Institut de Cancérologie de l'Ouest
unicancer PAYS DE LA LOIRE

Short title : **EITHICS**
Ref ICO : ICO-2019-04
N° EudraCT : 2019-001463-75

PROTOCOL EITHICS

A) CLINICAL STUDY IDENTIFICATION			
STUDY TITLE :	Feasability Pilot Study: Exploration by PET/CT with [18F]-DPA-714 of inflammation and the presence of M2 macrophages in triple-negative breast cancers		
SHORTAGE :	**EITHICS**		
COORDINATOR :	Dr Caroline ROUSSEAU		
METHODOLOGIST :	Dr Loïc CAMPION		
NUMBER OF ESTIMATED CENTERS :	3	**NUMBER OF PATIENTS**	12

B) PROMOTOR IDENTIFICATION	
ORGANISME NAME :	Service Promotion de Recherche Clinique de l'Institut de Cancérologie de l'Ouest.
CONTACT PERSON :	**NADIA ALLAM** **Tél : 02 40 67 98 26**

C) GENERAL STUDY INFORMATION	
INDICATION :	Triple-negative breast cancer, surgery first
METHOD :	Prospective, non-randomized phase II imaging study
PRIMARY ENDPOINT :	Study of correlation between M1/2 macrophagic polarization determined by immunohistochemistry on tumor and PET/CT fixation with [18F]-DPA-714 (qualitative and texture analysis) in patients with initially unresectable triple-negative breast cancer
SECONDARY ENDPOINT :	• To determine the correlation between the TSPO genotyping (HAB, MAB and LAB) and [18F] DPA-714 uptake on PET/CT • To evaluate the relevance of early and late phase PET/CT acquisitions with [18F]-DPA-714 • To evaluate the performance of three types of imaging studies: PET/CT with [18F]-DPA-714 (early and late phase), PET/CT with 18FDG and diffusion-weighted MRI in the characterization of triple-negative breast cancer • Characterization of triple-negative breast cancer • To evaluate potential toxicity of [18F]-DPA-714.

INCLUSION CRITERIA :	1. Patient aged over 18 years 2. Patient with a triple-negative breast cancer defined by following criteria: - Estrogen receptors < 10% - **AND** progesterone receptors <10% - **AND** non-amplified or non-overexpressed HER2 3. Patient with indication of surgery first for the primary tumor 4. PS 0 or 1 5. Effective contraception in women of childbearing age 6. Patient covered by a social security program 7. Obtaining the patient's signed written consent
NON-INCLUSION CRITERIA :	1. Patient with NO triple-negative breast cancer 2. Patient with inflammatory breast cancer 3. Patient with distant metastases 4. Patient treated by following anti-cancer medication: chemotherapy, immunotherapy, biological response modifying drugs, hormonal therapy and radiotherapy BEFORE performing biopsy and PET exam. 5. Diabetes under treatment with blood glucose >10 mmol/L 6. Patient with antibiotics and/or steroidal and/or non-steroidal anti-inflammatory drugs 30 days prior to PET with [18F]-DPA-714 7. Contra-indication to MRI (presence of metallic implants or prostheses, severe claustrophobia, pacemaker…) 8. Patient deprived of liberty, subject to judicial protection measures (safeguard of justice, curatorship) or under legal guardianship 9. Pregnant or potentially pregnant woman or currently breastfeeding 10. Agitation, inability to remain immobile in a supine position for at least one hour, or known history of claustrophobia.
MAIN EVALUATION CRITERIA :	M1/M2 Macrophagic polarization will be determined by the ratio between cellular expression of macrophagic marker M2 CD163 and pan-macrophagic marker CD68. The count of tumor associated macrophages (TAM) will be done on at least four hot spots and the mean will be used to establish the count of TAM per tumor. The polarization will be determined, based on the mean of all results obtained on all tumors, distinguishing: ⇒ tumors with a high ratio CD163/CD68 corresponding to M2 polarization of tumors ⇒ tumors with a low ratio CD163/CD68 corresponding to M1 polarization of tumors Thus, we will observe the difference in uptake level (SUV) on PET [18F] DPA-714 according to the high or low CD163/CD68 ratio in immunohistochemistry

SECONDARY EVALUATION CRITERIA :	• Genotyping of TSPO will be performed at the end of the study • Comparison of early and late PET/CT [18F]-DPA-714 acquisitions will be made by confronting qualitative and quantitative parameters of both acquisitions. • Comparison of performances of PET/CT with [18F]-DPA-714 to PET/CT with 18FDG and to diffusion-weighted breast MRI to characterize breast cancers TN will be conducted through texture analysis of the images. • Tumoral characterization will be performed by applying an immunohistochemical panel on surgical sample. The data of macrophagic polarization will be correlated to recent molecular classifications, defining three subtypes of triple-negative carcinomas, which can be characterized by immunohistochemical analysis: ⇒ Carcinoma of luminal/androgen receptor type (non "basal-like"): expression of androgen receptors and of transcriptional regulator FOXA1 ⇒ Carcinomas of basal type with weak immune response (and M2 macrophagic polarization) and neurogenesis: expression of basal-like marker CK5, of neuronal marker UCHL1/PGP9.5 and of schwannien marker PS100 ⇒ Carcinomas of basal type with adaptative immune response (defined by an absence of M2 macrophagic polarization, a complete lymphoid differentiation and an overexpression of immunity check-points): expression of basal-like marker CK5, complete B differentiation (plasmocytic marker Syndecan-1 (CD138); presence of tertiary lymphoid structures with dendritic net follicular marker CD21 and high endothelium venules marker MECA79). Tolerance of [18F]-DPA-714 will be verified by measuring of vital signs and patient surveillance two hours following the injection of radiopharmaceutical. All toxicity to [18F]-DPA-714 will be reported. The NCI Common Toxicity Criteria version 5.0 reference, will be used (annex 5).

D) DESCRIPTION OF STUDY INTERVENTION	
EXPERIMENTAL PROCEDURE : PET/CT with [18F]-DPA-714 [18F] DPA-714, is an expression specific radiopharmaceutical of TSPO, translocation protein of 18kDa, recognized as a highly sensitive biomarker of inflammation and macrophage activation in vivo. The radiopharmaceutical [18F]-DPA-714 will be prepared in accordance with good manufacturing practice rules within the facilities of the Center for Radiopharmaceutical Studies (CERRP), which is an annex of the Hospital Pharmacy, and affiliated with the radiopharmacy of the University Hospital (CHU) of Tours. Upon receipt, and in accordance with national regulations, each radiopharmacist and/or nuclear medicine physician will ensure the traceability of radioactive products and will oversee the storage and administration of the medications.	
THERAPEUTIC SCHEME :	No premedication or other treatment will be administered before or after the PET with [18F]-DPA-714 The administered activity is 3,5 MBq/kg ± 10%, with a minimum of 150 MBq and a maximum of 400 MBq. Injection of [18F]-DPA-714 as an IV bolus under the PET scanner with syringe flushing: after CT images and before PET images acquisition. Acquisition of dynamic PET images in List mode in prone position for 30 minutes. 60 minutes after the injection of the [18F]-DPA-714, acquisition in the prone position centered on the thorax and axillary fossae for approximately 15 minutes, followed by a whole-body acquisition for about 20 minutes.
TREATMENT DURATION :	The PET scan with [18F]-DPA-714 lasts approximately 3h to 3h30

E) STATISTICAL CONSIDERATION	
SAMPLE SIZE CALCULATION :	As this is a "proof of concept" exploratory and descriptive pilot study, the number of patients included was set at 12 patients over 1 year.

STATISTICAL ANALYSIS	For the TEP [18F]-DPA714 acquisitions, a qualitative analysis of the images will be performed during each examination by at least one nuclear medicine physician who is unaware of the results of the other imaging examinations. A centralized semi quantitative analysis of lesions as well as on tumor/background-signal based on the biodistribution of radiopharmaceutical will be performed (using a dedicated console DOSISOFT) The "gold standard" for lesion assessment will be defined by data from pathology and molecular biology. Continuous variables will be presented by the mean+/-standard deviation and qualitative variables by distribution of categories, as global and by subgroups of interest (hormonal receptors, histology type, SBR grade, mitotic index) The groups of interest will be compared using the following statistical tests: • Continuous variables: - if group variable with 2 categories: Wilcoxon non-parametric test - if group variable with more than 2 categories: Kruskal-Wallis non-parametric test • Qualitative variables: test exact de Fisher: The correlation between continuous variables will be calculated by Rho coefficient and Spearman test. Particular care will be taken to minimize any missing data on the 12 patients Patients are considered evaluable if they have undergone PET with [18F]DPA-714 and surgery. Patients who have not undergone PET with [18F]DPA-714 and/or surgery are not evaluable and will therefore be replaced

F) EXPECTED DURATION OF THE TRIAL	
INCLUSION PERIOD :	12 month
TREATMENT EPRIOD :	NA
FOLLOW UP PERIOD :	3 month
ESTMATED DURATIONUP TO THE ANALYSIS OF PRIMARY ENDPOINT:	3 month
OVERALL STUDY DURATON, INCLUDING FOLLOW UP:	15 month

SUMMARY TABLE OF INVESTIGATIONS & TREATMENTS

VISITES	Inclusion assesment	TEP [18F]DPA-714	SURGERY	After Surgery visit
Dates of visits	**d-30 to d0**	**d0**	**d0 à d60 (h)**	
Inclusion/non-inclusion criteria	**X**			
Signed informed consent	**X**			
Registration	**X**			
CLINICAL EXAM				
Antecedents/disease history	**X**			
Clinical exam, weight, height, PS (OMS)	**X**			
Vital signs (pulse, BP, temperature)		**X(d)**		
Toxicity		**X(e)**		**X(e)**
LABORATORY TESTS				
Biochemestry	**X(c)**			
Blood pregnancy test	**X(f)**			
Tumoral markers : ACE, CA 15-3	**X**			
Genotyping of TSPO protein	**X(a)(g)**			
IMAGING STUDIES				
PET with ^{18}FDG	**X(a) (h)**			
Diffusion-weighted bilateral breast & axillary fossae IRM	**X(a)**			
STUDY EXAM				
PET with [18F] DPA-174		**X(b) (h)**		
HISTOLOGICAL EXAM				
Initial biopsy : standard histology	**X**			
Surgical sample : standard histology			**X**	
Surgical sample: inflammatory markers + characterization of tumoral subtype (centralized analyses in Nantes)			**X**	
AUTORADIOGRAPHY OF TUMORAL SAMPLE				
In vitro Autoradiography with [3H]DPA-714 tumoral sample or surgical sample			**X**	

(a) After signature of informed consent
(b) After signature of informed consent and inclusion
(c) Biochemistry: bilirubin, GPT, GOT, Alkaline Phosphatases, Gamma GT, creatine, urea, blood glucose.
(d) Vital signs surveillance 2 hours before and following the tracer injection
(e) Follow-up of AE and SAE will be performed 28 days following PET with [18F] DPA-174
(f) Blood pregnancy test should be realized 72 hours preceding Sathe PET with [18F] DPA-174 exam
(g) Sample sending at the end of study for centralised analysis
(h) PET WITH ^{18}FDG, PET with [18F] DPA-174 and surgery should be realized within the shortest delay possible (maximum 2 moth)