CLINICAL TRIAL INFORMATION:

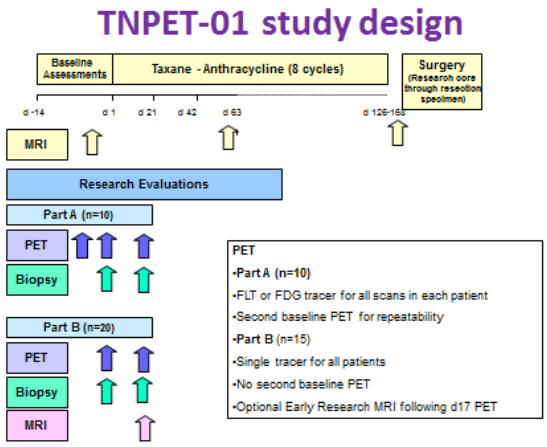
Protocol Full Title	A randomised phase II trial of [18F]fluorothymidine and the standard tracer [18F]Fluorodeoxyglucose in the assessment of systemic therapy response in triple negative breast cancer and their utility compared to conventional MRI imaging response, early ADC change and biopsy derived biomarkers.
Protocol Short Title	TNPET01

EudraCT number: 2011-004220-34

REC Number: 11/L0/1492

Co-Sponsors Kings College London/Guys and St Thomas' NHS foundation Trust

Medical condition or disease under investigation	**Imaging response biomarker study in triple negative breast cancer**
Purpose of clinical trial	Phase II early imaging response biomarker study using PET-CT imaging for monitoring on treatment change in triple negative breast cancer
Primary objective	**Part A:** To confirm PET scan SUV measurement repeatability using [^{18}F]FDG and [^{18}F]FLT tracers **Part B:** To evaluate PET imaging using ^{18}F]FLT or [^{18}F]FDG) as methods for evaluating response to systemic therapy in primary triple negative breast cancer with respect to MRI response at 3 cycles
Secondary objective (s) (Combined Part A and B data)	1. Ascertain the optimal scan initiation time after [^{18}F]FLT and [^{18}F]FDG tracer administration in patients with triple negative breast cancer 2. To correlate PET imaging response in breast and axillary lymph nodes with residual cancer burden (RCB) at definitive surgery 3. To correlate PET imaging response using each tracer with blood and biopsy derived biomarkers. 4. Non invasive assessment of Ki and k1 from this data set 5. To obtain performance estimates for the ability of the Part B tracer (FDG or FLT) to report MRI response derived from integration of Apparent Diffusion Coefficient (ADC) and size change data at 3 cycles 6. To obtain exploratory performance estimates for early MRI size and ADC evaluation on Diffusion Weighted MRI sequences after 1 cycle to report RECIST response at 3 cycles and RCB at definitive surgery 7. To correlate MRI imaging ADC change with blood and biopsy derived proliferation biomarkers and apoptosis biomarkers 8. To confirm the safety of [^{18}F]FLT in patients with breast cancer.
Trial Design	Single centre, non-therapeutic randomised open label Phase II trial with two parts
Trial Interventions	Part A • 10 participants randomly allocated to FDG or FLTtracer will have two PET-CT scans separated by a minimum of 24 hours performed at baseline prior to chemotherapy. Participants will have a third scan at day 17±3 following the first cycle of

	chemotherapy to assess SUV response to treatment Part B • 15 patients will be scanned once prior to commencing chemotherapy and again at day 14-21 post cycle 1 using the single tracer selected for progression to Part B according to end of Part A criteria . • Optional study specific MRI scan performed at the end of cycle 1 (day 17±3) for early size change and apparent diffusion coefficient (ADC) evaluation. All particpants (A and B) will have a research core biopsy performed prior to chemotherapy, following their day 17±3 PET scan and through the definitive resection specimen at the time of surgery. All participants may have an optional research blood sample 
Sample Size	25
Summary of eligibility criteria	Inclusion • Female age 18 to 70 years • Stage II-III biopsy proven early breast cancer for which primary chemotherapy is recommended. • HER2 negative primary tumours (IHC 0 or 1+, or IHC 2+ and FISH non-amplified (ratio of *Her2* to chromosome 17 of more than 2.0) • ER negative primary invasive breast cancer (Allred <3) • ECOG PS of 0 or 1

	• Primary tumour size >2cm • Eligible for neoadjuvant chemotherapy according to departmental protocols • Able to comply with treatment plans, scheduled visits, all study PET imaging and biopsy procedures and follow-up • Agree to use a medically acceptable birth control during the duration of their chemotherapy if of childbearing age. Exclusion • Any prior treatment for the breast cancer • Patients who are pregnant or breast feeding • Evidence of metastatic disease at diagnosis precluding neoadjuvant chemotherapy. • Requirement for concurrent radiotherapy treatment • Serious medical condition or concurrent medical illness likely to compromise ability to complete chemotherapy course. • Anticoagulation requirement which would preclude serial biopsy • Diabetes Mellitus • Any other problems that may make the patient unable to tolerate the PET scans or translational biopsies • Investigational Medicinal Product in the previous 28 days
IMP, dosage and route of administration	Intravenous radiotracer administration at time 0 on day of PET imaging (3 sessions per patient Part A, 2 per patient Part B). Single IMP per patient [^{18}F]-fluorothymidine (FLT): maximum 200 MBq FLT: 6.5 mSv per administration The study IMP [^{18}F]-fluorothymidine is supplied by: The PET Imaging Centre St. Thomas' Hospital London SE1 7EH UK MIA(IMP) No. 11387 1. [^{18}F]-fluorodeoxyglucose (FDG):

	maximum 200 MBq of FDG: 4mSv per administration The study IMP [^{18}F]-FDG is MetaTrace FDG Solution for injection. Marketing authorisation number: PL 27150/001
Active comparator product(s)	Not applicable
Start Date	November 2012
End date	Surgery date for final patient

Population of subjects

Part A

A total of 11 patients were randomised within part A. 5 participants scanned using the FDG and 4 participants randomised to FLT completed both baseline scans and were included in the analyses. In the FLT group 1 participant withdrew consent following her first PET scan and a further patient was withdrawn from the study without receiving any allocated research interventions due to FLT production failure.

		FLT (n=4)	FDG (n=5)	Overall (n=9)
Age (years)	Mean (s.d.)	40.3 (10.7)	43.6 (5.0)	42.1 (7.7)
	Median (min-max)	39 (30-53)	44 (36-49)	44 (30-53)

Part B

A total of 16 patients were included for Part B, 5 datasets progressed through from Part A and 11 were screened. 1 patient withdrew consent after randomisation and 1 patient was incorrectly randomised as she was ineligible and therapy withdrawn.

A total of 22 patients were recruited (combined parts A and B). 3 patients were withdrawn (1 FLT production failure, 1 patient choice, 1 HER2+ consent)

BASELINE CHARACTERISTICS:

Age range for Part A- 30-53, mean age 42 Age range for Part B- 36-60, mean age 47

Endpoints	Part A (n=10) Final patient completing pre chemotherapy test-retest imaging in Part A. Tracers will be expected to achieve SUV repeatability of within ±15% and SUV reduction of 20-40% in at least 50% of MRI defined responders evaluable at the point the last patient is entered into part A. If these criteria are not met for a single tracer the alternative tracer will proceed to Part B of the study. If both tracers meet the criteria the tracer with the highest proportion of MRI defined responders with a drop in SUV of >20% will be selected to go through to part B. In the event of equal proportions the decision will be based on consensus between the team on which tracer performs the best overall. All consenting patients in part B will be followed using this single tracer. The study will terminate if neither tracer meets these criteria. Part B (n=15) On confirmation of tracer repeatability and after approval as a result of the Part A analysis the database will continue forward for a single tracer and Part A data contribute for Part B endpoint analysis. End of Study The end of the trial is the date of surgery of the last patient participating in the trial. This will be either completion of the last patients surgical visit if no IMP-related AE's have been seen of until any IMP-related AE monthly follow-up visits have been completed
Sample Size	25

End Point #1Statistical Analyses

Part A (participant recruitment completed) delivers the first phenotype specific repeatability constraints for the most commonly reported standardised uptake parameters (SUV); maximum (SUVmax), mean (SUVmean), peak (SUVpeak) and lean body mass corrected peak (SULpeak),

assessed at conventional (90 minutes) and exploratory (120 and 180 minute) acquisition time points. The TNBC SUV intrinsic variability was 12-24% in both tracers and is dependent on scan acquisition time and SUV parameter. Based on the Part A data the FDG tracer progressed to the second phase, Part B, to provide the first TNBC phenotype specific response data at a post-cycle 1 time point.

The data suggests SUV change can predict later residual cancer burden and that >40% threshold change will be required to differentiate RCB 0-1 vs 2-3 response, a change that exceeds current EORTC/PERCIST recommendations for solid tumour chemotherapy response prediction. The study will inform future use of early FDG-PET as an exploratory biomarker in window of opportunity and novel therapy neo-adjuvant trials in TNBC

3.End Point #2Statistical Analyses

Part B data analysis is in progress. The emerging data suggests SUV change can predict later residual cancer burden and that >40% threshold change will be required to differentiate RCB 0-1 vs 2-3 response, a change that exceeds current EORTC/PERCIST recommendations for solid tumour chemotherapy response prediction. The study will inform future use of early FDG-PET as an exploratory biomarker in window of opportunity and novel therapy neo-adjuvant trials in TNBC

1.Adverse events information;

The protocol specified that any AE, SAE, IME or SAR deemed related to chemotherapy as per the Guys and St Thomas Breast Unit Protocols and the relevant SmPC did not require reporting within this protocol. Elective admissions to hospital for procedures which were planned and documented in the medical records at the time of consent (e.g. planned surgical procedure chemotherapy administration) are not SAEs, did not require SAE reporting.

Non-serious adverse event.

None reported