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FINAL STUDY REPORT

Full title of the trial:	**P**et imaging as a biomarker of **E**verolimus **A**dded value in hormone **R**efractory postmenopausa**L** women
Short title of the trial:	PEARL
EudraCT Number:	2012-004860-22
Sponsor Protocol Number:	IJB-BCTL:20120306
ClinicalTrials.gov Number:	NCT02028364
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Report date	17/06/2022

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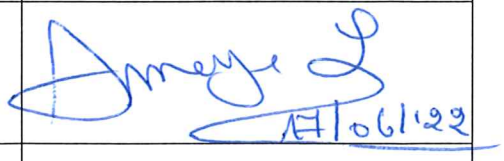
Lieveke Ameye	Statistician	
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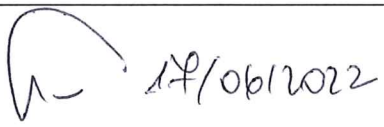
Andrea Gombos	MD, Study Chair	

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1. TRIAL INFORMATION

PHASE	Phase II
STUDY DESIGN	This study was a prospective phase II single arm, open label, multicentre study evaluating early FDG-PET/CT response as a predictor of treatment response to exemestane (25 mg daily orally) and everolimus (10 mg daily orally) in subjects refractory to non-steroidal aromatase inhibitors (NSAI) in locally advanced and metastatic breast cancer (MBC) Pilot phase The aim of the pilot phase was to verify the hypothesis that roughly 1 in 5 subjects is an FDG-PET/CT non-responder and to determine the optimum timing for the second FDG-PET/CT assessment (2 weeks versus 4 weeks after initiation of treatment). Thus, subjects underwent 3 FDG-PET/CT scans. Concordance-analysis using Kappa coefficient (c.f. Koch and Landis scale) between FDG-PET-responses seen at 2 versus 4 weeks was performed. In case of concordance rate of more than 90% week 2 FDG-PET/CT will be chosen for the main study. In the case of non-concordance we will choose the timepoint where the mean absolute change in SUV_{max} will be the highest. This decision rule will be applied blinded from any clinical data so that these subjects can be used in the main study. The pilot phase was conducted and recruited 27 evaluable subjects (instead of the 30 subjects initially planned. Accrual was stopped after 30 evaluable subjects included but 3 subjects were denoted inevaluable afterwards). All subjects received everolimus 10 mg daily plus exemestane 25 mg daily and treatment has been continued until disease progression or unacceptable toxicity or consent withdrawal, whichever came first. Main study The aim of the pilot phase was to characterize FDG-PET/CT responses in locally advanced or metastatic breast cancer subjects treated with everolimus and exemestane and to establish which is the most suitable time point for the early-FDG-PET/CT (D14 vs D28 after the initiation of treatment). Results of this pilot phase led to the following findings: - The metabolic non-response rate on Day 14 and Day 28 was similar: 59% (16/27) (Kappa coefficient for concordance: 0.85 (95% CI, 0.64 to 1); mean SUV decrease at day 14: 26%, at day 28: 32%-p-0.03). The assessment of response was blinded from any clinical data so that these subjects can be used for the main study - Subject population highly representative of real life regarding the number of previous treatments, metastatic sites and toxicity profile of everolimus. The sample size is too small to perform any subgroup analysis at this stage

	- Median PFS in all 27 subjects: 6.6 months, the disease control rate at the first RECIST evaluation was 66%: (18% of subjects had partial response and 48% stable disease). The median duration of everolimus treatment was 6 month (range: 1-17 month). There was not enough statistical power with this sample size to assess PFS in FDG-PET-responders and non-responders separately, hence the need to proceed to the expansion phase After the completion of the pilot study the results were reviewed and the decision to proceed to the full subject enrolment of the main study was taken. The investigators remained blinded to these results. It was decided to continue the day 14 FDG-PET/CT for the second phase, given the high concordance between the two time points used in the pilot phase and the aim to reduce at maximum treatment exposure and costs. According to the observed metabolic non-response rate in the pilot phase (59%), 19 more evaluable subjects are needed to complete the trial (meaning approximately 30 subjects screened) and to answer the primary endpoint. Again, all subjects received everolimus 10 mg daily plus exemestane 25 mg daily. In this instance, subjects had 2 FDG-PET/CT: at baseline and at the time point determined by the results of the pilot study, i.e. at the end of week 2 (D14). Note that the clinician were blinded to the FDG-PET/CT result and the FDG-PET/CT physicians to the clinical follow-up. Study treatment continued until disease progression, unacceptable toxicity or consent withdrawal. Further treatment after progression was at the investigators discretion.
STUDY RATIONALE	Given the higher level of adverse events in trials combining aromatase inhibitors with mTOR inhibitors, selecting patients who will achieve a benefit and thus justify the burden of treatment is of upmost importance. It is clear that this drug combination has a role in endocrine resistant patients. What is unclear is in which patients the clinical benefit rationalizes the potential toxicity. What is problematic is that unlike HER2+ BC, there is an absence of established biomarkers in this population that will identify patients that will sustain this benefit and thus it is unclear whom to expose to this increased toxicity. FDG-PET/CT offers a possible solution to early identification of patients who will NOT benefit from treatment with exemestane and everolimus. Thus a phase II trial evaluating the use of FDG-PET/CT as a biomarker in subjects with ER+ locally advanced and MBC treated with exemestane plus everolimus will answer the question of whether subjects who will NOT derive benefit from this combination of therapy can be selected early in the therapeutic process using FDG-PET/CT metabolic response thus minimizing unnecessary toxicities in these patients and high costs to society.

OBJECTIVES	Primary objective To evaluate if early metabolic response (MR) using FDG-PET/CT is associated with progression free survival (PFS) in ER+, HER2 negative ABC or MBC subjects treated with exemestane plus everolimus. Secondary objective To evaluate if early metabolic response (MR) using FDG-PET/CT is associated with overall survival (OS) (limited to a 3 year-follow-up) in ER+, HER2 negative locally advanced or MBC subjects treated with exemestane plus everolimus.
ENDPOINTS	For the pilot phase: • Primary endpoint: Proportion of FDG-PET/CT metabolic non responders/FDG-PET/CT metabolic responders For the main phase: • Primary endpoints : Progression free survival (PFS) (based on RECIST 1.1 criteria) • Secondary endpoints : Overall survival (OS)
INCLUSION CRITERIA	Subjects must meet ALL of the following criteria to be eligible for this study: 1. Adult women (≥18 years of age) with locally advanced or metastatic breast cancer not amenable to curative treatment by surgery or radiotherapy. 2. Histological or cytological confirmation of estrogen-receptor positive (ER+), HER2 negative breast cancer. 3. Postmenopausal female defined as: a. Age ≥ 55 years and one year or more of amenorrhoea b. Age <55 years and one year or more of amenorrhoea, with an estradiol assay <20pg/ml c. Surgical menopause with bilateral oophorectomy 4. Breast cancer that is refractory to non-steroidal aromatase inhibitors (NSAI) (i.e. anastrozole or letrozole) defined as: a. Recurrence while on, or within 12 months of end of adjuvant treatment with anastrozole or letrozole; OR b. Progression while on, or within one month of end of anastrozole or letrozole or treatment for locally advanced or metastatic breast cancer. 5. FDG-PET/CT measurable disease defined as: at least one target lesion fulfilling following criteria: a. Size ≥1.5cm; AND b. FDG-PET/CT avid lesion with uptake above the background liver uptake as described below: i.e. with a marked accumulation of FDG, at least 1.5-fold greater than liver SUV mean + 2 SDs (in 3cm spherical ROI in normal right lobe of liver). If liver is abnormal, target lesion should have uptake > 2.0 x SUV mean of blood pool in 1cm diameter ROI in descending thoracic aorta. NB: - The target lesion can be a bone metastasis if it fulfils the above mentioned criteria.

	- In the case the target lesion is a lymph node, the small axis should be ≥ 1.5 cm. 6. Radiological or clinical evidence of recurrence or progression on last systemic therapy prior to enrolment. 7. Adequate bone marrow function as shown by: a. Haemoglobin (HgB) ≥ 9.0 g/dL b. ANC ≥ 1,500/mm^3 (≥1.5 x 10^9/L) c. Platelets ≥ 100,000/mm^3 (≥ 100x 10^9/L) 8. Adequate liver function as shown by: a. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤2.5xULN (or ≤ 5 if hepatic metastases are present) b. Total serum bilirubin ≤ 1.5 x ULN (≤ 3 ULN for subjects known to have Gilbert Syndrome) 9. Adequate renal function as shown by Serum creatinine≤ 1.5 x ULN 10. Fasting serum cholesterol, triglycerides and glucose a. Fasting serum cholesterol ≤ 300 mg/dL or 7.75 mmol/L b. Fasting triglycerides ≤ 2.5 x ULN c. Fasting glucose < 1.5 x ULN 11. Eastern Co-operative Oncology Group (ECOG) performance status ≤ 2. 12. Written and signed informed consent obtained before any trial related activity. 13. Availability of a FFPE core of primary breast tumour 14. Possibility to obtain the mandatory blood samples for the translational research studies. 15. For subjects with accessible metastatic lesions, possibility to obtain the mandatory biopsy (FFPE and frozen) of a metastatic lesion
EXCLUSION CRITERIA	Subjects meeting any **ONE** of the following criteria are **NOT** eligible for the study: 1. HER2-overexpressing patients by local laboratory testing (IHC 3+ staining or in situ hybridization positive). 2. Subjects with only non-measurable lesions by FDG-PET/CT (e.g. pleural effusion, ascites etc.). 3. Symptomatic visceral disease for example liver, pulmonary metastases or lymphangitis carcinomatosis. 4. Known hypersensitivity to mTOR inhibitors, e.g. sirolimus (rapamycin). 5. Another malignancy within 5 years prior to enrolment, with the exception of adequately treated in-situ carcinoma of the cervix, uteri, basal or squamous cell carcinoma or non-melanomatous skin cancer. 6. Radiotherapy within four weeks prior to enrolment except in case of localized radiotherapy for analgesic purpose or for lytic lesions at risk of fracture, which can then be completed within two weeks prior to enrolment. Patients must have recovered from radiotherapy toxicities prior to enrolment. 7. Currently receiving hormone replacement therapy, unless discontinued prior to enrolment. 8. Symptomatic brain metastases or other central nervous system metastases which are not controlled by local treatments.

	9. Subjects receiving concomitant immunosuppressive agents or chronic corticosteroid use at the time of study entry except in cases outlined below: a. Topical applications (e.g. rash) b. Inhaled sprays (e.g. obstructive airways disease) c. Eye drops d. Local injections (e.g. intra-articular) e. Stable low dose of corticosteroids for at least two weeks before enrolment 10. Subjects with known HIV seropositivity. Screening for HIV infection at baseline is not required 11. Acute and chronic, active infectious disorders (except for Hepatitis B and Hepatitis C positive patients). 12. Active bleeding diathesis, or on oral anti-vitamin K medication (except low dose warfarin, LMWH and acetylsalicylic acid or equivalent, as long as the INR is ≤ 2.0). 13. Any severe uncontrolled medical conditions such as: a. Unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction ≤ 6 months prior to enrolment, uncontrolled cardiac arrhythmia b. Uncontrolled diabetes as defined by fasting glucose > 1.5 x ULN c. Acute and chronic, active infectious disorders and non-malignant medical illnesses that are uncontrolled or whose control may be jeopardized by the complications of this study therapy. d. Symptomatic deterioration of lung function 14. Subjects being treated with drugs recognized as being strong inhibitors or inducers of the isoenzyme CYP3A (Rifabutin, Rifampicin, Clarithromycin, Ketoconazole, Itraconazoleonazole, Voriconazole, Ritonavir, Telithromycin) within the last 5 days prior to enrolment 15. History of non-compliance to medical regimens. 16. Subjects unwilling or unable to comply with the protocol. 17. Concurrent anti-cancer treatment in another investigational trial, including hormonal therapy, immunotherapy or targeted agents other than those administered in this study.
INVESTIGATIONAL MEDICINAL PRODUCTS	Everolimus 10 mg orally daily given in conjunction with exemestane 25 mg orally daily until disease progression or treatment discontinuation for any other reasons.
INDICATION OF USE	Locally advanced or metastatic hormone receptor positive and HER2 negative breast cancer refractory to non-steroidal aromatase inhibitors (NSAI) (i.e. anastrozole or letrozole) and treated with everolimus and exemestane. NSAI “Resistance” defined as: a. Recurrence while on, or within 12 months of end of adjuvant treatment with anastrozole or letrozole; OR b. Progression while on, or within one month of end of anastrozole or letrozole or treatment for locally advanced or metastatic breast cancer
TARGETED POPULATION	Locally advanced or metastatic endocrine receptor positive and HER2 negative breast cancer refractory to non-steroidal aromatase inhibitors (NSAI) (i.e. anastrozole or letrozole)

PARTICIPATING COUNTRY	Belgium
PARTICIPATING SITES NUMBER	6 participating sites
LENGTH OF THE STUDY	• Actual start date of recruitment to the protocol: 12/02/2014 • Actual date stop date of recruitment to the protocol: 13/07/2018 • No long term follow-up planned
INDEPENDENT DATA MONITORING COMMITTEE	No
ANALYSIS STAGE & DATE	Final Date of final analysis: August 9th 2021
DATE OF GLOBAL END OF TRIAL	06/07/2021

2. SUBJECT INFORMATION

2.1. General information

Pilot phase

44 subjects were registered in the pilot phase, 35 subjects were enrolled and exposed to investigational medicinal products (IMPs). 27 subjects were evaluable.

The actual number of subjects registered in each age range for the whole trial is specified in the table 1.

Age categorical characteristic	Number of subjects
In Utero	0
Preterm newborn-gestational age>37 week	0
Newborns (0-27 days)	0
Infants and toddlers (28 days – 23 months)	0
Children (2 – 11 years)	0
Adolescents (12 – 17 years)	0
Between 18 and 65 years	36
From 66 years to 84 years	8
85 years and over	0
TOTAL	**35**

Table 1: Number of subjects registered by age category

Main phase

20 subjects were registered in the main phase, 20 subjects were enrolled and exposed to investigational medicinal products (IMPs). 20 subjects were evaluable.

The actual number of subjects enrolled in each age range for the whole trial is specified in the table 2.

Age categorical characteristic	Number of subjects
In Utero	0
Preterm newborn-gestational age>37 week	0
Newborns (0-27 days)	0
Infants and toddlers (28 days – 23 months)	0
Children (2 – 11 years)	0
Adolescents (12 – 17 years)	0
Between 18 and 65 years	15
From 66 years to 84 years	5
85 years and over	0
TOTAL	**20**

Table 2: Number of subjects enrolled by age category

2.2. Subject disposition

In total, 55 subjects were enrolled in the trial, 42 subjects completed the trial and 13 subjects did not complete the trial.

The reasons why some subjects did not complete the trial with the corresponding subjects' number are specified in the table 3.

Non-completion reasons	Number of subjects
Adverse event, not serious	4
Concurrent illness preventing further protocol treatment	
Consent withdrawn by subject	
No treatment (in eligible subjects)	
Physician decision	1
Progression before treatment	
Protocol violation	8

Table 3: Non-completion reasons with corresponding subjects' number.

3. STATISTICAL ANALYSIS

3.1. Number of subjects

PILOT phase

44 subjects considered for inclusion.

First subject in February 2014, last subject in June 2016.

- 9 ineligible:
  - 8 no PET measurable lesion (ID 10, 13, 15, 18, 23, 36, 38, 43)
  - 1 increase of liver function tests , another treatment started (ID 25)
- 2 ineligible after enrollment: HER2+ on baseline biopsy (results obtained after enrollment in the study). Subject treated until HER2 found positive. (ID 4, 6)
- 6 Not evaluable for PET (ID 1, 8, 16, 21, 31, 37)

=> 44 – 9 – 2 – 6 = **27 evaluable subjects**.

MAIN phase

First subject in June 2017, last subject in May 2018.

20 subjects considered for inclusion.

In all 20 subjects, the PET showed evaluable lesions according to study protocol.

Total

In total, we have 27 + 20 = **47 evaluable subjects**.

3.2. Baseline characteristics

N = 47 evaluable subjects

Age		
Mean/IQR (years)	57.1± 13.4	
ECOG PS		
0	23	49%
1	23	49%
2	1	2%
Histology		
Invasive ductal	38	83%
Invasive lobular	8	17%
Unknown	1	
Grade		
G1	8	22%
G2	17	47%
G3	11	31%
Unknown	11	
Current disease status		
Metastatic	45	96%
Lung or liver	35	74%
Bone only	6	13%
Other	4	9%
Locally advanced	2	4%
N Metastatic sites		
0	2	4%
1-2	24	51%
≥3	21	45%
KI-67 (primary)		
<10%	5	14%
10%-15%	9	24%
16%-25%	6	16%
>25%	17	46%
Unknown	10	
Prior CDK 4/6		
No	35	74%
Yes	12	26%
Number of lines CT in advanced setting		
0	20	43%
1	14	30%
≥2	13	28%
Number of lines ET in advanced setting		
0	2	4%
1	13	28%
≥2	32	68%
NSAI sensitive		
No	13	30%
Yes	34	70%

3.3. Treatment

N = 47 evaluable subjects

All 47 subjects started the treatment of everolimus and exemestane on the same day.

Duration everolimus treatment (in months)¶		
(=from start everolimus till last day everolimus)		
mean ± std	5.5 ± 5.2	
median (min-max)	4.1 (1.0 to 29.0)	
Duration exemestane treatment (in months)¶		
(= from start exemestane till last day exemestane)		
mean ± std	6.2 ± 5.5	
median (min-max)	5.0 (1.6 to 29.0)	
Subjects who continued exemestane after having stopped everolimus	13	28%
Subjects who continued exemestane more than a month after having stopped everolimus	5	11%
Subjects with treatment interruption of exemestane (protocol violation)	1	2%
Subjects with treatment interruption everolimus	23	49%
Subjects with dose reduction everolimus (from 10 till 5)**	21	47%

3.4. ^{18}F-FDG-PET/CT

Days between baseline PET and start treatment		
1 - 7 days	36	77%
8-14 days	9	19%
≥15 days	2	4%
Days between start treatment and PET D14		
12	2	4%
13	5	11%
14	32	68%
15	6	13%
16	1	2%
17	1	2%

Two subjects were excluded from the final metabolic response analysis because they stopped everolimus at 3 and 8 days respectively before the D14 ^{18}F-FDG-PET/CT was performed.

Thus, the final PFS analysis was performed on **45 subjects**. (Figure 1.A)

As initially defined in the protocol we used the so called consistent subject-based response (CONSIST) criteria for PET response assessment. A subject was considered to be a "responder" when a uniform SUVmax reduction of more than 25% was seen in all lesions. All cases not fulfilling this criterion were classified as "non-responders".

Considering previous data in the literature about breast cancer subjects treated with targeted therapies, we retrospectively decided to assess a lower cut-off than initially defined in the protocol to define metabolic non-response. We thought it would be worthwhile to test this because it was potentially more adapted to distinguish responding subjects from the non-responding ones in this particular population (everolimus being a targeted therapy). Consequently, we performed a post-hoc analysis defining as responders those subjects who showed a uniform reduction of 15% of all lesions.

3.5. ctDNA analysis

A total of **121 sequential plasma sampl**es were analyzed. The ctDNA analysis population comprised the following: 46 subjects at baseline, 42 subjects on D14, and 33 subjects at progression. Differences in the number of plasma samples analyzed at different time points are related to missing samples. (Figure 1.B)

Plasma ctDNA was sequenced to identify hotspot mutations in 40 cancer-specific genes (Table 4) with a coverage of 15000x using the commercially available next generation sequencing panel.

ctDNA was detected in 26 out of 46 subjects at baseline (56.5%) and in 14 out of 42 subjects on D14 (33.3%).

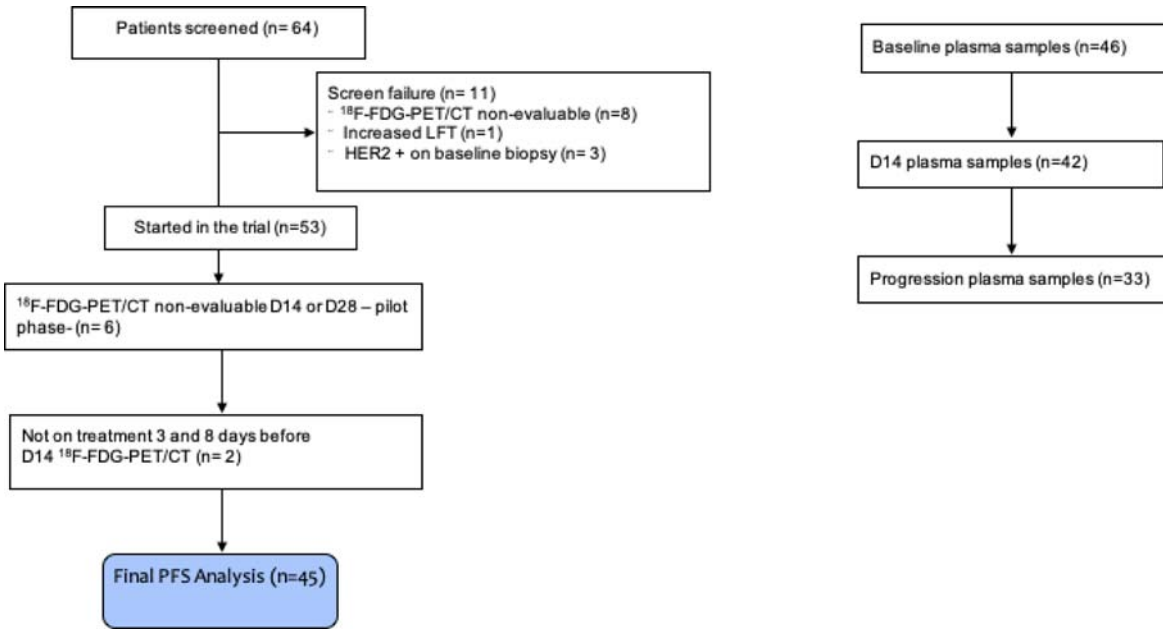

Figure 1

A – Subject selection for the main ^{18}F-FDG-PET/CT analysis; B – Number of sequential plasma samples analysed at key time- points. Four samples were missing on D14 and 13 at progression

AKT1	ALK	AR	BRAF	BTK	CTNNB1	DDR2	EGFR
ERBB2	ESR1	EZH2	FBXW7	FGFR1	FGFR2	FGFR3	FOXL2
GNA11	GNAQ	GNAS	HRAS	IDH1	IDH2	JAK2	JAK3
KIT	KRAS	MAP2K1	MAP2K2	MET	MPL	MTOR	NPM1
NRAS	PDGFRA	PIK3CA	PTEN	RAF1	RET	ROS1	TP53

Table 4 Targeted gene sequencing panel used for sequencing of plasma samples able to identify single nucleotide variants (SNVs) from 40 gene

3.6. Results

3.6.1. PFS according to ^{18}F-FDG-PET/CT response and ctDNA detection 14 days after the start of treatment with exemestane-everolimus

PFS is calculated as time to progression since date of early PET, in order to adjust for guarantee-time bias. (as all subjects needed an early PET assessment in order to be evaluable). Subjects without progression were censored at the date of last known to be progression-free.

3.6.1.1. ^{18}F-FDG-PET/CT response on D14 and subject outcome:

The metabolic non-response rate according to the criteria initially defined in the protocol (i.e., subjects with a SUVmax reduction of more than 25% in all lesions classified as responders) was **66.6%:** 30 subjects out of the 45 included in the final PFS analysis were non-responders. There was no statistically significant difference in outcome between those subjects who were considered responders and those who were not. The median PFS for ^{18}F-FDG-PET/CT response was 6.0 months compared to 3.1 months for non-response, but this difference was non-significant with a HR of 0.77 (95% CI, 0.40-1.50), p=0.44 (Figure 2.a). Results were similar in the intention to treat (ITT) population (n=47), including those two subjects who stopped everolimus before D14 ^{18}F-FDG-PET/CT (data not shown).

As mentioned above (3.4), the 25% threshold to define response has previously mainly been used in subjects receiving chemotherapy and to assess late response. We therefore retrospectively looked for a metabolic response cut-off that could be more adapted to a targeted treatment such as everolimus. By using 15% as a cut-off, probably a more accurate one for an early response evaluation already after 14 days of targeted therapy, the median PFS of responders (n=23) was 6.4 months versus 2.2 months of non-responders, p=0.0032, HR-0.38 (95% CI 0.20-0.72) (Figure 2.b).

3.6.1.2. Combined analysis of ^{18}F-FDG-PET/CT response and ctDNA detection on D14 and subject outcome.

As a next step, we assessed whether the combination of ctDNA detection and absence of metabolic response with ^{18}F-FDG-PET/CT on D14 can better identify those patients who will not derive benefit from the EXE-EVE combination than either method alone. ctDNA was detected in 26 out of 46 subjects at baseline (56.5%) and in 14 out of 42 subjects on D14 (33.3%)

The median PFS on EXE-EVE was only 1.8 months in subjects with no metabolic response and detectable ctDNA on D14 (Figure 2.e). By contrast, subjects with metabolic response and no detectable ctDNA at D14 had a median PFS of 4.4 months, p=0.0015 (Figure 2.e). The difference between these two groups of subjects is even more obvious when we use 15% reduction of SUVmax to define ^{18}F-FDG-PET response (median PFS 6.5 months versus 1.9 months, $p=6.9x10^{-4}$) (Figure 2.f).

3.6.1.3. ctDNA detection at baseline and on D14 and subject outcome.

Next, we analyzed whether ctDNA detection alone (yes versus no) at baseline and at D14 has an impact on subject outcome when treated with exemestane-everolimus. Median PFS in subjects with versus without detected ctDNA at baseline was 5 versus 4 months, p=0.050, HR-1.9 (95% CI: 0.99-3.6) (Figure 2.c). More importantly, median PFS in subjects with ctDNA detection at D14 was significantly lower than in subjects without ctDNA detection (2.1 versus 5 months, respectively, HR 2.5 [95% CI: 1.3-5.0 p=0.012] (Figure 2.d).

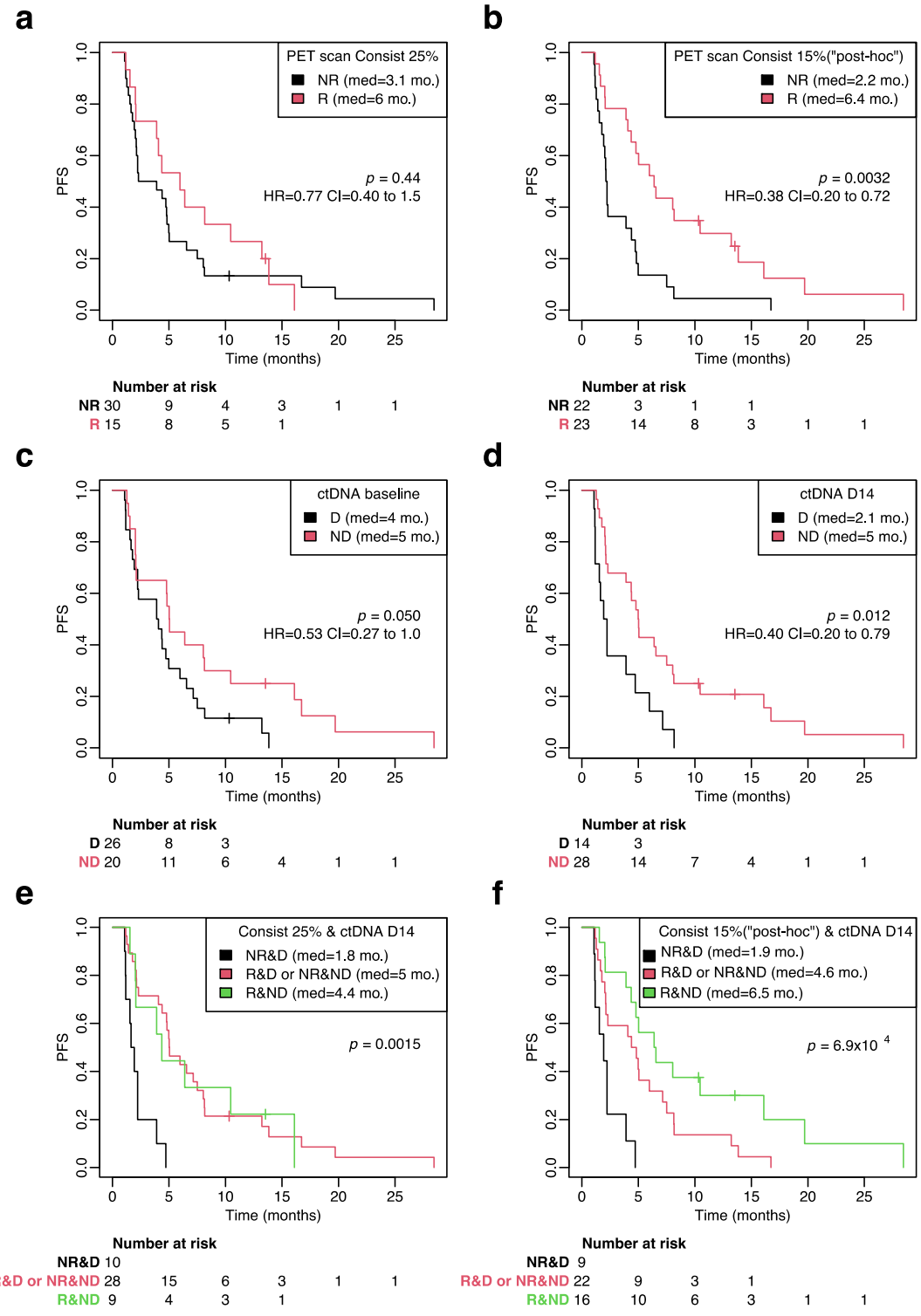

Figure 2

Kaplan-Meier plots of PFS according to ^{18}F-FDG-PET/CT response and ctDNA detection 14 days after the start of treatment with exemestane-everolimus.

a. PFS plots according to ^{18}F-FDG-PET/CT response on D14: subjects with > 25% homogenous decrease in maximum standardized uptake value (SUVmax) in all target lesions are considered as “responders”.

b. PFS plots according to ^{18}F-FDG-PET/CT response on D14: subjects with a > 15 % homogenous decrease in maximum standardized uptake value (SUVmax) in all target lesions are considered as “responders”. This is a “post-hoc” analysis, initially not scheduled by the study protocol.

c. PFS according to ctDNA detection at baseline (40-gene targeted gene sequencing panel was realized in all plasma samples).

d. PFS according to ctDNA detection after 14 days of treatment with exemestane-everolimus.

e. PFS according to the combined analysis of ctDNA detection and ^{18}F-FDG-PET/CT response when subjects with > 25% homogenous decrease in maximum standardized uptake value (SUVmax) in all target lesions are considered as “responders”

f. Combined analysis of ctDNA detection and ^{18}F-FDG-PET/CT response when subjects with >15% homogenous decrease in maximum standardized uptake value (SUVmax) in all target lesions are considered as “responders”. The 15% cut-off is a “post-hoc” analysis, initially not scheduled by the study protocol.

Log-Rank P values and LogRank Hazard ratios and 95% Confidence intervals for LogRank Hazard ratios for each test are displayed in each corresponding panel. For each group the number at risk are presented under the X axis.

Consist criteria of ^{18}F-FDG-PET/CT response is described Methods section, R= ^{18}F-FDG-PET/CT responder; NR= ^{18}F-FDG-PET/CT non-responder; ctDNA = circulating tumor DNA; D=ctDNA detected; ND=ctDNA not detected; D14 = 14 days after starting treatment with exemestane-everolimus

3.6.2. PFS according to subgroups (univariate analysis)

When we performed a univariate analysis of the above-described parameters and other clinical and biological characteristics, young age (≤60 years), ctDNA detection at D14, and the absence of metabolic response using the threshold of 15% had a significantly negative impact on outcome **(Figure 3)**

	HR	CI	*p*
Age (<=60 vs >60)	0.51	0.27 to 0.96	0.032
ECOG (0 vs 1–2)	1.4	0.8 to 2.5	0.23
N metastases (1–2 vs 3+)	0.91	0.49 to 1.7	0.76
Visceral meta (N/Y)	0.79	0.31 to 2	0.88
Prior anti–CDK4/6 (N/Y)	1.0	0.75 to 1.4	0.91
N prior chemotherapies (0–1 vs 2+)	1.3	0.68 to 2.5	0.43
NSAI sensitive (N/Y)	1.0	0.76 to 1.4	0.86
No metabolic response D14 (CONSIST 25%)	1.3	0.67 to 2.5	0.44
No metabolic response D14 (CONSIST 15%)	2.6	1.4 to 4.9	0.0032
ctDNA detected baseline (N/Y)	1.9	0.99 to 3.6	0.050
ctDNA detected D14 (N/Y)	2.5	1.3 to 5	0.012

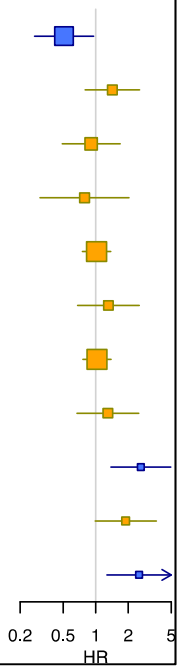

Figure 3

Impact of clinical and pathological characteristics, ^{18}F-FDG-PET/CT response and ctDNA detection on PFS (univariate analysis)

Blue boxes represent parameters which have significant impact on PFS. Yellow boxes represent parameters which doesn't have significant impact on PFS. Horizontal lines represent CI. X axis represents HR. CI: 95% confidence interval, p: p value, HR: hazard ratio

ECOG = Eastern Cooperative Oncology Group performance status, ranging from 0 to 5, and

indicating that the subject is at 0, fully active able to carry on all pre-disease performance without restriction; at 1,restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature; at 2,

2 - ambulatory and capable of all self-care but unable to carry out any work activities; up about more than 50% of waking hours;

N metastasis = number of metastatic organ sites involved,

Visceral meta = presence of visceral metastasis, N=no, Y=yes,

N prior chemotherapies = number of prior chemotherapy regimens used for the treatment of advanced breast cancer,

NSAI = non-steroidal aromatase inhibitor; sensitive is defined as relapse ≥ 2 years after the end of a NSAI in adjuvant setting or ≥ 6 months treatment in metastatic setting,

Consist 25% = <25% homogenous decrease in maximum standardized uptake value (SUVmax) in all target lesions are considered as "non-responders",

Consist 15% = <15% homogenous decrease in maximum standardized uptake value (SUVmax) in all target lesions are considered as "non-responders"

3.7. Publication

The results of the trial were published in NPJ Breast Cancer:Andrea Gombos, David Venet, Lieveke Ameye et al, FDG positron emission tomography imaging and ctDNA detection as an early dynamic biomarker of everolimus efficacy in advanced luminal breast cancer. NPJ Breast Cancer. 2021 Sep 21;7(1):125. doi: 10.1038/s41523-021-00331-8.

4. SAFETY ANALYSIS

4.1. General information

Serious adverse events (SAEs) and non-serious adverse events (AEs) were collected from the first administration of study treatments until 28 days after the last dose of study treatments.

All subjects exposed to everolimus and exemestane i.e. 55 subjects were taken into account in the safety analysis.

9 subjects were affected by serious adverse events.

50 subjects were affected by non-serious adverse events.

The total number of deaths until 28 days after EOT is 4, from whom 1 resulting from adverse events and 3 from progression.

Notes:

1. The adverse event and serious adverse event assessment method was systematic.
2. The MedDRA version used was the version 22.1.
3. In this protocol:
   Progression of underlying malignancy is not reported as an AE if it was clearly consistent with the suspected progression of the underlying cancer. Hospitalization due solely to the progression of underlying malignancy should NOT be reported as an SAE/AE. Clinical symptoms of progression may be reported as AEs or SAEs if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.
   If there is any uncertainty about an AE being due to the disease under study, it should be reported as an AE or SAE. Clinical symptoms of underlying malignancy, even if they meet a seriousness criteria, should not be reported as SAE unless the investigator considers them as more severe than expected.

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4.2. Serious Adverse Events overview

The table hereunder presents all serious adverse events sorted by MedDRA System Organ Class (SOC) and MedDRA Preferred Terms (PT).

MedDRA Primary SOC *MedDRA PT*	Number of subject affected	All SAE occurrences	SAE occurrences causally related to everolimus	SAE occurrences causally related to exemestane	Number of fatalities number	Number of fatalities causally related to treatment
Blood and lymphatic system disorders						
Febrile neutropenia	1	1				
Cardiac disorders						
Cardiac failure	1	1				
Myocardial ischaemia	1	1	1			
Gastrointestinal disorders						
Abdominal pain	1	1	1			
Diarrhoea	1	1	1			
Nausea	1	1	1			
General disorders and administration site conditions						
Mucosal inflammation	1	1	1			
Infections and infestations						
Bronchopulmonary aspergillosis	1	1	1			
Pyelonephritis	1	1	1			
Psychiatric disorders						
Anxiety	1	1				

MedDRA Primary SOC *MedDRA PT*	Number of subject affected	All SAE occurrences	SAE occurrences causally related to everolimus	SAE occurrences causally related to exemestane	Number of fatalities number	Number of fatalities causally related to treatment
Respiratory, thoracic and mediastinal disorders						
Bronchopneumopathy	1	1	1			
Interstitial lung disease	1	1	1		1	1
Lung disorder	2	2	2			
Pneumonitis	2	2	2			

4.3. Non-Serious Adverse Events overview

The frequency threshold for reporting non-serious adverse events is 0 %.

The below table presents all non-serious adverse events sorted by MedDRA System Organ Class (SOC), MedDRA Preferred Terms (PT).

MedDRA Primary SOC *MedDRA PT*	Number of subject affected	All AE occurrences	AE occurrences causally related to everolimus and/or exemestane
Blood and lymphatic system disorders			
Anaemia	13	14	8
Hyperleukocytosis	1	1	
Neutropenia	1	1	1
Thrombocytopenia	4	4	2
Ear and labyrinth disorders			
Vertigo	1	1	
Endocrine disorders			
Diabetes insipidus	1	1	
Hypothyroidism	1	1	
Eye disorders			
Dry eye	1	1	
Ocular hyperaemia	1	1	
Gastrointestinal disorders			
Abdominal pain	1	1	
Abdominal pain upper	6	6	
Anal fissure	2	2	1
Anorectal discomfort	1	1	
Constipation	7	7	3
Diarrhoea	14	15	13
Dry mouth	1	1	1
Dyspepsia	2	2	2
Dysphagia	1	1	1
Gastritis	1	1	1
Gastroenteritis	1	1	
Gastrointestinal disorder	2	2	
Haemorrhoids	3	3	1
Nausea	7	8	5
Oesophagitis	2	2	2
Proctalgia	1	1	
Rectal haemorrhage	2	2	
Stomatitis	8	9	9

MedDRA Primary SOC *MedDRA PT*	Number of subject affected	All AE occurrences	AE occurrences causally related to everolimus and/or exemestane
Toothache	1	1	
Vomiting	3	4	2
General disorders and administration site conditions			
Asthenia	11	11	10
Chest pain	1	1	
Face oedema	1	1	
Fatigue	27	28	23
General physical health deterioration	1	1	1
Hyperthermia	1	1	1
Inflammation	1	1	1
Influenza like illness	1	1	1
Malaise	1	1	1
Mucosal inflammation	28	36	36
Oedema peripheral	6	7	3
Pain	3	3	1
Peripheral swelling	1	1	1
Pyrexia	5	5	2
Immune system disorders			
Drug hypersensitivity	1	2	
Infections and infestations			
Bartholin's abscess	1	1	
Conjunctivitis	3	3	2
Cystitis	2	3	2
Erysipelas	1	1	1
Folliculitis	3	3	2
Genital herpes simplex	1	1	
Gingivitis	1	2	2
Localised infection	1	1	1
Lung infection	1	1	
Infections and infestations			
Mastitis	1	1	
Nasopharyngitis	2	2	1
Pharyngitis	1	1	1
Pneumonia	2	2	2
Pneumonia haemophilus	1	1	
Respiratory tract infection	1	1	
Rhinitis	5	5	3

MedDRA Primary SOC *MedDRA PT*	Number of subject affected	All AE occurrences	AE occurrences causally related to everolimus and/or exemestane
Sinusitis	3	3	1
Tracheitis	1	1	
Urinary tract infection	5	5	2
Wound infection	1	1	
Injury, poisoning and procedural complications			
Fall	1	1	
Limb injury	1	1	
Skin injury	1	1	
Investigations			
Alanine aminotransferase increased	3	3	2
Aspartate aminotransferase increased	3	3	2
Blood creatinine increased	2	2	2
Transaminases increased	5	6	4
Weight decreased	6	6	6
Weight increased	1	1	1
Metabolism and nutrition disorders			
Decreased appetite	19	20	16
Diabetes mellitus	1	1	1
Dyslipidaemia	3	3	3
Hypercholesterolaemia	4	4	4
Hyperglycaemia	7	7	7
Hypertriglyceridaemia	1	2	2
Hypocalcaemia	2	2	1
Hypokalaemia	3	3	1
Hypomagnesaemia	1	1	
Hyponatraemia	1	1	1
Vitamin B complex deficiency	1	1	1
Musculoskeletal and connective tissue disorders			
Arthralgia	7	7	4
Back pain	5	5	
Bone pain	9	10	1
Myalgia	3	3	2
Osteonecrosis of jaw	1	1	
Pain in extremity	4	4	1
Pain in jaw	1	1	

MedDRA Primary SOC *MedDRA PT*	Number of subject affected	All AE occurrences	AE occurrences causally related to everolimus and/or exemestane
Nervous system disorders			
Ageusia	2	2	2
Carpal tunnel syndrome	1	1	
Dizziness	1	1	
Dysgeusia	3	4	4
Headache	10	10	1
Paraesthesia	1	1	
Peripheral sensory neuropathy	1	1	
Taste disorder	1	1	1
Psychiatric disorders			
Anxiety	2	2	
Insomnia	5	5	3
Sleep disorder	1	1	1
Renal and urinary disorders			
Acute kidney injury	1	1	
Pollakiuria	1	1	
Renal pain	1	1	1
Reproductive system and breast disorders			
Breast pain	2	2	
Vulvovaginal dryness	1	1	
Vulvovaginal inflammation	1	1	1
Respiratory, thoracic and mediastinal disorders			
Asthma	1	1	
Bronchospasm	1	1	
Cough	12	13	7
Dysphonia	2	2	1
Dyspnoea	6	8	7
Dyspnoea exertional	2	21	2
Epistaxis	6	6	4
Interstitial lung disease	1	1	1
Lung disorder	2	2	2
Nasal dryness	1	1	1
Nasal ulcer	1	1	1
Oropharyngeal pain	2	2	1
Pleural effusion	1	1	
Pneumonitis	2	2	2
Productive cough	1	1	

MedDRA Primary SOC *MedDRA PT*	Number of subject affected	All AE occurrences	AE occurrences causally related to everolimus and/or exemestane
Skin and subcutaneous tissue disorders			
Acne	2	2	2
Alopecia	2	2	2
Dry skin	8	8	8
Eczema	2	2	2
Erythema	2	2	2
Hyperhidrosis	1	1	1
Nail toxicity	1	1	1
Onychalgia	1	1	
Onychoclasis	1	1	1
Palmar-plantar erythrodysaesthesia syndrome	1	1	1
Photosensitivity reaction	1	1	1
Pruritus	8	8	8
Rash	17	21	21
Rash maculo-papular	2	2	1
Skin fissures	1	1	1
Skin lesion	1	1	1
Skin ulcer	1	1	1
Vascular disorders			
Haematoma	1	1	
Hot flush	3	3	1
Hypertension	3	3	1
Lymphoedema	8	8	5

5. ADDITIONAL INFORMATION

5.1. Global substantial protocol amendments

The global substantial amendments to the protocol are summarised in the below table.

Amendment date	Description
23/05/2014	Protocol v2.0 (14/04/14): • Clarification of haematology baseline assessments • Clarification of PET timelines • Change of efficacy assessment time point form every 8 weeks to 12 weeks to be in accordance with the reimbursement conditions for Afinitor in combination with exemestane.
15/05/2017	Protocol v3.0 (28/04/17): • Add Pilot phase results (Second FDG PET/CT timepoint selected for the main phase) • Main phase sample size's recalculation • Modification of schedule of assessment • Modification of samples collection and translational researches • Clarification inclusion/exclusion criteria
20/06/2017	Protocol v4.0 (19/05/17): • Modification of inclusion criteria
07/12/20	Protocol v5.0 (14/08/20): • Modification of the end of study definition • Modification of the secondary objective • Addition of a sample • Clarification regarding TR analyses

5.2. Global interruption(s) and restarts

The global interruption(s) to the trial are summarised in the below table.

Interruption date	Description	Restart date
30/06/2016	Last subject included in the pilot phase of the study.	22/05/2017

5.3. Limitations and caveats

There were no limitations and caveats applicable to this summary of the results.