen doses during Screening and the study periods. While allergen challenge (number and allergen concentrations) will be customized per subject, the same number and concentrations of allergen extract will be inhaled during both study periods by the same subject. All** details will be described in the SOP **and WI**.<br><br>Baseline $FEV_1$ at least 70% of predicted value at Screening Day 2 (inclusive) and $FEV_1$ on Screening Day 3 within 10% (counted from the absolute value in ~~litres~~**liters**) of Screening Day 2 and ≥65% predicted (and ≥1.8 L). On Day 1 of Period 1, $FEV_1$ should be within 10% (again from absolute value in ~~litres~~**liters**) of Screening baseline $FEV_1$ (i.e. mean baseline $FEV_1$ Screening Day 2 and Screening Day 3; and ≥65% predicted (and ≥1.8 L). Baseline $FEV_1$ (in ~~litres~~**liters**) in both study periods should be within 10% and ≥65% predicted (and ≥1.8 L). |
| **Section 8.4.2.2, Prior and concomitant medications** | All **lung and anti-allergic** medications **and vaccines** taken from 3 months prior to screening until EOS or started during the course of the study **and all other medications taken from screening until EOS or started during the course of the study** will be recorded on the Concomitant Medications page. |
| **Section 8.4.3.1, Exhaled nitric oxide** | Fractionated nitric oxide (FeNO) will be measured from exhaled air by Niox Vero® device (Circassia, Oxford, United Kingdom) according to guidelines as detailed in our SOP/**WI**. |
| **Section 8.4.3.2, Exhaled breath condensate (EBC)** | Exhaled breath condensate will be collected according to our SOP/**WI** using an Ecoscreen condenser (CareFusion, San Diego, CA, United States). |

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| | Analysis will be performed in **the** AMC according to local SOP. In addition, FP-025 concentration may be exploratively quantified in (some of) the samples. |
| **Section 8.4.3.3, Nasal lavage (NAL)** | Analysis will be performed in **the** AMC. |
| **Section 8.4.3.4, Nasal brush (NAB)** | Processing, storage and analysis of the NAB samples will be described in the **respective** SOPs/**WIs**. Analysis will be performed in UMCG. |
| **Section 8.4.3.5, IOS and Spirometry** | Spirometry<br>Lung function tests will be performed with a standardized/calibrated spirometer according to the SOPs/**WIs** in line with current guidelines.<br><br>IOS<br>IOS measurements will be performed approximately **4-**5 minutes before ~~spirometry pre-~~**all spirometric measurements during and after** allergen challenge ~~and post-~~**, in any case at baseline, after** diluent**,** and ~~subsequently~~ at 0.5, 1, 2, **3,** 4, 5, 6, 7, 8 h post-allergen challenge and at recovery (post-salbutamol). **If logistics allow, IOS measurements will also be performed approximately 4-5 minutes before spirometric measurements scheduled on days without allergen challenge, or, in case of methacholine challenge, as defined in the respective SOPs/WIs.** |
| **Section 8.4.3.6, Methacholine challenge** | Methacholine challenge will be performed according to the SOP in line with current guidelines. Shortly, incremental doses of methacholine bromide will be inhaled for 2 minutes by tidal breathing at **approximately** 5 minutes intervals. The challenge will be discontinued if a fall in $FEV_1$ of ≥20% from post-diluent baseline has been reached, ~~of~~**or** until the highest concentration has been administered. If a fall in $FEV_1$ of ≥20% from baseline has been reached, a $PC20FEV_1$(Meth) will be calculated by extrapolation~~.~~ **as defined in the manual/SOP/WI**. After the challenge, subjects will be ~~dosed with~~**given** salbutamol to aid recovery~~.~~ **and lung function will be measured after approximately 15-30 minutes (as per manual/SOP/WI).** |
| **Section 8.4.3.7, Sputum induction** | Sputum induction will be performed according to guidelines as detailed in the ~~SOPs~~**SOP/WI**.<br><br>Sputum samples will be processed by a trained and qualified technician within 2 hours of induction onsite the respective research ~~centre~~**center**, according to the ~~SOPs~~**SOP/WI**. The cell pellets will be cytospinned and supernatants will be frozen in tubes pending analysis. Staining (DiffQui(c)k or Giemsa (when required)) of the cytospins and differential cell counts in addition to analysis of soluble biomarkers **from the supernatants** will be performed in **the** AMC **according to respective SOPs**. |
| **Section 8.4.4.6, Allergy testing** | Allergy testing will include at least the following allergens: house dust mite (D. Pteronyssinus/ **D. farinae**), animal dander (at least cat**,** ~~and~~ dog **and horse**), fungi **(Asp Fumigatus, Alternaria Alternata)**, and pollen (grass, trees**, Artemisia Vulgaris**). |
| **Section 8.4.4.7, Overall asthma symptoms** | **Overall asthma symptoms will be assessed using a visual analogue scale (VAS).** |

<table>
<tr><td>Section 8.4.6, Order of assessments</td><td>Blood biomarker sampling (eosinophils and biomarkers): Day 1; Day 10 (together with the safety lab); Day 11 (together with pre-dose PK measurements) and approximately 6-7 hours post-allergen challenge; Day 12; and at Follow-up (together with the safety lab).</td></tr>
<tr><td>Section 8.4.11, Total blood volume</td><td>Table 8-2: Blood volume
<table>
<tr><th></th><th>Vol x Frequency</th><th>Total (mL)</th></tr>
<tr><td>Blood Chemistry (incl. virus serology, pregnancy testing)</td><td>4.5 mL x <s>10</s>8 and 3.5 mL x 1</td><td>45.039.5 mL</td></tr>
<tr><td>Hematology</td><td>3.0 mL x 8</td><td>24.0 mL</td></tr>
<tr><td>Coagulation</td><td>2.7 mL x 8</td><td>21.6 mL</td></tr>
<tr><td>PK</td><td>6.0 mL x 15</td><td>90.0 mL</td></tr>
<tr><td>PD (inflammation markers and biomarkers)</td><td>4<s>9</s>3.0 mL x <s>9</s>11 (EDTA) and 3.5<s>.0</s> mL x <s>9</s>11 (serum) and 4.0 mL x 11(heparin)</td><td><s>81.0</s>115.5 mL</td></tr>
<tr><td>PD (PBMC)</td><td><s>20</s>18.0 mL x 4 (<s>heparine</s>heparin)</td><td>8072.0 mL</td></tr>
<tr><td>Total blood volume</td><td></td><td><s>341</s>362.6 mL</td></tr>
</table></td></tr>
<tr><td>Section 8.6.2, Rescue medication</td><td>On Screening Day 3, all <s>patients</s>subjects will be provided with a short acting beta2-agonist (100 µg salbutamol/dose). <s>Patients</s>Subjects will be instructed to use it throughout the study as rescue medication on an ‘as needed basis’.</td></tr>
<tr><td>Section 8.6.6, Study drug administration</td><td>In the event the study medication is taken at home the number of capsules taken will be recorded in <s>patient</s>a subject dairy.</td></tr>
<tr><td>Section 8.10.2, Safety and tolerability parameters</td><td>• Overall asthma symptoms (VAS)<br>• Lung function measurements ($FEV_1$, $FEV_1$/FVC, PEF, IOS)</td></tr>
<tr><td>Section 9.1.5, Follow-up of adverse events</td><td>Adverse events still ongoing <s>96 hours</s> after drug intake must be followed until resolution, or to a maximum of 28 days after study drug discontinuation.<br><br>If an AE is present at the follow-up visit/ end-of-study visit, it should be followed to resolution or stabilization unless the subject is lost to follow-up. Resolution means the subject has returned to baseline state of health or the Investigator does not expect any further improvement or worsening of the event.</td></tr>
<tr><td>Section 9.2.1.2, Pregnancy</td><td>All initial reports of pregnancy, including pregnancy outcome (follow-up) in female subjects or in partners of male subjects, must be reported to the QPS Safety Unit (SU) by the investigator within 24 hours of his/her knowledge of the event using a Pregnancy Form.<br><br>The investigator will contact the subject at the expected time of delivery for follow-up. Abnormal pregnancy outcomes (e.g., spontaneous or induced abortion, stillbirth, neonatal death, congenital abnormality, birth defect) are considered SAEs and must be reported using the Serious Adverse Event Form.</td></tr>
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| **Section 9.2.2.1 Before study drug initiation** | Serious adverse events occurring after signature of the Informed Consent and up to study drug initiation must be reported to QPS ~~PV~~**Safety** unit only if they are considered by the investigator to be related to study- mandated procedures. |
| **Section 9.2.2.3, After study drug discontinuation** | Therefore, these treatment-emergent SAEs are entered both in the ~~Foresee Pharmaceuticals Co., Ltd~~**QPS** drug safety and clinical databases, and must be reconciled before study closure. |
| **Section 9.2.2.4, Reporting procedures** | All SAEs must be reported by the investigator to QPS ~~PV~~**Safety** unit within 24 hours of the investigator's knowledge of the event.<br><br>These SAE forms must be e-mailed to QPS ~~PV~~**Safety** unit (see contact details page 4). The investigator must complete the SAE form in English (unless otherwise specified) and assess the relationship to study drug.<br><br>Follow-up information about a previously reported SAE must also be reported within 24 hours of receiving it. QPS ~~PV~~**Safety** unit may contact the investigator to obtain further information.<br><br>Suspected (considered related to the study drug) and Unexpected (not previously described in the reference safety document), Serious Adverse Reactions (SUSARs) will be expedited by QPS ~~PV~~**Safety** unit to Health Authorities, ECs/IRBs and investigators, as appropriate. SUSARs will not be subject to systematic unblinding. |
| **Section 9.2.2.5, Procedure for unblinding of serious suspected adverse reactions** | **The unblinding procedure for serious suspected adverse reactions is defined in the safety management plan.** |
| **Section 9.2.3, Follow-up of serious adverse events** | New SAEs occurring at any time after the End-of-Study or after the 28-day follow-up period after study drug discontinuation (whichever comes first) may be reported to QPS ~~PV~~**Safety** unit within 24 hours of the investigator's knowledge of the event, if felt appropriate by the investigators. |
| **Section 10.4, Safety and tolerability parameters** | The medical history is coded using the MedDRA version 20.1 **or higher** and listed.<br><br>All AEs and SAEs are coded using the MedDRA version 20.1 **or higher**.<br><br>The number and percentage of ~~patients~~**subjects** who experienced AEs coded with the same preferred term and SOC will be summarized by treatment group (in descending order according to the incidence in the investigational study drug group). |
| **Section 10.7, Baseline characteristics and concomitant medications** | Summary statistics (mean, median, SD, min, max, number of available observations) will be provided for continuous demographic variables (e.g., age, height, weight). Individual ~~patient~~**subject** listings of demographic data will be provided.<br><br>Qualitative demographic characteristics (gender, race) will be summarized by counts and percentages. Other baseline ~~patient~~**subject** characteristics (medical history, physical examination clinical findings, previous medications, inclusion/exclusion checklist) will only be listed. |

# 3 TABLE OF CONTENTS

**List of tables**

# 4 LIST OF ABBREVIATIONS

| | |
|---|---|
| ADME | Absorption, Distribution, Metabolism, Excretion |
| AE | Adverse Event |
| AHR | Airway hyperresponsiveness |
| ALT | Alanine aminotransferase/serum glutamic pyruvic transaminase (SGPT) |
| AMC | Academic Medical Centre |
| AST | Aspartate aminotransferase/serum glutamic oxaloacetic transaminase (SGOT) |
| ATC | Anatomic Therapeutic Chemical |
| AUC | Area Under the plasma concentration Curve |
| $AUC_{0\text{-}12h}$ | AUC from 0-12 hours |
| $AUC_{0\text{-}24h}$ | AUC from 0-24 hours |
| $AUC_{0\text{-}inf}$ | AUC from 0-infinity |
| $AUC_{0\text{-}tau}$ | AUC from 0-tau |
| $AUC_{0\text{-}t}$ | AUC from 0 to the last measurable concentration (t) |
| AX | Area under the reactance curve (IOS) |
| BID | Twice a day (*Bis In Die*) |
| BMI | Body Mass Index |
| CHO | Chinese Hamster Ovary |
| $C_{max}$ | Maximum plasma concentration |
| CRO | Contract Research Organization |
| CV | Coefficient of Variation |
| CS | Corticosteroids |
| CYP | Cytochrome P450 |
| EAR | Early asthmatic response |
| EBC | Exhaled Breath Condensate |
| EC | Ethics Committee |
| ECG | Electrocardiogram |
| eCRF | Electronic Case Report Form |
| EOS | End-of-Study |
| EudraCT | European drug regulatory affairs Clinical Trials |
| FeNO | Fractional exhaled Nitric Oxide |
| $FEV_1$ | Forced expiratory volume in 1 second |
| $FEV_1$ $AUC_{0\text{-}3h}$ | Area under the time-FEV1 response curve from zero to 3 hours post-allergen |

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| $FEV_1$ $AUC_{3-8h}$ | Area under the time-FEV1 response curve from 3 to 8 hours post-allergen |
| $FEV_1$ $AUC_{0-8h}$ | Area under the time-FEV1 response curve from zero to 8 hours post-allergen |
| Fres | Resonant frequency (IOS) |
| FVC | Forced vital capacity |
| GCP | Good Clinical Practice |
| GGT | Gamma-glutamyltransferase |
| GLP | Good Laboratory Practice |
| GMP | Good Manufacturing Practice |
| HDL | High-density Lipoprotein |
| HDM | House dust mite |
| HIV | Human Immunodeficiency Virus |
| HR | Heart Rate |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonization |
| ILC | Innate Lymphoid Cell |
| IOS | Impulse-oscillometry |
| i.v. | Intravenous(ly) |
| IRB | Institutional Review Board |
| LAR | Late asthmatic response |
| LDL | Low-density Lipoprotein |
| LLOQ | Lower Limit of Quantification |
| MAD | Multiple Ascending Dose |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MMP | Metalloproteinase |
| MPO | Myeloperoxidase |
| NAB | Nasal Brush |
| NAL | Nasal Lavage |
| NOAEL | No Observed Adverse Effect Level |
| PBMC | Peripheral Blood Mononuclear Cell |
| $PC20FEV_1$(Hist) | Provocative concentration of histamine causing a 20% fall in $FEV_1$ |
| $PC20FEV_1$(Meth) | Provocative concentration of methacholine causing a 20% fall in $FEV_1$ |
| P-gp | P-glycoprotein |
| PK | Pharmacokinetics |

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| QC | Quality Control |
| RCE | Coefficient of excretion |
| RR | Respiration rate |
| R5 | Resistance of 5 Hertz (IOS) |
| R20 | Resistance of 20 Hertz (IOS) |
| R5-R20 | Frequency dependence of resistance (IOS) |
| SAD | Single Ascending Dose |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SI | Sputum Induction |
| SPT | Skin Prick Testing |
| SD | Standard Deviation |
| SOC | System Organ Class |
| SOP | Standard Operating Procedure |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| Tau | Dosing interval |
| TEAE | Treatment-Related Adverse Event |
| $t_{max}$ | Time to reach maximum plasma concentration |
| VAS | Visual Analogue Scale |
| WHO | World Health Organization |
| X5 | Reactance at 5 Hertz (IOS) |

# 5 PROTOCOL SYNOPSIS

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| TITLE | The effect of FP-025, a MMP-12 inhibitor, on allergen-induced airway responses, airway inflammation and aspects of airway remodeling in subjects with mild eosinophilic house dust mite (HDM)-allergic asthma |
| PHASE | IIa |
| CENTERS | 2 centers:<br>• QPS Netherlands B.V., Groningen, The Netherlands<br>• Academic Medical Centre (AMC), Depts. of Respiratory Medicine and Experimental Immunology, Amsterdam, The Netherlands |
| INVESTIGATIONAL DRUG and DOSAGE FORM | FP-025 in capsules of 50 mg each |
| COMPARATIVE DRUG and DOSAGE FORM | Matching placebo capsules |
| ROUTE OF ADMINISTRATION | Oral administration |
| OBJECTIVES | ***Primary objective:***<br>• To determine the effect of FP-025 versus placebo on the allergen (HDM)-induced late asthmatic response expressed as $FEV_1$ $AUC_{3\text{-}8h}$ in subjects with clinically stable, mild allergic asthma and blood eosinophilia.<br>***Secondary objectives:***<br>• To determine the pharmacodynamics of FP-025 versus placebo on additional markers of airway physiology, including (additional measures of) the early and late response, airway hyperresponsiveness and small airways function; inflammatory markers (including blood eosinophils and FeNO) following inhaled HDM-challenge in subjects with clinically stable, mild allergic asthma and blood eosinophilia.<br>• To determine the treatment effect (i.e. Day 1 versus Day 10) of multiple oral doses of FP-025 versus placebo on baseline parameters (such as blood eosinophils, FeNO and $PC20FEV_1$(Meth)/$PC20FEV_1$(Hist)).<br>• To determine the safety and tolerability of multiple oral doses of FP-025 versus placebo in subjects with clinically stable, mild allergic asthma and blood eosinophilia.<br>• To assess the pharmacokinetics of multiple oral doses of FP-025 following inhaled HDM-challenge in subjects with clinically stable, mild allergic asthma and blood eosinophilia. |

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| | ***Exploratory objectives:***<br>• To explore the effect of FP-025 versus placebo on:<br>o Cellular and soluble inflammatory biomarkers in nasal lavage (NAL) and sputum;<br>o Nasal gene and microRNA expression in nasal brush (NAB);<br>o (Inflammatory) biomarkers in exhaled breath condensate (EBC), blood (eosinophil activation markers and potentially others as well) and urine (lipid mediators);<br>o Functional in vitro responses of peripheral blood mononuclear cells (PBMC);<br>o Expression and activity of MMP-12, other MMP's and markers of tissue remodeling in blood, sputum and potentially in NAL. |
| ENDPOINTS | ***Primary endpoint:***<br>The primary endpoint of this study is the effect of study treatments on $FEV_1$ $AUC_{3\text{-}8h}$ during the LAR (FP-025 versus placebo).<br>***Secondary endpoints:***<br>• Pharmacodynamic endpoints include the effect of study treatments on allergen (HDM)-induced changes in:<br>o LAR expressed as max% fall in $FEV_1$ from post-diluent baseline;<br>o Early asthmatic response (EAR) expressed as $FEV_1$ $AUC_{0\text{-}3h}$;<br>o EAR expressed as max% fall in $FEV_1$ from post-diluent baseline;<br>o Joint HDM-induced airway response expressed as $AUC_{0\text{-}8h}$;<br>o Airway hyperresponsiveness expressed as $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) (Day 10-Day 12);<br>o Small airway parameters following HDM-challenge (i.e. R5, R20, R5-R20, AX, X5, Fres);<br>o Fractionated nitric oxide (FeNO);<br>o Blood eosinophils.<br>• Potential treatment effect (FP-025 versus placebo) on baseline parameters (i.e. Day 1 versus Day 10), including:<br>o Blood eosinophils;<br>o $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist);<br>o FeNO.<br>• Safety parameters include physical examination, clinical signs/symptoms reporting (MedDRA), (S)AEs, vital signs, lung function measurements, overall asthma symptoms, ECG and clinical safety laboratory outcomes (blood/urine). |

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| | • Pharmacokinetic parameters of FP-025 in blood (plasma) include $C_{max}$, $t_{max}$, and $AUC_{0\text{-}tau}$.<br>***Exploratory endpoints:***<br>Exploratory pharmacodynamic parameters of FP-025 (Day 10 versus Day 12 = treatment effect on HDM-induced (airway) responses; and Day 1 versus Day 10 = treatment effect on baseline parameters) include:<br>• Cellular and soluble markers of inflammation in nasal lavage (NAL) and sputum, i.e.: eosinophil and lymphocyte counts, total cell counts, levels of ECP, MPO, IL-1b, FGF, VEGF and IL-5 and relative coefficient of excretion (RCE: ratio A2M in sputum/NAL relative to that in serum divided by the ratio of albumin sputum/NAL relative to that in serum);<br>• Genome-wide gene and microRNA expression and DNA methylation in nasal brush (NAB) samples;<br>• Biomarkers of oxidative stress and cellular activation in exhaled breath condensate (EBC);<br>• Cellular (eosinophil activation) and soluble inflammatory mediators (IL-5) in blood;<br>• Lipid mediators in urine (to be determined; leukotrienes);<br>• Peripheral blood mononuclear cell (PBMC) counts and functional parameters (cytokine release; Elispot assay);<br>• Expression and activity of MMP-12, other MMP's and markers of tissue remodeling in blood, NAL and sputum;<br>• Potentially longer lasting treatment effects 14 days following Period 2 (FP-025 versus placebo) (spirometry (baseline $FEV_1$), $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) and airway and systemic biomarkers (i.e. blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum). |
| DESIGN | A randomized, placebo-controlled, double-blind, 2-way cross-over, 2-centre study |
| TREATMENTS | Subjects will receive 400 mg FP-025 (8x 50 mg capsules, BID) or matching placebo (8 capsules, BID) from Day 1 (evening = first dose) to Day 12 (morning = last dose); a total of 22 doses per study period during two study periods. |
| SUMMARY OF STUDY DESIGN | Prior to the study, there will be a Screening period consisting of 3 clinic visits (within approximately 6 weeks) to test subject eligibility.<br>On Screening Day 1, the following procedures will take place: signing of the informed consent, a check of the inclusion/exclusion criteria, medical history and demographics, physical examination, measurements of vital signs, height and |

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| | weight, calculation of body mass index (BMI), blood sampling for blood eosinophils and clinical safety laboratory assessments, ECG, allergy testing and screening for drug and alcohol use, smoking (i.e. cotinine), pregnancy and HIV, Hepatitis B and Hepatitis C (i.e. serology). At the discretion of the Principal Investigator (PI) or sub-investigators, some Screening Day 1 assessments (e.g. safety lab/pregnancy test/drug screen/cotinine screen/physical examination) may be moved to and/or repeated on Screening Day 2 and/or Screening Day 3.<br><br>Within approximately 40 days after Screening Day 1, potentially eligible subjects will start with more specific screening procedures (on 2 separate days (Screening Day 2 and Screening Day 3), preferably within 5 days, but maximally within 8 days). Subjects will be called by a physician or research staff within approximately 1 week prior to Screening Day 2 to check their asthma stability/medication intake/general health status. On Screening Day 2, spirometry with subsequent methacholine/histamine challenge (for calculation of the $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist)) will be performed, followed by sputum induction (SI). If logistics allow, IOS will be added to spirometric measurements. On Screening Day 3, there will be an inhaled HDM challenge with airway response measurements (including IOS and spirometry) during and up to approximately 8 hours post-allergen. On completion of all response measurements following both inhalational challenges on Screening Day 2 and 3, subjects will receive rescue bronchodilator medication and lung function will be measured. Upon leaving the clinic, all subjects will be provided with rescue medication (short acting beta2-agonist) to be used on an 'as needed basis' throughout the study. In addition, after each inhalational challenge, subjects will receive emergency instructions, and will be provided rescue medication (Ventolin). Within approximately 48 hours after Screening Day 3, subjects will be called by a physician or research staff to check their asthma status/rescue medication use and general health.<br><br>Following a washout period of at least 3 weeks and up to approximately 7 weeks, eligible subjects will be enrolled into the study and randomized on Day 1 if their asthma is within continuation criteria of screening (as assessed by history, (rescue) medication use, baseline $FEV_1$ and $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist)). The study will consist of two identical study periods of 12 treatment days each, separated by a washout period of at least 3 weeks (and up to approximately 7 weeks). Approximately 36 eligible subjects will be enrolled, to yield 32 evaluable subjects who will be treated with both FP-025 (400 mg BID) or placebo in a cross-over design from the evening of Day 1 till the morning of Day 12 (22 doses per study period in total). Based on a double-blind randomized schedule, 16 |

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| | subjects will receive placebo in Period 1 and FP-025 in Period 2 and 16 subjects will receive FP-025 in Period 1 and placebo in Period 2. Both study periods will follow the same schedule of procedures.<br><br>Subjects will be called by a physician or research staff within approximately 1 week prior to Day -1 of each study period to check their asthma stability/ medication intake/ general health status. On Day -1, the checks performed on Screening Day 1 (i.e. medical history (includes asthma medication use, Adverse Events and asthma stability check)), physical examination, vital signs, weight, ECG, blood collection for clinical safety laboratory assessments and drug, alcohol, cotinine and pregnancy testing) will be repeated to reconfirm eligibility. Subjects stay overnight in the unit at the discretion of the PI or subinvestigator.<br><br>On Day 1, after a short checklist, the following tests will be performed for baseline purposes, i.e. blood and urine sampling (both for routine safety and biomarkers), FeNO, IOS (if logistics allow), spirometry, EBC, NAL, NAB, methacholine/histamine challenge (with calculation of $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) for stability/eligibility check) and SI.<br><br>On Day 1 of Period 1, subjects should still have stable asthma (based on continuation criteria as applicable in the allergen challenge: i.e. history/medication use/spirometry and $PC20FEV_1$(Meth)/$PC20FEV_1$(Hist)) in order to be randomized by a blinded pharmacist and start with the study drug administration. Should a subject not comply to the stability criteria, (s)he may be rescheduled at the discretion of the (co)PI or co-Investigator, depending on the underlying cause.<br><br>Starting on Day 1 with an evening dose, FP-025 or placebo will be given over 12 days (BID from Day 2 up to and including Day 11, final morning dose on Day 12) to ensure stable FP-025 levels during Days 10-12 (outcome measure days).<br><br>To aid compliance, during Day 2-9, subjects will either receive study medication onsite the clinic (observed dosing) or take their study medication at home or combinations of these. In the event study medication will be taken at home (between 6-10 am/pm), the subject will be contacted twice a day on dosing days to receive confirmation of study medication intake. In addition, from Screening Day 3 onwards and during study, subjects will be keeping track of their rescue medication use and AEs in a diary.<br><br>On Day 5 (+/- 1 day) subjects will return to the clinic for safety monitoring, study requirements compliances and observed study medication intake during morning or evening doses. |

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| | In the evening of Day 9, depending on their place of residence and asthma status, subjects will come onsite to the clinic to check and ensure asthma stability +/- undergo additional checks (safety lab/cotinine/alcohol/drug screen; as indicated in flowchart) before subsequent tests on Days 10-12.<br><br>On Day 10 (i.e. approximately 24 hours pre-allergen), approximately 30-60 minutes after first daily dosing, subjects undergo the same procedures as on Day 1 (i.e. safety/stability check, blood and urine collection (biomarkers), FeNO, EBC, NAL, NAB, IOS (if logistics allow), spirometry, methacholine/histamine challenge and SI. Subjects stay overnight in the unit. On Day 11, approx. 30-60 minutes after the morning dose, an inhaled allergen (HDM) challenge will be performed with airway response measurements (IOS and spirometry). During this study day, repeated PK sampling and blood biomarker sampling will be performed. EBC, NAL and NAB will be performed at approximately 6.5 hours post allergen challenge. Subjects stay overnight in the unit. On Day 12 (approximately 24 hours post-allergen), 30-60 minutes after the last study medication (i.e. morning) dose, the same procedures as on Day 10 will be repeated. Furthermore, clinical safety laboratory assessments will be performed. Clinically stable subjects will receive written instructions, including emergency numbers, and rescue medication prior to leaving the clinic. Within approximately 72 hours after Day 12 of each study period, subjects will be called by a physician to check their asthma/medication intake/AEs and general health. If deemed necessary by the (co)PI, there may be another check on the subjects before and/or after each study period and in any case following drop-out (either by phone or if needed and in case of drop-out, onsite the clinic).<br><br>Fourteen (14) days (± 2 days) after final administration of FP-025 or placebo on Day 12 of Period 2, a follow-up visit will be scheduled. This visit will not only serve as a follow-up visit (e.g. checking weight and AEs, performing physical examination, ECG and pregnancy test, checking vital signs and collecting blood for clinical safety laboratory assessments), the 'duration of efficacy' will also be explored by performing the same procedures as performed on Day 1 and Day 10 (i.e. blood and urine samplings (safety/biomarkers), FeNO, EBC, NAL, NAB, IOS (if logistics allow), spirometry, methacholine/histamine challenge and SI). |
| STUDY POPULATION | Approximately 100 male and female HDM-allergic asthmatics (between 18 and 55 years of age at Screening, inclusive) will be screened to yield approximately 36 eligible subjects to be enrolled, to yield 32 evaluable subjects. |
| INCLUSION CRITERIA | The following criteria must be met by all subjects considered |

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| | for study participation:<br>1. Females or males, between 18 and 55 years of age at Screening, inclusive, on the day of signing the Informed Consent Form (ICF).<br>2. Apart from a clinically stable asthma and HDM-allergy, subjects should be generally healthy with no history of a clinically relevant medical condition that in the opinion of the investigator might interfere with successful study conduct and no clinically relevant abnormalities on medical history, physical exam, vital signs, laboratory parameters or ECG at Screening.<br>3. Subject has a BMI ≥ 18.0 kg/m$^2$ and ≤ 32.0 kg/m$^2$ (and weighs ≥50 kg).<br>4. Subjects have been diagnosed with asthma *cf* GINA guidelines.<br>5. Subjects should have established allergy for HDM (serum HDM-specific IgE or positive SPT at Screening or documented within 1 year pre-screening).<br>6. No severe exacerbation of asthma within past 1 year requiring hospital admission and/or treatment with oral corticosteroids; no (never) intensive care admissions for asthma or intubation).<br>7. $FEV_1$ should be ≥70% of predicted on Screening Day 2.<br>8. On Screening Day 2, $PC20FEV_1$(Meth) should be <16 mg/mL if methacholine chloride is used (or adjusted by a factor of 1.2 if methacholine bromide is used). If histamine is used, $PC20FEV_1$(Hist) should be <16 mg/mL.<br>9. Baseline blood eosinophils should be ≥150 cells/µL at Screening or documented within 3 months before Screening Day 1.<br>10. Subjects should have a documented airway late response to inhaled HDM on Screening Day 3.<br>11. Subjects of childbearing potential must be willing to use adequate contraception (double-barrier) or must refrain from intercourse.<br>12. Female subjects of non-childbearing potential must have had ≥ 12 months of spontaneous amenorrhea (with follicle-stimulating hormone [FSH] ≥ 30 mIU/mL). Surgically sterile women are defined as those who have had a hysterectomy, bilateral ovariectomy (for 'benign' reasons), or bilateral tubal ligation.<br>13. All female subjects should have a negative pregnancy test at Screening and on Day -1.<br>14. Negative alcohol breath test on Screening Day 1 and Day -1.<br>15. Negative cotinine test on Screening Day 1 and Day -1.<br>16. Negative urine drug screen for recreational and other drugs on Screening Day 1 and Day -1. |

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| | 17. Subjects are non-smokers. A non-smoker is defined as an individual who has abstained from smoking for at least 1 year prior to Screening Day 1. Number of years smoked x number of packs per day should be <5 pack years.<br>18. Subject should be willing and able to perform the lung function tests and other study-related procedures and comply with study protocol requirements.<br>19. Subject should provide a signed and dated informed consent. |
| EXCLUSION CRITERIA | Subjects will be excluded if they meet any of the following criteria:<br>1. Subject has any active and/or chronic (physical or mental) condition requiring maintenance (pharmaco)therapy or which otherwise precludes subject from safe or adequate study participation (ineligibility will be assessed by the PI).<br>2. Subject has a history of cancer (exception: localized basalioma or cervix carcinoma *in situ*).<br>3. Subject had any major (nasal) surgery in the 6 months before Screening Day 1.<br>4. Subject is pregnant or lactating.<br>5. Subject is using immunotherapy that according to the PI may interfere with the study (e.g. in case of immunotherapy with HDM or when subject is in the updosing phase of any immunotherapy).<br>6. Subject regularly used alcohol (intake of >21 units/wk for males and >14 units/wk for females) and/or recreational drugs within the last 6 months prior to screening.<br>7. Subject had any respiratory (viral) infections (e.g. common cold) within 3 weeks of Screening Day 1 or on Day -1.<br>8. Subject is using maintenance asthma therapy or long-acting bronchodilators or any other anti-asthma or anti-allergic medications (as detailed in the protocol) other than infrequent use of SABA *prn* only.<br>9. Subject is using prohibited medications as detailed in the protocol.<br>10. Multi-sensitized symptomatic subjects with seasonal (pollen) allergies should be included outside of the relevant allergen season and/or should not be in frequent contact with the relevant allergen during the study.<br>11. Subject has any known allergic response for the medications used or known severe allergic reactions or anaphylaxis (to food/medications/insect venoms).<br>12. Subject participated in medical studies in the past 3 months (non-biologicals) or in the past 6 months (biologicals).<br>13. Subject is anticipated not to comply with study medication or other aspects of the study (at the discretion of the investigator). |

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| BLOOD SAMPLING FOR PK | Blood sampling for PK will take place at time points indicated in Table 5-1. |
| PHARMACODYNAMIC PARAMETERS | The following pharmacodynamic parameters for FP-025 will be evaluated/measured:<br>***Primary parameter:***<br>Spirometry:<br>• Area under the time-$FEV_1$ response curve from 3-8 hours post-allergen ($FEV_1$ $AUC_{3-8h}$)<br>***Secondary parameters:***<br>Spirometry:<br>• Area under the time-$FEV_1$ response curve from 0-3 hours post-allergen ($FEV_1$ $AUC_{0-3h}$)<br>• Max% fall in $FEV_1$ from baseline between 0-3 hours post-allergen<br>• Max% fall in $FEV_1$ from baseline between 3-8 hours post-allergen<br>• Area under the time-$FEV_1$ response curve from 0-8 hours post-allergen ($FEV_1$ $AUC_{0-8h}$)<br>Methacholine/histamine challenge:<br>• Provocative concentration of methacholine or histamine causing 20% fall of the $FEV_1$ from baseline ($PC20FEV_1$(Meth) or $PC20FEV_1$(Hist))<br>Impulse-oscillometry (IOS) post-allergen challenge, mainly:<br>• Resistance at a frequency of 5 hertz (R5)<br>• Resistance at a frequency of 20 hertz (R20)<br>• Frequency dependence of resistance (R5-R20)<br>• Resonant frequency (Fres)<br>• Reactance at 5 hertz (X5)<br>• Area under reactance curve (AX)<br>Exhaled air:<br>• Fractional exhaled nitric oxide concentration in exhaled air (FeNO) in parts per billion (ppb)<br>Blood<br>• Eosinophil counts<br>***Exploratory parameters:***<br>Impulse-oscillometry (IOS) post-methacholine/histamine challenge<br>Nasal lavage (NAL):<br>• Inflammatory and structural cell counts (cytospin):<br>o Squamous and ciliated nasal epithelial cells<br>o Total cells<br>o Neutrophils<br>o Eosinophils |

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| | ○ Macrophages<br>○ Lymphocytes<br>○ Mast cells<br>• Supernatant (soluble markers):<br>○ Cytokines (IL-1b, *a.o.*) and chemokines (e.g. Th2/ILC2 pathway and linked to eosinophils like IL-5, IL-33, *a.o.*)<br>○ Cellular activation markers (ECP, MPO, tryptase, *chymase*)<br>○ Markers of tissue remodeling *(*FGF, VEGF, MMP-12 *and other MMP's, to be determined)* and leakage markers (e.g. albumin, alpha2-macroglobulin)<br><br><u>Sputum:</u><br>• Inflammatory and structural cell counts (cytospin):<br>○ Squamous and ciliated bronchial epithelial cells<br>○ Total cells<br>○ Neutrophils<br>○ Eosinophils<br>○ Macrophages<br>○ Lymphocytes<br>○ Mast cells<br>• Supernatant (soluble markers; but not limited to):<br>○ Soluble cell activation markers (but not limited to): ECP, MPO, tryptase, *chymase*, TNF-α, IL-1α/β, IL-6, CXCL-8, IL-5, CXCL-1, CCL4, CCL20, scIgA, MMP-9, MMP-12, IL-33, TIMP-1, elastase<br>○ Soluble airway remodeling markers (but not limited to): *hyaluronan, tenascin C,* FGF 1/2*,* MMP-9, MMP-12, MMP's (zymogram), IL-13, fractalkine, periostin, TGF-ß, *YKL-40 (chitinase-3-like-1 protein), ADMA,* VEGF<br>○ Soluble inflammatory leakage markers: alpha2-macroglobulin, albumin<br>○ Cytokines and chemokines of Th2/ILC2 and other mainstream pathways and those linked to eosinophils (but not limited to): IL-1α/β, IL-1RA, MIP-1α/β, IL-2, IL-4, IL-5, IL-10, IL-13, IL-17, IL-18, GMCSF, IFN-γ, IP-10, TNF-α, IL-33<br><br><u>Nasal brush (NAB):</u><br>• Genome-wide gene expression<br>• microRNA expression<br>• DNA methylation<br><br><u>Exhaled breath condensate (EBC) (not limited to):</u> |

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| | • Chloro-tyrosine, bromo-tyrosine, nitro-tyrosine, tyrosine, di-tryrosine, arginine, ADMA, malondialdehyde, *potentially also leukotrienes.*<br><br><u>Blood (not limited to):</u><br>• Eosinophil activation markers (flow cytometry: CD69, CD11b and CD62L)<br>• IL-5<br>• Alpha2-macroglobulin<br>• Albumin<br>• Periostin<br>• *MMP's (zymogram)*<br><br><u>Urine (not limited to):</u><br>• *Lipid mediators (to be determined)*<br><br><u>PBMC (to be decided):</u><br>• Cytokine release after HDM exposure<br>• *Anti-oxidant response (to xanthine oxidase/xanthine)*<br><br>Parameters in italic are to be discussed and analysis will be decided upon after initial analyses have been performed. |
| SAFETY PARAMETERS | The following safety parameters will be measured:<br>• Physical examination<br>• (Optional) asthma symptoms evaluation (a VAS scale may be used for this purpose)<br>• Clinical signs/symptoms reporting (MedDRA)<br>• (S)AEs<br>• Vital signs (HR, BP, temperature +/- $SpO_2$)<br>• Lung function measurements ($FEV_1$, $FEV_1$/FVC, PEF, IOS)<br>• ECG<br>• Clinical safety laboratory outcomes (e.g. blood: hematology, chemistry, clotting parameters and urinalysis) |
| PHARMACOKINETIC PARAMETERS | The following pharmacokinetic parameters will be analyzed/calculated:<br>• Maximum plasma concentration ($C_{max}$);<br>• Time to $C_{max}$ ($t_{max}$);<br>• Area under the concentration-time curve from time zero to tau ($AUC_{0\text{-}tau}$). |
| STATISTICAL METHODOLOGY | **Sample size justification:**<br>The sample sizes for this study are based on previous allergen challenge studies performed by the same researchers as described in literature and previous local experiences including several outcomes (airway physiology and several (diluted) biomarkers), taking into account additional variability due to a 2 centre setup. |

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| | **Safety and tolerability**<br>Safety and tolerability will be evaluated by descriptive statistics and listings of adverse events/signs using MedDRA, and listings of vital signs, safety laboratory tests, spirometry and ECGs.<br><br>**Pharmacokinetics**<br>PK parameters in plasma will be summarized using descriptive statistics. All PK parameters will be determined using non-compartmental analysis of plasma concentration-time profiles.<br><br>**Pharmacodynamics**<br>The following analyses will be performed (but not limited to):<br><br>Primary:<br>• LAR ($FEV_1$ $AUC_{3\text{-}8h}$): differences between FP025 and placebo<br><br>Secondary, differences in (FP025 versus placebo):<br>• EAR: as $FEV_1$ $AUC_{0\text{-}3h}$ post-allergen and in max%fall from post-diluent baseline 0-3 h post-allergen<br>• LAR: max%fall from post-diluent baseline 3-8 h post-allergen<br>• Joint HDM-induced airway response expressed as $FEV_1$ $AUC_{0\text{-}8h}$ post-allergen<br>• Changes in allergen-induced AHR: i.e: $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) pre-post allergen (Day 10 versus Day12)<br>• Small airway parameters measured by IOS during LAR and during EAR and over 0-8 h post-allergen challenge (to be determined)<br>• Changes in allergen-induced airway and systemic biomarkers (i.e. eosinophils (blood) and FeNO (exhaled air) (Day 10 versus Day12)<br>• Changes in blood eosinophils, FeNO and PC20FEV1(Meth) or $PC20FEV_1$(Hist) Day 1 versus Day 10 (potential treatment effect)<br><br>Exploratory (allergen-induced changes (Day 10 versus Day 12) and potential treatment effects (Day 1 versus Day 10) on the following parameters, but not limited to):<br>• Treatment effects: differences in changes in the following parameters (Day 1 versus Day 10): spirometry (baseline $FEV_1$), $PC20FEV_1$(Meth)/$PC20FEV_1$(Hist) and airway and systemic biomarkers (i.e. in blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum).<br>• Allergen-induced changes in exploratory inflammatory biomarkers (Day 10 versus Day 12) (i.e.: cellular and soluble biomarkers in sputum / NAL; biomarkers in EBC; in blood/urine/ex vivo |

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| | • Potentially longer lasting treatment effects 14 days following Period 2 (spirometry (baseline $FEV_1$), $PC20FEV_1$(Meth)/$PC20FEV_1$(Hist) and airway and systemic biomarkers (i.e. in blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum).<br>• To relate biomarkers and physiological changes in different compartments (central airways/small airways/upper airways).<br>• To explore the effect of study medications on gene-transcriptomics from NAB pre- versus post-allergen.<br>• IOS measurements following inhaled allergen and eventual methacholine/histamine challenges to assess the effect of study medication on the peripheral airways and for additional safety.<br>• Only the MedDRA (safety) and PK parameters will be reported descriptively; the others will be analyzed either by paired t-testings or by ANOVA. |

**Table 5-1: Visit and Assessment Schedule**

| **Study Period** | **Screening within 6 weeks (followed by a washout period of 3-7 weeks)[a]** | | | **Treatment Period 1 and 2 (consecutive days) (washout period of 3-approximately 7 weeks between periods)** | | | | | | | | | | **EOS/Follow-up** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Day** | **S1** | **S2** | **S3** | **-1** | **1[b]** | **2-4** | **5 (± 1 day)** | **6-8** | **9** | **10[c]** | **11** | **12[c]** | **<7 days after dosing on Day 12** | **14 ± 2 days after last dosing** |
| Informed consent | X | | | | | | | | | | | | | |
| Height | X | | | | | | | | | | | | | |
| Weight | X | | | X | | | | | | | | | | X |
| BMI | X | | | | | | | | | | | | | |
| In-/exclusion criteria[d] | X | X | | X | | | | | | | | | | |
| Reconfirmation of Eligibility[e] | | X | X | X | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | | |
| Medical history | X | | | X | | | | | | | | | | |
| Physical examination | X | | | X | | | | | | | | | | X |
| Allergy testing[f] | X | | | | | | | | | | | | | |
| Vital signs[g] | X | | | X | | | | | X | | X | | | X |
| 12-lead ECG[g] | X | | | X | | | | | X | | | | | X |

| **Study Period** | **Screening within 6 weeks (followed by a washout period of 3-7 weeks)[a]** | | | **Treatment Period 1 and 2 (consecutive days) (washout period of 3-approximately 7 weeks between periods)** | | | | | | | | | | **EOS/Follow-up** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Day** | **S1** | **S2** | **S3** | **-1** | **1[b]** | **2-4** | **5 (± 1 day)** | **6-8** | **9** | **10[c]** | **11** | **12[c]** | **<7 days after dosing on Day 12** | **14 ± 2 days after last dosing** |
| Clinical safety laboratory tests in blood and urine (including blood eosinophil count, coagulation and hematology)[g] | X | | | X | | | | | X | | | X | | X |
| Virus serology (i.e. hepatitis B and C, HIV) | X | | | | | | | | | | | | | |
| Urine drug screen[g] | X | | | X | | | | | X | | | | | |
| Alcohol breath test[g] | X | | | X | | | | | X | | | | | |
| Cotinine screen[g] | X | | | X | | | | | X | | | | | |
| Pregnancy test[h] | X | | | X | | | | | | | | | | X |
| Inhaled allergen (HDM) challenge | | | X | | | | | | | | X | | | |
| FeNO in exhaled air | | | | | X | | | | | X | | X | | X |
| EBC | | | | | X | | | | | X | X | X | | X |
| Nasal lavage and nasal brush[i] | | | | | X | | | | | X | X | X | | X |
| IOS[j] | | X | X | | X | | | | | X | X | X | | X |
| Spirometry[k] | | X | X | | X | | | | | X | X | X | | X |
| Methacholine/histamine challenge | | X | | | X | | | | | X | | X | | X |

| **Study Period** | **Screening within 6 weeks (followed by a washout period of 3-7 weeks)[a]** | | | **Treatment Period 1 and 2 (consecutive days) (washout period of 3-approximately 7 weeks between periods)** | | | | | | | | | | **EOS/Follow-up** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Day** | **S1** | **S2** | **S3** | **-1** | **1[b]** | **2-4** | **5 (± 1 day)** | **6-8** | **9** | **10[c]** | **11** | **12[c]** | **<7 days after dosing on Day 12** | **14 ± 2 days after last dosing** |
| (Optional) overall asthma symptoms[l] | | | | | X | | | | | X | X | X | | |
| Randomization[m] | | | | | X | | | | | | | | | |
| Sputum induction | | X | | | X | | | | | X | | X | | X |
| Blood collection for inflammation markers and biomarkers[n] | | | | | X | | | | | X | X | X | | X |
| Urine collection for biomarkers (i.e. lipid mediators) | | | | | X | | | | | X | (X) | X | | X |
| Oral dosing of FP-025 or placebo (twice daily from Day 2 onwards)[o] | | | | | X | X | X | X | X | X | X | X | | |
| PK blood sampling[p] | | | | | | | | | | | X | | | X |
| Ambulant visits | X | (X) | (X) | | | (X) | X | (X) | (X) | | | | (X) | X |
| Residence in clinic[q] | | (X) | (X) | X | X | | | | (X) | X | X | X | | |
| Dispense diary | | | X | | | | | | | | | | | |
| Subject instructions and/or diary review[r] | | | X | | | | X | | | | X | | | |

| **Study Period** | **Screening within 6 weeks (followed by a washout period of 3-7 weeks)[a]** | | | **Treatment Period 1 and 2 (consecutive days) (washout period of 3-approximately 7 weeks between periods)** | | | | | | | | | | **EOS/Follow-up** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Day** | **S1** | **S2** | **S3** | **-1** | **1[b]** | **2-4** | **5 (± 1 day)** | **6-8** | **9** | **10[c]** | **11** | **12[c]** | **<7 days after dosing on Day 12** | **14 ± 2 days after last dosing** |
| Check treatment compliance (e.g. observed dosing, diary and phone calls) | | | | | X | X | X | X | X | X | X | X | | |
| Check health status | | (X) | (X) | X | X | | | | (X) | X | X | X | | |
| Phone call[s] | | X | X | X | | X | X | X | (X) | | | | X | |
| Adverse events | <---------- Continuously ----------> | | | | | | | | | | | | | |
| Concomitant medications (including rescue medication)[t] | <---------- Continuously ----------> | | | | | | | | | | | | | |

a. At the discretion of the PI or sub-investigator, Screening Day S1 some assessments (e.g. safety lab//pregnancy test/drug screen/cotinine screen/physical examination) can be moved to and/or repeated on Screening Day S2 and/or Screening Day S3. Screening Day S2 should start within approximately 40 days after Screening Day S1. Screening Day S3 preferably should start within 5 days after Day S2 but maximally within 8 days after Day S2.
b. FeNO, EBC, NAL, NAB, spirometry, methacholine/histamine challenge and SI to be performed in the morning (pre-dose). On Day 1, spirometry will be performed immediately after FeNO (since eligibility criterion).
c. All procedures, except ECG on Day 10, are to be started 30-60 minutes following first daily dose. On Day 10 procedures should be performed 24+/- 1 hour before starting inhaled allergen challenge (Day 11) and on Day 12 procedures should be performed 24+/-1 hour after (the start of) inhaled allergen challenge.
d. Only on Screening Day S1, Screening Day S2 and Day-1 of Treatment Period 1.
e. By Medical history/Medication use/$FEV_1$ and $PC20FEV_1$(Meth)/$PC20FEV_1$(Hist).
f. Not needed if allergic status can be based on historically demonstrated allergy, either by SPT or serology; otherwise by serum allergen specific IgE measurement (or SPT) at Screening.
g. Assessment on Day 9 may also be performed prior to all other tests on Day 10.
h. Female subjects only: serum pregnancy test at Screening, urine pregnancy test at Day -1 and EOS.
i. Day 1: pre-dose, Day 10: 24 +/-1 hour before starting inhaled allergen challenge, Day 11: approx. 6.5 hours after inhaled allergen challenge, Day 12: 24+/-1 hour after the start of inhaled allergen challenge.

j. IOS to be performed approximately 4-5 mins before all spirometric measurements during inhaled allergen challenge, in any case at baseline, after diluent, and t=0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hours after inhaled allergen challenge, and after rescue medication. If logistics allow, IOS will also be performed approximately 4-5 minutes before spirometric measurements on days without inhaled allergen challenge.
k. Screening Day S3 and Day 11: spirometry to be performed t=0 (i.e. pre-allergen and 10 mins post-diluent) and 10, 20, 30, 45 minutes and t=1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hours after inhaled allergen challenge. If required for safety, additional spirometry to be performed e.g. at t=2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 hours after inhaled allergen challenge. If at Screening, there is a fall of 10-15% from baseline at 8 hours post-allergen, $FEV_1$ can be measured up to 9 h post-allergen.
l. To be performed before and after inhaled allergen challenge and methacholine/histamine challenge and after administration of ventolin.
m. To be performed after eligibility check (including $FEV_1$) and methacholine/histamine challenge with subsequent $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) calculation on Day 1 of Treatment Period 1.
n. On Day 12 and at Follow-up, collected together with blood collection for clinical safety. On Day 11 together with pre-dose PK blood collection and approximately 6-7 hours post-allergen challenge. Additional PD blood sampling for PBMC collection only on Day 1 and Day 10.
o. First dose in the evening of Day 1. Dosing on Day 2-4 and Day 6-8 is performed at home. Dosing on Day 5 (+/- 1 day) and Day 9 is at home or in the clinic. Last daily dose on Day 11 to be taken after last PK blood sample. Last dose in the morning of Day 12. Dosing times: at home: 6-10 am/pm; at the unit: 7-9 am/pm.
p. Collection times PK Day 11: (before first daily dose (t=0 hours), 1, 2, 4, 6, 8 and 9 hours post first daily dose). If clashing with other measurements then the efficacy measurements will prevail. At Follow-up, collected together with blood collection for clinical safety and biomarkers.
q. Subjects have to stay overnight from Day -1 to Day 1 and from Day 10 to Day 12 for each period. An overnight stay from S2 to S3 is optional as is an overnight stay from Day 9 to Day 10. Residence is preferred, but depending on the personal situation of the subject (at the discretion of the investigator, including living far or close from the clinic) and his/her medical condition a subject may or may not stay overnight. Subjects can only be dismissed from the unit at the discretion of the investigator and with rescue medication, personalized written instructions on how and when to use the rescue medication and emergency phone numbers.
r. On Screening Day S3 and Day 11, instruction will be given prior to inhaled allergen challenge. The diary will be reviewed during every visit to the clinic.
s. Phone call by a physician or research staff will be performed within approximately 1 week prior to Screening Day S2, within approximately 48 hours after Screening Day S3, within approximately 1 week prior to Day -1 of each treatment period, twice a day during the treatment periods when study medication is taken at home, within approximately 72 hours after Day 12 of each treatment period and within approximately 1 week after dosing on Day 12 of each period. If deemed necessary by the (co)PI more frequent phone calls or check-up visits may be scheduled during Screening/study.
t. From 3 months prior to Screening.

# 6 INTRODUCTION AND RATIONALE

## 6.1 Introduction

FP-025 is a novel non-hydroxamate small molecule selective inhibitor of Matrix Metalloproteinase 12 (MMP-12). It has 90-fold selectivity over the next closest family member (MMP-2) and 2-3 orders of magnitude selectivity over the seven other MMP family members (MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, and MMP-14). MMP-12 has been shown to play an important role in several aspects of chronic inflammatory airway diseases like asthma and COPD, including airway inflammation, airway hyperresponsiveness, (small) airways remodeling and disease severity [1-7]. In addition, FP-025 attenuated allergic inflammation and improved lung function in two murine models of allergic inflammation (OVA and HDM models) and even showed normalization of lung histology in the HDM model.

**Non-Clinical Toxicology**

FP-025 was evaluated in a series of non-GLP and definitive GLP preclinical toxicology studies, including single- and repeat-dose toxicity studies and a core battery of genotoxicity tests. In both preclinical species (rat and dog), FP-025 showed a very benign toxicity profile. In rats, FP-025 was well tolerated at doses up to 1000 mg/kg/day (500 mg/kg BID) for 28 days. There were no test article related changes in any of the parameters evaluated. In dogs, FP-025 was well tolerated at doses up to 400 mg/kg/day (200 mg/kg BID) for 28 days. There were no test article related changes in any of the parameters evaluated.

In general, $C_{max}$ and AUC values in the pivotal repeat-dose toxicity studies increased with dose, but the increase was much less than dose proportional at the mid to high dose levels, suggesting saturation of exposure. There was no significant gender difference in drug exposure in either species.

FP-025 was negative in the *in vitro* Ames-test. In the i*n vitro* Chromosome Aberration assay, FP-025 was negative to the induction of chromosome aberration in CHO cells in the absence of rat S9 metabolic activation, but was equivocal to the induction of chromosome aberration in CHO cells in the presence of rat S9 metabolic activation. As a result, a follow-up *in vivo* study in rats was conducted to generate the weight of evidence following ICH guideline S2 (R1) on genotoxicity testing and data interpretation for pharmaceuticals intended for human use. The *in vivo* rat micronucleus and comet assay was conducted to assess the genotoxic potential of FP-025 based upon its ability to induce DNA damage in liver cells of rats using the principles of the Comet assay and to evaluate the *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte cells in rat bone marrow. FP-025 at doses up to and including 2000 mg/kg/day for 3 consecutive days was concluded to be negative in the Comet assay, and negative in the Micronucleus assay. The findings from this study was also supported by the *in vivo* mouse Micronucleus assay, where FP-025 did not induce the formation of micronuclei up to the dose of 1000 mg/kg/day in BALB/c mice.

**Preclinical Pharmacokinetics and ADME**

The absorption, distribution, metabolism, and excretion data described herein support the clinical development of FP-025 for chronic respiratory diseases. Overall, FP-025 exhibits a favorable ADME/PK/TK profile.

FP-025 is moderately absorbed in animal species. Oral bioavailability of FP-025 was approximately 36% in male mice. While the absolute oral bioavailability in rats has not been determined, the radiolabeled mass balance study in male and female rats suggested high oral absorption with >70% of administered radioactivity excreted in the bile following an oral dose. The half-life of FP-025 was relatively short in rats and dogs, generally ranging from 1 - 6 hours, which supported twice daily dosing in toxicity studies.

The pharmacokinetics of FP-025 was comparable between males and females in mice, rats, and dogs. FP-025 was absorbed at a moderate to slow rate ($t_{max}$ ~ 1- 8 hours) in animals. $t_{max}$ showed a tendency to increase with increasing oral doses. In the toxicity species, absorption increased with increase of dose, but was generally less than dose proportional, and showed signs of exposure saturation (especially in $C_{max}$) at high dose levels (>300 mg/kg/day in rats, and >200 mg/kg/day in dogs). The toxicity species were generally well exposed to FP-025 with relatively high $C_{max}$ and AUC values. At the NOAEL toxicology dose (> 1000 mg/kg/day) for the 28-day repeat-dose rat study, the steady-state $C_{max}$ was 2,915 and 2,722 ng/mL for male and female rats, respectively, and $AUC_{0\text{-}24h}$ was 36,194 and 36,748 ng*h/mL for male and female rats, respectively. At the NOAEL toxicology dose (> 400 mg/kg/day) for the 28-day repeat-dose dog study, the steady-state $C_{max}$ was 7,923 and 9,388 ng/mL for male and female dogs, respectively, and $AUC_{0\text{-}24h}$ was 96,085 and 11,6058 ng*h/mL for male and female dogs, respectively. There was no significant change in systemic exposure ($C_{max}$ and AUC) upon repeat oral dosing.

FP-025 was bound to plasma proteins to the extent of approximately 99.9 % in the rat, dog, and human. Following a single oral administration of $^{14}$C-FP-025 to male and female rats, there was limited partitioning of the drug-derived radioactivity into blood cells with blood-to-plasma ratio of total radioactivity to be approximately 0.7-0.9 during initial time points (up to 2 hours post-dose). The blood-to-plasma ratio of total radioactivity, however, increased at the 8 and 24 hour time points, probably due to a different distribution of metabolites. *In vitro*, FP-025 showed low permeability with no significant efflux in a Caco-2 intestinal transport model, suggesting it is not likely a substrate of human P-glycoprotein (P-gp).

The tissue distribution of FP-025 was evaluated. Following a single oral dose of $^{14}$C-FP-025 (10 mg/kg) to male and female rats, the $^{14}$C-FP-025-derived radioactivity was found to distribute extensively to various tissues (brain, stomach, spleen, lung, heart, testis, muscle, liver, kidney, small intestine, large intestine and fat). The majority of the radioactivity in tissues exhibited the maximum radioactivity concentrations at 0.5 h, and then steadily decreased with increasing time (2, 8 to 24 h) post-dose. The radioactivity was most abundant in stomach, small intestine, and liver.

The *in vitro* intrinsic clearance of FP-025 was moderate to high in liver microsomes of the dog and human, and very high in the rat. When incubated *in vitro* with liver microsomes of the rat, dog, and human, FP-025 was shown to be extensively metabolized. Nine metabolites (M1-M9) were identified. All metabolites had a shorter retention time than the parent. The oxygenation, dehydrogenation, hydrogenation and dealkylation pathways were found. Qualitatively, there was not a significant species difference in the biotransformation of FP-025, suggesting that the toxicity species (rat and dog) are appropriate.

*In vivo*, following a single oral dose of $^{14}$C-FP-025 to male and female rats, 18 metabolites were identified and further characterized in the bile, plasma, urine and feces. Metabolism of FP-025 occurred via multiple pathways, including di-oxygenation and hydrogenation (M1); acetylation (M2); tri-oxygenation and hydrogenation (M3); dealkylation and hydrogenation (M4 and M6); dehydrogenation (M5); mono-oxygenation (M7 and M10); di-oxygenation

(M8); mono-oxygenation and glucuronidation (M9); dealkylation (M11); di-oxygenation and dehydrogenation (M12); di-oxygenation and hydrogenation (M13); hydrogenation (M14); dealkylation and di-hydrolysis (M15 and M17); mono-oxygenation and hydrogenation (M16); mono-oxygenation and dehydrogenation (M18). In the pooled bile samples, 12 metabolites (M4, M5, M6, M7, M8, M9, M10, M11, M12, M13, M14 and M16) were detected, but the parent was not detected. M7, M8, M9 and M10 were major metabolites. In the pooled plasma samples, 3 metabolites (M11, M15 and M17) and the parent were detected at 0.5 and 2 h. M11 was a major metabolite. In the pooled urine samples, M18 was detected, but no parent was detected. In the pooled feces samples, 10 metabolites (M1, M2, M3, M5, M7, M8, M10, M12, M13 and M18) were detected, but no parent was detected. M12 and M13 were major metabolites.

FP-025 was not an inhibitor of CYP2A6, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5, but showed weak inhibition of CYP1A2, CYP2B6, CYP2C8, and CYP2C9 with an $IC_{50}$ value of 32.87, 14.72, 20.6, and 28.44 µM, respectively. *In vitro* in human hepatocytes, FP-025 showed very limited potential of inducing CYP1A, 2B and 3A enzyme activity and gene expression at concentrations up to 10 µM. Preliminary reaction phenotyping data showed that CYP3A4 may be involved in the oxidative metabolism of FP-025. FP-025 showed no significant efflux in a Caco-2 assay, suggesting it is not likely a P-gp substrate.

Following oral administration, fecal excretion was the major route of elimination in male and female rats. Approximately 94-98% of the administered dose was excreted in feces, while only 3-4% was excreted in the urine. In bile duct cannulated male and female rats, biliary excretion was predominant with over 73% of the dose found in the bile. In general, the excretion of FP-025 was rapid and occurred largely within the first 48 hours post-dose.

**Clinical pharmacology**

A single ascending dose study of FP-025 (FP02C-14-001) was conducted in Taiwan as the first-in-human study. Pharmacokinetics of FP-025 following single doses was evaluated using two formulations – API-in-Capsule and ASD-in-Capsule. After receiving a single dose of FP-025 by oral administration, median $t_{max}$ ranged from 3.5 to 6.01 hours for FP-025 API-in-Capsule formulation and was 1.0 to 2.5 hours for FP-025 ASD-in-Capsule formulation. Mean $C_{max}$ and $AUC_{0-t}$ values of FP-025 API-in-Capsule increased in a greater than dose proportional manner over the dose range of 200 mg to 400 mg but the drug exposure of API-in-Capsule was increased in a less than dose proportional manner from 400 mg to 800 mg. Mean $C_{max}$, $AUC_{0-t}$, and $AUC_{0-inf}$ values of FP-025 ASD-in-Capsule increased in a greater than dose proportional manner over the dose range of 50 mg to 100 mg and in an approximately dose proportional manner from 100 to 450 mg. Based on the pharmacokinetics profiles of FP-025, ASD-in-Capsule formulation appeared to have better drug exposure and oral absorption compared to the API-in-Capsule formulation. Highest exposure to FP-025 was observed in the 450 mg ASD-in-Capsule group, with mean $C_{max}$ and $AUC_{0-inf}$ of 2570 ng/mL and 26000 ng*h/mL, respectively.

Subsequently, a multiple ascending dose/food effect study (FP02C-17-001) was conducted in the Netherlands. Healthy male subjects, and female subjects with non-childbearing potential, were administered 14 doses of FP-025 (or placebo, in a 6:2 ratio) orally using the ASD-in-capsule formulation in 8 days. Single doses were given on Days 1 and 8 of the study, whereas from Day 2 to Day 7, the subjects received twice-daily doses. The dose levels used in the study were 100 mg, 200 mg, and 400 mg. Moreover, 8 additional subjects received 200 mg single dose of FP-025 in two occasions, once after an overnight fast, and once after a high-fat, high-

calorie meal. The safety and tolerability, as well as the pharmacokinetics of FP-025, were evaluated.

Exposure of FP-025 increased with dose after multiple dosing. A 2- and 4- fold increase in dose from 100 mg BID to 200 mg BID and 400 mg BID resulted in 3.0- and 5.6- fold increases in steady-state AUC ($AUC_{0\text{-}12h}$ on Day 8). FP-025 is rapidly absorbed, with average $t_{max}$ between 1.5-2 hours. At the highest dose tested (400 mg BID), $C_{max}$ of 3710 ng/mL (~9 μM) and daily AUC of ~ 50,000 ng*h/mL were observed. The half-life of FP-025 was 6.6-8.4 hours. Compared to the pharmacokinetic profile obtained following an overnight fast, co-administration of FP-025 with a high-fat, high-calorie meal resulted in a delay of average $t_{max}$ from 1.0 to 2.5 hours, and a ~25% lowering of $C_{max}$. However, the overall exposure of FP-025, as estimated by $AUC_{0\text{-}inf}$, did not change significantly.

**Clinical safety**

The safety and tolerability profile in the single ascending dose study supported that single dose administration of FP-025 in both formulations (API-in-Capsule and ASD-in-Capsule) was well-tolerated in healthy volunteers. The only treatment-related adverse event (TEAE) was diarrhea, which was mild in severity and was observed in both the placebo and FP-025 API-in-Capsule formulation. No TEAE was reported with FP-025 ASD-in-Capsule formulation.

In the multiple ascending dose/food effect study, FP-025 oral doses were safe and well-tolerated at all groups tested. The most common (≥ 5%) TEAEs summarized by preferred term (PT) in all subjects were fatigue observed in 2 subjects (2/24, 8.33%), dizziness observed in 2 subjects (2/24, 8.33%), headache observed in 2 subjects (2/24, 8.33%), cough observed in 2 subjects (2/24, 8.33%), nasal congestion observed in 2 subjects (2/24, 8.33%), and oropharyngeal pain observed in 2 subjects (2/24, 8.33%).

With regard to severity, all TEAEs were mild in severity and only 1 (1/6, 16.67%) subject in the 100 mg of FP-025 group was given concomitant medication due to a study drug unrelated AE (toothache). None of the subjects had dose changed or discontinued the study due to AEs.

With regard to relationship of TEAEs to FP-025, 7 events reported in 5 subjects (5/24, 20.83%) were judged as possibly related to study drug by investigator. Of these 7 events, 2 events reported in 2 (2/6, 33.33%) subjects were in the 100 mg FP-025 group, which were fatigue and cough. Other 5 events reported in 3 (3/6, 50%) subjects were in the 200 mg FP-025 group, which were eye irritation twice and erythema in one subject, dizziness in one subject, and rash in one subject.

All TEAEs recovered or resolved spontaneously by the end of the study. No clinically significant abnormalities in hematology, urinalysis, clinical chemistry, vitals, and ECGs were observed.

Detailed information on clinical safety can be found in the Investigator's Brochure [8].

The current study will be conducted in compliance with the protocol, GCP and the applicable regulatory requirement(s).

## 6.2 Study Rationale

As part of the clinical development of FP-025 for the indication of chronic inflammatory respiratory diseases such as asthma, the efficacy of FP-025 needs to be tested. Inhaled allergen challenge is an established exacerbation model of asthma linking allergen-induced changes in airway inflammation to changes in airway physiology (bronchoconstriction, increases in airway hyperresponsiveness). Therefore, allergen challenge - often complemented with

(non)invasive airway samplings - has been broadly and successfully implemented into early drug development to explore clinical effectiveness of novel medications interfering with the T-helper 2 pathway [9]. Recent evidence has been provided for the involvement of the small airways in dual responders (i.e., with both an EAR and a LAR) following inhaled allergen [10]. Apart from airway hyperresponsiveness, small airways dysfunction has been associated with features of airway remodeling [11], therefore, the allergen challenge could be an ideal model to study the physiology, inflammatory and more structural changes of both the central and peripheral airways.

In an allergic sheep model, an MMP-12 inhibitor effectively protected against allergen-induced EAR and LAR [1]. In addition, inhaled HDM has been shown to increase sputum MMP-12 levels in allergic asthmatics and in chronic HDM-sensitized mice (Jordana model) inhibition of MMP-12 resulted in an improved lung function, long-lasting reduction of airway inflammation and apparent restoration of tissue remodeling (unpublished data).

Study aims: The current study is expected to provide a proof of mechanism on the anti-inflammatory (mainly, anti-eosinophilic), bronchoprotective, and possibly, on anti-remodeling potential (exemplified by effect on small airways and allergen-induced hyperresponsiveness) of FP-025 in subjects with HDM-allergic asthma.

In view of the well-known one airway concept where evidence has been provided for interaction between the upper and lower airways [12, 13] as an explanation for concomitant allergic rhinitis and asthma [14], it is anticipated that systemic therapeutic modalities may be beneficial for the treatment of CARAS (concomitant allergic rhinitis with asthma). This has been shown for other systemic approaches, including anti-leukotrienes (e.g. montelukast), biologicals targeting IgE, IL5 and IL4/IL13, and allergen-immunotherapy. Therefore, in this study, the effects of inhaled HDM and concomitant study treatments (FP025 versus placebo) on the upper airways will be explored by inflammatory biomarkers from nasal lavage (NAL) and transcriptomic analysis from nasal brushes (NAB).

### 6.2.1 Dose Selection

The active FP-025 dose level that will be used in this study, 400 mg BID, showed an overall safe and tolerable profile in an 8-day MAD study in healthy subjects conducted at QPS-Netherlands. In addition, the exposure achieved at this dose level is in line with exposure that has previously shown anti-inflammatory efficacy in preclinical models of asthma (unpublished data).

The duration of dosing (22 doses over 12 days) is based on the number of days to reach stable plasma concentrations irrespective of food effect (i.e. <5 days) plus another 7 days of dosing. This will allow us to investigate both the anti-inflammatory/bronchoprotective efficacy of FP-025 at stable plasma concentrations in a relevant human inflammatory model and the safety/tolerability of multiple dosings in the target population.

### 6.2.2 Allergen Challenge Model

Inhaled allergen challenge is a standardized and reproducible research tool reflecting several key features of asthma. Combined with non- or semi-invasive biomarker samplings, allergen challenge is a valuable tool to evaluate clinical effectiveness of novel medications interfering with the T-helper 2 pathway [9]. Inhaled allergen challenge can be safely conducted in dedicated research settings with ample expertise and experience with this disease model and the management of acute bronchoconstriction. Safety aspects and rescue medication will be managed and customized per subject by the investigator [9], and may include combinations of the following: administration of rescue medication (i.e. short-acting beta2-agonist) following

allergen challenge, written instructions, emergency phone numbers, a follow-up phone call and rescue medication; or any other safety measures as deemed necessary by the investigator.

### 6.2.3 Methacholine/Histamine Challenge

Currently, there is a ubiquitous shortage of the raw materials to produce methacholine, making it difficult to obtain the methacholine needed for the methacholine challenges in this study. In case of insufficient supply of methacholine, histamine will be used instead in this study.

Although interacting with different receptors, both histamine and methacholine are regarded as 'a direct agent' as outlined in the international guidelines by leading societies (ATS/ERS)), and both have been (interchangeably) used to assess nonspecific airway hyperresponsiveness according to the same protocol in standard clinical practice as well as in the context of the allergen bronchoprovocation test [15, 16]. Furthermore, both histamine (which was historically the first agent) and methacholine have been used to predict the allergen PC20 in a similar way [9, 16]. Therefore, switching to histamine will not harm the subject safety nor the study objectives, particularly as the allergen-induced airway response on several (bio)markers will be compared within subjects. To allow for scientifically sound within-subject comparisons, whenever possible, subjects will receive the same challenge agent (i.e. methacholine or histamine) throughout the study. Both methacholine and histamine challenges will be performed according to the QPS standard operating procedures/work instructions in line with the above-mentioned international guidelines.

# 7 STUDY OBJECTIVES

## 7.1 Primary objective

- To determine the effect of FP-025 versus placebo on the allergen (HDM)-induced late asthmatic response expressed as $FEV_1$ $AUC_{3\text{-}8h}$ in subjects with clinically stable, mild allergic asthma and blood eosinophilia.

## 7.2 Secondary objectives

- To determine the pharmacodynamics of FP-025 versus placebo on additional markers of airway physiology, including (additional measures of) the early and late response, airway hyperresponsiveness and small airways function; inflammatory markers (including blood eosinophils and FeNO) following inhaled HDM-challenge in subjects with clinically stable, mild allergic asthma and blood eosinophilia.
- To determine the treatment effect (i.e. Day 1 versus Day 10) of multiple oral doses of FP-025 versus placebo on baseline parameters (such as blood eosinophils, FeNO and $PC20FEV_1$(Meth)/$PC20FEV_1$(Hist)).
- To determine the safety and tolerability of multiple oral doses of FP-025 versus placebo in subjects with clinically stable, mild allergic asthma and blood eosinophilia.
- To assess the pharmacokinetics of multiple oral doses of FP-025 following inhaled HDM-challenge in subjects with clinically stable, mild allergic asthma and blood eosinophilia.

## 7.3 Exploratory objectives

- To explore the effect of FP-025 versus placebo on:
  - Cellular and soluble inflammatory biomarkers in nasal lavage (NAL) and sputum;
  - Nasal gene and microRNA expression in nasal brush (NAB);
  - (Inflammatory) biomarkers in exhaled breath condensate (EBC), blood (eosinophil activation markers and potentially others as well) and urine (lipid mediators);
  - Functional in vitro responses of peripheral blood mononuclear cells (PBMC);
  - Expression and activity of MMP-12, other MMP's and markers of tissue remodeling in blood, sputum and potentially in NAL.

# 8 STUDY DESIGN

## 8.1 Endpoints

### 8.1.1 Primary Endpoint

The primary endpoint of this study is the effect of study treatments on $FEV_1$ $AUC_{3\text{-}8h}$ during the LAR (FP-025 versus placebo).

### 8.1.2 Secondary Endpoints

- Pharmacodynamic endpoints include the effect of study treatments on allergen (HDM)-induced changes in:
  - LAR expressed as max% fall in $FEV_1$ from post-diluent baseline;
  - Early asthmatic response (EAR) expressed as $FEV_1$ $AUC_{0\text{-}3h}$;
  - EAR expressed as max% fall in $FEV_1$ from post-diluent baseline;
  - Joint HDM-induced airway response expressed as $AUC_{0\text{-}8h}$;
  - Airway hyperresponsiveness expressed as $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) (Day 10-Day 12);
  - Small airway parameters following HDM-challenge (i.e. R5, R20, R5-R20, AX, X5, Fres);
  - Fractionated nitric oxide (FeNO);
  - Blood eosinophils.
- Potential treatment effect (FP-025 versus placebo) on baseline parameters (i.e. Day 1 versus Day 10), including:
  - Blood eosinophils;
  - $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist);
  - FeNO.
- Safety parameters include physical examination, clinical signs/symptoms reporting (MedDRA), (S)AEs, vital signs, lung function measurements, overall asthma symptoms, ECG and clinical safety laboratory outcomes (blood/urine).
- Pharmacokinetic parameters of FP-025 in blood (plasma) include $C_{max}$, $t_{max}$, and $AUC_{0\text{-}tau}$.

### 8.1.3 Exploratory Endpoints

Exploratory pharmacodynamic parameters of FP-025 (Day 10 versus Day 12 = treatment effect on HDM-induced (airway) responses; and Day 1 versus Day 10 = treatment effect on baseline parameters) include:

- Cellular and soluble markers of inflammation in nasal lavage (NAL) and sputum, i.e.: eosinophil and lymphocyte counts, total cell counts, levels of ECP, MPO, IL-1b, FGF, VEGF and IL-5 and relative coefficient of excretion (RCE: ratio A2M in sputum/NAL relative to that in serum divided by the ratio of albumin sputum/NAL relative to that in serum);
- Genome-wide gene and microRNA expression and DNA methylation in nasal brush (NAB) samples;
- Biomarkers of oxidative stress and cellular activation in exhaled breath condensate (EBC);
- Cellular (eosinophil activation) and soluble inflammatory mediators (IL-5) in blood;
- Lipid mediators in urine (to be determined; leukotrienes);
- Peripheral blood mononuclear cell (PBMC) counts and functional parameters (cytokine release; Elispot assay);

- Expression and activity of MMP-12, other MMP's and markers of tissue remodeling in blood, NAL and sputum;
- Potentially longer lasting treatment effects 14 days following Period 2 (FP-025 versus placebo) (spirometry (baseline $FEV_1$), $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) and airway and systemic biomarkers (i.e. blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum).

## 8.2 Overall Study Design

This is a randomized, placebo-controlled, double-blind, 2-way cross-over, 2-centre study in male and female subjects with stable, mild HDM-allergic asthma.

Prior to the study, there will be a Screening period consisting of 3 clinic visits (within approximately 6 weeks) to test subject eligibility.

On Screening Day 1, the following procedures will take place: signing of the informed consent, a check of the inclusion/exclusion criteria, medical history and demographics, physical examination, measurements of vital signs, height and weight, calculation of body mass index (BMI), blood sampling for blood eosinophils and clinical safety laboratory assessments, ECG, allergy testing and screening for drug and alcohol use, smoking (i.e. cotinine), pregnancy and HIV, Hepatitis B and Hepatitis C (i.e. serology). At the discretion of the Principal Investigators (PI) or sub-investigators, some Screening Day 1 assessments (e.g. safety lab/pregnancy test/drug screen/cotinine screen/physical examination) may be moved to and/or repeated on Screening Day 2 and/or Screening Day 3.

Within approximately 40 days after Screening Day 1, potentially eligible subjects will start with more specific screening procedures (on 2 separate days (Screening Day 2 and Screening Day 3), preferably within 5 days but maximally within 8 days). Subjects will be called by a physician or research staff within approximately 1 week prior to Screening Day 2 to check their asthma stability/medication intake/general health status. On Screening Day 2, spirometry with subsequent methacholine/histamine challenge (for calculation of the $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist)) will be performed, followed by sputum induction (SI). If logistics allow, IOS will be added to spirometric measurements. On Screening Day 3, there will be an inhaled HDM challenge [9] with airway response measurements (including IOS and spirometry) during and up to approximately 8 hours post-allergen. On completion of all response measurements following both inhalational challenges on Screening Day 2 and 3, subjects will receive rescue bronchodilator medication and lung function will be measured. Upon leaving the clinic, all subjects will be provided with rescue medication (short acting beta2-agonist) to be used on an 'as needed basis' throughout the study. In addition, after each inhalational challenge, subjects will receive emergency instructions, and will be provided rescue medication (Ventolin). Within approximately 48 hours after Screening Day 3, subjects will be called by a physician to check their asthma status/rescue medication use and general health.

Following a washout period of at least 3 weeks and up to approximately 7 weeks, eligible subjects will be enrolled into the study and randomized on Day 1 if their asthma is within continuation criteria of screening (as assessed by history, (rescue) medication use, baseline $FEV_1$ and $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist)). The study will consist of two identical study periods of 12 treatment days each, separated by a washout period of at least 3 weeks (and up to approximately 7 weeks). Approximately 36 eligible subjects will be enrolled, to yield 32 evaluable subjects who will be treated with both FP-025 (400 mg BID) or placebo in a cross-over design from the evening of Day 1 till the morning of Day 12 (22 doses per study period in total). Based on a double-blind randomized schedule, 16 subjects will receive placebo in

Period 1 and FP-025 in Period 2 and 16 subjects will receive FP-025 in Period 1 and placebo in Period 2. Both study periods will follow the same schedule of procedures.

Subjects will be called by a physician or research staff within approximately 1 week prior to Day -1 of each study period to check their asthma stability/ medication intake/ general health status. On Day -1, the checks performed on Screening Day 1 (i.e. medical history (includes asthma medication use, Adverse Events and asthma stability check)), physical examination, vital signs, weight, ECG, blood collection for clinical safety laboratory assessments and drug, alcohol, cotinine and pregnancy testing) will be repeated to reconfirm eligibility. Subjects stay overnight in the unit at the discretion of the PI or subinvestigator.

On Day 1, after a short checklist, the following tests will be performed for baseline purposes, i.e. blood and urine sampling (both for routine safety and biomarkers), FeNO, IOS (if logistics allow), spirometry, EBC, NAL, NAB, methacholine/histamine challenge (with calculation of $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) for stability/eligibility check) and SI.

On Day 1 of Period 1, subjects should still have stable asthma (based on continuation criteria as applicable in the allergen challenge: i.e. history/medication use/spirometry and $PC20FEV_1$(Meth)/$PC20FEV_1$(Hist)) in order to be randomized by a blinded pharmacist and start with the study drug administration. Should a subject not comply to the stability criteria, (s)he may be rescheduled at the discretion of the(co)PI, depending on the underlying cause.

Starting on Day 1 with an evening dose, FP-025 or placebo will be given over 12 days (BID from Day 2 up to and including Day 11, final morning dose on Day 12) to ensure stable FP-025 levels during Days 10-12 (outcome measure days).

To aid compliance, during Day 2-9, subjects will either receive study medication onsite the clinic (observed dosing) or take their study medication at home or combinations of these. In the event study medication will be taken at home (between 6-10 am/pm), the subject will be contacted twice a day on dosing days to receive confirmation of study medication intake. In addition, from Screening Day 3 onwards and during study, subjects will be keeping track of their rescue medication use and AEs in a diary.

On Day 5 (+/- 1 day) subjects will return to the clinic for safety monitoring, study requirements compliances and observed study medication intake during morning or evening doses.

In the evening of Day 9, depending on their place of residence and asthma status, subjects will come onsite to the clinic to check and ensure asthma stability +/- undergo additional checks (safety lab/cotinine/alcohol/drug screen; as indicated in flowchart) before subsequent tests on Days 10-12.

On Day 10 (i.e. approximately 24 hours pre-allergen), approximately 30-60 minutes after first daily dosing, subjects undergo the same procedures as on Day 1 (i.e. safety/stability check, blood and urine collection (biomarkers), FeNO, EBC, NAL, NAB, IOS (if logistics allow), spirometry, methacholine/histamine challenge and SI. Subjects stay overnight in the unit. On Day 11, approx. 30-60 minutes after the morning dose, an inhaled allergen (HDM) challenge will be performed with airway response measurements (IOS and spirometry). During this study day, repeated PK sampling and blood biomarker sampling will be performed. EBC, NAL and NAB will be performed at approximately 6.5 hours post allergen challenge. Subjects stay overnight in the unit. On Day 12 (approximately 24 hours post-allergen), 30-60 minutes after the last study medication (i.e. morning) dose, the same procedures as on Day 10 will be repeated. Furthermore, clinical safety laboratory assessments will be performed. Clinically stable subjects will receive written instructions, including emergency numbers, and rescue medication prior to leaving the clinic. Within approximately 72 hours after Day 12 of each

study period, subjects will be called by a physician to check their asthma/medication intake/AEs and general health. If deemed necessary by the (co)PI, there may be another check on the subjects before and/or after each study period and in any case following drop-out (either by phone or if needed and in case of drop-out, onsite the clinic).

Fourteen (14) days (± 2 days) after final administration of FP-025 or placebo on Day 12 of Period 2, a follow-up visit will be scheduled. This visit will not only serve as a follow-up visit (e.g. checking weight and AEs, performing physical examination, ECG and pregnancy test, checking vital signs and collecting blood for clinical safety laboratory assessments), the 'duration of efficacy' will also be explored by performing the same procedures as performed on Day 1 and Day 10 (i.e. blood and urine samplings (safety/biomarkers), FeNO, EBC, NAL, NAB, IOS (if logistics allow), spirometry, methacholine/histamine challenge and SI).

For details regarding the timing of specific procedures, see Table 5-1.

## 8.3 Inhaled allergen (HDM) challenge

The allergen challenge will be conducted using a standardized technique with incremental doses of inhaled house dust mite (HDM) extract as described in the literature [9] and as further detailed in the standard operating procedure (SOP) and work instruction (WI). Underneath a short description of the allergen challenge methodology is outlined; however, the SOP will be leading as it will contain additional details on the equipment, HDM extract, additional safety and consistency measures.

At Screening, an inhaled HDM challenge will be conducted for dose-finding purposes. Before the test, baseline $FEV_1$ measurements will be conducted (for safety+/-stability/reproducibility). The allergen challenge will be preceded by inhalation of the allergen's diluent. Post-diluent $FEV_1$ should not drop by more than 10% from baseline. Subsequently, eligible subjects will inhale serial doubling concentrations of HDM extract by tidal breathing with the nose clipped during 2 minutes at approximately 12-15 minutes intervals. Ten minutes after each HDM dose, $FEV_1$ will be measured in duplicate and the highest, technically satisfactory $FEV_1$ will be implicated in the analysis and will be expressed as %fall from post-diluent baseline. Once the $FEV_1$ drops by at least 20% from post-diluent baseline, no further allergen will be administered and the airway response will be further measured at 20, 30, 45, 60, 90, 120 minutes and (at least) at 3, 4, 5, 6, 7, and 8 hours post-allergen (if during screening, at 8 hours post-allergen the $FEV_1$ drops between 10-15% from post-diluent baseline, additional $FEV_1$ measurements may be conducted until 9 hours post-allergen (8.5 and 9 hours). In case of a drop of $FEV_1$ of at least 15% from post-diluent baseline during this time-span, subject is considered a dual responder and can be included into the study. For the sake of safety, additional spirometric measurements and/or measurements of peripheral oxygenation (SpO2) may be added at the discretion of the investigator.

An early asthmatic/allergic airway responses (EAR) will be defined as a fall in $FEV_1$ of at least 20% from post-diluent baseline occurring within 10-20 min of an allergen dose, while a late asthmatic/allergic airway response (LAR) will be defined as a drop in $FEV_1$ of at least 15% from post-diluent baseline occurring within 8 hours post-allergen (or ultimately 9 hours at Screening, i.e. if at 8 hours post-allergen, there is a drop of >10% and < 15%). During the study, IOS recordings (see Section 8.4.3.5) will precede spirometric measurements, and hence, will be performed within approximately 4-5 min before actual spirometry time points at pre-, during and post-allergen challenge.

After completion of the last spirometric response measurement (i.e., usually at 8 hours post-allergen), subjects will receive salbutamol to enable the $FEV_1$ to return within approx. 10%

from pre-challenge baseline. Clinically stable subjects may be sent home with written instructions, rescue medication and emergency phone numbers. Subjects with a LAR will be contacted for a phone call check up by research staff usually within 24-48 hours after the challenge day, or otherwise at the discretion of the investigator. Subjects with a dual airway response will be eligible for the study, provided that all other in- and exclusion criteria are met. Potentially eligible subjects who just fail to meet the criteria of HDM-induced airway responses may be re-challenged at the discretion of the Principle Investigator. Subjects with concomitant other sensitizations will be instructed to refrain as much as possible from exposure to co-sensitizing allergens. Pollen-sensitized symptomatic subjects will be challenged outside of the relevant pollen season.

During both study periods, generally, the 3 highest incremental doses of HDM extract will be used that provoked (an EAR and subsequently) a LAR during Screening. If during screening the EAR (and subsequent LAR) was reached following inhalation of 1 or 2 allergen doses, an additional lower dose (to complete in total 3 doses) may be added at the discretion of the investigator to ensure consistency of the procedure across subjects. However, safety will always prevail, meaning that in some cases, subjects will receive fewer allergen doses during Screening and the study periods. While allergen challenge (number and allergen concentrations) will be customized per subject, the same number and concentrations of allergen extract will be inhaled during both study periods by the same subject. All details will be described in the SOP and WI.

During the allergen challenge and subsequent measurements (i.e. from the start of the challenge until post-bronchodilator spirometry), subjects will stay inside the unit to prevent exposure to environmental triggers.

Continuation criteria allergen challenge:
Baseline $FEV_1$ at least 70% of predicted value at Screening Day 2 (inclusive) and $FEV_1$ on Screening Day 3 within 10% (counted from the absolute value in liters) of Screening Day 2 and ≥65% predicted (and ≥1.8 L). On Day 1 of Period 1, $FEV_1$ should be within 10% (again from absolute value in liters) of Screening baseline $FEV_1$ (i.e. mean baseline $FEV_1$ Screening Day 2 and Screening Day 3; and ≥65% predicted (and ≥1.8 L). Baseline $FEV_1$ (in liters) in both study periods should be within 10% and ≥65% predicted (and ≥1.8 L).

Furthermore, $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) on Day 1 of Period 1 should be within 1.5 doubling doses of Screening and on Day 1 of Period 2 within 1 doubling dose of Day 1 of Period 1. Should the subject's values on Day 1 of either study period fall outside these ranges, subject may be rescheduled depending on the cause and at the discretion of the investigator.

## 8.4 Study Assessments

### 8.4.1 **Informed Consent**

After adequate explanation of the aims, methods, objectives of the study and potential hazards of the study drug, a written informed consent from each individual participating in this study will be obtained.

### 8.4.2 **Baseline Characteristics and Concomitant Medications**

#### 8.4.2.1 Baseline Characteristics

The following subject characteristics and baseline assessments are documented as part of the screening:

- Medical history (all relevant medical history will be documented)

- Demographics including sex, date of birth (month and year), ethnicity, weight, and height.
- Vital signs (refer to Section 8.4.4.2)
- Physical examination (refer to Section 8.4.4.5)
- Hematology and clinical chemistry and urinalysis (refer to Section 8.4.4.8)
- Virus serology (refer to Section 8.4.10)
- Allergy testing (refer to Section 8.4.4.6)
- 12-lead ECG (refer to Section 8.4.4.4)

Eligible subjects will furthermore be screened for the following baseline assessments/inclusion criteria:

- Spirometry (refer to Section 8.4.3.5)
- Methacholine/histamine challenge (refer to Section 8.4.3.6)

Additionally, at Screening, sputum induction (Section 8.4.3.7) will be conducted for training purposes.

#### 8.4.2.2 Prior and Concomitant Medications

All lung and anti-allergic medications and vaccines taken from 3 months prior to screening until EOS or started during the course of the study and all other medications taken from screening until EOS or started during the course of the study will be recorded on the Concomitant Medications page.

Concomitant Medications initiated, stopped, up-titrated or down-titrated for an AE will be recorded on a specific Concomitant Medications page.

A summary of allowed and prohibited Concomitant Medications is given in Section 8.5.3.1.

### 8.4.3 **Pharmacodynamic Assessments**

#### 8.4.3.1 Exhaled nitric oxide

Fractionated nitric oxide (FeNO) will be measured from exhaled air by Niox Vero® device (Circassia, Oxford, United Kingdom) according to guidelines as detailed in our SOP/WI.

#### 8.4.3.2 Exhaled breath condensate (EBC)

Exhaled breath condensate will be collected according to our SOP/WI using an Ecoscreen condenser (CareFusion, San Diego, CA, United States). Subjects will breathe calmly into the Ecoscreen collector for 10 (and maximally 20) minutes until at least 2 mL of EBC has been collected. Immediately following collection, EBC will be stored at -80°C pending analysis of inflammatory markers. Analysis will be performed in the AMC according to local SOP. In addition, FP-025 concentration may be exploratively quantified in (some of) the samples.

#### 8.4.3.3 Nasal lavage (NAL)

Nasal lavage (NAL) will be collected, processed and analyzed according to previously standardized methodology detailed in the SOPs.

NAL will be performed in the same nostril throughout the study (any exceptions will be clarified in the source documents).

Processing, storage and analysis of the NAL samples will be described in the SOPs. Analysis will be performed in the AMC.

8.4.3.4 Nasal brush (NAB)

Nasal cells will be collected by a nasal brush (NAB) according to the SOP. NAB will be performed in the same nostril throughout the study (any exceptions will be clarified in the source documents).

Processing, storage and analysis of the NAB samples will be described in the respective SOPs/WIs. Analysis will be performed in UMCG.

8.4.3.5 IOS and Spirometry

Spirometry:
Lung function tests will be performed with a standardized/calibrated spirometer according to the SOPs/WIs in line with current guidelines. For baseline purposes (i.e., pre-testing and post-diluent), several $FEV_1$ maneuvers will be performed to yield at least 3 technically satisfactory $FEV_1$ measurements for the determination of the baseline value.

IOS:
Airway resistance and reactance will be measured using a Vyntus Impulse Oscillometry System (IOS) (CareFusion, San Diego, CA, United States) according to the SOP. IOS measurements will be performed approximately 4-5 minutes before all spirometric measurements during and after allergen challenge, in any case at baseline, after diluent, and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8 h post-allergen challenge and at recovery (post-salbutamol). If logistics allow, IOS measurements will also be performed approximately 4-5 minutes before spirometric measurements scheduled on days without allergen challenge, or, in case of methacholine/histamine challenge, as defined in the respective SOPs/WIs.

8.4.3.6 Methacholine/Histamine Challenge

Methacholine/histamine challenge will be performed according to the SOP in line with current guidelines. When methacholine bromide is not available, histamine should be used for the challenge. Whenever possible, subjects should receive the same challenge agent (i.e. methacholine or histamine) throughout the study.

Shortly, incremental doses of methacholine bromide/histamine will be inhaled for 2 minutes by tidal breathing at approximately 5 minutes intervals. The challenge will be discontinued if a fall in $FEV_1$ of ≥20% from post-diluent baseline has been reached, or until the highest concentration has been administered. If a fall in $FEV_1$ of ≥20% from baseline has been reached, a $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) will be calculated by extrapolation as defined in the manual/SOP/WI. After the challenge, subjects will be given salbutamol to aid recovery and lung function will be measured after approximately 15-30 minutes (as per manual/SOP/WI).

8.4.3.7 Sputum Induction

Sputum induction will be performed according to guidelines as detailed in the SOP/WI. Following administration of salbutamol (to protect against airway narrowing), baseline $FEV_1$ will be recorded. Subsequently, hypertonic saline (NaCl 4.5%) will be aerosolized by an ultrasonic nebulizer during 5 minutes and inhaled by tidal breathing with the nose clipped, as detailed in the SOP. Three (3) cycles of 5 minutes NaCl 4.5% inhalations will be conducted; if after the last inhalation no or not enough sputum has been obtained, the investigator can decide to do one more inhalation (max 5 mins). After each inhalation cycle, subjects will be asked to blow their nose, to rinse their oral cavity with water and subsequently to expectorate sputum into a clean plastic jar without clearing their throat. $FEV_1$ measurements (in duplicate) will be performed after each inhalation before engaging on the next inhalation cycle. If needed, and at

the discretion of the investigator, subjects will be administered additional bronchodilator after finalizing the procedure.

Sputum samples will be processed by a trained and qualified technician within 2 hours of induction onsite the respective research center, according to the SOP/WI. The cell pellets will be cytospinned and supernatants will be frozen in tubes pending analysis. Staining (DiffQui(c)k or Giemsa (when required)) of the cytospins and differential cell counts in addition to analysis of soluble biomarkers from the supernatants will be performed in the AMC according to respective SOPs.

### 8.4.4 Safety and Tolerability Assessments

The definitions, reporting, and follow-up of AEs and SAEs are described in Section 9. Table 5-1 provides an overview of all time points on which safety assessments will be performed.

#### 8.4.4.1 Adverse Events

Adverse events will be recorded according to the SOP/Work instruction of the clinic.

#### 8.4.4.2 Vital Signs

Vital signs measured during the Screening and the study will include heart rate (HR), respiration rate (RR), blood pressure (BP), body temperature (Temp) and peripheral oxygen saturation (SpO2) using adequately maintained and calibrated automatic equipment.

BP and HR measurements will be recorded in supine position after having rested for a 5-minute period. Respiration rate will be measured manually by counting the number of breaths over a full minute. Body temperature will be assessed tympanically. SpO2 will be measured by a pulse oximeter.

#### 8.4.4.3 Weight, Height and BMI

The subject's body weight will be measured using a validated balance according to the SOP. Body weight will be recorded with 1 decimal. The subject's height is measured without wearing shoes. The BMI will be calculated from the weight and height recorded at screening. BMI ($kg/m^2$) = weight (kg)/height ($m^2$).

#### 8.4.4.4 12-lead ECG

Subjects will be in a supine position for 5 minutes prior to the recording. ECGs will be evaluated and classified as normal/abnormal. In case of "abnormal", the abnormality has to be described. HR, RR, PR, QRS, QT and QTc (Bazett and Fridericia) will be reported on the eCRF. Bazett ($QT/RR^{1/2}$) and Fridericia ($QT/RR^{1/3}$) are automatically calculated by the ECG apparatus and provided on the print-out. RR will be calculated in the database. For the screening ECG and the baseline ECG, three recordings will be made with a 1-minute interval between recordings. For the evaluation of the corrected QT intervals, the average of the 3 recordings will be taken as baseline. All other recordings will be single (one recording of at least 3 complexes).

#### 8.4.4.5 Physical Examination

Physical examination will be performed consisting of inspection, percussion, palpation, and auscultation. Clinically relevant findings that are observed at the screening must be recorded on the relevant Medical History eCRF page. Clinically relevant findings found after signing of the informed consent form (ICF) and meeting the definition of an AE (new AE or worsening of previously existing condition) must be recorded on an AE page of the eCRF.

#### 8.4.4.6 Allergy testing

Allergy assessment will be conducted at screening by serum total and specific IgE or a skin prick test. Allergy testing will include at least the following allergens: house dust mite (D. Pteronyssinus/ D. farinae), animal dander (at least cat, dog and horse), fungi (Asp Fumigatus, Alternaria Alternata), and pollen (grass, trees, Artemisia Vulgaris).

A positive test for HDM either in serum or by SPT documented in the past 12 months of screening will be acceptable and hence will not need to be repeated.

#### 8.4.4.7 Overall Asthma Symptoms

Overall asthma symptoms will be assessed using a visual analogue scale (VAS). A standard VAS includes a scale of 100 mm. When a scale of another size is used, the values will be adjusted to match the standard VAS size of 100 mm.

#### 8.4.4.8 Laboratory Assessments

Clinically significant laboratory abnormalities must be reported by the investigator as an AE or SAE as appropriate (see Section 9).

The samples will be taken under fasted conditions. Subjects will not be allowed to eat or drink (except water) for a period of 4 h prior to blood sampling. As a rule, the blood samples will be taken from the subject by puncture of a vein in the cubital or the antebrachial region. The samples will be sent to the local certified laboratory in accordance with specifications.

**Table 8-1: Summary of Clinical Laboratory Tests**

| Hematology | Chemistry | Urinalysis | Others |
|---|---|---|---|
| Hemoglobin<br>Hematocrit<br>RBC count<br>Platelet count<br>WBC count<br>Neutrophils (total)<br>Eosinophils<br>Monocytes<br>Basophils<br>Lymphocytes<br>Mean corpuscular volume (MCV) | BUN<br>Creatinine<br>Fasting glucose<br>Sodium<br>Potassium<br>ALT<br>AST<br>GGT<br>Bilirubin (total, direct and indirect)<br>Alkaline phosphatase<br>Albumin<br>Cholesterol (total)<br>LDL<br>HDL<br>Triglycerides<br>Lactate dehydrogenase<br>Total protein | Urobilinogen<br>Nitrites<br>pH<br>Glucose[a]<br>Protein[a]<br>Blood[a]<br>Ketones[a]<br>Microscopy[b]<br>Specific gravity<br>Bilirubin<br>Leukocytes | Serum HDM-specific IgE<br>Serum pregnancy test[c]<br>FSH[d]<br>Serology (HIV, hepatitis B and hepatitis C)[e]<br>Coagulation (PT, INR, PTT, thrombocytes)<br>Urine drug screen<br>Alcohol breath test<br>Cotinine test |

[a] Dipstick.
[b] Only if urine dipstick is positive for blood or protein.
[c] Only during screening, other pregnancy tests will be done with urine.
[d] Required of postmenopausal females only during screening.
[e] Only at Screening

### 8.4.5 Pharmacokinetic Assessments

#### 8.4.5.1 Timing for Sampling

Blood PK sampling will be conducted during Day 11 and at Follow-up, as detailed in Table 5-1.

#### 8.4.5.2 Procedures for Sampling

About 6.0 mL blood for the PK samples will be collected via vena puncture or via an intravenous (i.v.) catheter following the local standard procedures. Information on equipment and further details on the procedures on the sampling are documented in a separate instruction manual.

#### 8.4.5.3 Labeling

The tubes will be pre-labeled and will carry the following information:

- Foresee Pharmaceuticals Co., Ltd
- Type of sample, e.g., blood, urine, etc.
- Study number
- Sample number
- Study day and scheduled time of sampling
- Subject number

#### 8.4.5.4 Shipping Procedures

The site staff will take care of the shipment of the samples. Samples must be sent to QPS Netherlands B.V. (see contact details on page 2) at time intervals agreed with the sponsor. The samples must be packed securely together with completed shipment forms in polystyrene-insulated shipping containers together with enough dry ice to last for 48 hours. More details on shipping procedures can be found in the Laboratory Manual.

#### 8.4.5.5 Bioanalysis

The concentrations of FP-025 in plasma will be determined using a validated LC-MS/MS assay. The LLOQ is 5.0 ng/mL. Concentrations will be calculated by interpolation from a calibration curve. Quality control samples will be analyzed throughout the study. Their measured concentrations will be used to determine between-run, overall precision, and accuracy of the analyses. No analysis will be done for placebo samples. Therefore, a designated person in the laboratory will be unblinded. In order to minimize the risks associated with unblinding study staff, this person from the laboratory is not involved in any other aspect of the study.

### 8.4.6 **Order of Assessments**

In case several study procedures are scheduled at the same time point, the order will be detailed in the workflow sheet. During allergen challenge, spirometry measurements will be conducted at the indicated time – being the primary parameter.

Approximately 6.5 h post-allergen challenge, the following assessments will be conducted in this order: blood sampling (eosinophils/biomarkers); EBC; NAL; NAB, followed by the IOS and spirometry at 7 hours post-allergen.

Blood biomarker sampling (eosinophils and biomarkers): Day 1; Day 10 (together with the safety lab); Day 11 (together with pre-dose PK measurements) and approximately 6-7 hours post-allergen challenge; Day 12; and at Follow-up (together with the safety lab).

During the study periods, all protocol related-assessments will be conducted at the same time of the day +/- 1 hour. This means that, e.g. if the methacholine/histamine challenge has been conducted on Day 1 at 10:00, then on Day 10 and 12 this should be done between 9-11 am (starting time); applicable for both study periods.

### 8.4.7 Allowed Time Windows for PD, Safety and PK Assessments

Time deviations allowed are tabulated below:

| Scheduled time for blood sample collection for PD, safety and PK | Allowed time deviation |
|---|---|
| ≤ 4 hours after study drug administration | ± 5 min |
| > 4 hours and ≤24 hours after study drug administration | ± 10 min |
| Follow-up | ± 2 days |
| Scheduled time for ECGs and blood pressure measurements | Allowed time deviation |
| ≤ 4 hours after study drug administration | ± 15 min |
| > 4 hours and ≤24 hours after study drug administration | ± 30 min |
| Follow-up | ± 2 days |
| Scheduled time for exhaled air, EBC, NAL, NAB, IOS, spirometry, methacholine/histamine challenge and SI | Allowed time deviation |
| ≥30 minutes and ≤ 60 minutes after study drug administration | ± 60 min |

In case deviations are more than allowed, a comment should be provided in the eCRF.

### 8.4.8 Drug and Cotinine Screen

For a urine drug screening, the following compounds will be assessed: amphetamine, barbiturates, benzodiazepines, cocaine, marijuana, methadone, methamphetamine, morphine, phencyclidine, and tricyclic antidepressants. To check if a subject has been smoking, cotinine levels will be assessed.

### 8.4.9 Pregnancy Test

A serum pregnancy test will be performed at Screening. A urine pregnancy test will be performed at Day -1 of each study period and at Follow-up. The results of the test performed on Day -1 must be available prior to dosing.

### 8.4.10 Serology

At screening, virus serology will be assessed (HIV, hepatitis B and hepatitis C).

### 8.4.11 Total Blood Volume

The total volume of blood to be taken per subject during the entire course of the study will be as follows:

**Table 8-2: Blood Volume**

| | Vol x Frequency | Total (mL) |
|---|---|---|
| Blood Chemistry (incl. virus serology, pregnancy testing) | 4.5 mL x 8 and 3.5 mL x 1 | 39.5 mL |
| Hematology | 3.0 mL x 8 | 24.0 mL |
| Coagulation | 2.7 mL x 8 | 21.6 mL |
| PK | 6.0 mL x 15 | 90.0 mL |
| PD (inflammation markers and biomarkers) | 3.0 mL x 11 (EDTA) and 3.5 mL x 11 (serum) and 4.0 mL x 11(heparin) | 115.5 mL |
| PD (PBMC) | 18.0 mL x 4 (heparin) | 72.0 mL |

| | | |
|---|---|---|
| **Total blood volume** | | 362.6 mL |

### 8.4.12 Blood Sample Storage

Blood samples will be stored for 15 years for possible future research (genetic analysis excluded).

## 8.5 Study Population

The subject population will include adult male and female asthmatic subjects who satisfy all entry criteria. Any assessments may be repeated at the discretion of the (Principle or co-) investigators.

### 8.5.1 Inclusion Criteria

The following criteria must be met by all subjects considered for study participation:

1. Females or males, between 18 and 55 years of age at Screening, inclusive, on the day of signing the Informed Consent Form (ICF).
2. Apart from a clinically stable asthma and HDM-allergy, subjects should be generally healthy with no history of a clinically relevant medical condition that in the opinion of the investigator might interfere with successful study conduct and no clinically relevant abnormalities on medical history, physical exam, vital signs, laboratory parameters or ECG at Screening.
3. Subject has a BMI $\geq$ 18.0 kg/m$^2$ and $\leq$ 32.0 kg/m$^2$ (and weighs $\geq$50 kg).
4. Subjects have been diagnosed with asthma *cf* GINA guidelines.
5. Subjects should have established allergy for HDM (serum HDM-specific IgE or positive SPT at Screening or documented within 1 year pre-screening).
6. No severe exacerbation of asthma within past 1 year requiring hospital admission and/or treatment with oral corticosteroids; no (never) intensive care admissions for asthma or intubation).
7. $FEV_1$ should be $\geq$70% of predicted on Screening Day 2.
8. On Screening Day 2, $PC20FEV_1$(Meth) should be <16 mg/mL if methacholine chloride is used (or adjusted by a factor of 1.2 if methacholine bromide is used). If histamine is used, $PC20FEV_1$(Hist) should be <16 mg/mL.
9. Baseline blood eosinophils should be $\geq$150 cells/µL at Screening or documented within 3 months before Screening Day 1.
10. Subjects should have a documented airway late response to inhaled HDM on Screening Day 3.
11. Subjects of childbearing potential must be willing to use adequate contraception (double birthbarrier) or must refrain from intercourse.
12. Female subjects of non-childbearing potential must have had $\geq$ 12 months of spontaneous amenorrhea (with follicle-stimulating hormone [FSH] $\geq$ 30 mIU/mL). Surgically sterile women are defined as those who have had a hysterectomy, bilateral ovariectomy (for 'benign' reasons), or bilateral tubal ligation.
13. All female subjects should have a negative pregnancy test at Screening and on Day -1.
14. Negative alcohol breath test on Screening Day 1 and Day -1.
15. Negative cotinine test on Screening Day 1 and Day -1.
16. Negative urine drug screen for recreational and other drugs on Screening Day 1 and Day -1.
17. Subjects are non-smokers. A non-smoker is defined as an individual who has abstained from smoking for at least 1 year prior to Screening Day 1. Number of years smoked x number of packs per day should be <5 pack years.

18. Subject should be willing and able to perform the lung function tests and other study-related procedures and comply with study protocol requirements.
19. Subject should provide a signed and dated informed consent.

### 8.5.2 Exclusion Criteria

Subjects will be excluded if they meet any of the following criteria:

1. Subject has any active and/or chronic (physical or mental) condition requiring maintenance (pharmaco)therapy or which otherwise precludes subject from safe or adequate study participation (ineligibility will be assessed by the PI).
2. Subject has a history of cancer (exception: localized basalioma or cervix carcinoma *in situ*).
3. Subject had any major (nasal) surgery in the 6 months before Screening Day 1.
4. Subject is pregnant or lactating.
5. Subject is using immunotherapy that according to the PI may interfere with the study (e.g. in case of immunotherapy with HDM or when subject is in the updosing phase of any immunotherapy).
6. Subject regularly used alcohol (intake of >21 units/wk for males and >14 units/wk for females) and/or recreational drugs within the last 6 months prior to screening.
7. Subject had any respiratory (viral) infections (e.g. common cold) within 3 weeks of Screening Day 1 or on Day -1.
8. Subject is using maintenance asthma therapy or long-acting bronchodilators or any other anti-asthma or anti-allergic medications (as detailed in the protocol) other than infrequent use of SABA *prn* only.
9. Subject is using prohibited medications as detailed in the protocol.
10. Multi-sensitized symptomatic subjects with seasonal (pollen) allergies should be included outside of the relevant allergen season and/or should not be in frequent contact with the relevant allergen during the study.
11. Subject has any known allergic response for the medications used or known severe allergic reactions or anaphylaxis (to food/medications/insect venoms).
12. Subject participated in medical studies in the past 3 months (non-biologicals) or in the past 6 months (biologicals).
13. Subject is anticipated not to comply with study medication or other aspects of the study (at the discretion of the investigator).

### 8.5.3 Diet, Activities, and Other Restrictions

#### 8.5.3.1 Concomitant Medication

During screening and study, subjects may only use a short-acting beta2agonist (SABA) on an infrequent, as needed basis for their asthma. More frequent intake of SABA will be allowed following allergen challenge as per instructions by the investigator with close monitoring. As an on-demand analgetic, only paracetamol is allowed on infrequent basis.

The use of other types of concomitant medication is only allowed at the discretion of the investigator and in consent with the sponsor. If concomitant medication is needed during the study, this medication must be recorded on the eCRF, stating its generic name, date/time of administration, dose, route and duration, as well as the indication.

The following medications are not allowed (and should be stopped) before screening and should be refrained from throughout the study:

- Systemic corticosteroids (<16 weeks and ≤ 1x per year in the previous 3 years);
- Inhaled corticosteroids or combinations with bronchodilators (<6 weeks);

- Nasal corticosteroids (<4 weeks);
- Cutaneous corticosteroids: only acceptable if infrequent use and small body surfaces – to be evaluated on an individual basis by the investigator/agreed by sponsor.
- Anti-IgE (<6 months);
- Allergen immunotherapy for HDM (never; for other allergens <3 months of start and only sublingual immunotherapy for pollen at stable levels for >3 months);
- Long-acting beta agonists (LABA) < 48 hours or ultraLABA ( <2 weeks);
- Long-acting muscarinic agonists (LAMA) (< 3 weeks);
- LABA/LAMA combinations (<3 weeks);
- Short-acting beta agonists (SABA) (< 6 hours; SABA *prn* is allowed if at stable doses and if on average no more than 2x daily during 1 month pre-screening);
- Short-acting muscarinic antagonists (ipratropium bromide) (<48 h);
- Leukotriene modifiers including Leukotriene receptor agonists (Montelukast/Zafirlukast/Pranlukast) (<2 weeks);
- Cromoglycate (oral or topical) (<2 weeks);
- Theophylline/PDE4 inhibitors (<2 weeks);
- Antihistamines (< 1 week);
- Living vaccines (<3 months);
- Other vaccines or medications at the discretion of the investigator and with mutual consent of the sponsor;
- Other study medication (<3 months);
- Biologicals (<6 months);
- Prior dosing of FP025 (< 6 months).

#### 8.5.3.2 Alcohol and Party Drugs

Drinking of alcoholic beverages is not permitted from 48 hours prior to Day -1 until the last PK sample of that treatment period has been collected. During wash-out period and between treatment periods, 1 unit of alcohol per 24 hours is allowed. Use of party drugs is not permitted during the study.

#### 8.5.3.3 Physical Activities

During Screening and study periods, subjects should refrain from excessive physical exercise and strenuous sports activities (e.g. endurance sports) at least 48 hours before onsite testing. After allergen challenge strenuous exercise should be discouraged (for at least 48 hours). Subjects should have generally a good night rest and should not be working in irregular night shifts during the study periods (i.e. from Day 1 to Day 12).

#### 8.5.3.4 Travelling

During each study period, subjects will be discouraged to travel. Any long term travel plans (especially traveling abroad) should be discussed with site study staff.

#### 8.5.3.5 Dietary Aspects

Drinking of xanthine-containing beverages, such as coffee/tea/cocoa/Coca-Cola, is not permitted 12 hours before onsite tests until the end of testing. For Day 10-12, this means no xanthine-containing beverages after approximately noon on Day 9 through Day 12 after testing. Decaffeinated drinks including green tea or rooibos tea are allowed during the study.

#### 8.5.3.6 Smoking

Use of any tobacco- or nicotine-containing product is prohibited until Follow-up. Furthermore, subjects will be instructed to as much as possible avoid passive (second-hand) smoking and other irritants (e.g. open fires/chemical paints/etc.) during the study.

#### 8.5.3.7 Concomitant allergies

Symptomatic subjects with allergies to animals (e.g. cats) should not be exposed to these animals during the study. Subjects with symptomatic pollen allergies will be challenged outside of the relevant season.

#### 8.5.3.8 Contraception

During the study, adequate (double-barrier) contraception should be used by male participants and/or their female partners. Acceptable methods of contraception include (but are not limited to): a condom, vasectomy, diaphragm, cervical/vault cap, oral contraceptives, intra-uterine device, spermicidal jelly, sexual abstinence.

Females participating in the study must either be postmenopausal with no menses for at least 12 months or surgically sterile (hysterectomy, bilateral ovariectomy or bilateral tubal ligation) or agree to use double birth barrier of contraception. Acceptable methods of contraception include (but are not limited to): diaphragm, cervical/vault cap, oral contraceptives, intra-uterine device, sexual abstinence, condom, vasectomy of the sexual partner (performed at least 90 days prior to Screening and medically assessed as successful).

## 8.6 Study Drugs

Study drugs include the investigational drug FP-025 and matching placebo administered during the study.

### 8.6.1 **Investigational Drug and Matching Placebo**

Foresee Pharmaceuticals Co., Ltd will provide FP-025 and matching placebo (Table 8–3Table 8–3). FP-025 is available for clinical trial use in the form of capsules. Placebo is available as matching FP-025 for administration.

**Table 8–3: Study drug**

| Study drug | FP-025 | Placebo |
|---|---|---|
| Form | ASD-in-Capsule, 50 mg (size 0) | Capsule (size 0) |
| Supplier | Foresee Pharmaceuticals Co., Ltd. | Foresee Pharmaceuticals Co., Ltd. |
| Manufacturer | WuXi AppTec Co., Ltd.<br>Site address: 299 FuTe Zhong Road, Waigaoqiao Free Trade Zone, Shanghai, 200131, P.R. China<br>Email: joe_tu@wuxiapptec.com<br>Tel: +86-21-20663130 | WuXi AppTec Co., Ltd.<br>Site address: 299 FuTe Zhong Road, Waigaoqiao Free Trade Zone, Shanghai, 200131, P.R. China<br>Email: joe_tu@wuxiapptec.com<br>Tel: +86-21-20663130 |
| Storage condition | Refrigerator | Refrigerator |

### 8.6.2 Rescue Medication

On Screening Day 3, all subjects will be provided with a short acting beta2-agonist (100 µg salbutamol/dose). Subjects will be instructed to use it throughout the study as rescue medication on an 'as needed basis'. No other rescue medications will be permitted.

### 8.6.3 Study Drug Preparation

All study drugs will be prepared (i.e., packaged and labeled in individual doses) at QPS Netherlands B.V. by an unblinded pharmacist, or his/her designee. In order to minimize the risks associated with unblinding study staff, the pharmacist/designee is not involved in any other aspect of the study. All study drugs will be dispensed by the investigator or a person under his supervision.

### 8.6.4 Study Drug Packaging

Foresee Pharmaceuticals Co., Ltd will be responsible for the supply of the following drugs:

- Oral capsules, each containing 50 mg of FP-025;
- Matching placebo.

The study drug will be packed and dispatched in containers. A batch release certificate will be provided, stating that the batches have been manufactured according to GMP. Study drugs are provided as capsules and supplied to the study center as a bulk shipment. Under the supervision of the pharmacist, the study drug will be packed per subject for each dosing occasion, according to the randomization list.

### 8.6.5 Study Drug Labeling

The labeling complies with the applicable (local) laws and regulations. At the minimum, the following information will be stated on the labels:

- Study number
- FP-025 (strength) or placebo
- Name, address and telephone number of sponsor, CRO or investigator
- Name of the investigator
- Batch number
- Subject identification/treatment number
- Quantity of dosage units
- Route of administration (e.g., oral), pharmaceutical dosage form and quantity of dosage units
- Directions for use
- Storage conditions
- Retest or expiry date
- "For clinical trial use only" or similar wording
- "Keep out of reach of children"

### 8.6.6 Study Drug Administration

Dosing will take place twice daily: i.e., in the unit between 7:00 and 9:00 in the morning and in the evening and if at home between 6:00 and 10:00 in the morning and in the evening. Study medication is taken with a glass of water (approximately 240 ml, or extra if needed). In the event the study medication is taken at home the number of capsules taken will be recorded in a subject dairy. The subject will also be instructed to store the medication in the refrigerator and to secure limited access to the study medication. In total, subjects will receive 22 doses of 400 mg FP-025 (8x 50 mg capsules, BID) or 22 doses of matching placebo (8 capsules, BID) during two study periods.

On PK sampling day (Day 11 and Follow-up), subjects are allowed to eat a light (not fat) breakfast.

The time of administration of study medication and the initials of the person supervising the administration will be recorded in the eCRF.

### 8.6.7 Storage and Return of the Study Drug

Upon receipt of the study medication, the responsible pharmacists will inspect all study drugs for completeness. Subsequently, he/she must immediately return the enclosed acknowledgement of receipt form; duly completed and signed (the date of receipt must be noted).

The pharmacists are responsible for storage of the study drug at the study site in an appropriate lockable room at adequate temperature (see Table 8–3). The study drug will be stored according to the instructions provided by the Sponsor. Only the pharmacist or his/her assistant, who are otherwise not involved in the study, will handle the study drug.

A Drug Preparation Protocol as well as a Drug Accountability Record must be kept current and should contain the following information:

- Subject number for whom the drug was prepared
- Initials and date of the person who prepared the study drug
- Name of the person who dispensed the study drug
- Date(s) on which drug was prepared and quantity of the drug prepared
- Quantity of drug provided to the subject for home use
- Number of capsules returned
- The inventory must be available for inspection by the monitor

After sponsor confirmation, all unused investigational material (drugs and packaging) must be returned to the Sponsor after a drug accountability check by the monitor, listing the following:

- All FP-025 and placebo administered
- All unused FP-025
- All FP-025 returned at the end of the study, and the date of return/destroyed by the site

The pharmacist will be responsible for the inventory and accountability of all Clinical Trial Material, exercising accepted pharmaceutical practices. An accurate, timely record of the Clinical Trial Material will be maintained. Only after completion of the study (to avoid breaking the blind), the Clinical Trial Material and the inventory will be available for inspection by the designated representatives of Foresee Pharmaceuticals Co., Ltd upon request. The original Drug Preparation Protocol and Drug Accountability Record are considered as source data and will be archived at the site.

## 8.7 Study Drug Discontinuation and Study Withdrawal

### 8.7.1 Study Drug Interruption or Discontinuation

The investigator must discontinue the study drug if continued administration of the study drug is believed to be contrary to the best interests of the subject.

The premature discontinuation of study drug might be triggered by an Adverse Event (AE), a diagnostic or therapeutic procedure, an abnormal assessment (e.g., ECG or laboratory abnormalities), or for administrative reasons (in particular withdrawal of the subject's consent). The reason for study drug discontinuation must be documented in the eCRF and the sponsor must be informed. If the reason for discontinuation from study drug is an abnormal result on a laboratory test, vital sign, ECG recording, or physical examination, this information will be

recorded as an AE in the eCRF. The subject will remain under the supervision of the investigator until satisfactory health has returned.

### 8.7.2 Study Discontinuation

Subjects may prematurely discontinue the study at any time. Premature discontinuation from the study is to be understood as:

- Subject did not undergo the EOS examination

and/or

- Subject is missing pivotal study related assessments

The premature discontinuation of the study might be triggered by non-compliance with the study-related procedures, worsening of the underlying disease (asthma), an AE, withdrawal of the subject's consent, administrative reasons, or because the subject is lost to follow up. A subject will be considered as lost to follow-up if, and only if, he or she cannot be reached after exhausting all means of contact.

The reasons for premature discontinuation of the study must be documented in the eCRF and the Sponsor must be informed.

Drop-outs may be replaced but only upon mutual agreement between the investigator and the Sponsor, unless withdrawal is due to a drug-related AE. The replacing subject will receive the same treatment as was assigned to the subject whom he/she replaces.

### 8.7.3 Subject's Follow-up after Study Discontinuation

The subjects will be advised that participation in these investigations is voluntary. Furthermore, the subjects may request that from the time point of withdrawal no more data will be recorded and that all biological samples collected in the course of the study will be destroyed.

In the case of premature discontinuation after study drug intake, the assessments scheduled for the EOS examination will be performed as soon as possible after study drug intake.

## 8.8 Treatment Exposure and Compliance

Records of study drug used, dosages administered, and intervals between visits are kept during the study. Study drug accountability is performed on an ongoing basis by the study staff and checked by the monitor during site visits and at the completion of the study.

During the study, observed dosing will apply. Drug administrations in the clinic will be done under direct medical supervision by site staff. A hand and cavity check will be done immediately after each drug administration. When the drug is taken at home, compliance will be checked using a diary with on demand (video) phone calls. The measurement of plasma levels of FP-025 during the analytical phase will serve as a further check of compliance.

## 8.9 Treatment Assignment and Blinding

### 8.9.1 Assignment of Subject Numbers

Subjects will receive a 4-digit number on Day 1 before the start of procedures/assessments. The assignment of number and code for subject identification is based on the obligation for anonymity. Screen-failures will be tracked and recorded on a screening log, which will be discussed during site initiation visit.

Subjects enrolled at QPS will be assigned a subject number in a rising, sequential manner, beginning with 1001, regardless of gender. Subjects enrolled at the AMC will be assigned a subject number in a rising, sequential manner, beginning with 2001, regardless of gender.

Subjects who replace discontinuing subjects who have started procedures/assessments on Day 1 will receive the number of this subject +100, e.g., subject 1106 will replace subject 1006, subject 1206 will replace subject 1106, etc. The substitute subject will receive the treatment assigned to the withdrawn subject.

In the case of discontinuation before Day 1 procedures/assessments have started, the substitute subject will receive the same number as the subject he/she replaces.

### 8.9.2 Treatment Assignment

Subjects will be randomly allocated to start with either placebo or FP-025 in Period 1. The randomization code is stored securely. It is accessible only to authorized persons who are not involved in the conduct and analysis of the study, until time of unblinding. Individual sealed randomization codes are kept by the appropriate QPS representative, and these are used for emergency unblinding only.

Randomization is controlled by the study drug packaging. The number on the medication container equals the randomization number. Subjects must be randomized in a consecutive order starting with the lowest medication container number.

Randomization will be performed by the Biostatistics department of QPS Qualitix Taiwan, according to QPS Qualitix Taiwan SOPs. The randomization code and the randomization list will be generated using SAS version *9.3*.

For the purpose of an interim analysis (see Section 10.9), the randomization code of the subjects who have completed the study will be unblinded and made available for data analysis. Unblinding of the randomization code will also apply after study closure, i.e., when the study has been fully completed, the protocol deviations determined and the clinical database declared complete, accurate, and locked.

### 8.9.3 Double-blinding

The study is performed in a double-blinded fashion. The investigational drug and its matching placebo are indistinguishable and medication containers will be packaged in the same way. The randomization code will be kept strictly confidential. It is accessible only to the pharmacist on site, who is not involved in the conduct and analysis of the study, and will keep the randomization code/scheme strictly confidential.

The investigator and research staff, the subjects, the monitors and the sponsor's staff will remain blinded to the treatment until study closure. For the interim analysis (see Section 10.9), only the independent statistician will be unblinded to the study treatment of those subjects who have completed the study and have been included in the interim analysis.

### 8.9.4 Emergency Procedure for Unblinding

The investigator receives emergency envelopes for each subject containing the identity of the study drug dispensed. The investigator and the study staff must remain blinded to the subject's treatment assignment, even if the subject refuses to participate in any study procedures or experiences an AE. The identity of the study drug may be revealed only if the subject experiences a medical emergency whose management would be improved by the knowledge of the blinded treatment assignment.

The occurrence of any code break during the study must be clearly justified and explained by the investigator. Before opening the emergency envelope, every attempt must be made by the investigator to discuss the intended code break with Foresee Pharmaceuticals Co., Ltd. In all cases, the sponsor must be informed as soon as possible before or after the code break.

Any code break must be documented on the emergency envelope and in a detailed report with the date and time of the code break and signed by the investigator. This report is to be attached to the eCRF.

At each monitoring visit, the monitor checks the emergency envelopes.

## 8.10 Study Parameters

### 8.10.1 **Pharmacodynamic Parameters**

The following pharmacodynamic parameters for FP-025 will be evaluated/measured:

***Primary parameter:***

Spirometry:

- Area under the time-$FEV_1$ response curve from 3-8 hours post-allergen ($FEV_1$ $AUC_{3-8h}$)

***Secondary parameters:***

Spirometry:

- Area under the time-$FEV_1$ response curve from 0-3 hours post-allergen ($FEV_1$ $AUC_{0-3h}$)
- Max% fall in $FEV_1$ from baseline between 0-3 hours post-allergen
- Max% fall in $FEV_1$ from baseline between 3-8 hours post-allergen
- Area under the time-$FEV_1$ response curve from 0-8 hours post-allergen ($FEV_1$ $AUC_{0-8h}$)

Methacholine/histamine challenge:

- Provocative concentration of methacholine or histamine causing 20% fall of the FEV1 from baseline (PC20FEV1(Meth) or $PC20FEV_1$(Hist))

Impulse-oscillometry (IOS), mainly:

- Resistance at a frequency of 5 hertz (R5)
- Resistance at a frequency of 20 hertz (R20)
- Frequency dependence of resistance (R5-R20)
- Resonant frequency (Fres)
- Reactance at 5 hertz (X5)
- Area under reactance curve (AX)

Exhaled air:

- Fractional exhaled nitric oxide concentration in exhaled air (FeNO) in parts per billion (ppb)

Blood

- Eosinophil counts

***Exploratory parameters:***

Impulse-oscillometry (IOS) post-methacholine/histamine challenge

Nasal lavage (NAL):

- Inflammatory and structural cell counts (cytospin):
  - Squamous and ciliated nasal epithelial cells
  - Total cells
  - Neutrophils
  - Eosinophils
  - Macrophages
  - Lymphocytes
  - Mast cells
- Supernatant (soluble markers):
  - Cytokines (IL-1b, *a.o.*) and chemokines (e.g. Th2/ILC2 pathway and linked to eosinophils like IL-5, IL-33, *a.o.*)
  - Cellular activation markers (ECP, MPO, tryptase, *chymase*)
- Markers of tissue remodeling *(*FGF, VEGF, MMP-12 *and other MMP's, to be determined)* and leakage markers (e.g. albumin, alpha2-macroglobulin)

Sputum:

- Inflammatory and structural cell counts (cytospin):
  - Squamous and ciliated bronchial epithelial cells
  - Total cells
  - Neutrophils
  - Eosinophils
  - Macrophages
  - Lymphocytes
  - Mast cells
- Supernatant (soluble markers; but not limited to):
  - Soluble cell activation markers (but not limited to): ECP, MPO, tryptase, *chymase*, TNF-α, IL-1α/β, IL-6, CXCL-8, IL-5, CXCL-1, CCL4, CCL20, scIgA, MMP-9, MMP-12, IL-33, TIMP-1, elastase
  - Soluble airway remodeling markers (but not limited to): *hyaluronan, tenascin C,* FGF 1/2*,* MMP-9, MMP-12, MMP's (zymogram), IL-13, fractalkine, periostin, TGF-ß, *YKL-40 (chitinase-3-like-1 protein), ADMA,* VEGF
  - Soluble inflammatory leakage markers: alpha2-macroglobulin, albumin
  - Cytokines and chemokines of Th2/ILC2 and other mainstream pathways and those linked to eosinophils (but not limited to): IL-1α/β, IL-1RA, MIP-1α/β, IL-2, IL-4, IL-5, IL-10, IL-13, IL-17, IL-18, GMCSF, IFN-γ, IP-10, TNF-α, IL-33

Nasal brush (NAB):

- Genome-wide gene expression
- microRNA expression
- DNA methylation

Exhaled breath condensate (EBC) (not limited to):

- Chloro-tyrosine, bromo-tyrosine, nitro-tyrosine, tyrosine, di-tryrosine, arginine, ADMA, malondialdehyde, *potentially also leukotrienes*.

Blood (not limited to):

- Eosinophil activation markers (flow cytometry: CD69, CD11b and CD62L)
- IL-5
- Alpha2-macroglobulin
- Albumin

- Periostin
- *MMP's (zymogram)*

Urine (not limited to):

- *Lipid mediators (to be determined)*

PBMC (to be decided):

- Cytokine release after HDM exposure
- *Anti-oxidant response (to xanthine oxidase/xanthine)*

Parameters in italic are to be discussed and analysis will be decided upon after initial analyses have been performed.

### 8.10.2 Safety and Tolerability Parameters

Pre-treatment baseline is defined as the last value measured prior to (the first) study drug intake (vital signs, ECG, laboratory parameters, safety lung function), unless otherwise specified.

The following parameters have been defined as parameters regarding safety and tolerability:

- Physical examination
- Overall asthma symptoms (VAS)
- Clinical signs/symptoms reporting (MedDRA)
- (S)AEs
- Vital signs (HR, BP, temperature +/- $SpO_2$)
- Lung function measurements ($FEV_1$, $FEV_1$/FVC, PEF, IOS)
- ECG
- Clinical safety laboratory outcomes (e.g. blood: hematology, chemistry, clotting parameters and urinalysis).

### 8.10.3 Pharmacokinetic Parameters

The PK parameters for FP-025 in plasma will be derived by non-compartmental analysis of the plasma concentration-time profiles.

The following pharmacokinetic parameters will be analyzed/calculated:

- Maximum plasma concentration ($C_{max}$);
- Time to $C_{max}$ ($t_{max}$);
- Area under the concentration-time curve from time zero to tau ($AUC_{0\text{-}tau}$)

# 9 SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

## 9.1 Adverse Events

### 9.1.1 Definitions of Adverse Events

An Adverse Event (AE) is any adverse change from the subject's baseline condition, i.e., any unfavorable and unintended sign including an abnormal laboratory finding, symptom or disease that occurs during the course of the study, whether or not considered related to the study drug. A treatment-emergent AE is any AE temporally associated with the use of a study drug, whether or not considered related to the study drug.

Adverse events include:

- Exacerbation of a pre-existing disease.
- Increase in frequency or intensity of a pre-existing episodic disease or medical condition.
- Disease or medical condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
- Events considered by the investigator to be related to study-mandated procedures and/or requiring any medical intervention.
- Abnormal assessments, e.g., ECG, vital signs or physical examination findings, must be reported as AEs if they represent a clinically significant finding that was not present at baseline or worsened during the course of the study.
- Laboratory test abnormalities must be reported as AEs if they represent a clinically significant finding, symptomatic or not, which was not present at baseline or worsened during the course of the study or led to dose reduction, interruption or permanent discontinuation of study drug.

Adverse events do not include:

- Medical or surgical procedure, e.g., surgery, endoscopy, tooth extraction, transfusion. However, the event leading to the procedure is an AE. If this event is serious, the procedure must be described in the SAE narrative.
- Pre-existing disease or medical condition that does not worsen.
- Situations in which an adverse change has not occurred, e.g., hospitalizations for cosmetic elective surgery or for social and/or convenience reasons.
- Overdose of either study drug or concomitant medication without any signs or symptoms. However, overdose must be mentioned in the Study Drug Log.

### 9.1.2 Intensity of Adverse Events

The intensity of clinical AEs is graded on a three-point scale: mild, moderate, severe, and reported on specific AE pages of the eCRF.

If the intensity of an AE worsens during study drug administration, the AE will be closed and a new AE with enhanced severity will be generated in the eCRF. If the AE lessens in intensity, no change in the severity is required.

If an AE occurs during a washout or placebo run-in phase and afterwards worsens during the treatment phase, a new AE page must be filled in with the intensity observed during study drug administration.

**Mild**: Event may be noticeable to subject; does not influence daily activities; usually does not require intervention.

**Moderate**: Event may make subject uncomfortable; performance of daily activities may be influenced; intervention may be needed.

**Severe**: Event may cause noticeable discomfort; usually interferes with daily activities; subject may not be able to continue in the study; treatment or intervention is usually needed.

A mild, moderate or severe AE may or may not be serious. These terms are used to describe the intensity of a specific event (as in mild, moderate, or severe myocardial infarction). However, a severe event may be of relatively minor medical significance (such as severe headache) and is not necessarily serious. For example, nausea lasting several hours may be rated as severe, but may not be clinically serious. Fever of 39 °C that is not considered severe may become serious if it prolongs hospital discharge by a day (see Section 9.2.1.2). Seriousness rather than severity serves as a guide for defining regulatory reporting obligations. On the other hand, a mild chest tightness – turning out to be a myocardial infarction requiring hospitalization will be termed an SAE (serious adverse event).

These definitions do not apply to clinically significant and asymptomatic laboratory test abnormalities or abnormal assessments (e.g., ECG findings) considered as AEs. The investigator should tick non-applicable on the AE page of the eCRF to qualify the intensity of the AE.

### 9.1.3 Relationship to Study Drug

Adverse events should be assessed by the investigators as to whether or not there is a reasonable possibility of causal relationship to the study drug and reported as either related or unrelated.

**Related to study drug**: This category applies to any AE (serious or not) that appears to have a reasonable possibility of causal relationship to the use of the study drug (i.e., a relationship cannot be ruled out). Guidelines to determine whether an event might be considered related include (but are not limited to) the following:

- The event occurred in close temporal relationship to study drug administration.
- The event abated (diminished) or disappeared when treatment with the study drug was down-titrated, interrupted, or discontinued.
- The event re-occurred when treatment was re-introduced.
- Environmental factors such as clinical state and other treatments could equally have caused the event.

Related AEs should be considered as one of the following categories:

| | |
|---|---|
| Definite | A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (de-challenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory re-challenge procedure if necessary. |
| Probable | A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition. |

| | |
|---|---|
| Possible | A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear. |

**Unrelated to study drug**: This category applies to any AE (serious or not) that does not appear to have a reasonable relationship to the use of study drug (see above guidelines).

Unrelated AEs should be considered as one of the following categories:

| | |
|---|---|
| Unlikely | A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations. |
| Unrelated | A clinical event, including laboratory test abnormality, which is clearly not related to drug administration. |

#### 9.1.4 Reporting of Adverse Events

All AEs occurring after signing the informed consent form and up to Follow-up must be recorded on specific AE pages of the eCRF.

For randomized subjects only, all adverse events that occur after the consent form is signed but before randomization must be reported by the investigator if they are the result of a protocol specified intervention.

#### 9.1.5 Follow-up of Adverse Events

Adverse events still ongoing after drug intake must be followed until resolution, or to a maximum of 28 days after study drug discontinuation. Follow-up will consist of a phone call or any other relevant procedures (e.g. repeating of a laboratory value or inspection or referral to a specialist, if deemed necessary by the investigator).

If an AE is present at the follow-up visit/ end-of-study visit, it should be followed to resolution or stabilization unless the subject is lost to follow-up. Resolution means the subject has returned to baseline state of health or the Investigator does not expect any further improvement or worsening of the event.

## 9.2 Serious Adverse Events

### 9.2.1 Definitions

#### 9.2.1.1 Serious Adverse Events

A Serious Adverse Event (SAE) is defined by the International Conference on Harmonization (ICH) guidelines as any AE fulfilling at least one of the following criteria:

- Fatal.
- Life-threatening.
- Requiring subject's hospitalization or prolongation of existing hospitalization.
- Resulting in persistent or significant disability or incapacity.
- Congenital anomaly or birth defect.
- Medically significant or requires intervention to prevent at least one of the outcomes listed above.
- Life-threatening refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.

- Important medical events that may not immediately result in death, be life-threatening, or require hospitalization may be considered as SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions above.

The reference safety document to assess whether or not an SAE should be reported by the sponsor to Health Authorities, ECs/IRBs and investigators in an expedited fashion is the Investigator's Brochure.

#### 9.2.1.2 Pregnancy

All initial reports of pregnancy, including pregnancy outcome (follow-up) in female subjects or in partners of male subjects, must be reported to the QPS Safety Unit (SU) by the investigator within 24 hours of his/her knowledge of the event using a Pregnancy Form.

The investigator will contact the subject at the expected time of delivery for follow-up. Abnormal pregnancy outcomes (e.g., spontaneous or induced abortion, stillbirth, neonatal death, congenital abnormality, birth defect) are considered SAEs and must be reported using the Serious Adverse Event Form.

#### 9.2.1.3 Hospitalization - Prolongation of Existing Hospitalization

Hospitalization is defined as an overnight stay in a hospital unit and/or emergency room.

An additional overnight stay for monitoring of an AE defines a prolongation of existing hospitalization. Allergen-challenge related overnight stays without the need for maintenance asthma therapy (e.g. prednisone), will not qualify for an SAE.

The following is not considered an SAE and should be reported as an AE only:

- Treatment on an emergency or outpatient basis for an event not fulfilling the definition of seriousness given above and not resulting in hospitalization.

The following reasons for hospitalizations are not considered AEs, and therefore not SAEs:

- Extra overnight stay before or following study related procedures/including challenge testing which do not require intervention other than with bronchodilators.
- Hospitalizations for cosmetic elective surgery, social and/or convenience reasons.
- Standard monitoring of a pre-existing disease or medical condition that did not worsen, e.g., hospitalization for coronary angiography in a patient with stable angina pectoris.
- Elective treatment of a pre-existing disease or medical condition that did not worsen, e.g., hospitalization for chemotherapy for cancer, elective hip replacement for arthritis.

#### 9.2.1.4 Serious Adverse Events Related to Study-mandated Procedures

Such SAEs are defined as SAEs that appear to have a reasonable possibility of causal relationship (i.e., a relationship cannot be ruled out) to study-mandated procedures (excluding administration of study drug) such as discontinuation of subject's previous treatment during a washout period, or complication of a mandated study related challenge test or invasive procedure (e.g., blood sampling, HDM challenge test).

### 9.2.2 **Reporting of Serious Adverse Events**

#### 9.2.2.1 Before Study Drug Initiation

Serious adverse events occurring after signature of the Informed Consent and up to study drug initiation must be reported to QPS Safety unit only if they are considered by the investigator to be related to study- mandated procedures.

#### 9.2.2.2 During Study Drug Administration

All SAEs regardless of causal relationship must be reported, including those related to study-mandated procedures. These SAEs occurring during study drug administration, i.e., between study drug initiation and EOS after study drug discontinuation, are defined as treatment emergent SAEs.

These SAEs are reported on SAE forms and also on AE pages in the eCRF. Therefore, they are entered both in the drug safety and clinical databases, and must be reconciled before study closure.

#### 9.2.2.3 After Study Drug Discontinuation

New SAEs, including those related to study-mandated procedures, occurring during study drug administration, i.e., between study drug initiation and EOS, must be reported.

All SAEs occurring after study drug discontinuation must be recorded on an SAE form and as AEs in the eCRF. Therefore, these treatment-emergent SAEs are entered both in the QPS drug safety and clinical databases, and must be reconciled before study closure.

#### 9.2.2.4 Reporting Procedures

All SAEs must be reported by the investigator to QPS Safety unit within 24 hours of the investigator's knowledge of the event.

All SAEs must be recorded on SAE forms, irrespective of the study drug received by the subject, whether or not this event is considered by the investigator to be related to study drug.

These SAE forms must be e-mailed to QPS Safety unit (see contact details page 4). The investigator must complete the SAE form in English (unless otherwise specified) and assess the relationship to study drug.

Such preliminary reports will be followed by detailed descriptions that should include copies of hospital case reports, autopsy reports, hospital discharge summaries and other documents when requested and applicable. Follow-up information about a previously reported SAE must also be reported within 24 hours of receiving it. QPS Safety unit may contact the investigator to obtain further information.

Suspected (considered related to the study drug) and Unexpected (not previously described in the reference safety document), Serious Adverse Reactions (SUSARs) will be expedited by QPS Safety unit to Health Authorities, ECs/IRBs and investigators, as appropriate. SUSARs will not be subject to systematic unblinding.

#### 9.2.2.5 Procedure for Unblinding of Serious Suspected Adverse Reactions

The unblinding procedure for serious suspected adverse reactions is defined in the safety management plan.

### 9.2.3 **Follow-up of Serious Adverse Events**

Serious adverse events still ongoing at the End-of-Study visit must be followed until resolution or stabilization or until the event is otherwise explained.

New SAEs occurring at any time after the End-of-Study or after the 28-day follow-up period after study drug discontinuation (whichever comes first) may be reported to QPS Safety unit within 24 hours of the investigator's knowledge of the event, if felt appropriate by the investigators.

Such information will only be entered into the drug safety database and hence will not affect study closure.

# 10 STATISTICAL METHODOLOGY AND ANALYSES

## 10.1 Statistical Analysis Plan

The effect of FP-025 on primary and secondary outcome parameters will be statistically tested using paired tests comparing observations in the placebo period versus observations during active treatment with FP-025. Presence of period and carry-over effects will be evaluated using a two-group two-period within-subject analysis of variance.

A statistical analysis plan (SAP) will be written and finalized before the final study closure, i.e., database closure and unblinding of the randomization code of the study. The SAP will provide full details of the individual analyses, the data displays and the algorithms to be used for data derivations. A SAP for safety, tolerability and PD will be written by QPS Qualitix Taiwan. A PKAP for the PK analyses will be written by QPS LLC. All analyses on efficacy outcomes including their relationships or relationships between PK and PD/clinical/biomarker outcomes will be overseen by the independent statistician (Prof A.H. Zwinderman).

The SAP will include the link of major protocol deviations/violations to the analysis sets.

Any deviations from the original statistical plan will be described and justified in the final report.

## 10.2 Analysis Sets

Three different analysis sets will be defined. Subjects who withdraw from the study, or who have missing data, will be included in the statistical analyses provided that they are eligible for inclusion in the analysis population as described below.

**All-treated set**: This analysis set includes all randomized subjects who received study drug (at least one dose).

**Safety set**: This analysis set includes subjects from the all-treated set who had at least one safety assessment post-baseline. The safety set will be employed in the analysis of tolerability and safety variables.

**Per-protocol set**: This analysis set comprises all subjects included in the All-treated set who did not violate the protocol in a way that might affect the evaluation of the effect of the study drug on the primary endpoint, i.e., without major protocol violations or deviations. The Per-protocol set will be employed in the analysis of PK variables.

### 10.2.1 Sample Size

Approximately 100 male and female HDM-allergic asthmatics (between 18 and 55 years of age at Screening, inclusive) will be screened to yield approximately 36 eligible subjects to be enrolled, to yield 32 evaluable subjects.

The sample sizes for this study are based on previous allergen challenge studies [9] and previous local experiences including several outcomes (airway physiology and several (diluted) biomarkers), taking into account additional variability due to a 2 centre setup.

### 10.2.2 Procedure for accounting for missing, unused, and spurious data

All analyses will be performed on data available at the time point considered. In summary tables, the number of subjects with missing data will be presented unless otherwise specified. In the calculation of percentages, subjects with missing data if not included in an analysis will not be considered in numerator or denominator unless otherwise specified.

## 10.3 Pharmacodynamic Parameters

All $FEV_1$ values post-allergen will be expressed as %fall from post-diluent baseline ($FEV_1$) and the highest, technically satisfactory $FEV_1$ value per pre-defined time point will be included into analysis. This excludes additional time points from analysis (e.g. if between a time interval additional spirometry has been performed for safety reasons – these values do not need to be included into analysis). $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) will be calculated as defined in the SOP. Analyses of all (including additional) PD parameters and any relationships between parameters will be detailed in the SAP.

A definition of the PD parameters is described in Section 8.10.1.

The following analyses will be performed (but not limited to):

Primary:

- LAR ($FEV_1$ $AUC_{3\text{-}8h}$): differences between FP025 and placebo

Secondary, differences in (FP025 versus placebo):

- EAR: as $FEV_1$ $AUC_{0\text{-}3h}$ post-allergen and in max%fall from post-diluent baseline 0-3 h post-allergen
- LAR: max%fall from post-diluent baseline 3-8 h post-allergen
- Joint HDM-induced airway response expressed as $FEV_1$ $AUC_{0\text{-}8h}$ post-allergen
- Changes in allergen-induced AHR: i.e: $PC20FEV_1$(Meth) or $PC20FEV_1$(Hist) pre-post allergen (Day 10 versus Day12)
- Small airway parameters measured by IOS during LAR and during EAR and over 0-8 h post-allergen challenge (to be determined)
- Changes in allergen-induced airway and systemic biomarkers (i.e. eosinophils (blood) and FeNO (exhaled air) (Day 10 versus Day12)
- Changes in blood eosinophils, FeNO and PC20FEV1(Meth) or $PC20FEV_1$(Hist) Day 1 versus Day 10 (potential treatment effect)

Exploratory (allergen-induced changes (Day 10 versus Day 12) and potential treatment effects (Day 1 versus Day 10) on the following parameters, but not limited to):

- Treatment effects: differences in changes in the following parameters (Day 1 versus Day 10): spirometry (baseline $FEV_1$), $PC20FEV_1$(Meth)/$PC20FEV_1$(Hist) and airway and systemic biomarkers (i.e. in blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum).
- Allergen-induced changes in exploratory inflammatory biomarkers (Day 10 versus Day 12) (i.e.: cellular and soluble biomarkers in sputum / NAL; biomarkers in EBC; in blood/urine/ex vivo
- Potentially longer lasting treatment effects 14 days following Period 2 (spirometry (baseline $FEV_1$), $PC20FEV_1$(Meth)/$PC20FEV_1$(Hist) and airway and systemic biomarkers (i.e. in blood/urine/exhaled air (FeNO, EBC), NAL/NAB; sputum).
- To relate biomarkers and physiological changes in different compartments (central airways/small airways/upper airways).
- To explore the effect of study medications on gene-transcriptomics from NAB pre- versus post-allergen.
- IOS measurements following inhaled allergen and eventual methacholine/histamine challenges to assess the effect of study medication on the peripheral airways and for additional safety.
- Only the MedDRA (safety) and PK parameters will be reported descriptively; the others will be analyzed either by paired t-testings or by ANOVA.

All output will be reported using tables and/or figures including descriptive statistics. Individual results will be listed and plots (versus baseline) will be generated.

PK values will be used to evaluate safety but also to ensure that efficacy data can be adequately interpreted and eventually to compare PK between asthmatic subjects and healthy subjects (in the previous MAD study).

## 10.4 Safety and Tolerability Parameters

Definitions of the safety and tolerability parameters are described in Section 8.10.2.

The safety set is used to perform all safety analyses.

The medical history is coded using the MedDRA version 20.1 or higher and listed.

All AEs and SAEs are coded using the MedDRA version 20.1 or higher.

The treatment-emergent AEs are tabulated by system organ class (SOC), and individual preferred terms within each SOC by treatment group. The number and percentage of subjects who experienced AEs coded with the same preferred term and SOC will be summarized by treatment group (in descending order according to the incidence in the investigational study drug group). Adverse events will also be tabulated by severity and by relationship to study drug. Summary tables will be accompanied by individual subject listings broken down by treatment group, including pre-dose events.

SAEs will be listed and summarized similarly to AEs.

Reasons for death will only be listed.

Reasons for premature discontinuation of study drug will be listed and summarized by frequency tables.

ECG variables, vital sign measurements, lung function measurements and laboratory measurements will be summarized at each time point using mean, median, standard deviation (SD), min, max, number of available observations, and change from baseline. Individual patient listings of ECG data, vital sign data, lung function data and laboratory measurements will be provided.

Standard numeric laboratory parameters are presented in the units supplied. If needed, a conversion will be made to standard units.

## 10.5 Pharmacokinetic Parameters

The PK parameters (i.e., $C_{max}$, $t_{max}$, $AUC_{0\text{-}tau}$) will be calculated on the basis of the actual blood sampling time points. Please refer to Section 8.4.5.1 for details on the usage of timing of sampling.

### 10.5.1 **Pharmacokinetic Statistical Analysis**

A definition of the PK parameters is described in Section 8.10.3. PK parameters will be described descriptively.

The per-protocol analysis set will be used for all PK analyses. No analysis will be done for placebo samples. Individual subject listings will be provided. Mean and individual plasma concentration-time profiles for FP-025 will be presented graphically.

PK parameters will be summarized using arithmetic mean, SD, geometric mean, median, minimum, maximum, CV%, and two-sided 95% confidence limits of the geometric mean.

## 10.6 Exposure to Study Drug

A listing with information about the drug administration will be provided.

## 10.7 Baseline Characteristics and Concomitant Medications

Summary statistics (mean, median, SD, min, max, number of available observations) will be provided for continuous demographic variables (e.g., age, height, weight). Individual subject listings of demographic data will be provided.

Qualitative demographic characteristics (gender, race) will be summarized by counts and percentages. Other baseline subject characteristics (medical history, physical examination clinical findings, previous medications, inclusion/exclusion checklist) will only be listed.

Distributions of these characteristics will be compared between the treatment groups only descriptively. No statistical inference will be performed.

Previous and concomitant medications will be coded by the sponsor according to the WHO drug code and the ATC class code. Previous medications will be summarized by tabulating the number and percentages of patients treated.

## 10.8 Exploratory Analyses

Exploratory data-driven analyses can be performed at a later time point, i.e. after finalizing the CSR and can be added as an addendum later on.

## 10.9 Interim Analysis

After at least 11 subjects have successfully completed both treatment periods (with fully analyzable data sets of airway responses and sputum cytospins), an interim analysis will be conducted in order to compute the conditional power of a significant difference between FP-025 and placebo treatment on the LAR as well as on the sputum eosinophil counts (reflecting target engagement), at the planned end of the trial in the presence of sufficiently high PK levels and at the original expectation of the effect of FP-025 on the LAR. If the conditional power is less than 25%, the trial may be stopped for futility. However, such a decision will depend on the size of the FP-025 treatment-effect on secondary and exploratory outcomes.

In addition, the size of a potential carry-over effect of active treatment in the first treatment period into effects of treatment in the second treatment period will be investigated. If the size of the carry-over is larger than expected, the statistical analysis for the remaining subjects may be amended.

No adjustment of the sample size is made because with the intended sample size of 32 evaluable subjects, the power of the original effect is over 95% and this remains the case with the addition of the interim analysis.

In the absence of any clinically relevant AEs, recruitment and study conduct will continue during (preparation of) the interim analysis.

## 10.10 Clinical Study Report

Safety and tolerability parameters as well as PD (efficacy) and PK parameters will be evaluated in a Clinical Study Report. The CSR will include the data and the analysis of both the primary

and secondary parameters; exploratory parameters and analyses may be added in an addendum to the CSR later on.

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# 12 PROCEDURES AND GOOD CLINICAL PRACTICE

## 12.1 Procedures

### 12.1.1 **Protocol Amendments**

Any substantial change to a protocol has to be considered as an amendment as soon as these documents have been submitted to ECs/IRBs or Health Authorities. Therefore, an amendment could occur before or after the approval of these documents by ECs/IRBs or Health Authorities. Each amendment must be documented in writing and approved by Foresee Pharmaceuticals Co., Ltd. It should be reviewed by the Principal Investigator(s).

Adaptations of the core subject Information and Informed Consent requested by ECs/IRBs are not considered as amendments, as long as they do not significantly change the core document or affect the protocol.

#### 12.1.1.1 Non-substantial Amendment

Administrative or logistical minor changes require a non-substantial amendment. Such changes include but are not limited to changes in study staff or contact details (e.g., Foresee Pharmaceuticals Co., Ltd instead of CRO monitors) or minor changes in the packaging or labeling of study drug.

The implementation of a non-substantial amendment could be done with or without (according to national regulations) notification to the appropriate ECs/IRBs and Health Authorities. It does not require their approval or to be signed by the investigators.

#### 12.1.1.2 Substantial Amendment

Significant changes require a substantial amendment. Significant changes include but are not limited to: new data affecting the safety of subjects, change of the objectives/ parameters of the study, eligibility criteria, dose regimen, study assessments/procedures, treatment or study duration, with or without the need to modify the core Subject Information and Informed Consent.

Substantial amendments are to be approved by the appropriate ECs/IRBs and in some countries by the Health Authorities. The implementation of a substantial amendment can only occur after formal approval by the appropriate ECs/IRBs and/or Health Authorities and must be signed by the investigators.

#### 12.1.1.3 Urgent Amendment

An urgent amendment might become necessary to preserve the safety of the subjects included in the study. The requirements for approval should in no way prevent any immediate action being taken by the investigators or Foresee Pharmaceuticals Co., Ltd in the best interests of the subjects. Therefore, if deemed necessary, an investigator can implement an immediate change to the protocol for safety reasons. This means that, exceptionally, the implementation of urgent amendments will occur before submission to and approval by ECs/IRBs and Health Authorities.

In such cases, the investigator must notify Foresee Pharmaceuticals Co., Ltd within 24 hours. A related substantial amendment will be written within 10 working days by Foresee Pharmaceuticals Co., Ltd and submitted to the appropriate ECs/IRBs and Health Authorities.

### 12.1.2 **Monitoring**

The monitor will contact and visit the investigator regularly and will be allowed, on request, to have access to all source documents needed to verify the entries on the eCRF and other protocol related documents; provided that subject confidentiality is maintained in agreement with local regulations. It will be the monitor's responsibility to inspect the eCRF at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. Foresee Pharmaceuticals Co., Ltd monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of the main efficacy, safety and tolerability parameters. Additional checks of the consistency of the source data with the eCRFs are performed according to the study-specific monitoring plan.

The investigator must ensure that subjects' anonymity will be maintained. On eCRFs or other documents submitted to Foresee Pharmaceuticals Co., Ltd, subjects should not be identified by their names, but by the subject number. The investigator must keep a subject identification code list showing the randomization number, the subject's name, date of birth and address or any other locally accepted identifiers. Documents identifying the subjects (e.g., subjects' signed informed consent forms) should not be sent to Foresee Pharmaceuticals Co., Ltd and must be kept by the investigator in strict confidence.

The investigator and co-investigators agree to cooperate with the monitor(s) to ensure that any issue detected in the course of these monitoring visits is resolved. If the subject is hospitalized or dies in a hospital other than the study center, the investigator or co-investigator is in charge of contacting this hospital in order to document this SAE.

The investigator will supply Foresee Pharmaceuticals Co., Ltd on request with any required background data from the study documentation or clinic records. This is particularly important when eCRFs are illegible or when errors in data transcription are suspected. In case of special problems and/or governmental queries, it is also necessary to have access to the complete study records, provided that subject confidentiality is protected.

An initiation visit will be performed before the first subject is included. Monitoring visits and contacts will occur at regular intervals thereafter, according to a frequency defined in the study-specific monitoring plan. A close-out visit will be performed after study closure.

### 12.1.3 **Data Management**

#### 12.1.3.1 Data Collection

A Subject Screening and Enrollment Log will be completed for all eligible or non-eligible subjects with the reasons for exclusion.

For each subject enrolled, regardless of study drug initiation, an eCRF must be completed and signed by the Principal Investigator. This also applies to those subjects who fail to complete the study. If a subject withdraws from the study, the reason must be noted on the eCRF. Case report forms are to be completed on an ongoing basis.

Designated investigator staff will enter the data required by the protocol into the electronic Case Report Forms. Designated investigator site staff will not be given access to the EDC system until they have been trained. The Investigator must certify that the data entered into the Electronic Case Report Forms are complete and accurate.

Furthermore, subjects will be completing diaries. In these diaries subjects will record 1) asthma control (good – fair – uncontrolled); 2) any asthma triggers (allergens, environmental,

physical); 3) rescue medication (number of puffs); 4) any AEs (changes in physical condition; 5) drug intake (during study), how many capsules and time point of administration.

12.1.3.2 Database Management and Quality Control

Data from the source documents will be entered into the eCRF as specified in QPS Netherlands SOPs. After completion of the eCRF, each subject will be electronically approved (signed) by the investigator.

The CRA will perform source data verification according to the monitoring plan and the SOPs of the CRS department. The CRA will use the eCRF system to track the monitoring queries and their resolution by the site.

The entered data is systematically checked by Data Management of the CRS department, QPS Netherlands, according to specifications described in the Data Management Plan and the applicable SOPs. The Data Management Plan will include the definition of major and minor protocol deviations/violations.

After the eCRF has been declared complete and accurate, the eCRF will be locked, after written approval of the Sponsor. Any changes to the eCRF after that time require a database unlock and can only be made after receipt of written approval of the Sponsor.

### 12.1.4 Recording of Data and Retention of Documents

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents will be classified into two different categories: investigator's file, and subjects' clinical source documents.

The investigator's file will contain the protocol/amendments, financial disclosure forms, eCRFs and data clarification and query forms, EC/IRB and Health Authority approval with correspondence, informed consent, drug records, participating staff curriculum vitae and authorization forms, screening and enrolment logs, delegation log and other appropriate documents/correspondence as per ICH/Good Clinical Practice (GCP) and local regulations.

Subjects' clinical source documents include, but are not limited to, subjects' hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory, ECG, lung function, and other special assessment reports, consultant letters, diaries etc.

These two categories of documents must be kept on file by the investigator and site for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). No study document should be destroyed without prior written approval from Foresee Pharmaceuticals Co., Ltd. Should the investigator wish to assign the study records to another party, or move them to another location, Foresee Pharmaceuticals Co., Ltd must be notified in advance.

When source documents are required for the continued care of the subject, appropriate copies should be made for storing outside of the site.

### 12.1.5 Audit

Foresee Pharmaceuticals Co., Ltd may conduct audits of clinical research activities in accordance with internal standard operating procedures (SOPs) to evaluate compliance with the principles of GCP and ICH related guidelines.

Health Authorities may also wish to conduct an inspection (during the study or after its completion). Should an inspection be requested by Health Authorities, the investigator must inform Foresee Pharmaceuticals Co., Ltd immediately that such request has been made.

The investigator will permit such audits by Foresee Pharmaceuticals Co., Ltd. or Health Authorities and facilitate them by providing access to the relevant source documents.

### 12.1.6 Handling of Study Drug(s)

Foresee Pharmaceuticals Co., Ltd will supply all study drug(s) to the site according to local regulations. Drug supplies must be kept in an appropriate, secure area and stored according to the conditions specified on the drug labels. The site must maintain an accurate record of the shipment and dispensing of study drug(s) on an accountability form, which must be given to the monitor at the end of the study. An accurate record of the date and amount of study drug(s) dispensed to each subject must be available for inspection at any time.

All drug supplies are to be used only for this protocol and not for any other purpose. The responsible person must not destroy any drug labels, or unused drug supply. Upon termination of the study, the monitor will collect used and unused drug subject kits. They will be sent to the warehouse, where the sponsor or its deputy will check drug accountability. In certain circumstances, used and unused drug containers can be destroyed at the site once drug accountability is final and checked by the sponsor or its deputy and written permission for destruction has been obtained from Foresee Pharmaceuticals Co., Ltd.

### 12.1.7 Publication of Study Results

In accordance with standard editorial and ethical practice, Foresee Pharmaceuticals Co., Ltd will support publication of the data by the investigators and eligible staff conform ICMJE recommendations. This will be done under the responsibility of Foresee Pharmaceuticals Co., Ltd. in close collaboration with the investigators. The Principle investigators (ZD, RL) and co-investigators (KAE, MvdB) will be granted co-authorship and will work with Foresee Pharmaceuticals on any future publications.

### 12.1.8 Disclosure and Confidentiality

By signing the protocol, the investigator agrees to keep all information provided by Foresee Pharmaceuticals Co., Ltd in strict confidence and to request similar confidentiality from his/her staff and the EC/IRB. Study documents provided by Foresee Pharmaceuticals Co., Ltd (investigators' brochures, protocols, eCRFs and other protocol-related documents) will be stored appropriately to ensure their confidentiality. The information provided by Foresee Pharmaceuticals Co., Ltd to the investigator may not be disclosed to others without direct written authorization from Foresee Pharmaceuticals Co., Ltd, except to regulatory bodies as required including EC/IRB, the auditor from the Health Authority, a representative of the sponsor and to the extent necessary to obtain informed consent from subjects who wish to participate in the trial.

### 12.1.9 Premature Termination or Suspension of the Study

Both Foresee Pharmaceuticals Co., Ltd and the investigator reserve the right to terminate the study at any time.

If a study is prematurely terminated or suspended, Foresee Pharmaceuticals Co., Ltd will promptly inform the investigators, the ECs/IRBs and Health Authorities, as appropriate, and provide the reason(s) for the termination or suspension.

If the study is prematurely terminated or suspended for any reason, the investigator in agreement with Foresee Pharmaceuticals Co., Ltd should promptly inform the enrolled subjects and ensure their appropriate treatment and follow-up.

In addition, if the investigator terminates or suspends a study without prior agreement of the sponsor, the investigator should promptly inform Foresee Pharmaceuticals Co., Ltd and the

EC/IRB, and should provide the sponsor and the EC/IRB with a detailed written explanation of the termination or suspension.

If the EC/IRB terminates or suspends its approval/favorable opinion of a study, the investigator should promptly notify Foresee Pharmaceuticals Co., Ltd and provide Foresee Pharmaceuticals Co., Ltd with a detailed written explanation of the termination or suspension.

## 12.2 Good Clinical Practice

### 12.2.1 **Ethics and Good Clinical Practice**

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" (as amended in Tokyo, Venice, Hong Kong, Somerset-West, Edinburgh, Washington DC, Tokyo, Seoul and Fortaleza) and with the laws and regulations of the country in which the clinical research is conducted.

All studies must follow the ICH GCP Guidelines and, if applicable, the Code of Federal Regulations.

### 12.2.2 **Quality Control and Quality Assurance**

Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

The Sponsor can decide to conduct an audit at the clinical study center. The audit will be conducted with the aim to ensure that the clinical study is performed and data are generated, documented (recorded) and reported according to the protocol and in compliance with GCP and applicable regulatory requirements. An external party may be contracted for this purpose.

On request of the sponsor, QA may perform a study audit including the data management process.

### 12.2.3 **Ethics Committee / Institutional Review Board**

The investigator will submit this protocol and any related documents provided to the subject (such as subject information used to obtain informed consent) to an Ethics Committee (EC) or Institutional Review Board (IRB). Approval from the committee must be obtained before starting the study, and should be documented in a dated letter to the investigator, clearly identifying the trial, the documents reviewed and the date of approval. A list of members participating in the meeting must be provided, including the functions of these members. If study staff were present, it must be clear that none of these persons voted.

Modifications made to the protocol after receipt of the EC/IRB approval must also be submitted as amendments by the investigator to the EC/IRB in accordance with local procedures and regulations (see Section 12.1.1).

### 12.2.4 **Informed Consent**

It is the responsibility of the investigator to obtain written informed consent from each individual participating in this study after adequate explanation of the aims, methods, and objectives of the study and potential hazards of the study drug. The investigator must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time for any reason. Appropriate forms for documenting written informed consent will be provided to the sites prior to the study.

The Informed Consent and Subject Information will be provided in the local language.

### 12.2.5 **Compensation to Subjects and Investigators**

Foresee Pharmaceuticals Co., Ltd will provide an insurance in order to indemnify (legal and financial coverage) the investigator/center against claims arising from the study, except for claims that arise from malpractice and/or negligence. The compensation of the subject in the event of study-related injuries will comply with the applicable regulations.

# 13 STRUCTURED RISK ANALYSIS

A risk analysis (TSP603.02) will be made by the Division CP of QPS Netherlands B.V.