Clinical Study Report Synopsis **GE Healthcare**
GE-135-004

Title: A Phase 2, Open-Label, Test-Retest Study to Assess the Reproducibility of Quantitative Measurements of ^{18}F Uptake By Solid Tumors Using PET Imaging Following Intravenous Administration of AH111585 (^{18}F) Injection

This is an exact copy of the synopsis from the final clinical study report for the study GE-135-004. The final clinical study report (document identifier GE-135-004 CREP) was authorized for use on 08-Jan-2014 (Version 2.0).

<table>
<tr><td rowspan="3">Name of Sponsor/Company:
GE Healthcare Ltd. and its Affiliates
Name of Finished Product:
Fluciclatide (^{18}F) Injection
Name of Active Ingredient:
[^{18}F]Fluciclatide</td><td rowspan="3">Individual Study Table Referring to Part of Dossier in Which the Individual Study or Study Table is Presented:

Volume:

Reference:</td><td rowspan="3">(For National Authority Use only)</td></tr>
<tr><td colspan="3">Title of Study: A Phase 2, Open-Label, Test-Retest Study to Assess the Reproducibility of Quantitative Measurements of ^{18}F Uptake By Solid Tumors Using PET Imaging Following Intravenous Administration of AH111585 (^{18}F) Injection
Note: AH111585 (^{18}F) Injection was replaced in this report by Fluciclatide (^{18}F) Injection as these terms are interchangeable</td></tr>
<tr><td colspan="3">Investigators and Study Centers: Twelve centers; 6 in United Kingdom, 2 in India, 3 in Korea and 1 in Sweden</td></tr>
<tr><td colspan="4">Investigators and Centers for Independent Evaluation of Images:
Independent evaluation of images was undertaken to meet the primary endpoint analysis requirements at ACR Image Metrix, LL, 1818 Market Street, Suite 1600, Philadelphia PA, 19013 USA.
Independent evaluation of images was undertaken to meet the secondary endpoint analysis requirements at Uppsala University Hospital, Department of Oncology, Uppsala, Sweden.</td></tr>
<tr><td colspan="4">Publication (reference): None</td></tr>
<tr><td colspan="2">Study Period: 23 June 2009 to 23 September 2011</td><td colspan="2">Phase of Development: Phase 2</td></tr>
<tr><td colspan="4">Objectives:
Primary:
To assess the test-retest reproducibility of [^{18}F]fluciclatide uptake by solid tumors following intravenous administration of Fluciclatide (^{18}F) Injection.
Secondary:
• To assess the image quality and lesion detectability of [^{18}F]fluciclatide as a function of administered activity, by temporal segmentation.
• To assess the safety of ≥2 administrations, each of a maximum of 370 MBq, of Fluciclatide (^{18}F) Injection in subjects with solid primary or metastatic tumors.
• To assess the relationship between ^{18}F uptake by solid tumors and $\alpha_v\beta_3$ expression and other histological markers of angiogenesis in pre- or post- imaging biopsy material.</td></tr>
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Study Design:

This was a phase 2, open-label, test-retest reproducibility study in subjects with solid tumors. The aim of the study was to assess the reproducibility of quantitative measurements of ^{18}F uptake by tumors using Positron Emission Tomography (PET) imaging following intravenous administration of AH111585 (also known by the USAN approved name Fluciclatide (^{18}F) Injection). The target population was adult subjects with solid primary or metastatic tumors ≥2 cm in diameter. Malignancies could include, but were not limited to, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), glioblastoma multiforme (GBM), melanoma, sarcoma, head and neck, and breast cancers.

Efficacy was assessed using correlation analysis between parameters derived from PET data acquired from 2 imaging sessions performed 3 to 8 days apart on the same tumor. Because the optimal time point post-injection is dependent on the kinetics of the tracer, up to three [^{18}F]fluciclatide PET scans were acquired at each imaging session to ensure that data were acquired at the optimal time point post-injection for all the tumor types investigated. The duration of each whole-body [^{18}F]fluciclatide PET acquisition was approximately 25 minutes. The imaging acquisition protocol was the same for both imaging sessions. The administered activity of the 2 injections in an individual subject was to be identical (to ±10%) and was not to exceed 370 MBq. Subjects were considered evaluable if they received 2 administrations of Fluciclatide (^{18}F) Injection and underwent the corresponding PET acquisitions, and tumors demonstrated detectable levels of ^{18}F uptake in the PET scans.

In this study, it was planned that up to 10 subjects (the immunohistochemistry [IHC] group) would receive a biopsy between 8 and 1 week(s) prior to the first scan or <8 weeks after the second scan. The tissue obtained by biopsy was planned to be used to quantitatively assay for a battery of angiogenic and oncologic biomarkers ($\alpha_v\beta_3$, CD31, microvessel density [MVD], by IHC for $\alpha_v\beta_3$ integrin or CD31, protein kinase B [AKT], p-AKT, mitogen-activated protein kinase [MAPK], p-MAPK, vascular endothelial growth factor [VEGF] and VEGF-receptor [VEGFr]). Analysis for test-retest reproducibility was planned to be done separately for the IHC group and the major group of 35 subjects. The IHC group was to be used to correlate the tracer uptake with angiogenesis marker expression. However, only 1 subject had a biopsy sample taken within the protocol window, and this biopsy sample was not analyzable. Thus, no IHC studies were performed and the planned analyses for this sub-study were not performed.

All subjects receiving a dose of Fluciclatide (^{18}F) Injection were included in the safety analysis.

Selection of Subjects:

Inclusion Criteria:

(1) The subject had been diagnosed with at least one solid primary or metastatic tumor ≥2.0 cm in diameter, including but not limited to NSCLC, RCC, GBM, melanoma, sarcoma, head and neck, and breast cancers.
(2) The subject was undergoing therapy for a malignant tumor with non-curative intent.
(3) The subject had received clinical routine imaging diagnostic work-up (e.g. Computed Tomography [CT] with/without contrast, Magnetic Resonance Imaging [MRI] with/without contrast, bone scintigraphy, X-ray, mammography, [^{18}F]fluorodeoxyglucose [FDG] PET) within 8 weeks prior to the first [^{18}F]fluciclatide PET scan.
(4) The subject had the following baseline serum biochemistry and hematology values: blood urea nitrogen value (BUN) and serum creatinine value of ≤1.5 X the upper normal limit; prothrombin time and activated partial thromboplastin time within normal limits; platelet count of >75,000 x 10^6/L; and hemoglobin value of >9 g/dL.
(5) The subject had a clinically acceptable (as judged by the investigator) physical examination at screening and was capable of self-care, i.e. Eastern Cooperative Oncology Group (ECOG) performance status was 0 to 2.
(6) The subject was ≥18 years old.
(7) Female subjects needed to be either surgically sterile (had a documented bilateral oophorectomy and/or documented hysterectomy), post-menopausal (cessation of menses for more than 1 year), or, if of childbearing potential, the results of a serum pregnancy test performed at screening must have been negative and confirmed -6 to -1 hours prior to each administration of Fluciclatide (^{18}F) Injection by a urine pregnancy test. Female subjects of reproductive potential were also to employ an effective method of birth control. Barrier contraceptives had to be used throughout the study in both sexes.
(8) The subject was able and willing to comply with study procedures, and signed and dated informed consent was obtained.
(9) Endocrine therapy was permitted provided that it had been initiated ≥1 month prior to the first [^{18}F]fluciclatide imaging session and the treatment continued at least through the second [^{18}F]fluciclatide

imaging session.

Exclusion Criteria:

(1) The subject had received another investigational medicinal product (IMP) within 30 days before the first administration of Fluciclatide (^{18}F) Injection, would receive another IMP during or between the 2 administrations of Fluciclatide (^{18}F) Injection, or planned to receive an IMP within 1 week after the second administration of Fluciclatide (^{18}F) Injection.
(2) The subject was previously included in this study.
(3) The subject had any contraindication to any of the study procedures, products used or its constituents.
(4) The subject had known hyper- or hypo-coagulation syndromes. Such coagulopathies included but were not limited to Von Willebrand disease, Protein C deficiency, Protein S deficiency, Hemophilia A/B/C, Factor-V Leiden, and Bernard-Soulier syndrome.
(5) The subject had a known diagnosis of mental incapacitation and it affected his or her ability to consent.
(6) The subject had received chemotherapy within 2 weeks, or received radiotherapy, surgery, or any other treatment against cancer (with the exception of endocrine therapy) within 4 weeks prior to the first [^{18}F]fluciclatide PET scan.
(7) The subject was scheduled to undergo chemotherapy, radiotherapy, surgery or any other treatment against cancer (with the exception of endocrine therapy) between the first and second [^{18}F]fluciclatide PET scans.
(8) The subject's target tumor had been biopsied < 4 weeks prior to the first [^{18}F]fluciclatide PET scan (not applicable for the IHC group) OR The subject was scheduled to undergo biopsy for the target tumor between the first and second [^{18}F]fluciclatide PET scans.
(9) Subjects in the IHC group could not have had the target tumor biopsied ≤1 week prior to the first [^{18}F]fluciclatide PET scan.
(10) The subject had intra-hepatic tumors only (without extra-hepatic tumor).
(11) The subject had a known diagnosis of human immunodeficiency virus (HIV); hepatitis B-or C-type virus infection.
(12) The subject suffered from claustrophobia.
(13) The subject was being treated with heparin or warfarin.
(14) The subject was unable to lie down for 90 minutes.
(15) The subject was lactating.

Number of Subjects (planned and analyzed):

Up 45 evaluable subjects were planned to be included in up to 15 centers.
In total, 70 subjects signed informed consent and were enrolled in this study. Forty-nine subjects received at least one administration of Fluciclatide (^{18}F) Injection and were included in the safety population. Thirty-nine subjects were included in the full analysis set.

Treatment of Subjects

Investigational Medicinal Product: Fluciclatide (^{18}F) Injection consists of the radiolabelled compound [^{18}F]fluciclatide together with excipients. The substance was formulated in phosphate buffered saline containing ethanol and sodium p-aminobenzoate as a ready to use sterile solution. [^{18}F]Fluciclatide is a cyclic peptide radiolabelled with fluorine-18 (^{18}F) for detection using a PET scanner.
Duration of Treatment: Each full analysis set subject received 2 independent administrations of Fluciclatide (^{18}F) Injection.
Imaging: After each administration of Fluciclatide (^{18}F) Injection, whole body PET imaging was performed at 40-115 minutes post-injection. Up to three [^{18}F]fluciclatide PET scans were acquired at each imaging session. The duration of each whole body PET acquisition was approximately 25 minutes.

Endpoints

Primary Efficacy Endpoint:

Assessment of the test-retest reproducibility was based on measurements obtained for each targeted tumor and the extent of the difference between Standardized Uptake Value (SUV) measurements of [^{18}F]fluciclatide uptake following Fluciclatide (^{18}F) Injection administration and PET imaging on 2 separate days within an interval of 3-8 days.

Secondary Endpoints:

- Image quality and lesion detectability were assessed by blinded image evaluation to determine the acceptable diagnostic efficacy for a minimum administered activity (for a specified scanning duration)
- The safety profile of repeated administration of Fluciclatide (^{18}F) Injection in subjects with solid primary or metastatic tumors was assessed. Safety endpoints included the occurrence of one or more adverse events (AEs) from the first administration of Fluciclatide (^{18}F) Injection to up to 6 weeks after the second

administration of Fluciclatide (^{18}F) Injection.

- Correlation of measures of [^{18}F]fluciclatide uptake in tumors obtained from PET images to the expression of $\alpha_v\beta_3$ and other angiogenesis and oncologic markers by means of immuno-histologic analysis of tumor tissue samples (IHC group) was performed.

Safety:
Physical examinations, vital signs, serum biochemistry, hematology, coagulation parameters, 12-lead electrocardiogram, anti-fluciclatide antibody evaluation, pre-treatment and post-treatments events (AEs and serious AEs [SAEs]).

Statistical Analyses:
The outcome measure for the statistical analysis of the test-retest reproducibility was the relative differences of SUVs between the 2 [^{18}F]fluciclatide scans. The primary analysis was based on the construction of 95% confidence intervals for the mean and variance of the relative differences. Additional analyses included Bland-Altman plots.
The outcome measures for the statistical analysis of temporal segmentation included qualitative image quality, quantitative image quality and lesion detectability. Temporal segmentation means the extraction of multiple whole-body (WB) PET images from a single acquisition, which simulate WB images acquired using a fraction of the administered activity, more specifically a randomly chosen 25, 50 and 75% of the original 100% PET image (acquired following administration of ~370 MBq). All temporal segmentation analysis was performed on the first WB scan from imaging session 1 (if available; alternatively from Imaging Session 2). Subject data were excluded if the scan start time deviated from 40 minutes by more than 10 minutes.
The sample size estimate was based on the requirement for a 95% confidence interval for the true relative difference. An evaluable subject for the purpose of the primary analysis was one for whom the 2 [^{18}F]fluciclatide scans had been completed, and were of acceptable quality. No interim analysis was planned or conducted.

Summary of Results
Efficacy:
Including data from both imaging sessions, SUV_{peak} values in the primary analysis data set ranged from 1.44 to 10.46 g/mL at 40 minutes, 1.55 to 11.06 g/mL at 65 minutes and 1.41 to 11.55 g/mL at 90 minutes (using a PERCIST volume of interest (VOI) and an OSEM reconstruction). The mean (±SD) relative differences of SUV between the 2 imaging sessions were 2% (±16%) at 40 minutes, 2% (±17%) at 65 minutes and 5% (±22%) at 90 minutes (in the primary lesion analysis data, using a PERCIST VOI SUV_{peak} and OSEM reconstruction). A similar degree of test-retest reproducibility was also seen for filtered back projection (FBP), threshold VOI and exploratory data sets.
In the SUV data for individual tumor types, there was generally a larger systematic error and less variability than in the primary analysis (all-tumor) data set. The mean (±SD) relative differences of SUV between the 2 imaging sessions ranged from 4.2 (±9.4%) to 9.1% (±9.5%) for breast cancer, 2.6 (±8.7%) to 8.4% (±17.5%) for colorectal cancer, and 8.6 (±5.5%) to 12.9% (±12.7%) for melanoma (in the primary lesion analysis data using a PERCIST VOI SUV_{peak} and OSEM reconstruction). A similar level of test-retest reproducibility was also seen for FBP, threshold VOI and exploratory data sets.
Conformance to the imaging protocol was excellent, resulting in highly reproducible acquisition times in both imaging sessions.
Qualitative and quantitative analysis from the temporal segmentation study showed there was little visual difference between the 100% and 75% data sets. However, with increasing fractional administered activity of Fluciclatide (^{18}F) Injection, the variability of the liver SUV progressively decreased, indicating a progressively increasing signal-to-noise ratio. Image quality scores for the 75% data set exhibited substantially better agreement with those for the full data set than did image quality scores for either the 50% or the 25% data set.
Exploratory analysis found no discernible relationship between lesion size and the relative differences in test-retest SUVs. Reproducibility was lowest for tumors in the lung pleura, probably as a consequence of physiological motion.
Approximately half of the lesions identified in the standard of care (SoC) data exhibited measurable [^{18}F]fluciclatide uptake. This may indicate that levels of $\alpha_v\beta_{3/5}$ integrin expression vary from tumor to tumor.
Safety:
Eighteen subjects reported 47 treatment-emergent AEs during the study. One AE led to subject withdrawal but was not related to IMP. There were 7 SAEs, one of which led to subject withdrawal and 2 of which resulted in death, during the study; no SAEs or deaths were related to IMP.
Overall, there were no significant changes in any of the safety parameters monitored in this study, including

hematology and clinical laboratory variables, vital signs, and ECG, in the follow-up period. No clinically important trends indicative of adverse safety signals were noted.
Fluciclatide (^{18}F) Injection was safe and well tolerated by all subjects dosed in this study in which the majority of subjects received 2 doses on separate days.

Conclusions:

This was a Phase 2, open-label study assessing the test-retest reproducibility of [^{18}F]fluciclatide in subjects with solid tumors. A total of 49 subjects received at least one administration of Fluciclatide (^{18}F) Injection and were included in the safety population. Thirty-nine subjects were included in the full analysis set (i.e., all subjects who received 2 administrations of Fluciclatide (^{18}F) Injection, underwent 2 evaluable PET imaging sessions, and had tumors demonstrating detectable levels of ^{18}F uptake).
The primary objective was to assess the test-retest reproducibility of [^{18}F]fluciclatide uptake by solid tumors following intravenous administration of Fluciclatide (^{18}F) Injection on separate days within an interval of 2 to 9 days. Across all tumors analyzed, using a PERCIST VOI SUVpeak and OSEM reconstruction, the mean relative differences (systematic error) in SUVpeak values for all tumor types combined were small at all 3 time points (2% to 5%), while the variability in the relative differences (SD of 16%, 17%, and 22%) were consistent with the precision expected in a multicenter study with good calibration (Doot et al, 2012).
The secondary efficacy objectives were to assess the image quality and lesion detectability as a function of administered activity of [^{18}F]fluciclatide, by temporal segmentation and to assess the relationship between ^{18}F uptake by solid tumors and $\alpha_v\beta_3$ expression and other histological markers of angiogenesis in pre- or post-imaging biopsy material.
Qualitative and quantitative analysis from the temporal segmentation study showed there was little visual difference between the 100% and 75% dose data sets. However, with increasing fractional administered activity of Fluciclatide (^{18}F) Injection, the variability of the liver SUV progressively decreased, indicating a progressively increasing signal-to-noise ratio. Image quality scores for the 75% data set exhibited substantially better agreement with those for the full data set than did image quality scores for either the 50% or the 25% data set.
The planned assessment of the relationship between [^{18}F]fluciclatide uptake by solid tumors and $\alpha_v\beta_3$ expression and other histological markers of angiogenesis in pre- or post-imaging biopsy material was not performed. Only 1 subject had a biopsy sample taken within the protocol-specified window and this biopsy sample was not analyzable.
Overall, 18 subjects (37%) experienced a total of 47 treatment-emergent AEs. Most of the AEs were mild or moderate in intensity and were not related to IMP. Two deaths were reported as the outcome of SAEs during the study; one due to underlying progression of carcinoma of the cervix and one due to underlying progression of carcinoma of head of pancreas. An additional 5 non-fatal SAEs were reported. None of the deaths or non-fatal SAEs was related to administration of IMP. No clinically significant trends in changes in hematology and clinical laboratory variables, vital signs, or ECG were observed.
The following overall conclusions can be drawn from this study:

Efficacy

The mean relative differences (systematic error) in SUVpeak values for all tumor types combined were small at all 3 time points (2% to 5%), while the variability in the relative differences (SD) were 16%, 17%, and 22%.
A review of the PET imaging literature suggests that a precision value of 20% for SUV test-retest measurement is consistent with a multicenter study with good calibration.

Safety

Intravenous administration of Fluciclatide (^{18}F) Injection was generally safe and well tolerated in this study in which most of the subjects received 2 doses, on separate days, at an interval of 2 to 9 days.