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Trial Synopsis

(according to ICH E3)

In-vivo Imaging of nicotinic acetylcholine availability in neurodegenerative diseases using the radioligand 2-[^{18}F]F-A-85380 and positron-emission-tomography (PET)

(Nicotinic PET in neurodegenerative diseases)

Open-label, non-randomized, single center study

Name of Finished Product / Name of Active Substance:

2-[^{18}F]fluoro-3-(2(S)-azetidinylmethoxy)pyridine (2-[^{18}F]F-A-85380)

Indication / Diagnosis:

Neurodegenerative diseases such as Alzheimer's disease (AD), mild cognitive impairment (MCI), Multiple sclerosis (MS), Parkinson's disease (PD) and Huntington disease (HD)

Phase of Development:

0/I – Proof of Mechanism

EudraCT-Number:

2007-004979-19

Version 1.0 from October 27th,2017

First Patient in: 26.02.2009
End of Trial: 01.11.2016

Coordinating Investigator

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Signatures

The undersigned authors agree to the content of this final report by their signatures. The clinical trial was conducted according to the principles of the Declaration of Helsinki, Good Clinical Practice (GCP) and the applicable laws.

Legal representative of the sponsor and principal investigator	Univ.-Prof. Dr. med. O. Sabri	27.10.2017 Date
Further authors:	Dr. med. P. Meyer	27.10.17 Date
	Dr. rer. med. F. Zientek	27.10.2017 Date

Contents

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2 Used IMP(s)	3 Used active ingredient(s)
2-[^{18}F]F-A-85380	2-[^{18}F]fluoro-3-(2(S)-azetidinylmethoxy)pyridine

4 Individual table of trials

Not applicable

5 Title of study

In-vivo Imaging of nicotinic acetylcholine availability in neurodegenerative diseases using the radioligand 2-[^{18}F]F-A-85380 and positron-emission-tomography (PET)

6 Principal Investigator	7 Trial site
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The trial was performed as a single-center trial:

8 Publications

None so far.

9 Study period (in years)

First patient in: 26.02.2009

Last patient out: 26.03.2015

10 Development Phase

It was a phase 0/1 trial.

11 Objectives

Main issue:

- In-vivo quantification of α4β2 nicotinic acetylcholine receptor availability
- To find diagnostic potential patterns of altered in-vivo α4β2 nicotinic acetylcholine receptor binding in various neurodegenerative diseases.

2-[^{18}F]F-A-85380 (2-[^{18}F]fluoro-3-(2(S)-azetidinylmethoxy)pyridine) is a suitable diagnostic PET radiopharmaceutical for imaging of the human brain. 2-[^{18}F]F-A-85380 binding in the human brain is detected by positron emission tomography (PET) imaging and reflects the presence of α4β2 nicotinic acetylcholine receptors (α4β2-nAChR). Thus, we investigated whether there are alterations of the α4β2-nAChR availability, most likely representing neurodegenerative changes, in patients with neurodegenerative diseases such as Alzheimer's disease (AD, n=3), Parkinsonian syndrome, including Parkinson's disease, essential tremor and corticobasal degeneration (n=35), Huntington's disease (HD, n=2) and multiple sclerosis (MS, n=2) and compared them with healthy controls (HC, n=38).

Intermediate objectives:

To assess whether these patterns of altered α4β2-nAChR binding in neurodegenerative diseases are related to clinical parameters (such as age and/or gender), useful for early diagnosis and prediction of clinical outcome, are useful for increasing our understanding of individual drug therapy, are associated with environmental parameters and genetic variables.

12 Methods

The α4β2-nAChR PET in neurodegenerative diseases trial was performed as an open-label, single-centre, non-randomized, prospective cohort comparison with application (single or if applicable up to 3 times) of a diagnostic agent for the detection of a potential difference of α4β2-nAChR availability in patients with neurodegenerative diseases compared to healthy volunteers.

13 Overall number of trial participants

Planned number:	250	patients with neurodegenerative diseases
	50	healthy controls
Enrolled and investigated subjects:	42	patients with neurodegenerative diseases
	Out of these:	
	3 patients with Alzheimer's disease (AD)	
	35 patients with Parkinsonian syndroms/movement disorders (Parkinson's disease, PD; corticobasal degeneration, CBD; essential tremor, ET)	
	2 patients with Huntington's disease (HD)	
	2 patients with multiple sclerosis (MS)	
	38	healthy controls (HC)

The study was early terminated. The permission of the Federal Office for Radiation Protection expired on November 1th 2016. There was no request for extension. The final cohort of investigated study participants is composed of 42 patients with neurodegenerative diseases and 38 healthy controls. Originally 250 patients with neurodegenerative diseases and 50 healthy controls were planned to be studied with PET. The final number of study subjects was especially a result of the highly selective criteria of patient enrolment. Although the planned maximal number of study patients and healthy controls was not reached, the number of successfully enrolled and investigated subjects in this trial is larger than usually in other clinical monocentric neuroreceptor PET trials and the key questions can be answered

successfully also considering earlier own data of former nicotinic PET studies of patients with neurodegenerative disorders. The final sample size will be considered conscientiously for analysis.

14 Indication and main criteria for inclusion

Healthy volunteers:

1. Males/females aged older than or equal to 18 years of age
2. Able to understand the information provided on purpose and conduct of the clinical study
3. Have signed the informed consent to participate in the study
4. No history of or current neuropsychiatric or neurological diseases
5. No relevant intracerebral structural lesions
6. No cholinergic medication
7. Adequate visual and auditory abilities to complete neuropsychological testing, as assessed by the recruiting investigator

Patients:

1. Males/females aged older than or equal to 18 years of age up to 90 years of age
2. Patients with neurodegenerative diseases (dementia, movement disorders, white matter diseases) diagnosed according to the disease specific diagnostic criteria (DSM-V, ICD-10)
3. Capable of understanding the information provided on purpose and conduct of the clinical study and able to give meaningful informed consent by himself / herself
4. Have signed the informed consent to participate in the study
5. Adequate visual and auditory acuity to complete neuropsychological testing, as assessed by the recruiting investigator
6. Interruption of cholinergic medication for up to 2 weeks before PET

15 Main Criteria for Exclusion

Healthy volunteers:

1. Evidence of any significant psychiatric or neurological illness from history, clinical or para – clinical findings
2. Nicotine abuse
3. Relevant pathological findings in the brain MRI
4. Intake of cholinesterase inhibitors

Patients:

1. Intake of cholinesterase inhibitors within 2 weeks before PET
2. Nicotine abuse

All subjects:

1. Pregnancy/ Breastfeeding
2. Absence of effective contraception for women with childbearing potential
3. Knowledge of alcohol or drug abuse/dependence
4. Previous significant occupational exposure to ionizing radiation or in whom, within the last 10 years, radioactive substances or when ionizing radiation was applied for the purposes of research. According to § 24 Abs. 1 Nr. 6 StrlSchV / § 28b Abs. 1 Nr. 6 RöV)
5. Any significant disease or unstable medical condition (e.g. unstable angina, myocardial

infarction or coronary revascularization in the preceding 12 months, cardiac failure, chronic renal failure, chronic hepatic disease, severe pulmonary disease, blood disorders, poorly controlled diabetes, chronic infection)

6. Laboratory parameters that are outside the normal range and are considered clinically significant by the investigator
7. Criteria which in the opinion of the investigator preclude participation for scientific reasons, for reasons of compliance, or for reasons of the volunteer's safety
    - Participants in whom magnetic resonance imaging (MRI) is contraindicated.
8. Patient / Volunteer is in custody by order of an authority or a court of law

Information on the trial product

2-[^{18}F]F-A-85380 will be manufactured by the radiochemists of the radiopharmacy of the UHL-NM, starting from [^{18}F]fluoride and a commercial available non-radioactive compound (2-Nitro-3-[1-tert-butoxycarbonyl-2(S)-azetidinylmethoxy]pyridin) according to *Good Manufacturing Practice* (GMP). [^{18}F]Fluoride is produced by the cyclotron at the UHL-NM, using standard procedures.

Manufacturing of 2-[^{18}F]F-A-85380 will follow GMP standards including determination of bacterial endotoxins and chemical and radiochemical purity prior to human administration. Sterility tests will be conducted as control of the production process after release of the product according to the established procedure of UHL-NM. Manufacturing will be notified to the competent local pharmaceutical supervisory authority. 2-[^{18}F]F-A-85380 will be produced at a high specific activity. The final product will be formulated as solution for intravenous injection in 10 mL buffer for injection. The radioactivity of the final patient dose will be measured before injection using a suitable counter, as locally established.

Each batch of 2-[^{18}F]F-A-85380 produced must meet criteria for colour, clarity, purity, specific activity and pH before being released. Analyses will be conducted by the responsible radiopharmacist, and will be documented in the batch documentation maintained at the radiopharmacy.

The mean dose administered to trial subjects by intravenous injection was 367,1 MBq in 10 mL saline.

16 Duration of application

The trial duration per patient was 1 day per PET investigation (up to 3 PET investigations per subject) as well as approximately 2 days for clinical Follow Up over a period of two years.

For further details on the documentation and visit schedule see the following flowchart (Table 1: flowchart containing trial visits and examinations).

Flowchart:

Table 1: flowchart containing trial visits and examinations

Procedures	**Screening**	**Visit 1: [^{18}F]-FA-85380 PET**		
		pre	**during**	**post**
Time window	**– 60 to 0d**			
Screening procedures				
Written informed consent[1)]	X	X		
Demographics	X	X		
Educational level + history of smoking	X	X		
Medical and surgical history	X	X		
Physical examination (incl. height, weight)	X	X		
Neuropsychiatric examination	X	X		
Allen´s test	X	X		
Check of inclusion/exclusion criteria	X	X		
Visit: [^{18}F]-FA-85380 PET				
Administration of [^{18}F]-FA-85380, start of cerebral PET imaging[2)]			X	
Arterial blood sampling		X	X	
Safety evaluations				
Vital signs (BP, HR) if necessary	X	X	X	X
Adverse events		X	X	X
Concomitant medication	X	X	X	X

1) Written informed consent before all other activities
2) 0 – 90 min and 360 - 420 min post injection (p.i.).

17 Information on comparators

Not applicable

18 Evaluation criteria

18.1 Efficacy

Uptake and binding in pre-specified brain regions (described by standard variables of PET imaging data evaluation) were used to investigate the suitability of 2-[^{18}F]F-A-85380 to image and quantify the α4β2-nAChR availability in patients with neurodegenerative diseases by PET as compared to healthy volunteers.

Parametric images of the observed distribution volumes (V_T), represent the quantitative measures of α4β2-nAChR most informative to address the study questions.

Due to the relatively early stage of tracer development and its limited application in humans with neurodegenerative disorders, no confirmatory but exploratory analyses were intended.

No primary endpoint was defined. Due to its exploratory nature of this study, the paucity of data related to this trial and the relatively small number of study participants in each cohort, an explorative p<0.005, 30 voxels for voxelbased analyses were accepted as statistically significant. An adjustment for multiple testing was only performed if applicable.

18.2 Safety

During the course of study safety analyses were performed to prepare the Development safety Update Report (DSURs) and Annual Safety Reports (ASR).

Safety assessments via SAEs/ AEs:

SAEs and AEs would have been analysed from subject files (for DSUR/ASR and final analysis) when applicable regarding:

- Number of events per participant in total,
- Number of events per participant unrelated/ related to the IMPs, Rate of events with regard to seriousness/ outcome/ severity/ need of therapy/ ongoing course.

19 Statistical methods / analysis procedures

Aim of analysing the quantitative PET imaging data was

2. to identify those regions in the brain which show significant α4β2 nAChR differences between the various cohorts,
3. enabling to predict the cohort membership of the participants based on the V_T values in brain regions which were selected with regard to their possible involvement in these neurodegenerative diseases.

19.1 Data (pre)processing

Parametric images of the distribution volume (V_T), as quantitative measure of α4β2-nAChR availability, were calculated by full pharmacokinetic modelling from the dynamic PET (ECAT EXACT HR+-PET scanner; 0-90 min and 6-7 hours p.i.) following correction of the arterial input function for individual plasma protein binding and metabolites of the radiotracer (Logan-plot method; 60 min to 7 hours p.i.; 13 points).

Using SPM, the parametric images of quantitative PET data (V_T) was spatially normalized onto a standard MRI and smoothed with a Gaussian kernel of 8 to 12 mm. For group comparisons, parametric images (V_T) of each patient cohort was selected and compared with the cohort of healthy controls. SPM was carried out for the correlation analysis between the availability of cerebral α4β2 nicotinic receptors (V_T) and a clinical parameter (age and/or sex).

19.2 Statistical data analysis (exploratory)

Exploratory voxelbased analyses (SPM) of the parametric images of the distribution volume (V_T) as quantitative measure of the α4β2-nAChR availability were performed using two-tailed unpaired t-tests for group comparisons (p<0.005, extent threshold κ=30 voxels).

Secondary and safety endpoints
As described in section 18.2., the safety analyses were fully reflected by the annual safety reports. Taken together, there have been no AEs and no SAE in the course of the clinical trial. There was one of drop-out, but not related to safety issues.

20 Summary

20.1 Efficacy results

Comparisons between cohorts with various neurodegenerative disorders and healthy controls (HC): results from voxelbased analyses (SPM) of parametric images of the distribution volume (V_T) as quantitative measure of the α4β2-nAChR availability:

1. Huntington's disease (HD) vs. HC: In HD, there was significantly lower α4β2-nAChR-binding in the caudate nucleus, inferior frontal and temporal cortices ($p<0.001$, $\kappa=5$ voxels; FWE-corrected). There was no significant increase of V_T in HD compared with HC.
2. Essential tremor (ET) vs. HC(old): In ET, there was significantly lower α4β2-nAChR-binding asymmetric in the right insula, putamen, thalamus and inferior frontal cortex (rectus) ($p<0.001$, $\kappa=5$ voxels; uncorrected). There was no significant increase of V_T in ET compared with HC.
3. Alzheimer's disease (AD) vs. HC: In late-onset Alzheimer's disease, there was significantly lower α4β2-nAChR-binding in the right superior temporal cortex and surprisingly, higher α4β2-nAChR-binding in the left inferior temporal / fusiform cortex ($p<0.005$, $\kappa=30$ voxels; uncorrected).
4. Parkinson's disease (PD) vs. HC(old): In PD, there was a significant, widespread decrease of α4β2-nAChR-binding in subcortical (midbrain-substantia nigra, putamen), cortical (fronto-temporo-parieto-occipital and cingulate cortices, hippocampus-amygdala, insula) and cerebellar brain regions ($p<0.001$, $\kappa=5$ voxels; uncorrected). There was no significant increase of V_T in PD compared with HC.
5. Parkinson's disease (PD): male PD vs. female PD: In male PD, compared with female PD, matched for age, V_T was significantly lower in the caudate nucleus, pallidum/putamen, insula, fronto-occipital and anterior cingulate cortices and parahippocampus. There was no significant increase of V_T in male PD compared with female PD ($p<0.005$, $\kappa=30$ voxels; uncorrected).
6. Due to the lack of quantitative PET data and/or due to the low number of the patients in some cohorts (corticobasal degeneration [n=1], and multiple sclerosis [n=1]), group comparisons in these patient cohorts were not carried out.

Age and its relationship to α4β2-nAChR availability (V_T) in healthy controls (HC):
Healthy controls (HC): SPM-analyses

1. Correlation with age: there was a significant negative correlation between age and α4β2-nAChR-binding in the thalamus, anterior cingulate cortex and the hippocampus and cerebellum ($p<0.005$, $\kappa=30$ voxels; FWE-corrected) and the inferior fronto-temporo-parietal and insular cortices ($p<0.005$, $\kappa=30$ voxels; uncorrected). There was a significant positive correlation between age and V_T in the left insular cortex ($p<0.005$, $\kappa=30$ voxels; uncorrected).
2. HC-old vs. HC-young: In HC-old, compared to HC-young, there was significantly lower α4β2-nAChR-binding in the lingual cortex/hippocampus, anterior cingulate cortex, thalamus ($p<0.005$, $\kappa=30$ voxels; FWE-corrected), in the right fronto-temporo and insular cortices, cerebellum ($p<0.005$, $\kappa=30$ voxels; uncorrected). In HC-old, compared to HC-young, there was significantly higher α4β2-nAChR-binding in the left insular and left fronto-temporal cortices ($p<0.005$, $\kappa=30$ voxels; uncorrected).

20.2 Safety results

No AEs, SAEs and/or SUSARs occurred within the study during the entire course of the trial, no further analyses were performed.

21 Conclusion

Regarding the aims and hypotheses of this trial, we state that the investigation of parametric PET images of V_T, the gold standard to quantify the α4β2-nAChR availability, enables to identify distinct patterns of lower α4β2-nAChR binding in the human brain of patients with various neurodegenerative disorders as compared with healthy controls.

There is a widespread cortical and subcortical decrease of α4β2-nAChR availability in patients with mild to moderate Parkinson's disease. Of interest in male patients with PD, compared with female PD, there is lower α4β2-nAChR binding in the striato-limbic and paralimbic brain regions possibly expressing another type of motor and/or non-motor dysfunction in male and female PD that may be relevant for drug therapy. In patients with essential tremor, an important differential diagnosis of Parkinson's disease, there is lower α4β2-nAChR binding asymmetric in the right insula, putamen, thalamus and inferior frontal cortex (rectus) possibly reflecting non-motor dysfunction. In patients with Huntington's disease, though findings are limited due to the small study numbers, there is lower α4β2-nAChR availability in the caudate nucleus, inferior frontal and temporal cortices. In patients with mild, late-onset Alzheimer's disease, reduced α4β2-nAChR availability is restricted to the right superior temporal cortex and surprisingly higher α4β2-nAChR availability is present in the left fusiform and inferior temporal cortices. Although interpretation is limited due to the small numbers of study participants in this cohort, higher receptor binding may represent compensatory changes in AD. Normal aging is mainly associated with lower α4β2-nAChR binding in the thalamus, anterior cingulate cortex, hippocampus, cerebellum and fronto-temporo-parietal and insular cortices.

Taken together, the findings support that 2-[^{18}F]F-A-85380 is suitable for PET imaging of cerebral α4β2-nAChR in the living human brain. The radiotracer together with dedicated PET imaging might have the potential to serve as biomarker for cholinergic vulnerability in the early stage of various neurodegenerative disorders such as Parkinsonian syndromes (PD, ET), Huntington's disease and Alzheimer's disease. Furthermore it may help to explore the cholinergic vulnerability of healthy aging as well as identify sex-related differences in neurodegenerative disorders such as PD. Nicotinic PET imaging enriches our understanding of the pathophysiology of those neurodegenerative disorders. Our study findings encourage future PET trials using α4β2-nAChR-specific radioligands such as 2-[^{18}F]F-A-85380.

In conformity with the safety data of the annual safety reports, the application of the radioligand 2-[^{18}F]F-A-85380 as an in-vivo biomarker to quantify the availability of the brain α4β2 nicotinic acetylcholine receptors in neurodegenerative disorders is not associated with an increased safety risk for participants of the clinical trial. There were no drop-outs (for safety reasons), deaths, SAEs, AEs or SUSARs reported related to the investigational medicinal product or the clinical study procedures itself, indicating that the application of 2-[^{18}F]F-A-85380 as PET-tracer for diagnostic purposes is safe.

22 Appendices

22.1 CONSORT Flow Diagram

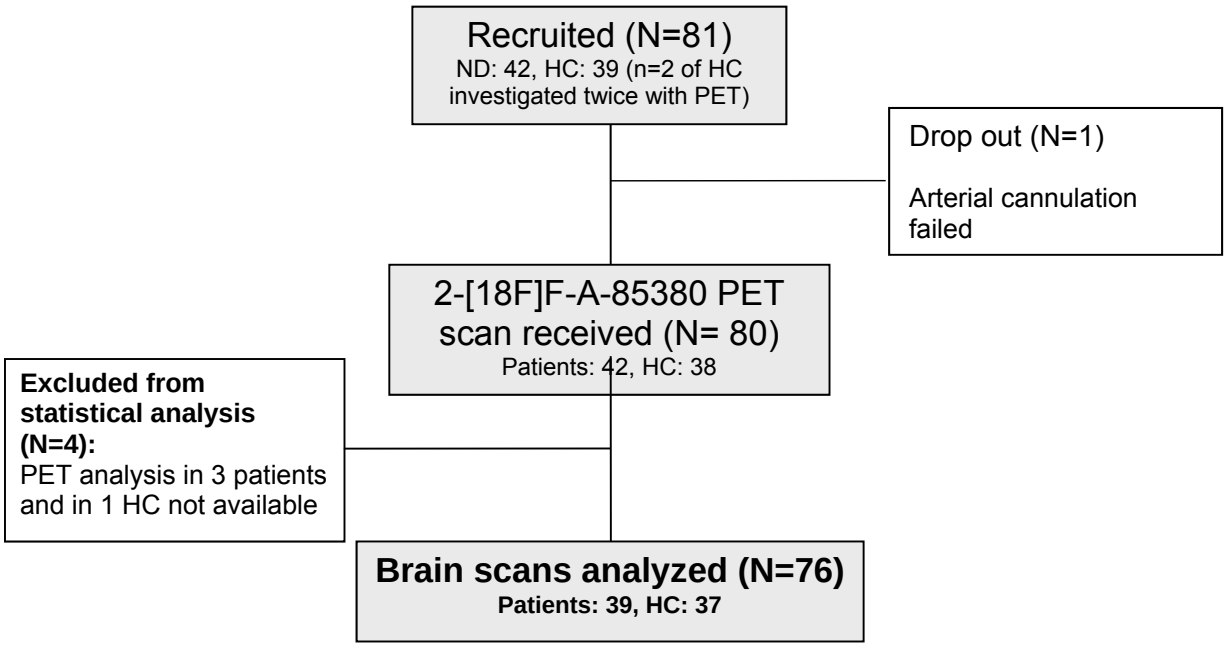

Figure 1: flow diagram (ND: neurodegenerative diseases, HC: healthy control)

22.2 Subject Characteristics of all study participants assessed with 2-[18F]F-A-85380 PET

	N	Age (mean±SD)	Gender (male : female)
Healthy controls (HC)[1)]	Total: 38	49 ± 22	18:20
-Young HC (< 30 years)	14	24 ± 3	4:10
-Old HC (≥ 30 years)	24	66 ± 9	14:10
Patients with neuro-degenerative diseases (ND)	Total: 42	66 ± 13	25:17
Movement disorders / Parkinsonian syndromes	35	68 ± 10	25:10
-Parkinson's disease (PD)[2)]	27	68 ± 11	19:8
-Essential tremor (ET)	7	67 ± 11	6:1
-Corticobasal degeneration (CBD)	1	65	0:1
Huntington's disease (HD)	2	62 ± 5	0:2

Multiple sclerosis (MS)[3)]	2	33 ±10	0:2
Alzheimer's disease (AD)[4)]	3	71 ± 19	0:3

Table 2: Study participants' characteristics;

1) PET performed twice in 2 HC; PET data of one HC not available.
2) PET in one patient not available.
3) PET data of one patient not available.
4) PET data of one patient not available.