Clinical Study Report **GE Healthcare**
GE-067-011

Title: A PRINCIPAL, PROSPECTIVE, OPEN-LABEL BIOPSY STUDY TO VALIDATE DETECTION OF CEREBRAL CORTICAL AMYLOID WITH FLUTEMETAMOL F 18 INJECTION IN NORMAL PRESSURE HYDROCEPHALUS (NPH) SUBJECTS

This is an exact copy of the synopsis from the final clinical study report for the study GE067-011. The final clinical study report (document-identifier: GE-067-011 CREP) was authorized for use by the Head of Global Medical on 13-Dec-2011 (Version 2.0).

2 SYNOPSIS

Name of Sponsor/Company: GE Healthcare Ltd. and its Affiliates **Name of Finished Product:** Flutemetamol F 18 Injection **Name of Active Ingredient:** [^{18}F]flutemetamol	**Individual Study Table Referring to Part of Dossier in Which the Individual Study or Study Table is Presented:** **Volume:** **Reference:**	**(For National Authority Use only)**

Title of Study: A principal, prospective, open-label biopsy study to validate detection of cerebral cortical amyloid with Flutemetamol F 18 Injection in normal pressure hydrocephalus (NPH) subjects

Investigators and Study Centers: Coordinating investigator Professor Juha Rinne, Turku PET Centre, Kiinamyllynkatu 4-8, 20520 Turku, Finland; 4 recruiting sites, 1 imaging site in Finland

Center for Independent Evaluation of Images: Image Review Center, GE Healthcare.

Publication (reference): None

Study Period: 31 May 2010 to 16 December 2010 | **Phase of Development:** 3

Objectives:

The objectives are as stated in the statistical analysis plan (SAP) rather than the protocol.

Primary: To determine the level of association between the quantitative estimates of brain uptake of [^{18}F]flutemetamol and the quantitative immunohistochemistry (IHC) estimates of amyloid levels in biopsy samples (standard of truth [SoT]) obtained during intracranial pressure measurement or shunt placement in patients who had suspected NPH. The quantitative estimates of brain uptake of [^{18}F]flutemetamol (standard uptake value ratio [SUVR]) from the biopsy site were to be made from the analysis of positron emission tomography (PET) images using the cerebellum as the reference region. The quantitative IHC estimates of amyloid levels were to be estimated for each biopsy sample using a monoclonal antibody (4G8) raised against amyloid β as the primary SoT.

Secondary:

1. To determine the level of association between SUVR and the quantitative estimates (area percentages) of amyloid β levels for the following regions based on cerebellum as the reference region:
   IHC measures (4G8):
   IHC estimates of amyloid β levels in the biopsy samples (4G8) compared to:
   - Contralateral SUVR estimates
   - Composite SUVR estimates
2. To determine the level of association between SUVR and the quantitative estimates (area percentages) of amyloid β levels for the following regions based on pons as the reference region:
   IHC measures (4G8):
   IHC estimates of amyloid levels in the biopsy samples (4G8) compared to:
   - Biopsy site SUVR estimates
   - Contralateral SUVR estimates
   - Composite SUVR estimates
3. To determine the levels of association between the blinded visual assessment of PET images (normal/abnormal amyloid levels) and the assessment of normal and abnormal amyloid levels from the quantitative histochemical (HC)/IHC estimates of amyloid levels in the biopsy samples. The blinded visual assessment of Flutemetamol F 18 Injection brain PET images was to be performed by 3 independent blinded readers trained in the evaluation of PET amyloid imaging.
4. To determine the inter-reader (between-reader) agreement of blinded visual image assessment, as measured by Fleiss' kappa (>70% = good; >80% = very good; >90% = excellent).
5. To determine the intra-reader (within reader) reproducibility of blinded visual image assessment, as measured by their percentage self-consistency.

Study Design: This was an open-label, multi-center, non-comparative study to determine the level of association between cortical tracer uptake (determined from [^{18}F]flutemetamol PET images) with the level of cortical amyloid determined from biopsy samples. Frontal lobe biopsies were obtained from 17 subjects during

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shunt placement for treatment of NPH. The level of amyloid in each biopsy sample was quantified using IHC and HC. Subjects who were scheduled for intracranial shunt placement were contacted to evaluate their interest in the study. If interested, these subjects were required to sign an informed consent form, meet entrance criteria, undergo a magnetic resonance imaging (MRI) scan, be administered Flutemetamol F 18 Injection, and undergo a PET scan. A follow up phone call was made to assess adverse events (AEs). Within 8 weeks of the PET imaging, the shunt procedure and biopsy followed by a computed tomography (CT) scan were performed following each site's standard practice.
The subject allocation to Flutemetamol F 18 Injection was non-randomized; each subject was dosed. Image processing and SUVR determination were conducted blinded to the IHC and HC results. Neurosurgeons performing the biopsy procedure were blinded to the PET images obtained.

Selection of Subjects:
Inclusion Criteria:

(1) Informed consent had been signed and dated by the subject and/or the subjects' legally acceptable representative, if applicable, in accordance with local regulations.
(2) The subject was at least 50 years of age.
(3) The subjects' general health was adequate to comply with study procedures.
(4) The subject had been scheduled for an intracranial pressure measurement or shunt placement procedure for the treatment of NPH.
(5) For women who were either surgically sterile (had had a documented bilateral oophorectomy and/or documented hysterectomy) or were postmenopausal (cessation of menses for more than 2 years), enrolment in the study without a pregnancy test at screening was allowed. For women of childbearing potential, the results of a serum and urine human chorionic gonadotropin pregnancy test (with the result known on the day of and before the Flutemetamol F 18 Injection administration) were negative.

Exclusion Criteria:

(1) The subject had a contraindication for MRI or PET.
(2) The subject was pregnant or lactating.
(3) The subject had participated in any clinical study using an investigational agent within 30 days of dosing, with the exception of the PET tracer, ^{11}C-labelled Pittsburgh Compound B ([^{11}C]PiB).
(4) The subject had a known or suspected hypersensitivity/allergy to [^{18}F]flutemetamol or to any of the excipients.

Number of Subjects (planned and analyzed):
Up to 30 subjects were planned to be recruited. However, the study was stopped due to administrative decision after 21 subjects had been recruited from 4 centers in Finland. Eighteen subjects completed the study; 17 of these subjects were included in the efficacy analysis population.

Treatment of Subjects :
Investigational Medicinal Product: Each subject received one intravenous (i.v.) dose of Flutemetamol F 18 Injection administered within approximately 40 seconds. The activity of a single administration of Flutemetamol F 18 Injection was approximately 185 Megabecquerels (MBq), corresponding to an effective dose of approximately 6 milliSieverts.
Imaging: PET imaging was conducted for 30 minutes starting approximately 90 minutes after the administration of Flutemetamol F 18 Injection.
Duration of Treatment: The study involved a maximum of 4 visits for each subject. All subjects were required to sign an informed consent form, undergo a 3D MRI before the Flutemetamol F 18 Injection PET scan, undergo a scheduled shunt procedure (during which a biopsy was obtained), and obtain a CT. A follow-up phone call approximately 24 hours after the Flutemetamol F 18 Injection was made to assess potential AEs. The shunt procedure was performed and the biopsy was obtained within 8 weeks of PET imaging. Serious AEs for which a causal relationship to Flutemetamol F 18 Injection could not be ruled out were to be followed until

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30 days of injection.

Endpoints:

Efficacy:

The primary endpoint was the SUVR at the site of the biopsy based on the cerebellum as the reference region. The secondary endpoints were:

- SUVR contralateral to, and at the approximate level of (i.e., mirroring), the biopsy site based on the cerebellum as the reference region;
- Composite SUVR based on the cerebellum as the reference region;
- Biopsy site SUVR based on the pons as the reference region;
- SUVR contralateral to, and at the approximate level of (i.e., mirroring), the biopsy site based on the pons as the reference region;
- Composite SUVR based on the pons as the reference region;
- Classification of a subject's PET images as normal or abnormal based on the blinded visual evaluation of PET images by each of 3 independent blinded readers trained in the evaluation of PET amyloid imaging.

Safety: AEs, clinical laboratory assessments (chemistry, hematology and coagulation), vital signs, electrocardiograms (ECGs), and physical/neurological examination.

Standard of Truth:

Each subject's biopsy sample was fixed and then cut into microscopic sections, which were mounted on microscope slides. The slides containing tissue sections were then exposed to the appropriate IHC or HC stain and associated reagents for the analysis of interest. The number of slides analyzed and the analytical method varied by stain used, as follows:

4G8 Immunohistochemistry: The SoT for the amyloid level in a subject's brain tissue was the percentage of total area of brain-tissue microscopic section staining positive for amyloid β after exposure to IHC reagents that targeted amyloid β through use of a monoclonal antibody raised against amyloid β (4G8). The original stain specified by the protocol for the primary analysis was IHC using the stain NAB228 (see the explanation for the change in Section 9.8). Up to 3 slides per subject were analyzed for percent area of tissue section staining amyloid-positive, using an automated slide reading process. The average result was taken to be the overall assessment for the biopsy specimen and thus the subject.

Thioflavin S: Up to 3 slides per subject were read under a microscope by a neuropathologist using fluorescent light, who examined each slide as having none, sparse, moderate or frequent amyloid plaque loads (modified Consortium to Establish a Registry for Alzheimer's Disease [CERAD] scoring system).

Bielschowsky silver stain: Up to 3 slides per subject were read under a microscope by a neuropathologist, who examined up to 5 fields of view (FOV) per slide and assessed each FOV as having none (scored as 0), sparse (1), moderate (2) or frequent (3) neuritic plaque loads (modified CERAD scoring system). The 5 FOV scores were averaged for each slide. The mean FOV scores generated from each slide (up to 3) were averaged to give an overall mean FOV score for the biopsy specimen. We assume that the overall mean FOV score is representative of the biopsy sample and therefore, of the brain region from which the biopsy was taken. If this overall mean score was >1.5 the subject was classified as abnormal; otherwise, he/she was classified as normal.

Overall Pathology Assessment: The above IHC and HC assessments were used to determine a single overall pathology assessment of each subject's biopsy sample as normal or abnormal based on the level of amyloid. Any abnormal assessment rendered the overall pathology assessment as abnormal; only if all IHC and HC assessments were normal was the subject's biopsy specimen classified as normal.

Statistical Analyses:

Primary Efficacy Analysis: In the primary analysis, the SUVRs from the subjects' biopsy sites were compared to the corresponding levels of amyloid determined from 4G8-IHC estimates of amyloid β in the biopsy samples. The Pearson correlation coefficient was calculated. The null hypothesis of no correlation

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between SUVR and the 4G8-IHC estimates of amyloid β in the biopsy sample was tested. A statistical significance level of 0.05 was used for the correlation. This relationship was also analyzed using a regression model with SUVR as the independent variable and the amyloid β level from the biopsy sample as the dependent variable.

Secondary Efficacy Analyses: The level of association between SUVR and the amyloid level in the corresponding biopsy sample was analyzed using a regression model similar to that used in the primary analysis for the additional comparisons:

	SUVR from PET imaging			
Standard of Truth	**Reference region for SUVR**	**Biopsy site**	**Contralateral to the biopsy site**	**Composite region**
IHC 4G8	**Cerebellum**	(primary) % of tissue section area staining positive for amyloid β	% of tissue section area staining positive for amyloid β	% of tissue section area staining positive for amyloid β
	Pons	% of tissue section area staining positive for amyloid β	% of tissue section area staining positive for amyloid β	% of tissue section area staining positive for amyloid β

Blinded visual assessments of Flutemetamol F 18 Injection PET images of the brain were performed by 3 independent blinded readers who had been trained in the evaluation of PET amyloid imaging. Each reader assessed each subject's PET image set as normal or abnormal for amyloid. Each subject's PET image assessment (normal or abnormal) was compared with the corresponding histopathological assessment of the subject's biopsy sample (as normal or abnormal). Results were provided in a two-by-two table with the sensitivity and specificity and 95% confidence intervals.

The inter-reader (between-reader) agreement and the intra-reader (within-reader) reproducibility were determined. A kappa coefficient with 95% confidence interval was determined for each reader comparison, a multiplicity coefficient was determined for all readers, and each reader's result was compared to the majority read. Intra-reader reproducibility was measured using a kappa coefficient.

A comparison of the pathology results obtained with the different stains (Bielschowsky silver stain, Thioflavin

Summary of Results

Efficacy: The SUVR at the biopsy site was significantly correlated with the biopsy specimen amyloid β level (expressed as the percentage of tissue-section area staining with 4G8). The Pearson correlation coefficient was 0.669 (p=0.0064). The full model was significant (R^2=0.45; p=0.0064) and the model factor for SUVR was significant with a slope of 1.3 and a p-value of 0.0064. In the secondary efficacy analyses, contralateral and composite cortical SUVR values based on the cerebellum as the reference regions correlated significantly with the percentage of biopsy specimen staining for antibody based on 4G8 as did the biopsy site SUVR results based on pons as the reference region.

In the blinded visual assessments of the PET images, using the overall pathology assessment as the reference standard for amyloid level, Reader 1 had 100% (4/4) sensitivity and Readers 2 and 3 and the majority read had 75% (3/4) sensitivity. All readers and the majority read showed 100% (13/13) specificity. When the Bielschowsky silver stained sections were used as the SoT, all readers and the majority read showed 100% (3/3)

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sensitivity; Reader 1 showed 93% (13/14) specificity whereas Readers 2 and 3 and the majority read showed 100% (14/14) specificity. When Thioflavin S stained sections were used as the SoT, Reader 1 showed 57% (4/7) sensitivity, and Readers 2 and 3 and the majority read showed 43% (3/7) sensitivity; all readers and the majority read showed 100% (10/10) specificity. The results suggest that raised [^{18}F]flutemetamol uptake ratios in PET images are highly specific for abnormal fibrillar amyloid β deposits in the brain.
The inter-reader agreement results showed very good agreement (κ=0.88). There was only one discrepancy: Reader 1 interpreted one PET image as abnormal and Readers 2 and 3 interpreted it as normal. The intra-reader reproducibility was 100%.
Safety: Two (11%) subjects experienced a total of 2 AEs. The AEs reported were mild in intensity. One of the AEs reported (nausea) was considered to be possibly related to Flutemetamol F 18 Injection. There were no SAEs or AEs leading to withdrawal or deaths during the study. All AEs reported resolved during the study period.
There were no clinically significant findings in serum chemistry, hematology or coagulation laboratory values from screening to after imaging or clinically significant findings in changes of vital signs, ECG or physical examination.

Conclusions:
Efficacy:

- The cortical uptake of [^{18}F]flutemetamol, as measured by SUVR, correlates significantly with the percentage of brain-tissue section area staining positive for amyloid β with 4G8.
- Blinded visual interpretations of [^{18}F]flutemetamol image showed both sensitivity of 43% to 100% and specificity of 93% to 100% for detection of amyloid. Importantly, the sensitivity if image reading compared to pathology as revealed by Bielschowsky silver staining (which marks neuritic plaques) was 100% for all readers and the specificity was 93% to 100%.
- The results of blinded reader agreement for [^{18}F]flutemetamol PET showed very good agreement (κ = 0.88) between readers with 2 of 3 readers being in full agreement on all images. Intra-reader reproducibility was 100%.
- These data confirm the high concordance of [^{18}F]flutemetamol PET imaging with histopathology, supporting its sensitivity to detect fibrillar amyloid β in the brain of living subjects with suspected NPH.

Safety:

- Single doses of [^{18}F]flutemetamol were generally well tolerated in NPH subjects.
- No clinically significant abnormalities were noted in clinical laboratory results or results from physical and neurological examinations. There were no clinically significant changes in ECG results.