Sofie Biosciences, Inc.

SOFIE

CLINICAL STUDY PROTOCOL

Study Title: A Phase 2, Multicenter, Single Arm, Open Label, Non-Randomized Study of [^{18}F]FAPI-74 PET in Patients with Gastrointestinal Cancers

Investigational Product: [^{18}F]FAPI-74

Sponsor: Sofie Biosciences, Inc
21000 Atlantic Boulevard
Suite 730
Dulles, VA 20166

IND No.: 162243

Protocol Number: 18FFAPI-2023P2

Protocol Version/Date: Version 6.0 November 18th, 2024

This protocol was designed and will be conducted, recorded, and reported in compliance with the principles of Good Clinical Practice (GCP) guidelines, as adopted by U.S. Federal Law.

1. PROTOCOL SUMMARY

1.1. Synopsis

Study Title:	A Phase 2, Multicenter, Single Arm, Open Label, Non-Randomized Study of [^{18}F]FAPI-74 PET in Patients with Gastrointestinal Cancers
Sponsor	Sofie Biosciences, Inc 21000 Atlantic Boulevard Suite 730 Dulles, VA 20166
Protocol Number **Version:** **Date**	18FFAPI-2023P2 V6.0 November18th, 2024
Study Description:	Prospective, multi-center, open label, non-randomized clinical trial to assess efficacy of [^{18}F]FAPI-74 to detect FAP expressing cells in patients diagnosed with gastrointestinal cancers, including hepatocellular carcinoma, cholangiocarcinoma, gastroesophageal (includes gastric, gastroesophageal junction and esophageal), pancreatic, and colorectal cancer. The [^{18}F]FAPI-74 PET scan will be acquired in patients with proven GI cancers after initial staging using institutional standard methods. The PET scan results will be compared to FAP immunohistochemistry (as the primary objective) and histopathology (as the secondary objective) of the biopsied or resected tissues. Lastly, an expanded pharmacokinetic profile of [^{18}F]FAPI-74 will be obtained in healthy volunteers.
Study Rationale	Fibroblast activation protein (FAP) is a type II transmembrane glycoprotein that has low to absent expression in healthy tissues but unique expression profile in multiple malignancies (cancer-associated fibroblasts, CAFs). FAPI-74 is one of the validated inhibitor small molecules that has high binding properties to FAP which renders it an important diagnostic marker in visualizing tumor, stroma, and the microenvironment (TME). An effective marker of FAP expressing cells, such as radiolabeled FAPI-74 ([^{18}F]FAPI-74), utilized in molecular imaging such as positron emission tomography (PET), would provide significant value in disease diagnosis as well as monitoring response to antineoplastic therapy.
Objectives:	Primary Objective: 1. Evaluate positive predictive values of [^{18}F]FAPI-74 PET images, to detect FAP-expressing cells using immunohistochemistry. Secondary Objectives: 1. Evaluate positive predictive values of [^{18}F]FAPI-74 PET images, to detect cancer cells using histopathology as truth standard. 2. Further characterize the safety profile of [^{18}F]FAPI-74 utilizing Common Terminology Criteria for Adverse Events (CTCAE v5). Exploratory Objective: [redacted]
Planned Total Sample Size	The study will be conducted in two cohorts. Cohort A consists of the evaluation of patients with cancers of the gastrointestinal (GI) tract with the goal of establishing efficacy parameters of [^{18}F]FAPI-74. Cohort B consists of an evaluation of the pharmacokinetic parameters and additional safety profile of [^{18}F]FAPI-74 in healthy volunteers A. Investigational Cohort: 60-150 cancer patients in support of the clinical protocol in Cancers of the GI tract. B. Healthy Cohort: Up to 6 healthy volunteers to assess pharmacokinetics, cardiac metrics, and treatment emergent events in participants unburdened with known malignancy. Note applicable to A: in order to have an appropriate number of subjects for each cancer type,

	there will be minimum of 10 enrolled per cancer type. Due to high incidence rate of colorectal cancer and to ensure appropriate sampling of various GI cancers, the maximum number of subjects of any cancer type in this study is capped at 60. Enrollment will be stopped when each tumor type includes at least 10 subjects and the total number of subjects is at least 60, with a maximum of 150 subjects. Due to low incidence rate of Cholangiocarcinoma, the analysis for Cholangiocarcinoma and Hepatocellular carcinoma will be combined under hepatobiliary cancer with minimum of 10 subjects total.
Primary Inclusion Criteria	A. Investigational Cohort. 1. Confirmed diagnosis of GI Cancer, including hepatocellular carcinoma, cholangiocarcinoma, gastroesophageal (includes gastric, gastroesophageal junction, and esophageal), pancreatic and colorectal cancer. 2. Available sample of malignancy (primary and/or metastatic sampling) for FAP testing or scheduled biopsy and/or surgical resection as per routine clinical care. NOTE: research only biopsy may be considered on a case-by-case basis at the discretion of SOFIE to address this inclusion criteria. 3. *No treatment received between tissue sample taken and [^{18}F]FAPI-74 PET. 4. Anatomic imaging (e.g., CT, MRI) obtained within ≤ 90 days of Study Day 1. 5. Age ≥ 18 years 6. Completed informed consent as determined per the IRB of record B. Healthy subject Cohort 1. Age ≥ 18 years. 2. Completed informed consent as determined per the IRB of record. 3. Lab results, which include C-reactive protein, white blood cell counts, neutrophil, counts, and platelet counts within normal institutional limits. 4. No known diagnosis of disease that could impact FAP expression. List includes: cancer diagnosis, fibroinflammatory disease, fibrotic disease (lung fibrosis, liver fibrosis, Crohn's disease). 5. For three of the subjects, a 4-6 hour fast prior to injection of study drug. *In the event where treatment is expected to have no impact on FAP expression or [^{18}F]FAPI-74 PET, at the discretion of sponsor and medical reviewer, the subject can be allowed to proceed onto the study.
Primary Exclusion Criteria	Same for study subjects for Investigational Cohort and Healthy Cohort 1. Pregnant as determined by a pregnancy test as per institutional guidelines for individuals of child-bearing potential. 2. Declining to use effective contraceptive methods during the study (for individuals of child-producing potential). 3. Need for emergent surgery that would be delayed by participation. 4. Bacterial, viral, or fungal infections requiring systemic therapy, that are expected to impact FAP expression in the opinion of the sponsor or their designee. 5. Serious co-morbidities and serious nonmalignant disease (e.g., hydronephrosis, kidney failure, liver failure, systemic or local inflammatory or autoimmune diseases or other conditions) that in the opinion of the investigator, physician of record and/or SOFIE could compromise patient safety and/or protocol objectives. 6. Known diagnosis of an autoimmune disorder that is expected to impact FAP expression in the opinion of the sponsor or their designee. 7. Patients receiving any other investigational medical product (including imaging agents) within the past 28 days. 8. Breastfeeding. Note: nursing parents are allowed if the potential participant commits to pumping breast milk and discarding it from injection to ≥ 24 hours from the time of the [^{18}F]FAPI-74 injection.

	9. Known hypersensitivity to any excipients used in [^{18}F]FAPI-74: trace amounts of sodium acetate, sodium ascorbate, ascorbic acid, ethanol, acetonitrile or AlCl. 10. Renal or liver function impairment* *Defined by liver impairments as AST>5x the upper limit of normal, ALT>5x the upper limit of normal, or bilirubin>1.5x the upper limit of normal. Renal impairment as defined by a creatinine clearance of <50 ml per min utilizing the Cockcroft Gault formula Note: hepatocellular carcinoma and cholangiocarcinoma subjects are exempt from the outlined liver impairment thresholds and these marker limits do not apply as exclusion criteria for these subjects. Above threshold indirect bilirubin levels in the setting of Gilbert syndrome or extragalactic conditions such as hemolytic anemia are allowable and not contraindications to the study.
Test Article Administration and Imaging	Intravenous administration of radiolabeled [^{18}F]FAPI-74 (7 mCi ± 2 mCi) as per institutional policies, administered prior to the initiation of the attenuation correction CT followed by the PET acquisition encompassing the participant's vertex through mid-thighs. Images are acquired at 60 minutes (± 10 minutes). In a subset of subjects a second imaging time point of 30 minutes (± 10 minutes) will be obtained.
Duration of Participation	**Investigational Cohort** Up to 25 calendar days from Study Day 1 • screening period ≤ 90 calendar days • imaging intervention: ≤ 4 calendar days • (if clinically indicated) surgical intervention ≤ 21 days of [^{18}F]FAPI-74 PET scan **Healthy Cohort** Up to 1 calendar day from Study Day 1 • screening period ≤ 30 calendar days • imaging intervention: 1 calendar day
Data Evaluation	Clinical Data Data from sites will be reported and entered in an Electronic Data Capture (EDC) system Imaging Data Imaging data reads will be conducted by a central image reading lab followed by quantitative recording of the data. Special Considerations: Lesions will be counted, classified with respect to their locations (liver, peritoneum, non-regional lymph nodes, lung, bone, and other), and their [^{18}F]FAPI-74 avidity will be semi-quantitatively analyzed through SUV_{max}. Standard-of-care imaging will be evaluated with established clinical criteria for the assessment of metastases. Tissue data Tissue samples (biopsy or resected tissue) are required from all patients to meet the primary endpoint of the study. No treatment is to take place between the time that tissue has been obtained and [18F]FAPI-74 image acquired. Formalin-fixed, paraffin-embedded (FFPE) tissue sections from biopsied or resected specimens will be evaluated by immunohistochemical methods for FAP expression levels along with histopathology analysis. In the event that no tissue becomes available from the patient subject or quality of tissue proves not yield a FAP IHC, [redacted] In addition, in the event where treatment is expected to have no impact on FAP expression or [^{18}F]FAPI-74 PET, at the discretion of sponsor and medical reviewer, the subject can be allowed to proceed onto the study.

Statistical Analysis Plan and Justification of Sample Size

Investigational Cohort

The sample size will be between 60 and 150 subjects in order to provide adequate testing properties overall [redacted].For this study, the primary efficacy endpoints are positive predictive value (PPV) by comparing the results from [^{18}F]FAPI-74 PET and immunohistochemistry in detecting FAP-expressing cells .For determination of the positive predictive value a test with appropriate levels of sensitivity is appropriate. This will be demonstrated with the following hypothesis test for sensitivity.

H0: Sensitivity ≤ Performance Goal (PG)

H1: Sensitivity > Performance Goal (PG)

The minimum sample sizes necessary to achieve desired power for sensitivity were computed for the hypothesis test above utilizing a one-sided significance level of 0.05 and assuming a prevalence of 75.0% of FAP-expressing cells using immunohistochemistry (gold Standard), and the following parametric assumptions,

- Performance Goal (PG) = 0.75
- True Sensitivity = 0.90

In a one-sided test for sensitivity, a sample size of 60 subjects achieves 84.15% power at the 0.05 one-sided significance level when the sensitivity under the null hypothesis is 0.75 and the sensitivity under the alternative hypothesis is 0.9, assuming a condition prevalence of 75.0%.

It is expected that the sample size will be larger than 60 based on the goal of having 10 subjects for each cancer type, with a maximum of 150 total subjects.

		Immunohistochemistry	
		Positive	Negative
[^{18}F]FAPI-74 PET	Positive	A	B
	Negative	C	D
Total		A + C	B + D

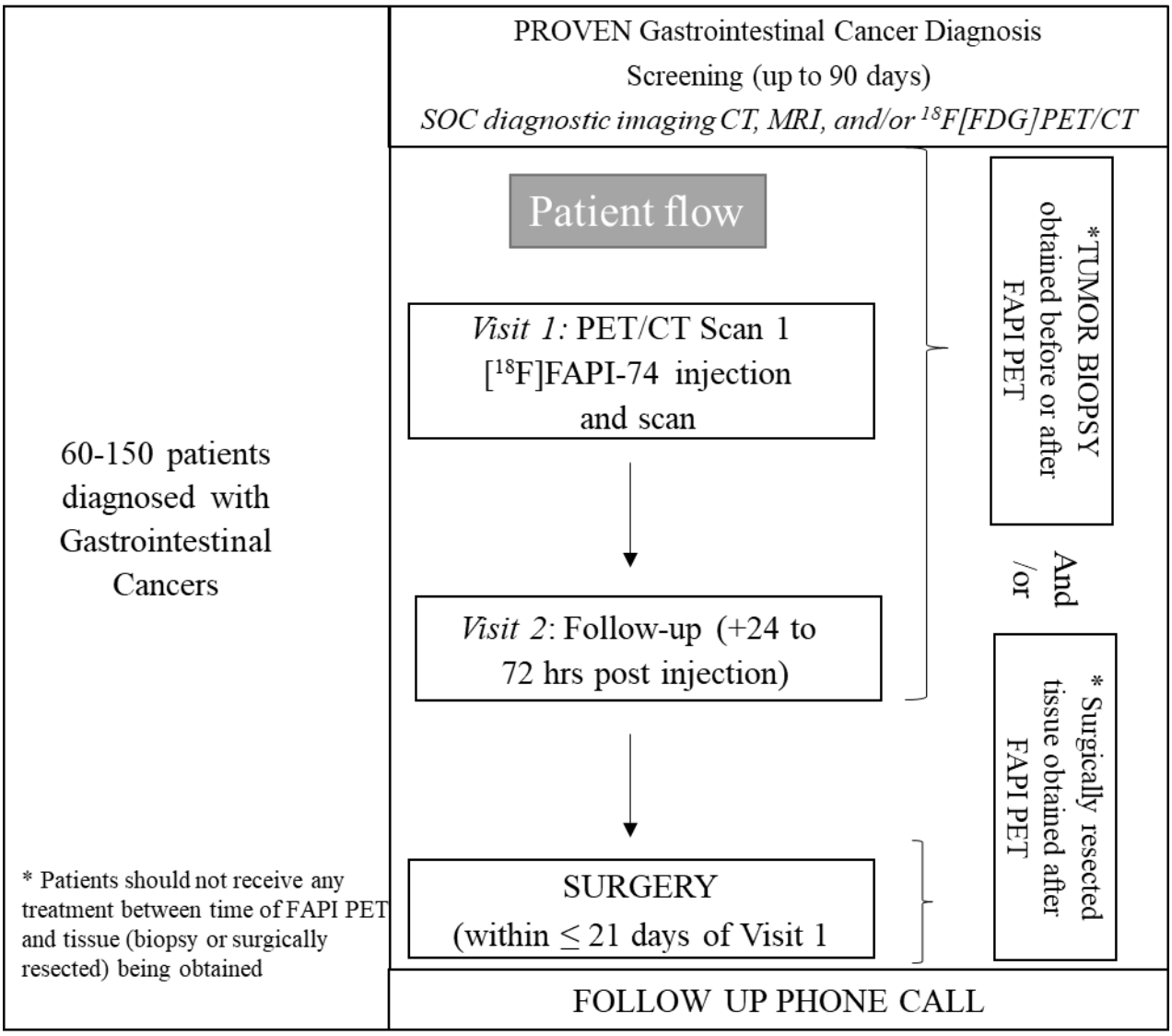
Positive Predictive Value (PPV) = A/ (A+B) Primary Endpoint

Sensitivity = A / (A + C)

The positive predictive value will be estimated and a 95% confidence interval about this estimate. The width of the confidence interval is determined based on the number of subjects in the A+B cells. Even when A+B totals is 35, a two-sided 95% confidence interval for a single proportion using the large sample normal approximation will extend 0.099 from the observed proportion for an expected proportion of 0.9. For larger A+B totals the Confidence interval width will be even more narrow. This demonstrates the estimation of PPV will have reasonable precision.

Intra- and inter-reader agreement of the scans using [^{18}F]FAPI-74 PET for the detection of FAP expressing cells will be summarized by kappa statistics (and 95% confidence intervals). Association between SUV_{max} from [^{18}F]FAPI-74 PET on cross sectional imaging and FAP expression from IHC will be summarized by correlation coefficients with 95% confidence intervals.

1.2. Schema for Investigational Cohort



1.3. Schedule of Activities- Investigational Cohort

Note: days are counted as calendar days unless otherwise specified. The day before study day 1 is Day -1.

Study Procedure	**Screening** ≤ 90 days of D1	**Visit 1** Study day 1	**Visit 2**[j] + 24 to 72 hours post-injection	**Surgery** ≤ 21 days of PET scan	**Surveillance Period** (14 days) [h]
Informed consent	X				
Complete medical history, including surgical history	X				
Standard of care imaging: CT, MR and/or [^{18}F]-FDG PET	X				
Concomitant medications	X	X	X		
Physical examination [a]	X		X[f]		
ECOG performance status	X	X			
Vital signs [b]	X	X	X[f]		
Serum chemistries [c]	X	X[k]	X		
CBC with differential		X[k]	X		
Tumor biomarkers (e.g., CA-19-9, CEA)[d]	X	As per standard of care			
Assessment of tumor burden[d]	X				
Urine pregnancy test[e]	X	X[k]			
Baseline signs and symptoms		X			
Study drug [^{18}F]FAPI-74 administration and PET scan		X			
Capturing administered treatments				X	X
AE assessment [i]		X	X		
Tissue obtained through core biopsy and/or surgical resection	X[g]	As per standard of care			

a: Includes height and weight. Height collected only during the screening window.
b: Temperature, blood pressure, respiratory rate and pulse rate. Obtain twice on day of imaging: once prior to injection and once post-injection prior to discharge
c: Glucose, Na, K, Cl, Carbonate (CO_2), Ca, BUN, creatinine, AST, ALT, ALP, total protein, albumin, and total bilirubin
d: Frequency and type of test as per institutional standard of care; tumor burden assessment is obtained from surgical, pathology and/or imaging reports
e: As per institution's practice. If urine pregnancy test is inconclusive, a serum pregnancy test must be performed with results reviewed prior to study drug administration
f: Only if necessary for adverse event assessment; symptom directed exam is acceptable
g: Tissue obtained through core biopsy and/or surgical resection. No treatment is to take place between time of FAPI scan and tissue being obtained. In the event of surgery, the subject is expected to receive surgery within ≤ 21 days of FAPI PET scan. IHC and histopathology will be at a central lab. Biopsy tissue obtained within 6 months of [^{18}F]FAPI-74 PET, without any treatment performed between the two events, can be accepted for analysis. At the discretion of the sponsor, with additional justification, tissue beyond 6 months can be considered for inclusion. In the event where treatment is expected to have no impact on FAP expression or [18F]FAPI-74 PET, at the discretion of sponsor and medical reviewer, the subject can be allowed to proceed onto the study
h: Passive follow-up only through chart review to collect information about post-surgical imaging, planned treatment, and outcomes. 14 days post-surgery for subjects who undergo surgery. 14 days post Visit 2 for subject who do not undergo surgery
i: AE assessments will be performed 24 hours post [^{18}F]FAPI-74 injection
j: Follow up visits may be performed through televisits. Subjects may utilize institution approved local labs/mobile clinics for blood draws as part of follow up assessment. Subjects may enter surgery <24 hours post-injection if the time of Visit 2 follow-up assessment is 10 half-lives or more post [^{18}F]FAPI-74 injection
k. Labs obtained during screening period, within 72 hours of Day 1, can be used as pre-FAPI-74 injection Day1 lab data.

1.4. Schedule of Activities- Healthy Cohort

Study Procedure	Screening ≤ 30 days of D1	Visit 1[h] Study day 1
Informed consent	X	
Complete medical history, including surgical history	X	
Concomitant medications	X	X
Physical examination [a]	X	
Vital signs [b]	X	X
Serum chemistries [c]	X	X[f]
CBC with differential	X	X[f]
Urine pregnancy test[e]	X	X[f]
Baseline signs and symptoms		X
Study drug [^{18}F]FAPI-74 administration and PET scan		X
Pharmacokinetic and ECG assessment	X[g] As per Section 6.1	

a: Includes height and weight. Height collected only during the screening window.
b: Temperature, blood pressure, respiratory rate and pulse rate. Obtain twice on day of imaging: once prior to injection and once post-injection prior to discharge
c: Glucose, Na, K, Cl, Carbonate (CO_2), Ca, BUN, creatinine, AST, ALT, ALP, total protein, albumin, total bilirubin, and C-reactive protein
e: As per institution's practice. If urine pregnancy test is inconclusive, a serum pregnancy test must be performed with results reviewed prior to study drug administration
f. Labs obtained during screening period, within 72 hours of Day 1, can be used as pre-FAPI-74 injection Day1 lab data.
g. 6 healthy volunteers will go through a Pharmacokinetic and ECG assessment according to outlined process in Section 6.1. These subjects will undergo a subset of screening as outlined above. These subjects will end their participation at the end of the PK and ECG assessment.
h. A phone call will occur within 24-48 hours to make sure the subject is feeling well and has had no Adverse Events.

Note: days are counted as calendar days unless otherwise specified. The day before study day 1 is Day -1

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3. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse Event
CFR	Code of Federal Regulations
eCRF	Electronic Case Report Form
CRO	Contract Research Organization
CBC	Complete Blood Count
CTCAE	Common Terminology Criteria for Adverse Events
EC	Ethics Committee
EDC	Electronic data capture, synonymous with the study database
FAP	Fibroblast activation protein
FAPI	Fibroblast-activation-protein inhibitors
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IHC	Immunohistochemistry
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
NAT	Neoadjuvant therapy (chemotherapy, radiation therapy, or both)
NPV	Negative Predictive Value
PET	Positron Emission Tomography
PPV	Positive Predictive Value
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
SUV	Standardized Uptake Value

4. INTRODUCTION

4.1. Background

An estimated 19.3 million new cancer cases and almost 10 million cancer deaths occurred worldwide in 2020. Cases of the digestive system exceeded 5 million (26%) and 3.6 million (36%) deaths (Sung et al., 2021). Prognosis of most tumors of the gastrointestinal system is poor; therefore, early detection and diagnosis are of great significance for treatment prognosis. The PET/CT imaging agent [^{18}F]FDG has been used in the diagnosis and staging of digestive tract malignant tumors (Akin et al., 2020). However, FDG applications have many limitations, especially in distinguishing between inflammation and malignancy, making its efficacy suboptimal for tumor delineation and identification of metastatic lesions (Chen et al., 2021; Hathi & Jones, 2019; Pang et al., 2021; Zhao et al., 2021)

Due to its low expression in healthy tissues, increased expression in wound healing, inflammation, and fibrosis, and high expression in CAFs in tumor-associated tissues (stroma) and some cancers, FAP has become an important and favorable therapeutic and diagnostic target in cancer. At present, FAP expression is quantified by mRNA or protein analysis from biopsies, an invasive technique that does not provide a whole-body assessment of target expression due to potential heterogeneity between primary tumors and metastatic lesions. Therefore, there is an unmet need to characterize FAP expression at the whole-body level to assess target avidity for FAP-directed therapies.

Based on preliminary data in patients with tumors of the gastrointestinal system, there is strong evidence for the potential efficacy of FAPI family of compounds in detecting primary and metastatic lesions. In two reviews by Peng et al. (Peng et al., 2022) and Yang et al. (Yang et al., 2021) the authors highlight the limitations of FDG PET/CT in imaging of tumors such as pancreatic cancer, gastric adenocarcinoma, highly differentiated hepatocellular carcinoma, renal cell carcinoma and detection of peritoneal metastasis. These articles highlight the existing publications which demonstrate positive preliminary data on FAPI family of compounds in primary and metastatic lesion detection with low background, making FAPI compounds a promising tool for non-invasive characterization, tumor staging and treatment monitoring.

The rare disease designation of a number of gastrointestinal cancers, such as CCC, HCC and gastric cancer, the anatomical and organ region relatedness of these cancers, along with the strong preliminary performance of FAPI compounds, especially in metastatic disease detection in liver and peritoneal carcinomatosis, provide a strong justification for a broad gastrointestinal tract evaluation of [^{18}F]FAPI-74 in these GI cancers for an effective and efficient clinical investigation.

4.2. Investigational Product: [^{18}F]FAPI-74

4.2.1. Development and Optimization

In healthy tissues, FAP expression is very low and often absent. Conversely, FAP expression is high across a wide variety of cancers and cancer-associated stromal tissues (Mona et al., 2022; *The Human Protein Atlas: FAP PATHOLOGY*) with high expression correlated with poor prognosis (Brennen et al., 2012). At present, FAP expression is quantified by mRNA or protein

analysis from biopsies, an invasive technique that does not provide a whole-body assessment of target expression due to potential heterogeneity between primary tumors and metastatic lesions. Therefore, there is an unmet need to characterize FAP expression at the whole-body level at a specific time point to establish reliable predictive biomarkers for FAP-directed therapies.

In response to this unmet need, many well-documented FAPI compounds and derivatives have been developed in recent years. This development has followed a continuous progression of improvements focusing on achieving high tumor retention, high signal-to-background, high tumor uptake, and low activity in normal organs. A second focus in development was on the shift from radiolabeling with [^{68}Ga] to labeling with [^{18}F] to overcome limitations posed by the short half-life of [^{68}Ga] and the small batch size of [^{68}Ga]-tracers. This work resulted in the identification of FAPI-74 as the preferred compound for radiolabeling with [^{18}F] that best meets these requirements.

Initial work on a PET imaging [^{68}Ga]-radiotracer targeting FAP produced the FAPI-01 and FAPI-02 compounds (Loktev et al., 2018). From this initial work, FAPI-02 showed superior performance with higher cellular uptake and retention over time. Further clinical work was conducted evaluating [^{68}Ga]FAPI-02, to perform diagnostic PET/CT scans in 3 patients with different cancer diagnoses: metastasized breast cancer, metastasized lung cancer, and metastasized pancreatic cancer (Loktev et al., 2018). All patients showed high uptake of the tracer in the primary tumor, as well as lymph node and bone metastases, with low to no tracer uptake in normal tissue (Loktev et al., 2018).

Subsequent work then focused on prolonging the tumor retention time, resulting in the development of twelve new FAPI compounds (Loktev et al., 2019). Through a series of experiments utilizing FAP-expressing cells and small animal imaging, these FAPI compounds were evaluated based on cell uptake, retention, specificity to FAP versus similar proteins (DPP4), biodistribution, and tumor to blood ratios. Results indicated that [^{68}Ga]FAPI-04 was the strongest candidate, with rapid internalization into FAP-expressing tumors and favorable tumor-to-organ ratios. Moreover, [^{68}Ga]FAPI-04 was a marked improvement over [^{68}Ga]FAPI-02 with a significantly improved tumor retention over 24 hours. The utility of FAPI-04 was further demonstrated in a groundbreaking study where 80 patients with 28 different types of cancer were successfully imaged with [^{68}Ga]FAPI-04, showing high image contrast and good tumor delineation (Kratochwil et al., 2019).

Despite these promising results with [^{68}Ga]FAPI-04, further optimization to enhance tumor retention while maintaining high image contrast was sought. Fifteen more FAPI compounds were developed and tested, with [^{68}Ga]FAPI-46 emerging as having marked improved performance, including better tumor uptake and higher tumor-to-organ ratios than FAPI-04, making [^{68}Ga]FAPI-46 the preferred [^{68}Ga]-compound for FAP PET imaging.

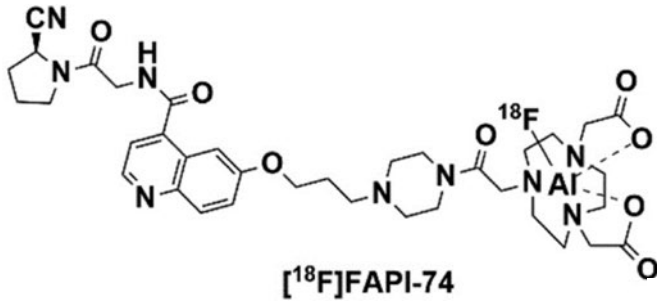
Although [^{68}Ga]FAPI-46 has demonstrated excellent characteristics in human studies, [^{68}Ge]/[^{68}Ga] generators can limit access to the FAPI compounds due to short half-life, requiring synthesis very close to the imaging site. In addition, capacity to support larger studies with [^{68}Ga]-labelled compounds could be challenging due to large centers with high patient throughput requiring several productions per day to meet potential demands. Aiming at broader applicability of FAP PET through [^{18}F]-radiolabeling of FAPI compounds, seven further FAPI compounds were evaluated. FAPI-74 was identified as the FAPI compound with the same

excellent pharmacological and radiopharmaceutical characteristics as FAPI-46 and suitable for radiolabeling with [^{18}F].

4.2.2. Investigational Product

The investigational product is [^{18}F]FAPI-74.

4.2.3. Structure



[^{18}F]FAPI-74

4.2.4. Synthesis overview

FAPI-74 precursor is a small organic molecule critical drug intermediate. It consists of a FAPI moiety conjugated with a NOTA chelator which is reacted with F-18, a positron emission radioisotope with a short half-life of 110 minutes, to produce the final drug product Al[^{18}F]FAPI-74 (equivalent to the product name [^{18}F]FAPI-74).

The manufacturing process of Al[^{18}F]FAPI-74 final drug product was adapted from the manual process developed by Giesel et al. (Giesel et al., 2021) and carried-out on a Trasis miniAllinOne radiosynthesizer.

Chemical Name:	*[^{18}F]Aluminum fluorine (S)-2,2`(7-(2-(4-(3-(4-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethylcarbamoyl)quinolin-6-yloxy) propyl)piperazin-1-yl)-2-oxoethyl)-1,4,7-triazonane-1,4-diyl)diacetic acid*
Pharmacological Class:	A PET radioligand for detecting Fibroblast Activation Protein
Dosage Formulation:	[^{18}F]FAPI-74 Injection (Final Drug Product)
Route of Administration:	Intravenous Administration

4.3. Nonclinical Overview

FAPI-74 is the result of continued development of the FAPI-02/-04/-46 compound series and utilizes the same scaffold. It was specifically selected for labeling with [^{18}F], which is desirable because of the short half-life of [^{68}Ga] and the limited batch size of [^{68}Ga]-tracers. Preclinical studies showed FAPI-74 as a very promising PET tracer with high binding specificity and selectivity to FAP-expressing cells, with high tumor-to-healthy tissue contrast, and physio-chemical properties comparable to the earlier FAPI derivatives, including FAPI-46, and FAPI-04 (Lindner 2021). [^{18}F]FAPI-74's impressive potential was robustly demonstrated in both cell

studies and in mice with FAP-expressing HT-1080 and PANC-1xenografts (Lindner 2021, Liu 2021).

The first proof to show FAPI-74 advantages was provided by an analysis of serum stability using [^{18}F]-FAPI-74. These results showed that at 4 hours only the intact radioactive drug was detected with neither radioactive degradation products nor free radioactivity being observed or measured. This provides evidence that FAPI-74 is stable *in vivo* and not affected by enzymes present in the human sera.

Upon successful cell culture experiments, Lindner et al. 2021 evaluated HT-1080-hFAP xenografted mice by small-animal PET imaging using FAPI-74 to assess *in vivo* and *ex vivo* biodistribution and *in vivo* binding specificity since specificity and effective discrimination between FAP-expressing tumor cells and normal organ cells is imperative. *In vivo* and *ex vivo* biodistribution data were highly congruent.

Pharmacokinetics of FAPi-74 have been assessed *in vitro* using cell-based assays to determine binding kinetics, and *in vivo* PET-based and *ex vivo* biodistribution studies in mice with HT-1080-hFAP xenografts. Lindner et al. (Lindner 2021) showed promising pharmacokinetic data for FAPI-74, with rapid binding, tumor uptake and clearance from non-target organs, stability in human sera and renal excretion.

A single dose, acute and extended, Good Laboratory Practice (GLP) compliant toxicology study was performed. A single dose of Al[^{19}F]FAPI-74 (166.7 µg/kg) was administered intravenously to male and female rats. Acute and delayed toxicity evaluations were performed. The dose was well-tolerated, and no adverse effects were noted. The human equivalent dose (HED) was preselected as 100 µg for a 60 kg adult which is 100 x of the no observed adverse effect level (NOAEL) established in rats.

4.4. Previous Clinical Studies Overview

Biodistribution and dosimetry studies have been performed [^{18}F]FAPI-74 in lung cancer patients showing high lesion-to-normal tissue contrast and the calculated effective dose from the PET component was low. Data is reported in Section 274 Summary of Clinical Safety **(Giesel et al., 2021)**

In addition, clinical PET/CT studies have been conducted and published with [^{18}F]FAPI-74 and [^{68}Ga]FAPI-74 demonstrating favorable tumor to background ratio, high SUVmax and ability to detect primary and metastatic disease in various cancer indications patients (Section 273 Summary of Clinical Efficacy). According to Supporting Letter Haberkorn, 12 patients have been imaged with [^{18}F]FAPI-74 and 97 patients with the gallium labeled product [^{68}Ga]FAPI-74 of the same molecular entity, adverse events were monitored and none were observed or reported in patients. In addition to the published data, under the SOFIE global distribution program where FAPI-74 precursor is provided to investigators globally in support of investigator initiated clinical trials, currently 17 clinical research studies are underway with a total of 470 patients. No serious adverse drug events have been reported from any of these studies.

4.5. Rationale for the Study

As a diagnostic radiopharmaceutical at microdose level, the very favorable dosimetry and non-toxic molecular design, a very robust benefit/risk and safety profile is expected and will be studied and confirmed during further product development.

5. STUDY OBJECTIVES AND OUTCOME MEASURES

5.1. Objectives

Primary Objective

- Evaluate positive predictive values of [^{18}F]FAPI-74 PET images, to detect FAP-expressing cells using immunohistochemistry

Secondary Objectives

- Evaluate positive predictive values of [^{18}F]FAPI-74 PET images, to detect cancer cells using histopathology as truth standard
- Further characterize the safety profile of [^{18}F]FAPI-74 in subjects utilizing Common Terminology Criteria for Adverse Events (CTCAE v5)

Exploratory Objective

[redacted]

5.2. Endpoints

5.2.1. Primary Endpoints

- Positive predictive value will be determined on a per-lesion basis for all lesions with tissue available for FAP analysis.
  - Lesions positive for FAP as detected by immunohistochemistry
  - Lesions positive for FAP as detected by [^{18}F]FAPI-74 PET imaging

5.2.2. Secondary Endpoints

- Positive predictive value will be determined on a per-lesion basis for all lesions with tissue available for histopathology.
  - Malignant lesions as detected by histopathology
  - Lesions positive for FAP as detected by [^{18}F]FAPI-74 PET imaging
- Incidence and severity of treatment emergent adverse events occurring within 24 hours.

5.2.3. Exploratory Endpoints

[redacted]

The selection of the primary objective for this study is based on [^{18}F]FAPI-74 having been developed to specifically target FAP expressing cells, allowing for a robust imaging mechanism in assessing the expression of FAP in cells. This objective is the fundamental validation of [^{18}F]FAPI-74 PET in FAP expression detection, confirmed through immunohistochemistry in the specific cancers being proposed.

As a secondary objective, we have proposed positive predictive value for [^{18}F]FAPI-74 in determining disease (cancer) through histopathology. Since FAP expression is in the TME, not primarily in the cancer cells themselves, we expect CAF in the vicinity of the tumor cells to show high expression of FAP, depending on type and stage of disease.

As per request from the FDA on July 19th, 2022 meeting pre-IND meeting, in a healthy volunteer cohort, we will conduct a pharmacokinetic study and an ECG study to detect QT interval prolongation and its relationship to drug concentration over the duration of the study.

6. STUDY DESIGN AND SCHEMATIC

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures are essential and required for study conduct.

6.1. Study Design

This is a prospective, multi-center, open label, non-randomized clinical trial to assess efficacy of [^{18}F]FAPI-74 to detect FAP expressing cells in patients diagnosed with gastrointestinal cancers, including hepatocellular carcinoma, cholangiocarcinoma, gastroesophageal (includes gastric, gastroesophageal junction and esophageal), pancreatic and colorectal cancer. The [^{18}F]FAPI-74 PET scan will be acquired in patients with proven GI cancers after initial staging using institutional standard methods. The PET scan results will be compared to FAP immunohistochemistry and histopathology of the biopsied or resected tissues.

Number of subjects planned

The study will be conducted in two cohorts. Cohort A consists of the evaluation of patients with cancers of the gastrointestinal (GI) tract with the goal of establishing efficacy parameters of [^{18}F]FAPI-74. Cohort B consists of an evaluation of the pharmacokinetic parameters and additional safety profile of [^{18}F]FAPI-74 in healthy volunteers

A. Investigational Cohort: 60-150 cancer patients in support of the clinical protocol in Cancers of the GI tract.

B. Healthy Cohort: Up to 6 healthy volunteers to assess pharmacokinetics, cardiac metrics, and treatment emergent events in participants unburdened with known malignancy.

Note applicable to A: in order to have an appropriate number of subjects for each cancer type, there will be minimum of 10 enrolled per cancer type. Due to high incidence rate of colorectal cancer and to ensure appropriate sampling of various GI cancers, the maximum number of subjects of any cancer type in this study is capped at 60. Enrollment will be stopped when each tumor type includes at least 10 subjects and the total number of subjects is at least 60, with a maximum of 150 subjects. Due to low incidence rate of Cholangiocarcinoma, the analysis for Cholangiocarcinoma and Hepatocellular carcinoma will be combined under hepatobiliary cancer with minimum of 10 subjects total.

Summary of Study Visits and Data Collection

Refer to Schedule of Events for full requirements for each visit as defined for Investigational Cohort and the Healthy Cohort

Investigational Cohort

Study day 1 refers to the day the (first) [^{18}F]FAPI-74 PET occurs, day 2 is the following calendar day, etc. Tissue is obtained from subjects through biopsy and/or surgical resection. Subjects are not to receive any treatment between [^{18}F]FAPI-74 PET acquisition and tissue being obtained. In the event that no tissue becomes available from the patient subject or quality of tissue proves not yield a FAP IHC, [redacted]

Screening: Screening assessments can be conducted at multiple visits provided they are within 90 days prior to study day 1, including anatomic imaging. Tests performed for standard of care may be used for screening purposes provided they are within the window. Tissue biopsy may be obtained during the screening period. Standard of care imaging provided with MR, CT and/or [^{18}F]FDG PET.

Visit 1: Defines Study Day 1. [^{18}F]FAPI-74 PET scan obtained.

Visit 2: 24 to 72 hours after [^{18}F]FAPI-74 injection. Safety assessment with safety labs and, if needed, a symptom directed physical examination.

Surgery: Surgical resection to occur within 21 days after [^{18}F]FAPI-74 PET scan.

Resected tissue should be collected as per laboratory manual. Surgical and pathology reports will be collected.

Biopsy tissue obtained within 6 months of [^{18}F]FAPI-74 PET, without any treatment performed between the two events, can be accepted for analysis. At the discretion of the sponsor, with additional justification, tissue beyond 6 months can be considered for inclusion.

Tissue samples will be assessed for FAP expression and by histopathology analysis.

Healthy Cohort

Screening: Screening assessments can be conducted at multiple visits to assure that the healthy volunteer meets all inclusion and exclusion criteria.

Visit 1: [^{18}F]FAPI-74 PET scan along with pharmacokinetic and electrocardiogram assessment performed.

Follow-up: A phone call will occur within 24-48 hours to perform a wellness check on the subject to ensure that they have had no Adverse Events. If Adverse Events are reported, they will be captured for the study and reported appropriately.

Pharmacokinetic and Electrocardiogram (ECG) assessment

6 healthy volunteer subjects will undergo Pharmacokinetic and ECG assessments as outlined below. Details are provided in the PK and ECG Manual. Note, this study design has been adopted from the pharmacokinetic and ECG assessment performed for [^{18}F]DCFPyL("Study of 18F-DCFPyl PET/CT Imaging in Patients With Suspected Recurrence of Prostate Cancer (CONDOR)," 2019).

A 4.5 mL blood sample will be collected at the following nine time points: pre-dose, 5±2 m,15±2 m, 30±5 m, 1±0.25 hr, 2±0.25 hr, 4±0.25 hr, 6±0.25 hr, and 8±0.25 hr post-dose. Urine samples will be collected over the following intervals: 0-2 hr, 2-4 hr and 4-8 hr post dose. Whole body PET/CT scans will be collected at three time points: up to 17 minutes after [^{18}F]FAPI-74 injection and prior to voiding, 1± 0.25 hr and 4± 0.25 hr post dose.

A 12-lead ECG will be taken at two intervals on the day of dosing, The pre-dose ECG should be evaluated prior to dosing by the Investigator or delegated team member. In the absence of clinically significant symptoms, the post-dose ECG may be reviewed by the Investigator, delegated team member, or per the site's usual clinical practice. If either ECG is machine interpreted as other than normal and clinically significant, the ECG must be evaluated by a cardiologist or qualified site designee for subject safety at a turn-around time as per the Investigator's discretion.

Procedure	Pre-Dose	0 hr	5±2m	15± 2m	30 ± 5m	1± 0.25 hr	2 ± 0.25 hr	3 ± 0.25 hr	4 ± 0.25hr	6 ± 0.25hr	8 ± 0.25hr
[^{18}F]FAPI-74 Dose Administration		X									
[^{18}F]FAPI-74 PET/CT		X				X			X		
Blood and Plasma Collection	X		X	X	X	X	X		X	X	X
Urine Collection		X					X		X		
ECG	X	X									

6.2. Subject Population

6.2.1.Sample Size

Up to 150 subjects with GI cancers, including gastrointestinal cancers, including hepatocellular carcinoma, cholangiocarcinoma, gastrioesophageal (includes gastric, gastroesophageal junction and esophageal), pancreatic and colorectal cancer.

6 additional healthy volunteers will be enrolled in support of the PK and ECG study.

Notes: in order to have an appropriate number of subjects for each cancer type, there will be minimum of 10 subjects enrolled per cancer type. Due to high incidence rate of colorectal cancer

and to ensure appropriate sampling of various GI cancers, the maximum number of subjects of any cancer type in this study is capped at 60. Due to low incidence rate of Cholangiocarcinoma, the analysis for Cholangiocarcinoma and Hepatocellular carcinoma will be combined under hepatobiliary cancer with minimum of 10 subjects total.

6.2.2. Inclusion Criteria

Investigational Cohort

- Confirmed diagnosis of gastrointestinal (GI) malignancy, including hepatocellular carcinoma, cholangiocarcinoma, gastroesophageal (includes gastric, gastroesophageal junction and esophageal), pancreatic and colorectal cancer
- Available tissue sample obtained through biopsy or to be obtained through scheduled biopsy and/or surgical resection
- No *treatment received between tissue sample taken and [^{18}F]FAPI-74 PET
- Anatomic imaging (e.g., CT, MRI) obtained within ≤ 90 days of study Day 1
- Age ≥ 18 years
- Completed informed consent as determined per the IRB of record

*In the event where treatment is expected to have no impact on FAP expression or [^{18}F]FAPI-74 PET, at the discretion of sponsor and medical reviewer, the subject can be allowed to proceed onto the study.

Healthy Cohort

- Age ≥ 18 years
- Completed informed consent as determined per the IRB of record
- Lab results, which include C-reactive protein, white blood cell counts, neutrophil, counts, and platelet counts within normal institutional limits.
- No known diagnosis of disease that could impact FAP expression. List includes: cancer diagnosis, fibroinflammatory disease, fibrotic disease (lung fibrosis, liver fibrosis, Crohn's disease)
- For three of the subjects, a 4-6 hour fast prior to injection of study drug

6.2.3. Exclusion Criteria

- Pregnant as determined by a pregnancy test as per institutional guidelines for individuals of child-bearing potential
- Declining to use effective contraceptive methods during the study (for individuals of child-producing potential)
- Need for emergent surgery that would be delayed by participation
- Bacterial, viral, or fungal infections requiring systemic therapy, that are expected to impact FAP expression in the opinion of the sponsor or their designee

- Serious co-morbidities and serious nonmalignant disease (e.g., hydronephrosis, kidney failure, liver failure, systemic or local inflammatory or autoimmune diseases or other conditions) that in the opinion of the investigator, physician of record and/or Sofie could compromise subject safety and/or protocol objectives
- Known diagnosis of an autoimmune disorder that is expected to impact FAP expression in the opinion of the sponsor or their designee
- Patients receiving any other investigational agent within the past 28 days
- Breastfeeding. Note: nursing parents are allowed if the potential participant commits to pumping breast milk and discarding it from injection to ≥ 24 hours from the time of the [^{18}F]FAPI-74 injection
- Known hypersensitivity to any excipients used in [^{18}F]FAPI-74: trace amounts of sodium acetate, sodium ascorbate, ascorbic acid, ethanol, acetonitrile or AlCl.
- Renal or liver function impairment*

*Defined by liver impairments as AST>5x the upper limit of normal, ALT>5x the upper limit of normal, or bilirubin>1.5x the upper limit of normal. Renal impairment as defined by a creatinine clearance of <50 ml per minute utilizing the Cockcroft Gault formula Note: hepatocellular carcinoma and cholangiocarcinoma subjects are exempt from the outlined liver impairment thresholds and these marker limits do not apply as exclusion criteria for these subjects. Above threshold indirect bilirubin levels in the setting of Gilbert syndrome or extragalactic conditions such as hemolytic anemia are allowable and not contraindications to the study.

6.2.4.Concomitant Medication

No other investigational medical products are allowed during this study, from eligibility determination to surgery. Participant may receive investigational medical products post-surgery for the purposes of another clinical trial.

Drugs used off-label consistent with institutional practice, standard of care, or practice of medicine do not meet the definition of investigational medical product.

Participants should receive full concomitant care during this clinical trial consistent with institutional practices and standard of care.

6.3. Study Intervention

[^{18}F]FAPI-74

FAPI-74 precursor is a small organic molecule critical drug intermediate. It consists of a FAPI moiety conjugated with a NOTA chelator which is reacted with F-18, a positron emission radioisotope with a short half-life of 110 minutes, to produce the final drug product Al[^{18}F]FAPI-74 (equivalent to the product name [^{18}F]FAPI-74).

The manufacturing process of Al[^{18}F]FAPI-74 final drug product was adapted from the manual process developed by Giesel et al. (Giesel et al., 2021) and carried-out on a Trasis miniAllinOne radiosynthesizer.

Dosing and Administration

Intravenous administration of radiolabeled [^{18}F]FAPI-74 (7 mCi ± 2 mCi) as per institutional policies, administered prior to the initiation of the attenuation correction CT followed by the

PET acquisition encompassing the participant's vertex through mid-thighs. Images are acquired at 60 minutes (± 10 minutes). In a subset of subjects a second imaging time point of 30 minutes (± 10 minutes) will be obtained.

Preparation, Handling, Storage, and Accountability

An accurate and current accounting of the precursor and/or study drugs will be maintained utilizing an ALCOA compliance drug accountability record (DAR) that will be verified by the Sponsor's study monitor.

Drug accountability will be documented using the study's drug accountability and receipt form (DARF) or the investigational site's template *if* deemed acceptable by the sponsor and/or designee. Documentation confirming review and approval of the Site's template must be provided by Sponsor or designee and filed within the ISF.

At minimum:

- Receipt: date received and signature/mark individual confirming receipt
- Dispensing: date, quantity of IMP (both in ml and mCi), study identification number and initials of participant, and signature/mark of individual confirming these dispensing details.
- Investigational medical product destroyed, where 'destroyed,' is equivalent to allowing decay for 10 half-lives and then destroying the physical component consistent with institutional policy & procedure.

The DAR will be monitored against study drug and supply shipments and ALCOA.

Drug components provided by the Sponsor or designee must be used only for this study. Accountability logs must be utilized to track drug components.

Only authorized prescribers may prescribe the investigational medical product. Authorized prescribers must be authorized users as per NRC.

A copy of the prescription must be kept on file in the participant's medical record or, if precluded by institutional policy, within the study binder.

Formulation and appearance

The final [^{18}F]FAPI-74 drug product (DP) is a clear, colorless or slightly yellow solution free from visible particles. The DP contains 1 mCi/ml to 110 mCi/ml of [^{18}F]FAPI-74 at the end of synthesis (EOS).

Storage and stability

Stored upright in lead-shielded container at 15 – 25 °C with use before expiration of 10 hours post end of synthesis (EOS). Handling of the investigational medical product is subject to radioactive material regulations and licensing requirements.

Preparation

[^{18}F]FAPI-74 is provided as ready to use vial or syringe. No further preparation or manipulation is required. The dose will be administered to the subject according to the site's standard operating practices.

Intervention Compliance

All interventions in this protocol are administered by licensed medical staff under the supervision of the study investigator. For this reason, adherence to protocol will be assessed through source document verification in the electronic health records for:

- Prescription, dispense, and administration of [18F]FAPI-74
- Scan settings (e.g., bed positions, field of view settings)

6.4. Study Intervention Discontinuation and Participant Discontinuation/withdrawal

6.4.1. Discontinuation of Study Interventions

Participants who withdraw after consenting to the clinical trial, but do not receive an injection of [^{18}F]FAPI-74, will not be followed for this clinical trial.

Participants who withdraw after receiving 1 injection of [^{18}F]FAPI-74 but who are not scanned will be followed as an intent-to-treat population for the purposes of safety assessments. If the participant withdraws full permission (i.e., revokes HIPAA authorization), the participant will not be followed further, but all data obtained until the date of HIPAA revocation will be used for the purposes of this study. A participant's intent must be well documented in the study record

Scenarios for study intervention discontinuation:

- **Participant declines further study intervention**. If a participant who has received at least one injection of [^{18}F]FAPI-74 declines further study intervention, the reason for declining further participation should be documented – specifically, if it is due to an adverse event.
- **Unforeseen medical event.** If a participant undergoes a medical event that compromises the subject's safety or study's endpoints, the participant may be withdrawn from treatment. The sponsor or investigator may make this determination. If a participant is withdrawn for this reason, the subject must be followed for a minimum of 24 hours from the last injection with investigational product.
- **Pregnancy**. If a participant declares pregnancy, the participant will be withdrawn from study procedures. The Sponsor will be notified within 1 business day of the event. The participant should be asked to consent to have the pregnancy followed (as appropriate).
- **Participant non-compliance**. If a participant does not comply with the required adverse event assessment (i.e., hematologic and serum chemistry assessments, follow-up assessments), study treatment may be discontinued to reduce risk to the participant.

6.4.2. Participant Discontinuation / Withdrawal from the Study

Subject participation in the study may be discontinued entirely (i.e., withdrawn from study without further follow-up or interaction) in the following circumstances:

- **Participants who decline to receive any study intervention after consenting** to the clinical trial are withdrawn from study and are not followed for this clinical trial.
- **Investigator non-compliance**. If an investigator is considered to be non-compliant with the study protocol or federal, state, local, or institutional requirements, the sponsor may end the study and withdraw subjects from trial.
- **Participant's refusal to continue**. Participants who decline further study follow-up (i.e., no longer wish to undergo study assessments) are withdrawn from the study. If the

participant withdraws full permission (i.e., revokes HIPAA authorization), the participant will not be followed further, but all data obtained until the date of HIPAA revocation will be used for the purposes of this study. The subject's intent must be well documented in the study record.

- **Termination of study**. If the study is terminated for any reason, the participant will be followed for a minimum of 24 hours from the last injection of [^{18}F]FAPI-74 for safety assessment, including hematologic and serum chemistries as prescribed per protocol.

The reason for participant discontinuation or withdrawal from the study will be recorded in the subject's study record and entered into the electronic data capture system (EDC).

6.4.3.Lost to Follow-up

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- **Imaging Visit**. If the participant fails to maintain the scheduled appointment for the [^{18}F]FAPI-74, and the investigational medical product is wasted, the site will contact the Sponsor or designee. The Sponsor or designee will determine if the participant should be removed from study or be allowed the opportunity to be rescheduled.
- **Safety follow-up**. The investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

6.4.4.Replacement Criteria

Participants who have withdrawn or are withdrawn from study may be replaced at Sponsor's discretion.

7. STUDY ASSESSMENTS AND PROCEDURES

An overview of the study schedule is provided in tabular form under "SCHEDULE OF Activities." Two Schedules of Activities have been provided, one for the Investigational Cohort and one for the Healthy Cohort. Note that only a subset of procedures below applies to the Healthy Cohort. Refer to the Schedule of Activities- Healthy Cohort for the master list of applicable procedures. The timing of each visit will be relative to the prior visit. Individual study procedures are described below in Section 7.1.

7.1. Study Procedures

7.1.1.Informed Consent

Informed consent will be obtained prior to undertaking any protocol-specific research-only procedures. All participants must provide informed consent as outlined by the IRB of record. No elements of consent will be waived and will be compliant with U.S. FDA federal code.

If written consent is used, the participant must receive a copy of the signed informed consent form. If oral consent is used, the participant must be provided documentation as outlined by the

IRB of record. Documentation of the consent process must be maintained in the participant's medical record or the participant's case history, dependent upon institutional policies.

7.1.2.Contraception

For individuals of child-bearing potential, effective contraception must be utilized during the study. Effective contraceptives include abstinence as well as barrier and oral contraceptives.

If participants are engaging in fertility sparing strategies (i.e., ova collection and banking, sperm collection and banking), this is to be done prior to injection of study agent.

Participants should not discontinue contraceptive strategies without speaking with their treating oncologists.

7.1.3.Medical and Surgical History

The following should be documented in the participant's case history and reported through the EDC:

- Medical history as documented in the medical record for the 5 years (e.g., such as non-rheumatoid arthritis, gout, edema). Medical history should provide explanation / justification for any active treatments and concomitant therapies taken at study entry.
- Known allergies
- History of myocardial infarction or other life-threatening event, regardless of time-frame.
- Race and ethnicity; if participant declines, this should be clearly noted in the case history.
- Any prior radiation and its indication.

7.1.4.Concomitant Medications

For the purposes of this protocol, a concomitant therapy is considered to be any of the following:

- Prescription medication from an authorized prescriber
- Over the counter medications
- Complementary or alternative medications, including herbal remedies or supplements

A concomitant medication log, separate from the participant's medical chart, will be maintained for the purposes of this trial. This log will be a source document and must meet ALCOA standards. Appropriate medical staff will review concomitant medications at each study visit with the subject so the medications can be reviewed if an untoward event occurs during the study.

All concomitant medications and supportive therapy administered from consent (i.e., routine medications) through end of must be reported using on the appropriate case report form (eCRF).

7.1.5.Routine and Symptom Directed Physical Examination

A physical examination is required per protocol for study eligibility during the screening window. At minimum, the physical examination includes examination of major body systems, including general appearance, skin, neck, head, eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system.

A symptom focused physical exam is performed as needed during the adverse event window 24

to 72 hours post-injection of [^{18}F]FAPI-74. This physical exam would be performed to further characterize an observed adverse event for study documentation. The adverse event or concern triggering symptom-focused physical exam should be clearly documented.

7.1.6.Vital Signs

For protocol, collected vital signs (VS) are blood pressure, heart rate, respiratory rate, temperature, and weight. Vital signs must be obtained after sitting at least two minutes; time seated and time obtained must be documented. Height is collected at screening only.

On day of [^{18}F]FAPI-74, a set of VS must be obtained prior to injection and post-injection prior to discharge.

7.1.7.Performance Status

Performance status will be assessed using ECOG performance status criteria as outlined in Appendix 2.

7.1.8.Clinical Laboratory Tests

Local laboratories will analyze all hematology, blood chemistry collected for the study. Samples will be analyzed at a facility meeting Clinical Laboratory Improvement Amendments (CLIA) requirements and/or using methods documented in a methods validation report.

7.1.9.CBC with differential

White blood cell count, red blood cell count, hematocrit, hemoglobin, platelets, absolute neutrophil count, lymphocytes, basophils, eosinophils, and monocytes will be collected per the schedule of events.

CBC w/diff must be collected prior to injection with [^{18}F]FAPI-74.

7.1.10. Serum Chemistry

Glucose (non-fasting), sodium, potassium, chloride, carbon dioxide, calcium, blood- urea-nitrogen (BUN), creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, albumin, and total bilirubin.

7.1.11. Pregnancy Test(s)

As per institutional policy, a urine pregnancy test will be performed for individuals of child-bearing potential at screening and prior to injection of [^{18}F]FAPI-74 as per institutional policies, but no more than 72 hours of dosing study drug.

If the urine pregnancy test is inconclusive, a blood test must be ordered and resulted as negative prior to injection of [^{18}F]FAPI-74.

An individual who has recently undergone an abortive procedure may still have elevated hCG levels. In this situation, documentation from a treating physician that the subject is not pregnant will suffice *in lieu* of a pregnancy test.

7.1.12. [^{18}F]FAPI-74 PET Scan

The imaging manual contains detailed technical information for the [^{18}F]FAPI-74 PET/CT scan.

7.1.13. Prior Preparation

Participants should be directed to hydrate well the day prior to scan. The scan does not require

fasting (except for three subjects in the Healthy Subject Cohort who will be instructed to fast 4-6 hours prior to injection of study drug), but participants are asked to abstain from alcohol ~ 1 day prior to scan. Participants should rest well the night before the scan.

7.1.14. Day of Scan

Obtain vitals (section 7.1.5) prior to initiating procedures to reduce stress-associated elevations.

[^{18}F]FAPI-74 is administered intravenously; intravenous access is per institutional standard of care for administration of PET tracer agents.

During the uptake period (60 minutes (±10 minutes), participants should be encouraged to hydrate and void frequently. Participants should be directed to void prior to being placed on the scanner.

Scanning will be initiated 60 minutes (±10 minutes) post-injection. Scanner coverage should be set up to extend from vertex to mid-thighs. Low dose CT is to be used for anatomical correlation.

In a subset of subjects a second imaging time point of 30 minutes (± 10 minutes) will be obtained.

7.1.15. Tissue Specimen Collection and Handling

Tissues collected at biopsy or surgery will be processed according to institutional standard for local histopathological assessment of pancreatic cancer.

See Laboratory Manual for detailed instructions for the preparation, packaging, and shipping instructions for tumor tissue that will be evaluated by IHC for FAP evaluation and histopathology.

7.1.16. Tumor Burden Assessment

Disease burden will be assessed using RECIST criteria by a central imaging core lab. Local imaging (e.g., CT, MRI, PET/CT) will be anonymized eliminating the PHI, codified with participant study ID, and will be provided to the Sponsor/CRO for third party imaging analysis. Imaging to be submitted is outlined in the Schedule of Activities.

7.2. Adverse Events

For the purposes of this study, the adverse event collection window is from the time of injection with [^{18}F]FAPI-74 through minimum 10 half-lives or 24 hours post-injection. If the 24-hour window ends on a holiday or non-clinic day (or the participant cannot return to clinic due to unforeseen circumstances, such as weather), the window may be extended until 72 hours post-injection.

Any adverse event suspected to be related to [^{18}F]FAPI-74 – regardless of collection window – should be assessed and reported to the Sponsor/CRO.

7.2.1.Definitions

This study defines terms as per the U.S. federal code at 21CFR§312.32.

7.2.2.Adverse event

Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

7.2.3.Life-threatening adverse event or life-threatening suspected adverse reaction

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of

either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

7.2.4.Serious adverse event or serious suspected adverse reaction

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.2.5.Suspected adverse reaction

Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.2.6.Unexpected adverse event or unexpected suspected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis.
Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

7.2.7.Severity Grading

Adverse event severity will be evaluated utilizing the CTCAE v.5 criteria. If there is a question regarding severity grading or harmonization, the Sponsor or CRO should be contacted.

7.2.8.Reporting Adverse Events

Routine adverse events

Adverse events that do not meet serious criteria (section 8.4) are considered routine adverse events. Routine adverse events are reported utilizing the appropriate eCRF page in the study database.

The investigator is reminded to comply with all institutional and local requirements as well as the requirements put forth by the IRB of record regarding reporting of routine adverse events.

Serious adverse events

Any adverse event meeting serious criteria (section 8.2) occurring within the collection window must be reported following the instructions in Appendix 1 – Serious Adverse Event Reporting. **The timeframe for reporting** the event to the sponsor/CRO **is 1 business day** of the clinical investigator becoming aware of the initial event or clinically significant follow-up information.

The Investigator must provide a causality assessment and must sign and date all SAE Report Forms.

If supporting documentation is included in the submission (e.g., hospital reports, consultant reports, death certificates, autopsy reports, etc.), all protected health information must be redacted. When appropriate, additional written reports and supporting documentation will be provided by the site for further clarification and information.

The investigator is reminded to comply with all institutional and local requirements as well as the requirements put forth by the IRB of record regarding serious adverse events.

7.2.9.Adverse events leading to study discontinuation

Participants who have withdrawn, or were withdrawn, from the study due to an adverse event (regardless of attribution) will be followed by the investigator until resolution or a new baseline, provided the participant consents to being followed.

7.2.10. Attribution to Investigational Medical Product and Study Procedure

The relationship of [^{18}F]FAPI-74 and the PET scan procedure to an adverse event will be assessed by the Investigator utilizing the FDA's December 2012 guidance *Safety Reporting Requirements for INDs and BA/BE Studies*.

The investigator must note if there is a reasonable possibility there is evidence to suggest a causal relationship between [^{18}F]FAPI-74 and the adverse event and if there is evidence to suggest a causal relationship between the PET scan procedure and the adverse event.

Within the reporting requirement under 21 CFR§312.32(c)(1)(i), FDA makes clear the meaning of reasonable possibility by providing the following examples of types of evidence that would suggest a causal relationship between the drug and the adverse event.

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture)
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that those events occur more frequently in the drug treatment group than in a concurrent or historical control group

Although the sponsor is responsible for determining whether there is a reasonable possibility that the drug caused the adverse event, taking into consideration the investigator's assessment, the FDA requires the clinical investigator provide the sponsor

with their assessment of causality for all serious adverse events.

7.2.11. Documentation

Case Histories / Participant Binders

Adverse events should be recorded within the participant's study record compliant with ALCOA. At minimum, information captured must include:

- Term, harmonized to CTCAE v. 5. Do not use a verbatim term or medical jargon – for example, use "white blood cell decreased," instead of leukopenia and "neutrophil count decreased," rather than neutropenia.
- Severity grade (as per CTCAE v. 5)
- Date, onset
- Date, end
- Outcome (e.g., without sequelae, with sequelae, ongoing)
- Attribution to study drug (unrelated, probable, definite)
- Attribution to the study procedure (i.e., PET/CT scan) (unrelated, probable, definite)

Attributions must be confirmed by the study's clinical investigator and documented by signature and date.

7.2.12. Laboratory Abnormalities

All abnormal laboratory results pre-categorized within the CTCAE v5 meeting grade 3 or 4 severity will be recorded and reported as AEs.

An abnormal laboratory result, pre-categorized within the CTCAE v5 meeting grade 1 or 2 severity, will be recorded and reported as an AE if the investigator deems the result clinically significant.

An abnormal laboratory result, not pre-categorized within the CTCAE v5 (e.g., increased eosinophils, decreased creatinine) will not be captured as an adverse event unless deemed clinically significant by the investigator. The investigator must deem the result "mild," "moderate," "severe," or "life threatening," with the clinically significant notation.

7.2.13. Clinical Syndrome/Diagnosis vs. Symptomatic Components

To the extent possible, individual symptomatic events (i.e., nausea, vomiting, laboratory abnormalities) observed during the course of the study should be reported as the overarching syndrome/diagnosis rather than the individual symptoms. For example, 'refeeding syndrome,' 'renal failure,' or 'hepatitis.' As appropriate, the laboratory abnormality itself (e.g., elevated creatinine) does not need to be recorded as an AE. Final determination of the appropriateness of recording (i.e., syndrome/diagnosis vs. symptom) is made by the clinical investigator.

7.2.14. Reporting Adverse Events to Participants

The Sponsor will review all reported serious adverse events and determine the appropriateness of changes to the informed consent document. The sponsor will notify investigators if re-consenting subjects is mandatory for all participants or only for those currently undergoing investigational procedures. If the sponsor does not believe re-consenting is mandatory, the investigator may require re-consent for those all participants or just those subjects undergoing

investigational procedures. Similarly, the sponsor will also defer to the IRB of record if it determines re-consent should be mandatory.

7.3. Unanticipated Problems

The phrase "unanticipated problems involving risks to subjects or others" is found but not defined in the HHS regulations at 45 CFR part 46. OHRP considers unanticipated problems, in general, to include any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)- approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

OHRP recognizes that it may be difficult to determine whether a particular incident, experience, or outcome is unexpected and whether it is related or possibly related to participation in the research. OHRP notes that an incident, experience, or outcome that meets the three criteria above generally will warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects or others

Only a small subset of adverse events occurring in human subjects participating in research will meet these three criteria for an unanticipated problem.

Furthermore, there are other types of incidents, experiences, and outcomes that occur during the conduct of human subjects research that represent unanticipated problems but are not considered adverse events. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased risk of harm, but no harm occurs. Examples of unanticipated problems (UP) provided by OHRP:

- Clinical trial data are stored on a laptop computer without encryption, and the laptop computer is stolen from the investigator's car on the way home from work.
- As a result of a processing error by a pharmacy technician, a subject enrolled in a multicenter clinical trial receives a dose of an experimental agent that is 10-times higher than the dose dictated by the IRB-approved protocol. While the dosing error increased the risk of toxic manifestations of the experimental agent, the subject experienced no detectable harm or adverse effect after an appropriate period of careful observation.
- Subjects with cancer are enrolled in a phase 2 clinical trial evaluating an investigational biologic product derived from human sera. After several subjects are enrolled and receive the investigational product, a study audit reveals that the investigational product administered to subjects was obtained from donors who were not appropriately screened and tested for several potential viral contaminants, including the human immunodeficiency virus and the hepatitis B virus.

7.4. Deviation from prescribed dose

In accordance with the NRC guidelines for diagnostic Nuclear Medicine, in the event of deviations from prescribed administered dose activity, the CRO/Sponsor should be contacted within 1 business day. This single intravenous (IV) injection should contain 7 mCi ± 2 mCi of [^{18}F]FAPI-74. The total volume administered will be between 0.1 and 10 mL. A protocol violation will be considered when [^{18}F]FAPI-74 dose administered is more than 20% of the prescribed activity.

If the dose deviation is reasonably associated with an adverse event (serious or non-serious), documentation for the adverse reaction must also be completed regardless of adverse reaction onset.

The investigator is reminded to comply with all institutional and local requirements as well as the requirements put forth by the IRB of record regarding protocol violations.

7.5. Pregnancy

A pregnancy occurring in a participant or in the partner of a participant during the course of the study (i.e., from study drug injection until end of study participation) is considered an unanticipated problem.

The investigator or site personnel must notify the Sponsor within 5 working days after the investigator or site personnel become aware of the pregnancy (see APPENDIX 1 – SERIOUS ADVERSE EVENT REPORTING for details). If an SAE occurs in conjunction with the pregnancy, then the reporting time frame for an SAE (within 1 business day) must be met.

The investigator is reminded to comply with all institutional and local requirements as well as the requirements put forth by the IRB of record regarding a pregnancy during trial participation.

7.6. Unanticipated Problem Reporting Adverse event as an unanticipated problem

An adverse event, or serious adverse event, also meeting the definition of an unanticipated problem will follow the adverse event reporting pathway.

7.7. Investigator-identified non-adverse event unanticipated problems

The investigator will provide information to the sponsor-investigator within 1 business day. The report will include the following information:

- Protocol identifying information: protocol title and number, investigator's name, and the participant study ID (if applicable)
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP
- A description of corrective actions that have been taken or are proposed in response to the UP or proposed changes to the protocol or investigational plan

The investigator is reminded to comply with all institutional and local requirements as well as the requirements put forth by the IRB of record regarding unanticipated problems.

7.8. Reporting Unanticipated Problems to Participants

The sponsor will review all unanticipated problems and determine the appropriateness of changes to the informed consent document. The sponsor will notify investigators if re-consenting subjects is mandatory for all participants or only for those currently undergoing investigational procedures.

The sponsor will defer to these IRBs if requested to change the risk section of the informed consent document. Similarly, the sponsor will also defer to the IRBs if it is determined re- consent should be mandatory.

8. STATISTICAL ANALYSIS

8.1. General

Descriptive statistics will be utilized for safety parameters. All subjects will be accounted for. The study population for all analyses will be defined as all subjects enrolled in the study who receive at least one dose of [^{18}F]FAPI-74. Subjects who exit the study prior to receiving study medication may be replaced.

The sample size will be between 60 and 150 subjects in order to provide adequate testing properties overall and [redacted]

For this study, the primary efficacy endpoints is positive predictive value (PPV) by comparing the results from [^{18}F]FAPI-74 PET and immunohistochemistry in detecting FAP-expressing cells .

For determination of the positive predictive value a test with appropriate levels of sensitivity is appropriate. This will be demonstrated with the following hypothesis test for sensitivity.

H0: Sensitivity ≤ Performance Goal (PG)

H1: Sensitivity > Performance Goal (PG)

The minimum sample sizes necessary to achieve desired power for sensitivity were computed for the hypothesis test above utilizing a one-sided significance level of 0.05 and assuming a prevalence of 75.0% of FAP-expressing cells using immunohistochemistry (gold Standard), and the following parametric assumptions,

- Performance Goal (PG) = 0.75
- True Sensitivity = 0.90

In a one-sided test for sensitivity, a sample size of 60 subjects achieves 84.15% power at the 0.05 one-sided significance level when the sensitivity under the null hypothesis is 0.75 and the sensitivity under the alternative hypothesis is 0.9, assuming a condition prevalence of 75.0%.

It is expected that the sample size will be much larger than 60 based on the goal of having 10 subjects for each cancer type, with a maximum of 150 total subjects. Due to low incidence rate of Cholangiocarcinoma, the analysis for Cholangiocarcinoma and Hepatocellular carcinoma will be combined under hepatobiliary cancer with minimum of 10 subjects total.

		Immunohistochemistry	
		Positive	Negative
[^{18}F]FAPI-74 PET	Positive	A	B
	Negative	C	D
Total		A + C	B + D

Positive Predictive Value (PPV) = A/ (A+B) Primary Endpoint

Sensitivity = A / (A + C)

The positive predictive value will be estimated and a 95% confidence interval about this estimate. The width of the confidence interval is determined based on the number of subjects in the A+B cells. Even when A+B totals is 35, a two-sided 95% confidence interval for a single proportion using the large sample normal approximation will extend 0.099 from the observed proportion for an expected proportion of 0.9. For larger A+B totals the Confidence interval width will be even more narrow. This demonstrates the estimation of PPV will have reasonable precision.

Additional exploratory data analyses will be conducted as deemed appropriate by the Sponsor.

8.2. Safety Analysis

All subjects who receive any amount of [^{18}F]FAPI-74 will be included in the final summaries and listings of safety data.

Frequencies of subjects experiencing at least one AE will be displayed by body system and preferred term according to MedDRA terminology. Detailed information collected for each AE will include: description of the event, duration, whether the AE was serious, intensity, relationship to study drug, action taken and clinical outcome intensity (severity) of the AEs will be graded according to the CTCAE v5.0.

Summary tables will present the number of subjects observed with AEs and corresponding percentages. The denominator used to calculate incidence percentages consists of patients receiving at least one dose of study medication. Within each table, the AEs will be categorized by MedDRA body system and preferred term. Additional subcategories will be based on event intensity and relationship to study drug.

Deaths and other SAEs will be tabulated.

Vital signs will be summarized using descriptive statistics.

Summary tables will be prepared to examine the distribution of laboratory measures over time.

8.3. Efficacy Analysis

8.3.1. Primary Efficacy Analyses

The primary efficacy endpoints will compare the results from [^{18}F]FAPI-74 PET and immunohistochemistry results. The table below displays the possible outcomes from the samples for each subject, with definitions for positive predictive values, sensitivity, and specificity.

	Immunohistochemistry

		Positive	Negative
[68Ga]FAPI-46 PET	Positive	A	B
	Negative	C	D
Total		A+C	B+D

Positive predictive value= A/A+B
Sensitivity= A / (A + C) Specificity = D / (B + D)

The primary analysis positive predictive value will be summarized using frequency counts and percentages as well as 95% asymptotic normal confidence intervals.

8.3.2.Secondary Efficacy Analyses

The first secondary efficacy endpoint will compare the results from [^{18}F]FAPI-74 PET and histopathology. This will be conducted in the same manner at the primary analysis that is a comparison to immunohistochemistry. In this case the gold standard result is the histopathology.

Intra- and inter-reader agreement of the scans using [^{18}F]FAPI-74 PET for the detection of FAP expressing cells will be summarized by kappa statistics (and 95% confidence intervals).
Association between SUV_{max} from [^{18}F]FAPI-74PET on cross sectional imaging and FAP expression from IHC will be summarized by correlation coefficients with 95% confidence intervals.

8.3.3.Exploratory Efficacy Analyses

[redacted]

8.4. Interim Analysis

Not applicable

8.5. Sample Size

60 to 150 subjects will be enrolled in the study.

In a one-sided test for sensitivity, a sample size of 60 subjects achieves 84.15% power at the 0.05 one-sided significance level when the sensitivity under the null hypothesis is 0.75 and the sensitivity under the alternative hypothesis is 0.9, assuming a condition prevalence of 75.0%.

It is expected that the sample size will be much larger than 60 based on the goal of having 10 subjects for each cancer type, with a maximum of 150 total subjects. Due to low incidence rate of Cholangiocarcinoma, the analysis for Cholangiocarcinoma and Hepatocellular carcinoma will be combined under hepatobiliary cancer with minimum of 10 subjects total.

See statistical analysis plan for full description of assumptions and analytical plan

9. SUPPORTING DOCUMENTATION AND OPERATIONAL

CONSIDERATIONS

9.1. Regulatory, Ethical, And Study Oversight Considerations

9.1.1.Informed Consent Process

Informed consent and other documents provided to participant

Consent forms describing in detail the study agent, study procedures, and risks will be given to the participant and written documentation of informed consent is required prior to starting research procedures. None of the elements of consent outlined in 21CFR§50.25 will be waived.

Authorization of use and disclosure of protected health information (PHI) must be obtained from each participant prior to performing any study-specific screening evaluations. The authorization for use and disclosure of PHI must contain the elements required by 45 CFR 164.508(b) for valid authorizations.

A copy of the signed document will be provided to the participant. The original signed informed consent document will be maintained in the participant's case history (i.e., study binder). Written documentation of the consent process, signed and dated by the person who obtained consent, must be completed, and maintained in the participant's case history (i.e., study binder).

Written documentation of the consent process must be completed for any reconsent, including the reason for the reconsent.

9.1.2.Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the Sponsor or designee. If the study is prematurely terminated or suspended, the Investigator will promptly inform participants actively undergoing study assessment as well as the IRB of record. The Investigator will provide the reason(s) for the termination or suspension to the IRB of record and should, as allowed by the IRB, provide the same information to the participant as appropriate.

Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

See also Protocol Section 7.

9.1.3.Participant and Data Confidentiality

All information regarding the nature of the proposed investigation provided by the Sponsor or

Study Monitor to the Investigator (with the exception of information required by law or regulations to be disclosed to the IRB, the subject, or the appropriate regulatory authority) must be kept in confidence by the Investigator.

9.1.4.Participant Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants.
Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, the Sponsor, or representatives of the IRB of record may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by IRB and Institutional regulations. Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be reported using the study's electronic database, which is 21CFR§11 compliant. **This will not include the participant's contact or identifying information** as defined by 45CFR§164.514(b)(2). Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by research staff will be secured and password protected.

Of note, **the participant's actual date of birth must not be used for any study documentation**. The participant's date of birth must be "reset" to 01 of the appropriate month. For example, a birthdate of 24 December 2000 would be 01 December 2000.

9.1.5.Research Use of Stored Human Specimens

Tissue samples will be obtained and stored as described in the Laboratory Manual.

9.1.6.Study Governance, Key Roles, and Accreditations

Study Lead

[redacted] Ph.D
[redacted] SOFIE
[redacted]
Phone: [redacted]

Study Medical Monitor

[redacted] M.D
[redacted]

[redacted]

Phone: [redacted]

9.1.7.Site Investigator Information

The investigator will provide appropriate documentation of qualification as outlined in form FDA 1572.

The investigator is responsible to ensure the site conducts this clinical trial is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312.

9.1.8.Delegation of Site Investigator Responsibilities

The Investigator should ensure that all persons involved in the conduct of the study are informed about the protocol, protocol amendments, study procedures, and study related duties.

9.1.9.Laboratory Accreditations

Clinical laboratories to be used for the purposes of hematology, serum chemistry, tumor markers, and pathologic analysis within this protocol must be listed on the Investigator's form FDA 1572. The form should be updated if additional clinical laboratories are utilized.

Licensure/accreditations and reference values and/or normal ranges for the test results must be provided to the Sponsor or designee.

9.1.10. Safety Oversight

Safety oversight will be under the direction of a Data and Safety Monitoring Committee composed of individuals with the appropriate expertise.

9.1.11. Clinical Monitoring

"Effective monitoring of clinical investigations by sponsors is critical to the protection of human subjects and the conduct of high-quality studies. Sponsors of clinical investigations involving human drugs, biological products, medical devices, and combinations thereof are required to provide oversight to ensure adequate protection of the rights, welfare, and safety of human subjects and the quality of the clinical trial data submitted to FDA. FDA's regulations require sponsors to monitor the conduct and progress of their clinical investigations. The regulations are not specific about how sponsors are to conduct such monitoring and are therefore compatible with a range of approaches to monitoring that will vary depending on multiple factors." Page 2 of FDA's *Guidance for Industry: Oversight of Clinical Investigations*

— A Risk-Based Approach to Monitoring (October 2013).

A risk-based monitoring approach will be employed for this clinical trial.

- Centralized monitoring, utilizing EDC data analysis for outliers and inconsistent data patterns.
- Remote monitoring visits. Preferably, RMV will utilize electronic source (i.e., access to electronic health records, HIPAA/HITECH approved source data review) but surrogate source may be utilized as deemed appropriate.
- Directed on-site monitoring. These will be performed as determined by the study sponsor or designee. All sites will undergo at least one on-site monitoring visit to determine compliance to protocol and investigational plan, adequacy of documentation practices and

good clinical practice.

Monitoring reports must be filed with the IRB of record unless prohibited by policy. If prohibited, a copy of the policy must be provided for both the ISF and trial master file.

9.1.12. Quality Assurance and Quality Control

This section will briefly describe the plans for quality management, the system for assessing the quality of processes within a system. Quality management encompasses quality assurance (QA) and quality control (QC).

Each site, both clinical and laboratory, should have SOPs for quality management that describe:

- How data and biological specimens (when applicable) will be evaluated for compliance with the protocol, ethical standards, regulatory compliance, and accuracy in relation to source documents.
- The documents to be reviewed (e.g., CRFs, clinic notes, product accountability records, specimen tracking logs, questionnaires, audio or video recordings), who is responsible, and the frequency for reviews.
- Who will be responsible for addressing QA issues (e.g., correcting procedures that are not in compliance with protocol) and QC issues (e.g., correcting errors in data entry).
- Staff training methods and how such training will be tracked.

If applicable, calibration exercises conducted prior to and during the study to train examiners and maintain acceptable intra- and inter-examiner agreement.

Regular monitoring and an independent audit, if conducted, must be performed according to ICH GCP.

9.1.13. Data Handling and Record Keeping Data collection & management

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the investigator. The investigator is responsible for ensuring the accuracy, completeness,

legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. An indelible ink that will ensure clarity of reproduced copies is required. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. All source documents must meet the FDA standard of ALCOA. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained and captured in a progress note and maintained in the participant's official electronic study record. Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into a 21 CFR Part 11-compliant data capture system provided by the CRO/Sponsor. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

9.1.14. Study document retention

Federal law at 21 CFR§312.62 mandates records required by 21CFR§312 (e.g., records of drug receipt and disposition, signed and dated consent forms, medical records including progress

notes of the physician, the individual's hospital chart(s), and the nurses' notes) must be retained by the investigator for at least 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

No study documents will be destroyed or moved to a new location without prior written approval from the Sponsor.

No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

These documents may need to be retained for a longer period, if required by local regulations.

9.1.15. Protocol Deviations

A protocol deviation is any noncompliance with the protocol, GCP, or procedure manuals (e.g., imaging manual, laboratory manual). The noncompliance may be either on the part of the participant, the investigator, or the study site staff.

As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6(R2):

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and record deviations contemporaneously.

All deviations must be addressed in study source documents and reported to the study monitor. Protocol deviations must be sent to the IRB of record per their guidelines. If the IRB of record has a policy prohibiting submission of deviations, a copy of this policy must be filed in both the ISF and trial master file.

The investigator and study staff are responsible for knowing and adhering to their IRB requirements.

9.1.16. Forward planned deviations

The Investigator is not allowed to institute changes to the protocol or investigational plan except when necessary to protect the safety, rights, or welfare of subjects.

- If a forward-planned deviation occurs to protect the safety, rights or welfare of subjects, the Investigator or designee must notify the Sponsor or Designee in writing within 1 business day. The deviation must specify the perceived risk and if, in the investigator's opinion, it impacted the study's safety or outcome data. Lastly, the Investigator or designee must provide an opinion if this change should be made for all participants or if it was a single occurrence.
- The notice of deviation must be filed with the IRB in accordance with its policy.
- The Sponsor or designee will provide a formal determination regarding the deviation. If the Sponsor or designee concurs with the deviation, a protocol clarification letter

and/or protocol amendment will be initiated. If the Sponsor or designee does not concur with the deviation, the determination will provide recommendations for similar situations for the participant's safety, rights, or welfare to be maintained while actively participating in the protocol.

9.1.17. Eligibility deviations

- Deviations from eligibility criteria are considered protocol violations
- These violations must be reported to the Sponsor or designee upon identification as well as to the IRB of record.
- The site is placed on accrual hold until a root cause analysis and corrective action plan is provided to the Sponsor or designee for review and it is approved.

9.1.18. Major deviations

Major deviations are those that are considered to negatively participant safety, rights, or welfare of a participant or that may negatively impact study results. These must be reported to the Sponsor or designee within 5 business days and must include a root cause analysis as well as a corrective action plan. Major deviations include, but are not limited to:

- Inadequate informed consent, which is defined as failing to follow the stipulations of the IRB of record, failing to adequately document informed consent was obtained prior to undertaking research-only procedures, filing to obtain informed consent as per the IRB of record, or failing to use the appropriate version of the informed consent
- Failing to submit an amendment (e.g., protocol, investigator's brochure) or revised informed consent document to the institutional review board of record within 60 days of receipt
- Falsification, fabrication, or other scientific misconduct
- Individual prescribing investigational medical product who is not authorized to do so
- Investigational agent administered to the incorrect subject
- Overdose of investigational medical product (as defined by Section 9.1)
- Failing to obtain continuing review (i.e., lapse of IRB approval)
- Failing to review suspected unexpected serious adverse reaction (SUSAR) reports

The Clinical Research Organization (CRO), LBR Regulatory Clinical and Consulting Services Inc., will maintain the master protocol deviation log on behalf of all investigational sites.

9.1.19. Publication and Data Sharing Publication(s)

The Sponsor recognizes the importance of and will fulfill its obligation for the disclosure of clinical research findings, timely and in appropriate forums. Any disclosure of results from the Sponsor's clinical trials will be submitted to the Sponsor for review to assure accuracy of information and to protect proprietary documents and materials. This review will not be unreasonably withheld and provided in writing within 30 days of receipt. Clinical trials conducted by more than one investigational site, multi-center studies, will be reported jointly with all subjects represented in the analyses.

9.1.20. Disclosure of Data

All information obtained as a result of this study or during the conduct of this study will be regarded as confidential. Disclosures (i.e., any release of information to any third party not noted herein) of any, not previously known to be public, information and/or results of the investigation for publication or by capsules or poster presentation shall not be made earlier than 30 days after submission of the proposed material to the sponsor for inspection, unless the sponsor consents to earlier disclosure. The Investigator will take appropriate cognizance of the sponsor's suggestions before disclosure for publication or presentation consistent with protection of the sponsor's right to its confidential data.

9.1.21. Conflict of Interest and Financial Disclosure

The independence of this study from any actual or perceived influence is critical. Any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed.

The Investigator shall provide the sponsor with sufficient accurate financial information to allow the Sponsor or designated Applicant to submit complete and accurate certification or disclosure statements as required under 21 CFR§54.

As per 21CFR§312.64(d) the Site Investigator will provide financial information for the 1572 investigator(s) and sub-investigator(s).

The Investigator shall promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

Investigators are reminded to file the appropriate documentation with their institution for review and management of conflict of interest.

9.2. Source Documentation

The Investigator must make study data accessible to the Sponsor, to other authorized representatives of the Sponsor, and to the appropriate regulatory authority inspectors. The eCRF for each subject will be checked against source documents at the study site by the Study Monitor.

Each participating site will maintain appropriate medical and research records for this trial, in compliance with federal code at 21CFR§312 as well as applicable regulatory and institutional requirements for the protection of confidentiality of participants. As part of participating in this study, each site will permit authorized representatives of the Sponsor and regulatory agencies to examine (and when permitted by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress, and data validity.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

It is not acceptable to use printed electronic case report form(s) as source documents or to

structure source documents exclusively from the data required for the study database. This creates a tunnel effect when evaluating participants for safety and/or outcomes.

It is not acceptable for the case report form or shadow chart to be the only record of a subject's participation in this clinical trial. This is to ensure that anyone who would access the subject's medical record has adequate knowledge that the subject is participating in a clinical trial of an investigational medical product.

9.2.1.Investigator's Site Files (Regulatory Binder)

The Investigator will maintain an investigator site file (ISF; regulatory binder) containing essential documentation identified by the Sponsor or designee. Essential documentation will be outlined through a table of contents provided by the Sponsor/designee as well as applicable documentation (i.e., monitor visit logs, screening logs, training logs, delegation logs, authorized prescriber log, sample SAE reporting forms, sample pregnancy reporting forms).

Copies of all reports to and correspondence with and from the IRB/EC will be provided to the Sponsor or their designee.

If not maintained through electronic software, the Investigator must maintain a correspondence file between the Investigator's site and the IRB of record. This correspondence must be made available for monitoring upon request.

The ISF documentation will align with the trial master file maintained by the sponsor or designee.

9.2.2.Required Documentation for Site Activation

The following source documentation must be collected from the Investigator and reviewed prior to shipment of the investigational medical product (study drug) to the Investigator.

- FDA form 1572
- Protocol signature page
- Investigator's brochure signature page

Ethics documents. The following documents must be provided to the sponsor or designee.

- IRB letter documenting favorable outcome, stipulations regarding consent, version of protocol reviewed, and version of investigator's brochure reviewed. If the IRB letter does not document stipulations of consent or protocol version, a copy of the IRB application with an attestation by appropriately delegated site personnel can be used.
- Stamped IRB-approved informed consent document, and stamped subject-facing materials must be provided. If the IRB does not utilize approval stamps for the consent and/or subject-facing materials, a copy of the policy or note to file must be filed in both the ISF and trial master file.
- A favorable site initiation report must be filed by the designated personnel. If site enrollment is to begin on the same day as the site initiation visit, the report must clearly state the time (24hr) the site was allowed to initiate enrollment and then be faxed or emailed to the Sponsor or designee. A fax receipt, or copy of the sent email, must be retained with appropriate time stamps. Best practice is for the Sponsor or designee to confirm receipt of the SIV report.

Operational documentation. The following information is required for

operationalization of the clinical trial and does not impact enrollment:

- The sponsor or designee must have copies of the appropriate federal, state, and local documentation regarding the receipt of radioactive materials by the Investigator's site
- Shipping address for investigational medical product as well as point-of-contact information.

9.3. Investigator Agreement

I have read the foregoing protocol *"A Phase 2, Multicenter, Single Arm, Open Label, Non-Randomized Study of [^{18}F]FAPI-74 PET in Subjects with Gastrointestinal Cancers "* and agree to conduct the study as described therein.

I will conduct this protocol as outlined therein and will make a reasonable effort to complete the study within the time designated. I will provide copies of the protocol and access to all information furnished by SOFIE Biosciences to study personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the drug and the study.

I understand that the study may be terminated, or enrollment suspended at any time by SOFIE Biosciences, Inc., with or without cause, or by me if it becomes necessary to protect the best interests of the study subjects.

_____

Investigator's Name, printed

_____ _____

Investigator's Signature date
(dd/mm/yyyy)

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11. APPENDICES

11.1. Appendix 1: Serious Adverse Event Reporting

CONTACT THE SOFIE BIOSCIENCES, INC. MEDICAL MONITOR WITHIN 24 HOURS OF LEARNING OF ANY SERIOUS ADVERSE EVENT. IF THE SERIOUS ADVERSE EVENT IS FATAL OR LIFE THREATENING, THE SPONSOR MUST BE INFORMED IMMEDIATELY.

For reporting of serious adverse events, Investigators must enter the SAE report form into database within 24 hours to the following:

To discuss SAE with the Medical Monitor, contact SOFIE Drug Safety Team
Email: SofieDrugSafety@mmsholdings.com

All reports of SAEs should be entered into the database within one working day. Follow-up information to SAEs must also be entered into the database within 24 hours as it becomes known **to the investigator.**

11.2. Appendix 2: Eastern Cooperative Group (ECOG) Performance Status Scale

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

* From ECOG, Robert Comis, MD, Group Chair

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11.3. Appendix 3: Response Evaluation Criteria in Solid Tumors (RECIST 1.1) QUICK REFERENCE

11.3.1. Eligibility

Only subjects with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint. Measurable disease is defined as the presence of at least one measurable lesion.

11.3.2. Methods of Assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

- CT is the best currently available and reproducible method to measure lesions selected for response assessment. MRI is also acceptable in certain situations (e.g., for body scans but not for lung).
- Lesions on a chest X-ray may be considered measurable lesions if they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- Clinical lesions will only be considered measurable when they are superficial and ≥10 mm in diameter as assessed using calipers. For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.
- Ultrasound (US) should not be used to measure tumor lesions.
- Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete response.
- Cytology and histology can be used in rare cases (e.g., for evaluation of residual masses to differentiate between Partial Response [PR] and Complete Response [CR] or evaluation of new or enlarging effusions to differentiate between Progressive Disease [PD] and Response/Stable Disease [SD]).
- Use of endoscopy and laparoscopy is not advised. However, they can be used to confirm complete pathological response.

11.3.3. Baseline Disease Assessment

All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

Measurable Lesions - Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm; when CT scans have slice thickness >5 mm, the minimum size should be twice the slice thickness).
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest X-ray.
- Malignant lymph nodes
    - To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness is recommended to be no greater than 5 mm). At baseline and in follow-up, only

the short axis will be measured and followed.

- Lytic bone lesions or mixed lytic-blastic lesions with identifiable soft tissue components that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered measurable if the soft tissue component meets the definition of measurability described above.
- 'Cystic lesions' thought to represent cystic metastases can be considered measurable if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same subject, these are preferred for selection as target lesions.

Non-Measurable Lesions - Non-measurable lesions are all other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with 10 to <15 mm short axis), as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Blastic bone lesions are non-measurable.

Lesions with prior local treatment, such as those situated in a previously irradiated area or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

11.3.4. Target Lesions

- All measurable lesions up to a maximum of two lesions per organ and five lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longest diameter) and be representative of all involved organs, as well as their suitability for reproducible repeated measurements.
- All measurements should be recorded in metric notation using calipers if clinically assessed.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters, which will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease. If lymph nodes are to be included in the sum, only the short axis will contribute.

11.3.5. Non-Target Lesions

All lesions (or sites of disease) not identified as target lesions, including pathological lymph nodes and all non-measurable lesions, should be identified as non-target lesions and be recorded at baseline. Measurements of these lesions are not required, and they should be followed as 'present', 'absent' or in rare cases, 'unequivocal progression'.

11.3.6. Response Criteria Evaluation of target lesions

Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters
Progressive Disease (PD)	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum on study (this may include the baseline sum). The sum must also demonstrate an absolute increase of at least 5 mm
Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD

Special notes on the assessment of target lesions

- Lymph nodes identified as target lesions should always have the actual short axis measurement recorded even if the nodes regress to below 10 mm on study. When lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met since a normal lymph node is defined as having a short axis of <10 mm.
- Target lesions that become 'too small to measure'. While on the study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small. However, sometimes lesions or lymph nodes become so faint on a CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure', in which case a default value of 5 mm should be assigned.
- Lesions that split or coalesce on treatment. When non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

11.3.7. Evaluation of non-target lesions

Complete Response (CR)	Disappearance of all non-target lesions and normalization of tumor marker levels. All lymph nodes must be non-pathological in size (< 10 mm short axis)
Non-CR/Non-PD	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
Progressive Disease (PD)	Unequivocal progression of existing non-target lesions. When patient has measurable disease. To achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status • When patient has only non-measurable disease. There is no measurable disease assessment to factor into the interpretation of an increase in non- measurable disease burden. Because worsening in non-target disease cannot be easily quantified, a useful test that can be applied is to consider if the increase in overall disease burden based on change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease. Examples include an increase in apleural effusion from 'trace' to 'large' or an increase in lymphangitic disease from localized to

	widespread.

New Lesions

The appearance of new malignant lesions denotes disease progression:

- The finding of a new lesion should be unequivocal (i.e., not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor, especially when the patient's baseline lesions show partial or complete response).
- If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.
- A lesion identified in a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and disease progression.

11.3.8. [^{18}F]FDG-PET

It is sometimes reasonable to incorporate the use of [^{18}F]FDG-PET scanning to complement CT in assessment of progression (particularly possible 'new' disease). New lesions on the basis of [^{18}F]FDG-PET imaging can be identified according to the following algorithm:

Negative [^{18}F]FDG-PET at baseline, with a positive [^{18}F]FDG-PET at follow-up is PD based on a new lesion.

No [^{18}F]FDG-PET at baseline and a positive [^{18}F]FDG-PET at follow-up:

- If the positive [^{18}F]FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.
- If the positive [^{18}F]FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal [^{18}F]FDG-PET scan).
- If the positive [^{18}F]FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

11.3.9. Time Point response

A summary of the overall response status calculation at each time point for patients who have measurable disease at baseline is presented in Table A1 below.

11.3.10. Table A1. Time point response: Subjects with target (+/–non-target) disease

Target lesions	Non-Target lesions	New Lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	NE	No	PR
PR	Non-PD/or not all evaluated	No	PR
SD	Non-PD/or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD

Any	Any	Yes	PD

CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease; NE = inevaluable.

When subjects have non-measurable (therefore non-target) disease only, Table A2 is to be used.

11.3.11. Table A2. Time point response: Subjects with non-target disease

Non-Target lesions	New Lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD[1]
Not all evaluated	No	NE
PD	Yes or No	PD
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease; NE = inevaluable.

[1] Non-CR / non-PD is preferred over 'Stable Disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials. To assign this category when no lesions can be measured is not advised

11.3.12. Confirmation

In non-randomized trials where response is the primary endpoint, confirmation of PR and CR is required to ensure responses identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials.

However, in all other circumstances, (i.e., in randomized phase 1I or III trials or studies where stable disease or progression are the primary endpoints), confirmation of response is not required since it will not add value to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies, which are not blinded.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6–8 weeks) that is defined in the study protocol.

11.3.13. Missing Assessments and Inevaluable Designation

When no imaging/measurement is done at all at a particular time point, the subject is not evaluable (NE) at that time point.

If only a subset of lesion measurements is made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would most likely happen in the case of PD.

11.4. Appendix 4 National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Version 5.0

The NCI CTCAE Version 5.0 can be found by going to the following web site: http://ctep.cancer.gov/reporting/ctc.html

Inquiries specifically regarding the Common Toxicity Criteria (CTC) should be addressed to: ncictephelp@ctep.nci.nih.gov