**Clinical Study Report Synopsis** **GE Healthcare**
**GE-135-003**

**Title:** A Phase 2, Open-label, Proof-of-concept Study to Assess the Ability to Detect Tumor and Angiogenesis via the Expression of $\alpha_v\beta_3$ Integrin Receptors by [$^{18}$F]fluciclatide PET Imaging

This is an exact copy of the synopsis from the final clinical study report for the study GE-135-003. The final clinical study report (document-identifier: GE-135-003 CREP) was authorized for use on 13-Jun-2013 (Version 1.0).

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| **Name of Sponsor/Company:** GE Healthcare Ltd. and its Affiliates<br>**Name of Finished Product:** Fluciclatide ($^{18}$F) Injection<br>**Name of Active Ingredient:** [$^{18}$F]Fluciclatide | **Individual Study Table Referring to Part of Dossier in Which the Individual Study or Study Table is Presented:**<br><br>**Volume:**<br><br>**Reference:** | **(For National Authority Use only)** |
| **Title of Study:** A Phase 2, Open-label, Proof-of-concept Study to Assess the Ability to Detect Tumor and Angiogenesis via the Expression of $\alpha_v\beta_3$ Integrin Receptors by [$^{18}$F]fluciclatide PET Imaging | | |
| **Investigators and Study Centers:** The study was conducted at 2 centers in the US and 1 center in the UK. | | |
| **Publication (reference):** None | | |
| **Study Period:** 13 December 2007 to 26 September 2011 | | **Phase of Development:** Phase 2 |

**Objectives**

**Primary Objective:**

- To correlate the magnitude of [$^{18}$F]fluciclatide uptake and retention with quantitative measurement of the levels of $\alpha_v\beta_3$ integrin expression in tumors.

**Secondary Objectives:**

- To correlate tumor perfusion and vascular permeability in tumor tissue (as measured by dynamic contrast enhanced computed tomography [DCE-CT]) with the magnitude of uptake and retention of [$^{18}$F]fluciclatide (note: DCE-CT is optional and will only be acquired at site 001 [M. D. Anderson Cancer Center]).
- To correlate [$^{18}$F]fluciclatide accumulation in tumors obtained from positron emission tomography (PET) images to the expression of vascular endothelial growth factor (VEGF); vascular endothelial growth factor receptor (VEGFr); protein kinase B (AKT) and phosphorylated AKT (p-AKT); mitogen-activated protein kinase (MAPK) and phosphorylated MAPK (p-MAPK); and microvessel density (MVD) in tumors (microvessels and tumor cells) by means of immuno-histologic analysis of tumor tissue samples.
- To obtain preliminary data on the feasibility of detection of both primary and metastatic tumor lesions in particular tumor types using [$^{18}$F]fluciclatide PET as compared to standard of care (SoC) modalities (e.g., [$^{18}$F]Fluorodeoxyglucose [FDG]-PET, contrast-enhanced [CE] static computed tomography [CT], magnetic resonance imaging [MRI], bone scintigraphy).
- To assess the safety of a single intravenous administration of a maximum activity of 370 MBq [$^{18}$F]fluciclatide in subjects with solid tumors.

**Study Design**

This was a phase 2, open-label, proof-of-concept study to assess the ability to detect tumors and angiogenesis via the expression of $\alpha_v\beta_3$ integrin receptors using [$^{18}$F]fluciclatide PET imaging. The plan was to include up to 30 evaluable subjects at up to 9 study centers. Subjects were considered evaluable if they underwent administration of Fluciclatide ($^{18}$F) Injection, dynamic and static PET imaging, and tumor tissue acquisition.

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The targeted population was adult subjects with solid tumors at initial diagnosis or diagnosis of recurrence who were scheduled to undergo resection or biopsy of the tumor as part of routine clinical treatment (see inclusion criteria for the list of target tumors).
Within 4 weeks of enrollment, subjects were scheduled for imaging using Fluciclatide ($^{18}$F) Injection. Prior to the PET imaging day, subjects underwent imaging procedures as required for SoC, e.g., CT, MRI, bone scintigraphy, or X-ray. Two types of PET imaging were performed: dynamic PET imaging over a single axial field of view (FoV) and static whole-body PET imaging composed of multiple contiguous views. Dynamic PET was acquired from immediately after IMP administration up to 60 minutes post-IMP administration. Static whole-body PET was acquired for a maximum of 60 minutes, starting approximately 60 minutes post-IMP administration. DCE-CT (optional) was performed prior to resection or biopsy of tumor tissue and could be performed directly after PET imaging or within 2 weeks after. Tissue samples were acquired within 2 weeks after PET. Samples for the integrin immunohistochemical (IHC) analyses were fresh frozen and stored at -80 $^{o}$C; samples for the IHC analyses of all other angiogenesis biomarkers were formalin fixed and embedded in paraffin. IHC analyses of all acquired samples were performed at a central laboratory and were completed after the close of enrollment. Analyses comprised standard hematoxylin and eosin staining and IHC staining to assess several biomarkers specific for oncology and angiogenesis quantitatively.
Efficacy was assessed using correlation analysis between parameters derived from PET and reference standards obtained either from IHC analysis of tumor tissue samples or SoC imaging. Measures of uptake and retention in tumors were obtained from the dynamic PET acquisitions. The efficacy evaluation was unblinded.
Tumors (or metastases) identified with SoC imaging were used for the primary efficacy analysis. The IHC results from analysis of the tumor tissues samples for $\alpha_v\beta_3$ integrin were correlated with the uptake and retention of [$^{18}$F]fluciclatide measured from PET images. Secondary analyses included correlations with additional angiogenesis biomarkers as well as comparison of tumors identified with [$^{18}$F]fluciclatide static whole body PET imaging with tumors identified with SoC imaging examinations.
Safety was assessed from the types and frequencies of adverse events (AEs), changes in vital signs, changes in electrocardiogram (ECG) parameters, changes in physical examination findings, and changes in clinical laboratory findings.

**Selection of Subjects**
**Inclusion Criteria:**

(1) The subject had been imaged diagnostically and was suspected of having a primary or metastatic target tumor lesion of 1 of the following types:
- High-grade glioma, including glioblastoma multiforme (GBM), anaplastic astrocytoma, and anaplastic oligodendroglioma
- Lung cancer, including SCLC and non-small cell lung cancer (NSCLC)
- Head and neck (H&N) tumors, including laryngeal squamous cell carcinoma, and well differentiated thyroid and oral cavity carcinoma
- Sarcoma
- Melanoma
- Renal cell cancer (RCC)

(2) The subject's tumor was ≥2.0 cm in diameter except for thyroid carcinoma (≥1.5 cm).
(3) The chosen target tumor was not within the liver.
(4) The subject was scheduled to undergo resection or biopsy of the target tumor as a result of routine clinical treatment and was scheduled to undergo or had received SoC diagnostic imaging work-up (following the study center's routine procedures), e.g., CT with or without contrast, MRI with or without contrast, bone scintigraphy, X-ray, or FDG-PET.
(5) The subject had not received any anti-angiogenic agents (e.g., bevacuzimab, sorafenib, sunitinib) within 60 days prior to PET imaging.

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| | **Reference:** | |

(6) The subject had the following baseline laboratory parameters: blood urea nitrogen (BUN) value and serum creatinine (sCr) value of ≤1.5 of the upper normal limit, prothrombin time (PTT) and aPTT within normal limits, platelet (Plt) count of ≥150,000 x $10^6$/L, and hemoglobin (Hgb) value of >9 g/dL.
(7) 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, such that the subject had a high chance to complete the study.
(8) The subject had had no open wounds within 10 days prior to study entry.
(9) The subject was ≥18 years old.
(10) Female subjects needed to be either surgically sterile (had a documented bilateral oophorectomy 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 within 24 hours must have been negative with the result known before administration of Fluciclatide ($^{18}$F) Injection. Female subjects of reproductive potential were also to employ an effective method of birth control. Barrier contraceptives had to be used by both sexes throughout the study.
(11) The subject was able and willing to comply with study procedures, and signed and dated informed consent was obtained.

**Specific inclusion criteria for subjects with suspected or previously diagnosed high-grade glioma:**

(1) The subject was suspected of having supratentorial malignant primary glioma (by biopsy or presenting MRI characteristics as determined by the subject's clinician) requiring further surgical resection as part of the recommended treatment plan for their newly diagnosed disease. These gliomas included GBM, anaplastic astrocytoma, and anaplastic oligodendroglioma.
(2) The subject had undergone recent biopsy of newly diagnosed high-grade glioma, had recovered from the effects of surgical biopsy, and baseline on-study MRI/CT was performed within 28 days of entry into the study.

**Specific inclusion criteria for subjects with suspected or previously diagnosed RCC:**

(1) The subject had been imaged diagnostically and was suspected of having a primary or metastatic RCC tumor lesion.
(2) The subject was scheduled to undergo resection or biopsy of the target tumor as a result of routine clinical treatment.

**Exclusion Criteria**

(1) The subject was lactating.
(2) The subject was being treated with heparin or Coumadin.
(3) The subject had received another investigational medicinal product (IMP) within 14 days before, or would have received an IMP within 1 week after administration of Fluciclatide ($^{18}$F) Injection.
(4) The subject was previously included in this study.
(5) The subject experienced substantial changes in their medical status before all essential study procedures (including all imaging procedures and surgical excision or biopsy) were performed.
(6) The subject had any contraindication to any of the study specified procedures, products used, or its constituents (e.g., X-ray contrast media).
(7) The subject had known hyper- or hypo-coagulation syndromes. Such coagulopathies include but are not limited to Von Willebrand disease, Protein C deficiency, Protein S deficiency, Hemophilia A/B/C, Factor-V Leiden, and Bernard-Soulier syndrome.
(8) The subject was unable to lie down for 125 minutes.
(9) The subject suffered from claustrophobia.
(10) The subject had known diagnosis of human immunodeficiency virus (HIV) infection.
(11) The subject had known diagnosis of hepatitis B or C infection.

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(12) The subject had known diagnosis of mental incapacitation and it affected his or her ability to consent.
(13) The subject had a history of serious hypersensitivity reaction to iodinated contrast media (not applicable unless the subject was undergoing a study specified procedure requiring contrast media).

**Number of Subjects (planned and analyzed)**

It was planned to enroll up to 30 evaluable subjects at up to 9 centers.
Thirty-three subjects were enrolled and 25 received Fluciclatide ($^{18}$F) Injection (the safety population). Of the 25 subjects who received Fluciclatide ($^{18}$F) Injection, 22 were evaluable for efficacy.

**Treatment of Subjects**

**Investigational Medicinal Product:** Fluciclatide ($^{18}$F) Injection was administered intravenously as a single dose with a maximum of 370 MBq (= 10 mCi), i.e., no more than 20 μg imaging agent. Administration was followed by a saline flush.
**Reference Standard:** Not applicable
**Duration of Treatment:** Subjects were included in the study for up to 10 weeks: from signing the informed consent at up to 4 weeks prior to PET imaging until completion of the final immunology testing at 6 weeks after PET imaging. PET imaging took about 2 hours. All standard of care imaging and histological procedures were completed within 4 weeks prior to and histological procedures within 2 weeks after PET imaging. DCE-CT imaging was completed within 2 weeks of PET imaging and prior to resection/biopsy.

**Endpoints**

**Primary Efficacy Endpoint:**

- Correlation of the quantitative uptake of [$^{18}$F]fluciclatide measured by PET with the quantitative measurement of staining intensity of IHC analysis of tumor tissue (resection or biopsy) samples for $\alpha_v\beta_3$ integrin.

**Secondary Efficacy Endpoints:**

- Comparison of tumor perfusion and vascular permeability in tumor tissue (as measured by DCE-CT) with the magnitude of [$^{18}$F]fluciclatide uptake and retention.
- Correlation of [$^{18}$F]fluciclatide accumulation in tumors obtained from PET images with the expression of $\alpha_v\beta_5$ integrin, CD31, VEGF, VEGFr-1, AKT and p-AKT, MAPK and p-MAPK, and MVD in tumors (microvessels and tumor cells) by means of IHC analysis of tumor tissue samples.
- Numbers of tumor lesions identified from [$^{18}$F]fluciclatide PET images, numbers of tumor lesions identified using SoC imaging, and the number of matches and mismatches in tumor lesion detection between [$^{18}$F]fluciclatide PET imaging and SoC imaging.

**Safety Endpoints:**

Safety endpoints included the occurrence of one or more treatment-emergent AEs from administration of Fluciclatide ($^{18}$F) Injection throughout the study period, and changes in blood chemistry (pre- to post- IMP administration), hematology, coagulation, vital signs, ECG, and injection site and physical examination findings.

**Statistical Analyses**

All subjects who were enrolled in the study and received Fluciclatide ($^{18}$F) Injection were included in the safety analysis. Subjects were included in the full analysis set (FAS) for efficacy analyses if they had non-missing data for static PET measurements and kinetic modeling PET measurement, both types from dynamic [$^{18}$F]fluciclatide PET imaging, and, for the primary analysis had interpretable IHC results of $\alpha_v\beta_3$ integrin staining intensity (OD measurements) and for the secondary analyses had interpretable and relevant IHC results for any of the protocol-specified angiogenesis biomarkers: $\alpha_v\beta_5$ integrin, CD31, VEGF, VEGFr-1, MAPK, p-MAPK, AKT, or p-AKT. Tabulations of summary statistics, graphical presentations, and statistical analyses were performed using SAS® software. The last pre-treatment observation was used as the baseline value for calculating post-treatment changes from baseline. Correlations between variables in the primary and secondary

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analyses were assessed using Pearson's correlation coefficient or Spearman's rank correlation; additional data were analyzed using descriptive statistics where applicable.

**Summary of Results**

Efficacy: The quantitative uptake and retention of [$^{18}$F]fluciclatide into target tumors measured by PET was correlated with the quantitative measurement of $\alpha_v\beta_3$ integrin staining intensity from the IHC analysis of tissue samples from those tumors (obtained from resection) as well as with the quantitative measurement of staining intensity from IHC analysis of tumor tissue samples for $\alpha_v\beta_5$ integrin, CD31, VEGF, VEGFr-1, AKT and p-AKT, p-MAPK, and MVD. Due to severe technical difficulties during the staining validation phase, the planned analysis of the MAPK biomarker was not performed. Two subjects had DCE-CT scans; the planned correlation of tumor perfusion and vascular permeability with uptake and retention of [$^{18}$F]fluciclatide was not performed due to the small number of scans that were performed. The mean percentage of tumor lesions per subject identified by both SoC imaging and [$^{18}$F]fluciclatide PET imaging ("matches") was 78.7% and the mean percentage of mismatches per subject was 21.3%.

Through post hoc analyses, Ki_inp-Patlak, VT_inp-Logan, SUVw_55, and SUVR_55_blood were selected as primary imaging parameters for PET and the 80% threshold volume of interest (VOI) was selected as the primary VOI type. All confidence intervals (CIs) for the correlations between $\alpha_v\beta_3$ OD measurements and the primary imaging parameters were centered at a positive value but also covered zero. Moderate positive correlations, with CIs that did not cover zero, were observed between $\alpha_v\beta_5$ integrin OD measurements for the FAS (N = 20) with Ki_inp-Patlak (r = 0.60). Correlations with $\alpha_v\beta_5$ integrin OD for subjects with RCC (N = 12) were moderately to very strongly positive (coefficients ranged from 0.57 to 0.90) and the CIs did not cover zero for the correlations with Ki_inp-Patlak, VT_inp-Logan, and SUVR_55_blood. AKT OD measurements for RCC (N = 12) and for chromophobe RCC (N = 4) demonstrated very strong positive correlations with SUVR_55_blood (r = 0.90, r = 1.00), respectively. No CI for the correlations between AKT OD measurements and the primary imaging parameters for RCC covered zero; nor did the very strong correlations with VT_inp-Logan and SUVR_55_blood for chromophobe RCC.

Safety: Overall, 13 subjects (52%) experienced a total of 30 treatment-emergent AEs. Five subjects had SAEs, including anaemia, idiopathic thrombocytopenic purpura, renal injury, GBM, pneumothorax and subcutaneous emphysema. The SAE glioblastoma multiforme led to the subject's death. The SAE idiopathic thrombocytopenic purpura was considered at least possibly related to the IMP; no other SAE or AE was considered at least possibly related to the IMP. Three subjects (12%) experienced treatment-emergent AEs with a highest intensity of severe, 5 (20%) with a highest intensity of moderate, and 5 (20%) with a highest intensity of mild.

No clinically significant trends were evident from hematology laboratory parameters, serum chemistry values, coagulation laboratory parameters, vital signs, ECGs, or physical or neurological examinations.

**Conclusions**

- The full set of 20 imaging parameters examined in this study was reduced to 4 primary parameters by classifying parameters according to similarities and selecting a representative parameter for each common methodology. Correlation coefficients using the 80% threshold VOI tended to be stronger. The results from the 80% threshold VOI were very similar to the results of the 80% threshold maximum VOI but were believed to be more robust due to the lower levels of noise.
- Correlation of the magnitude of [$^{18}$F]fluciclatide uptake and retention with quantitative measurement of the levels of $\alpha_v\beta_3$ integrin expression in tumors was negligible to weakly positive.
- Correlation of the magnitude of [$^{18}$F]fluciclatide uptake and retention with quantitative measurement of the levels of $\alpha_v\beta_5$ integrin expression in tumors was moderately positive for the FAS; correlation in melanoma and RCC subjects ranged from moderately positive to very strongly positive.
- Very strong positive correlations were observed between AKT OD measurements and the primary

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imaging parameters for subjects with RCC. None of the 95% CI intervals for these correlations covered zero.

- The mean percentage of tumor lesions per subject identified by both SoC imaging and [$^{18}$F]fluciclatide PET imaging was 79%, and ranged from 68% for clear-cell RCC to 81% for chromophobe RCC.
- A total of 30 treatment-emergent AEs were reported among the 13 subjects who had AEs during the study. Five subjects had SAEs, one of which resulted in death during the study (GBM); 1 SAE (idiopathic thrombocytopenic purpura) was considered possibly related to IMP.
- Overall, changes in the safety parameters monitored in this study, including hematology, serum chemistry, coagulation laboratory variables, vital signs, ECG, physical and neurological exams were clinically insignificant. No trend indicative of an adverse safety signal was observed.
- Fluciclatide ($^{18}$F) Injection was safe and well tolerated by the subjects in this study.