^{68}Ga-FAPI PET/CT to detect ongoing fibroblast activity in post-acute COVID-19 with respiratory impairment after hospital discharge: "The FAPI COVID-CLIMATE Study"

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Name	Signature	Date
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

6-MWT	**6 Minute Walking Test**
ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
DLCO	**Diffusing capacity of the Lungs for Carbon Monoxide**
DSMB	**Data Safety Monitoring Board**
ECM	**Extracellular Matrix**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
FAP	**Fibroblast Activation Protein**
FAPI	**Fibroblast Activation Protein Inhibitors**
GCP	**Good Clinical Practice**
GMP	**Good Manufacturing Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
HRCT	**High-Resolution Computed Tomography**
IB	**Investigator's Brochure**
IC	**Informed Consent**
ILA	**Interstitial Lung Abnormalities**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
MBq	**Megabecquerels**
PASC	**Post-Acute Sequelae of SARS-CoV-2 Infection**
PET/CT	**Positron emission tomography/computerized tomography**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**

Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
SUV	**Standardized Uptake Value**
TLF	**Total Lesion Fibrosis**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
VC	**Vital Capacity**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**
wlFAPI-MAV	**Whole Lung FAPI-Metabolic Active Volume**
wlTL-FAPI	**Whole Lung Total Lesion-FAPI**
wlSUV	**Whole Lung Standardised Uptake Value**

SUMMARY

Rationale: The pathogenesis of post-acute COVID-19 with respiratory complaints remains unknown. We aim to explore the pulmonary pattern and fibrosis activity in patients with post-acute COVID-19 with respiratory complaints using ^{68}Ga-FAPI Positron Emission Tomography-Computed Tomography (PET/CT) imaging.

Objective: To relate pulmonary fibroblast activity, measured by FAPI-PET/CT at least 3 months after hospital discharge, to interstitial lung abnormalities on HRCT at the same time point in post-acute COVID-19 patients with respiratory complaints.

Study design: This is a ZonMw funded single center prospective observational cohort study of post-acute COVID-19 patients with respiratory complaints.

Study population: We will recruit 20 adult patients with post-acute COVID-19 and respiratory complaints that will undergo ^{68}Ga-FAPI PET/CT imaging to establish pulmonary fibrosis activity.

Main study parameters/endpoints: To assess the degree of Fibroblast Activation Protein expression on ^{68}Ga-FAPI PET/CT at least 3 months after hospital discharge and to relate this to interstitial lung abnormalities on HRCT at the same time point.

1. INTRODUCTION AND RATIONALE

1.1 Background to pulmonary fibrosis in post-COVID-19 syndrome

The ongoing pandemic caused by the novel coronavirus (SARS-COV-2), which causes the disease known as COVID-19, is leading to another epidemic of post-acute patients with persistent health impairments. The majority of COVID-19 patients survive the infection and acute illness, however, the long-term health effects are largely unknown. A substantial proportion of patients experience long-term symptoms, ranging from pure respiratory symptoms to disabling fatigue and mental issues (1,2), referred to as long COVID-19 or post-acute sequelae of COVID-19 (PASC). It has been shown that the majority of COVID-19 survivors have some persistent interstitial lung abnormalities (ILA) on HRCT, most extensive in post-intensive care unit patients and patients with longer duration of hospitalization (3,4). However, both the evolution of these ILA and their nature are unknown, since they can reflect ongoing inflammation, residual areas of organizing pneumonia, or early fibrosis. This may result in persistent pulmonary complaints, with or without an abnormal lung function test, with up to 76% of the patients still having normal diffuse capacity (5). Although chest computed tomography (CT) has been shown to be a useful tool in identifying lung abnormalities in patients with PASC, it does not provide information about the pathogenesis and possible targets for intervention. Advanced molecular imaging to assess ongoing fibroblast activity has the potential to provide a pathophysiological model and, possibly, personalized targeted pharmacological interventions. The recently developed ^{68}Ga-FAPI PET tracer has this potential.

Fibroblast activation protein (FAP) alpha is a type II transmembrane protease with dipeptidyl peptidase and endopeptidase activity which is induced in fibroblasts upon activation and is negligible or absent in resting fibroblasts or other cell types (6). The recently developed radiolabeled quinoline-based positron emission tomography (PET) tracers binding to FAP, like ^{68}Ga-FAPI, demonstrate tracer uptake in various tumor entities (7). Expression of FAP as detected by ^{68}Ga-FAPI PET also correlates with fibrotic activity and disease progression in the lungs of patients with systemic sclerosis-associated interstitial lung disease (8). ^{68}Ga-FAPI PET can therefore aid in the understanding of the pathophysiology of PASC with persistent pulmonary complaints. With ^{68}Ga-FAPI PET we aim to quantify the fibroblastic activity in relation to the percentage of interstitial lung abnormalities on high resolution computed tomography (HRCT), lung function testing including diffusing capacity of the lungs for carbon monoxide (DLCO) and vital capacity (VC), fibrotic and inflammatory blood markers, systemic and upper respiratory tract cellular phenotypes as determined by single cell RNA sequencing, 6-minute walking test (6-MWT) and self-reported daily impairments measured by EQ-5D questionnaire of patients with PASC at >3 months after hospital

discharge and, in case of persisting symptoms, at 8-10 months after ^{68}Ga-FAPI PET/CT scan.

1.2 Study Aim

With ^{68}Ga-FAPI PET we aim to quantify the fibroblastic activity in relation to the interstitial lung abnormalities on high resolution computed tomography (HRCT), lung function including DLCO and VC, fibrotic and inflammatory blood markers, systemic and upper respiratory tract cellular phenotypes determined by single cell RNA sequencing, 6-MWT and self-reported daily impairments measured by EQ-5D questionnaire in post-acute COVID-19 patients.

1.3 Research Hypothesis

We hypothesize that increased fibroblast activity can be found on PET and interstitial lung abnormalities on HRCT in post-acute COVID19 patients with pulmonary complaints, with a match and/or mismatching pattern between both imaging modalities.

1.4 Rationale for Study

The quantification of pulmonary fibrosis activity using ^{68}Ga-FAPI PET has the ability to advance our understanding of the pathological processes that occur in post-acute COVID-19 patients with persistent pulmonary complaints. It is unknown whether the persistent respiratory impairments and fibrotic lesions on HRCT are the result of ongoing fibroblast activity. The findings from this study will provide us with much needed pathophysiological insight in post-acute COVID-19 and result in identification of patients that may benefit in the future from anti-fibrotic medication to enhance pulmonary recovery.

2. OBJECTIVES

Primary objectives

To relate pulmonary fibroblast activity, measured by FAPI-PET/CT at least 3 months after hospital discharge, to interstitial lung abnormalities on HRCT at the same time point (T_0) in post-acute COVID-19 patients with respiratory complaints.

Secondary objectives

To relate pulmonary fibroblast activity, measured by FAPI-PET/CT, to specific fibrosis and inflammatory blood markers, upper respiratory and systemic cell phenotypes, 6-MWT, lung function including DLCO and VC, and self-reported daily impairments measured by the EQ-5D questionnaire at the same time point (T_1) and to HRCT findings, EQ-5D and 6-MWT, in case of persistent respiratory complaints 8-10 months after timepoint T_0.

3. STUDY DESIGN

This is a prospective observational cohort study.

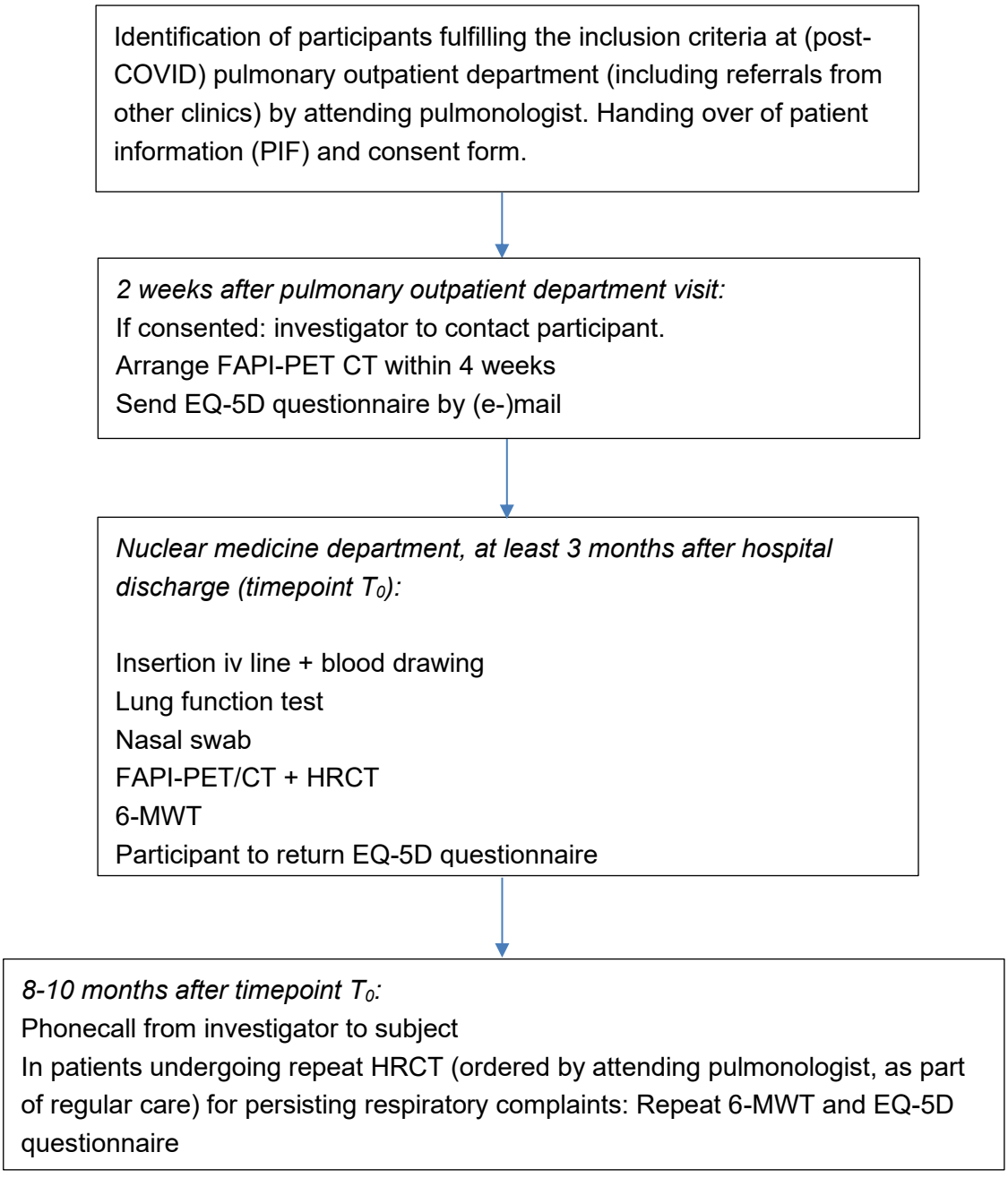

Figure 1. Flow chart of study procedures.

4. STUDY POPULATION

4.1 Population (base)

We will recruit 20 hospital-discharged adult patients with post-acute COVID-19 and respiratory complaints.

4.2 Inclusion criteria

- Male patients ≥18 years and female patients ≥20 years discharged from hospital after PCR-confirmed COVID-19 infection.
- Previous ICU or ward admission with high flow nasal oxygen (HFNO) or mechanical ventilation.
- Persistent respiratory complaints (shortness of breath) at least 3 months after hospital discharge.

4.3 Exclusion criteria

Subjects should not enter the study if any of the following criteria are fulfilled:

- Inability or unwilling to give informed consent.
- History of claustrophobia or feeling of inability to tolerate supine position for the PET/CT scans.
- Severe or significant comorbidity, defined as COPD GOLD stage II or higher, known interstitial lung disease and/or an ASA score (11) of IV and higher.
- Women who are pregnant or breastfeeding.

4.4 Sample size calculation

This is an explorative study and a power analysis cannot be applied to calculate the number of included patients. The total number will be 20 patients, as performed usually in these kinds of feasibility studies (12).

5. TREATMENT OF SUBJECTS

Not applicable.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

^{68}Ga–fibroblast activation protein inhibitor ([^{68}Ga]FAPI-46) is a quinoline-based radiopharmaceutical used for the in vivo detection of the fibroblast activation protein using positron emission tomography (PET). Chemical name is Gallium;(R)-2,2',2''-(10-(2-(4-(3-((4-(2-(2-cyano-4,4-difluoropyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-6-yl)(methyl)amino)propyl)piperazin-1-yl)-2-oxoethyl)1,4,7,10,tetraazacyclododecane-1,4,7-triyl)triacetate. [^{68}Ga]FAPI-46 is produced as a sterile, intravenously injectable solution of the radiopharmaceutical, containing 10% ethanol in 0.9% phosphate buffered sodium chloride (PBS) solution (v/v)

6.2 Summary of findings from non-clinical studies

Please see the Investigational Medicinal Product Dossier (IMPD).

6.3 Summary of findings from clinical studies

See section 13.1.

6.4 Summary of known and potential risks and benefits

See Section 13.1.

6.5 Description and justification of route of administration and dosage

^{68}Ga-FAPI has been administered to humans and demonstrated its ability to detect areas of fibrosis activity within different cancers at acceptable radiation doses (1.6 mSv) (13). No other available route of administration.

6.6 Dosages, dosage modifications and method of administration

Intravenous injection of 200 megabecquerels (MBq) ^{68}Ga-FAPI.

6.7 Preparation and labelling of Investigational Medicinal Product

^{68}Ga-FAPI will be manufactured locally in a GMP licensed facility adjacent to the PET scanners. Quality Control will be performed on a sample of the final product before releasing ^{68}Ga-FAPI for use against pre-established specifications including radiochemical and chemical purity, residual solvents, endotoxin content and pH. Sterility will also be confirmed post release.

6.8 Drug accountability

Not applicable.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

8.1 Study endpoints

8.1.1 Main study endpoint

To assess the degree of Fibroblast Activation Protein expression on ^{68}Ga-FAPI PET/CT at least 3 months after hospital discharge (timepoint T_0) and to relate this to interstitial lung abnormalities on HRCT at the same time point.

8.1.2 Secondary study endpoints

1. To relate the degree of Fibroblast Activation Protein expression at least 3 months after hospital discharge to DLCO and VC, ECM and inflammatory blood markers, systemic and upper respiratory tract cellular phenotypes, 6MWT and EQ-5D at the same time point.
2. To relate the degree of Fibroblast Activation Protein expression at least 3 months after hospital discharge to interstitial lung abnormalities on HRCT, 6MWT and EQ5D 8-10 months after timepoint T_0 in patients with persisting respiratory complaints.

8.2 Randomisation, blinding and treatment allocation

Not applicable.

8.3 Study procedures

8.3.1 PET Radiotracer ^{68}Ga-FAPI production

^{68}Ga-FAPI will be manufactured locally in a GMP licensed facility adjacent to the PET scanners. Quality Control will be performed on a sample of the final product before releasing ^{68}Ga-FAPI for use against pre-established specifications including radiochemical and chemical purity, residual solvents, endotoxin content and pH. Sterility will also be confirmed post release.

8.3.2 PET/CT imaging

To assess pulmonary fibrosis activity, we will perform ^{68}Ga-FAPI biodistribution studies in 20 post-COVID-19 patients using established protocols. After insertion of a periperheral intravenous catheter, each subject will undergo a PET and low dose CT (PET/CT) scan (Vision or QUADRA Biograph mCT, Siemens Medical Systems, Erlangen, Germany), from head to proximal lower limb 60 minutes following intravenous injection of 200 MBq ^{68}Ga-

FAPI. An HRCT scan will be performed immediately afterwards, on the same scanner. Total scanning time will be 15 minutes.
If HRCT, as ordered by attending pulmonologist as part of regular care, is repeated 11-13 months after hospital discharge for persisting pulmonary complaints, this data will be used for analysis.

PET analysis:

^{68}Ga-FAPI uptake as a measure of fibrosis activity in the lung will be evaluated by visual assessment of the active pulmonary ^{68}Ga-FAPI lesions . Increased ^{68}Ga-FAPI uptake will be compared with the muscle background uptake and defined as being positive when higher, and verified by follow-up with HRCT. For quantitative analysis, a specialized software platform from Hermes Hybrid 3D (Hermes Medical Solutions AB, Stockholm, Sweden) will be used. The mean and maximum standardized uptake value (SUV) and the metabolic active volume (MAV) normalized to body weight will be manually computed for the active pulmonary lesions and healthy tissues by drawing a 3-dimensional volume of interest. Multiplication of SUVmean and MAV results in total lesion FAPI (TL-FAPI). Whole-lung FAPI-MAV (wlFAPI-MAV) can then be calculated by summation of MAV over all lung areas. Whole lung mean SUV (wlSUVmean) will be calculated as the mean of the SUV values of all five pulmonary lobes. These parameters will allow us to calculate whole lung TL-FAPI (wlTL-FAPI) by multiplying wlFAPI-MAV with SUVmean.
SUVmax ratio for the pulmonary hotspot will be defined as the quotient of the SUVmax of this lesion and the normal muscular tissue, and target-to-background ratio (TBR) for active pulmonary lesions will be calculated according to the formula: TBR = tSUVmax/mSUVmean, where tSUVmax is the maximum SUV of lung lesion, and mSUVmean is the mean SUV of muscle.

HRCT analysis:

In the absence of a validated scoring system to evaluate HRCT scans for the presence of fibrosis in PASC, we will use a validated scoring system developed by Desai and Goh for evaluation of interstitial lung abnormalities on HRCT in patients with systemic sclerosis associated interstitial lung disease, idiopathic pulmonary fibrosis and idiopathic non-specific interstitial pneumonia (14,15). HRCT scans will be reviewed at five levels. At each level, four features of fibrosis will be quantified: the extent of interstitial lung disease, the relative proportions of interstitial lung disease made up of a reticular pattern and ground-glass opacification, the amount of emphysema, and the coarseness of fibrosis. Finally, an overall grade of interstitial lung disease depending on the relative extent of a reticular pattern will be given.

8.3.3 Questionnaire

For assessment of self-reported daily impairments, all subjects will be asked to complete the EQ-5D questionnaire. Completion of the EQ-5D takes 5 to 10 minutes. The EQ-5D is a generic classification system used to characterize current health states of individuals, for which “of the shelf” utilities from members of the general public are available in the literature. The EQ-5D consists of 5 domains (mobility; self-care; usual activity; pain/discomfort; anxiety/depression) and a Visual Analogue Scale (EQ-VAS). Subjects are asked to indicate their level of health by checking one of three levels of functioning for each domain. For the VAS, subjects draw a line from a box to the point on the thermometer like-scale corresponding to their health state, 0-100 (100 is best health state).
Outcome of the test is a unitless number between 0 and 1 that be calculated from an online calculator at the website of the developer of the test (euroqol.org), with 1 being the best possible outcome and 0 the worst.
Subjects will return the EQ-5D personally or via mail. The investigator will check whether all questionnaires are fully completed and will correct, if necessary, in consultation with the subject.
If HRCT, as ordered by attending pulmonologist as part of regular care for persisting pulmonary complaints, is repeated 8-10 months after timepoint T_0, 6 minute walking test will be performed again.

8.3.4 Blood sample collection

At the time of FAPI PET/CT, 30ml of blood for measurement of circulating Extracellular Matrix (ECM) markers and inflammatory parameters (C-reactive protein, leucocyte count, lymphocyte count, erythrocyte sedimentation rate) will be drawn from the intravenous catheter. ECM markers consist of several biomarkers that have been shown to predict lung function decline and mortality in pulmonary fibrosis (16). Serum samples will be labeled with a unique code which is made up of FAPI-COVID, the subject’s study number and date of drawing, stored at -80°C and sent for further analysis after collection of all samples. The samples will be accessible during the study period to the investigators. The encryption key is accessible to the coordinating investigator only.

8.3.5 Nasal epithelium collection

Nasal epithelium collection will be performed as follows. Subjects are asked for their favourable nostril to perform the procedure. Subjects are then asked to blow their nose and attempt to remove any mucous from the nose. 1 mL of Lidocaine (10mg/mL) is collected in a 1mL or 2 mL syringe. Subjects are asked to make the sound of the letter K repeatedly while

0.5mL of lidocaine is injected into the nostril (aiming toward the inferior turbinate) to numb the area to be brushed. The subjects are asked to lean forward and any remaining Lidocaine is collected onto a tissue. The steps are repeated with another 0.5mL of lidocaine. After a few minutes, using the speculum to open the nostril, the lateral area underneath the inferior turbinate is the brushed for 3 seconds and the brush is placed in 2 mL screw-cap Eppendorf tube containing 1.5 mL of RNAProtect Cell solution. The brush is then cut into the tube using a wire cutter cleaned with RNAse Zap and alcohol.

8.3.6 Physical exercise

All subjects will perform a 6-minute walking test at the same visit of the FAPI PET/CT. The test will be taken according to guidelines (17). Heartrate and peripheral oxygenation will be measured before and after the test. Outcome of the test is total walking distance in meters. The whole test takes approximately 10 minutes.
If HRCT, as ordered by attending pulmonologist as part of regular care, is repeated 8-10 months after timepoint T_0 for persisting pulmonary complaints, 6 minute walking test will be performed again.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.5 Replacement of individual subjects after withdrawal

Replacement for subjects after withdrawal from the study if the patients withdraws after inclusion.

8.6 Follow-up of subjects withdrawn from treatment

Patients that withdraw from the study will not be followed up.

8.7 Premature termination of the study

Not applicable.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed. Unexpected incidental findings on the ^{68}Ga-FAPI PET/CT will be noted and reported to the head practitioner of the patient who inform the patient as well.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

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

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

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

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events, except for the following SAEs:

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

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

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

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

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

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

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

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

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

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

9.3 Annual safety report

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

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

9.4 Follow-up of adverse events

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

9.5 Data Safety Monitoring Board (DSMB)

Considering the low risk associated with this study, a DSMB will not be instituted.

10. STATISTICAL ANALYSIS

Normally distributed quantitative variables will be presented with the mean, standard deviation and 95 % confidence interval. Non-normally distributed data will be presented with median and interquartile range. Outcomes of primary and secondary study parameters will be exploratory outcomes.

10.1 Primary study parameter(s)

Descriptive statistics will be used to analyze the degree of Fibroblast Activation Protein expression on ^{68}Ga-FAPI PET/CT, expressed as wlTL-FAPI and wlFAPI-MAV. Correlations between FAPI-PET/CT findings and HRCT findings will be assessed using Pearson's correlation coefficient or Spearman's rank-order correlation. A p-value of <0.05 will be considered significant.

10.2 Secondary study parameter(s)

The correlation between FAPI PET/CT findings and lung function (DLCO and VC), 6-MWT, EQ5D and HRCT fibrosis findings will be assessed using Pearson's correlation coefficient or Spearman's rank-order correlation. Statistical significance between consecutive tests will be assessed using analysis of variance, paired t-test or Wilcoxon signed-rank test, as appropriate. A p-value of <0.05 will be considered significant.

10.3 Other study parameters

Not applicable.

10.4 Interim analysis (if applicable)

Not applicable.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

Patients will be recruited at the post-COVID outpatient clinic in the UMCG, including referrals from pulmonologists in the north of the Netherlands. A participant information file (PIF) and consent form will be handed over to possible inclusions by the attending pulmonologist. The patient will return the consent form by mail to the principal investigator if the patient decides to participate.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable.

11.4 Benefits and risks assessment, group relatedness

The findings from this study will provide us pivotal biomechanistic insight in post-acute COVID-19. It will also result in identification of patients that may benefit in the future from anti-fibrotic medication to enhance pulmonary recovery. The risk of developing cancer as a consequence of the exposure to radiation in this study is very low.

11.5 Compensation for injury

The sponsor has a liability insurance which is in accordance with article 7 of the WMO.
The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

Participants will receive traveling costs in compensation and a gift card of 75 euro for their participation in the study.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

All Investigators and study site staff involved with this study must comply with the requirements of the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation with regard to the collection, storage, processing and disclosure of personal information and will uphold these Act's core principles. Access to collated participant data will be restricted to individuals from the research team treating the participants, representatives of the sponsor(s) and representatives of regulatory authorities.
Computers used to collate the data will have limited access measures via user names and passwords.
Published results will not contain any personal data that could allow identification of individual participants. RedCap will be used for data storage and analysis.

12.2 Monitoring and Quality Assurance

This study will be monitored yearly by a monitor from the UMCG monitoring pool (Johan Wiegers).

12.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

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

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject,

numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

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

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

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

12.6 Public disclosure and publication policy

Ownership of the data arising from this study resides with the study team. The study results will be submitted unreservedly for publication in a peer-reviewed journal or other scientific platform. Both positive and negative results will be disclosed. Published results will not contain any personal data that could allow identification of individual participants.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

There are some potential hazards of the investigations that we will perform as part of the trial. The main issues relate to exposure to ionising radiation and arterial line insertion.

a. Level of knowledge about mechanism of action

Ionising radiation can cause cancer which manifests itself after many years or decades. The estimated associated risk of developing fatal cancer is proportional to dose and also to the participant's age at the time of exposure. The risk for younger people is higher than for older people and the risk for females is higher than for males of the same age. According to report 26 of the Netherlands Commission on Radiation Dosimetry, radiation exposure for this study must be in category IIb at most. Since total radiation exposure in this study is 5 mSv, males must be at least 10 years old and females must be at least 20 years old to fall into category IIb (18).

A recent study scanned 25 patients following injection of between 198 and 299 MBq ^{68}Ga-FAPI-4 (19). With consideration to minimising dose, all participants in this study will receive a maximum of 200 MBq per IV administration of ^{68}Ga-FAPI-4 with a total dose of 1.6 mSv. All participants will receive an additional dose from the (low dose) CT-component of the scanner (an additional 0.7 mSv), including a baseline HRCT (2.7 mSv) so in total each PET/CT including HRCT will be associated with 5mSv of radiation exposure (see Supplement Radiation dose calculation). The follow-up chest HRCTs will be clinically performed.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

[^{68}Ga]FAPI-46 has been applied in several human studies in patients with various types of cancer (12,13,19–21) and pulmonary fibrosis (8). No adverse events have been reported in these studies.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Not for this study as we are looking into pulmonary fibrosis timeline in humans.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Not applicable.

e. Analysis of potential effect

Please see: Synthesis.

f. Pharmacokinetic considerations
Not applicable.

g. Study population
We will recruit 20 adult patients with post-acute COVID-19 and respiratory complaints that will undergo ^{68}Ga-FAPI PET/HRCT imaging to establish pulmonary fibrosis activity. This group will be divided in two subgroups:
Group 1: pulmonary complaints and abnormal lung function test.
Group 2: pulmonary complaints and normal lung function test.
The Total Research Protocol Dose (TRPD) per patient is 5 mSv.

h. Interaction with other products
Patients should not be included in other studies including ionizing radiation while being part of our study. No other pharmacodynamic interactions expected.

i. Predictability of effect
Not applicable.

j. Can effects be managed?
Potential risk is minimal at current radiation doses. Patients will be advised to avoid further ionizing radiation.

13.2 Synthesis
The absolute lifetime risk of developing cancer as a consequence of taking part in this study for the participant at highest risk, i.e. a 20-year-old female, is 0.04% (22). For comparison, the natural lifetime cancer incidence in the general population is about 50%. Taking part in this study increases the participant's chance of this happening to them from 50% to 50.04%. The average annual background radiation dose arising from natural sources of ionising radiation in the environment in the Netherlands is 1.7 mSv to 2.5 mSv (23). Participants in our study will receive the equivalent of about 2 to 2.5 times annual background radiation from natural sources.

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