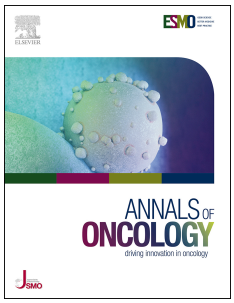

Early prediction of endocrine responsiveness in ER+/HER2-negative metastatic breast cancer (MBC): Pilot study with 18F-Fluoroestradiol (18F-FES) CT/PET

A. Gennari, E. Brain, A. De Censi, O. Nanni, R. Wuerstlein, A. Frassoldati, J. Cortes, V. Rossi, M. Palleschi, J.L. Alberini, F. Matteucci, A. Piccardo, G. Sacchetti, H. Ilhan, F. D'Avanzo, B. Ruffilli, S. Nardin, M. Monti, M. Puntoni, V. Fontana, L. Boni, N. Harbeck, on behalf of the ET-FES Collaborative Group

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Early prediction of endocrine responsiveness in ER+/HER2-negative metastatic breast cancer (MBC): Pilot study with 18F-Fluoroestradiol (18F-FES) CT/PET

Authors:
A Gennari[1,2], E Brain[3], A De Censi[4], O Nanni[5], R Wuerstlein[6], A Frassoldati[7], J Cortes[8,9], V Rossi[2], M Palleschi[10], JL Alberini[11], F Matteucci[12], A Piccardo[13], G Sacchetti[14], H Ilhan[15], F D'Avanzo[2], B Ruffilli[1], S Nardin[16], M Monti[5], M Puntoni[17], V Fontana[18], L Boni[18], N Harbeck[6] on behalf of the ET-FES Collaborative Group*

Institutions:
[1]Department of Traslational Medicine, University of Piemonte Orientale, Novara, Italy; [2]Division of Medical Oncology, Maggiore University Hospital, Novara, Italy
[3]Department of Medical Oncology, Institut Curie - Hôpital René Huguenin, Saint-Cloud, France
[4]Medical Oncology, E.O. "Ospedali Galliera, Genova, Italy
[5] Biostatistics and Clinical Trials Unit, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy
[6] Department of Obstetrics and Gynecology and CCC Munich, LMU University Hospital, Munich, Germany.
[7] Clinical Oncology, S. Anna University Hospital, Ferrara, Italy
[8]International Breast Cancer Center (IBCC), Pangaea Oncology, Quironsalud Group, Barcelona, Spain
[9]Faculty of Biomedical and Health Sciences, Department of Medicine, Universidad Europea de Madrid, Madrid, Spain
[10] Department of Medical Oncology, IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy
[11] Nuclear Medicine Department Centre Georges-Francois Leclerc, Dijon Cedex, France
[12] Nuclear Medicine Unit, IRCCS Istituto Romagnolo per lo studio dei tumori (IRST)- Dino Amadori, Meldola, Italy
[13] Department of Nuclear Medicine, E.O. "Ospedali Galliera, Genova, Italy
[14] Division of Nuclear Medicine Unit, Maggiore University Hospital, Novara, Italy
[15] Department of Nuclear Medicine, LMU University Hospital, Munich, Germany
[16] Medical Oncology Unit 1, IRCCS - Ospedale Policlinico San Martino, Genoa, Italy
[17]Clinical and Epidemiological Research Unit, University Hospital of Parma, Parma, Italy
[18] Department of Clinical Epidemiology, IRCSS Ospedale Policlinico San Martino, Genoa 20900, Italy.

* The individual names of the Collaborators are listed in the Appendix

Corresponding Author:
Prof Alessandra Gennari
Department of Traslational Medicine
University of Piemonte Orientale
Via P Solaroli, 17
28100 Novara, Italy
Email: alessandra.gennari@uniupo.it
Phone: +39 03213733822

ORCID: 0000-0002-0928-2281

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HIGHLIGHTS

- 18F-FES PET/CT may be used as a predictive tool of efficacy of ET to assess overall endocrine sensitivity.
- Endocrine sensitive patients (SUVmax≥2) treated with single agent ET have a prolonged overall survival.
- In endocrine sensitive patients PFS and OS related to the use of AI was significantly higher than ER directed agents.
- 18F-FES PET/CT can be used as a valid alternative to biopsy.

ABSTRACT

Background: 18F-FES PET/CT is considered an accurate diagnostic tool to determine whole-body endocrine responsiveness. In the ET-FES trial, we evaluated 18F-FES PET/CT as a predictive tool in ER+/HER2- metastatic breast cancer (MBC).

Methods: Eligible patients underwent a 18F-FES PET/CT at baseline. Patients with SUV≥2 received single agent ET until PD; patients with SUV<2 were randomized to single agent ET (Arm A) or chemotherapy (CT) (Arm B). Primary objective was to compare the activity of first line ET versus CT in patients with 18F-FES SUV <2.

Results: Overall, 147 patients were enrolled; 117 presented with 18F-FES SUV≥2 and received ET; 30 pts with SUV<2 were randomized to ET or CT. After a median follow up of 62.4 months, 104 patients (73.2%) had disease progression and 53 died (37.3%).

Median PFS was 12.4 months (95%CI 3.1-59.6) in patients with SUV <2 randomised to Arm A versus 23.0 months (95%CI 7.7-30.0) in Arm B, (HR = 0.71, 95%CI 0.3 - 1.7); median PFS was 18.0 months (95%CI 11.2-23.1) in patients with SUV≥2 treated with ET.

Median OS was 28.2 months (95%CI 14.2-NE) in patients with SUV <2 randomized to ET (Arm A) versus 52.8 months (95%CI 16.2-NE) in Arm B (CT). Median OS was not reached in patients with SUV≥2.

60-month OS rate was 41.6% (95%CI 10.4–71.1%) in Arm A, 42.0% (95%CI 14.0–68.2%) in Arm B and 59.6% (95%CI 48.6–69.0%) in patients with SUV≥2.

In patients with SUV≥2, 60-months OS rate was 72.6% if treated with aromatase inhibitors versus 40.6% in case of fulvestrant or tamoxifen (p<0.005).

Conclusions: The ET-FES trial demonstrated that ER+/HER2- MBC patients are a heterogeneous population, with different levels of endocrine responsiveness based on 18F-FES CT/PET SUV.

KEYWORDS

Endocrine sensitivity; Molecular Imaging; Randomized Clinical Trial; 18F-Fluoroestradiol PET/CT; Standardized Uptake Value (SUV)

BACKGROUND

Assessment of endocrine responsiveness of breast tumors is based on the positivity of expression of hormonal receptors (ER) in tumor samples at primary diagnosis. However, although 70% of breast cancers are apparently endocrine responsive, as defined by the expression of ER at the time of diagnosis, response to endocrine therapy (ET) is not homogeneous and may be altered by subsequent therapies. In the metastatic setting, overall response rates to single agent ET are in the range of 40 to 50%[1,2]; moreover, up to 30% of metastatic lesions completely or partially loose expression of ER[3]. Endocrine resistance may be attributable to several factors[3], including inadequate predictive ability of ER status, as currently assessed, tumor heterogeneity at the level of different metastatic lesions, and technical problems such as decalcification protocols in bone tissue; besides, it is almost impossible to biopsy all metastatic lesions in a patient.

Introduction of CDK 4/6 inhibitors in association with ET, has dramatically changed the treatment algorithm in ER positive MBC[4,5]; however, even in this case, it can be estimated that up to 30% of ER positive MBC patients will experience early treatment failure. Early identification of endocrine resistant patients could improve treatment options and tailor strategies, especially considering the most recent options in terms of endocrine-directed agents[4,6–8] and the availability of innovative target-directed drugs as well as new immune-conjugates[9–13].

The most recent ESMO guidelines recommend, whenever feasible, to perform a biopsy of metastatic lesions to confirm histology and evaluate ER and HER2 expression[4]. It also may be considered to re-biopsy a metastatic site after every progression to re-assess tumor biology. However, biopsy of a metastatic lesion may not be feasible or may have technical issues, and finally a single biopsy may not be representative of tumor heterogeneity.

Molecular imaging approaches, such as [^{18}F]-fluoro-estradiol Positron Emission Tomography (^{18}F-FES PET/CT) have been proposed as whole body imaging to assess overall ER expression at the different metastatic sites[14]. Available evidence, from retrospective experiences have suggested a correlation between ^{18}F-FES PET/CT uptake and the presence and the performance of ER in BC tissues and has been proposed as a predictive marker of endocrine sensitivity in patients treated with ET[15–22].

In this perspective, our primary research hypothesis is that the level of ^{18}F-FES uptake (SUV - Standard Uptake Value) at CT/PET scan may discriminate between endocrine sensitive disease with a high probability of achieving a response to ET and endocrine resistant tumors.

With these premises, we designed the ET-FES trial. Its primary aim was to prospectively assess the ability of ^{18}F-FES PET/CT to identify endocrine responsive patients, by means of a pivotal, phase II clinical trial.

METHODS

ET-FES is a prospective, pivotal, phase II randomised trial, conducted within the JTC-2011 ERA-NET TRANSCAN program, in 4 countries and 7 centers. Its primary aim was to evaluate if addition of ^{18}F-FES CT/PET to the standard diagnostic procedures allows tailored therapy in ER+/HER2- MBC, leading to an improved disease control.

The trial was conducted in accordance with the Good Clinical Practice Guidelines and approved by local Ethical Committees at each clinical site. Written informed consent was obtained from all the patients. EudraCT Number 2013-000287-29.

Study design:

Patients with ER+/HER2- MBC candidate to first line ET were evaluated with a ^{18}F-FES CT/PET scan at baseline, prior to start of treatment, in addition to standard diagnostic/staging procedures for disease assessment. ER and HER2 status was assessed on the primary tumor and/or metastatic site, when feasible. Patients with ^{18}F-FES SUV $\geq$ 2 were considered endocrine sensitive, according to prior exploratory studies[1–3] and treated with ET as clinically indicated, in accordance with current guidelines. Patients with a low ^{18}F-FES uptake (SUV < 2) were randomized to single agent ET until disease progression (Arm A) or single agent CT (Arm B). The choice of type of ET and CT was left

to the clinical judgment of the treating physician according to local clinical practice. In November 2016, with the increasing use of mTOR and CDK 4/6 inhibitors in the treatment of ER+/HER2- MBC, the study protocol was amended (Version 2, 03/11/2016) to include the possibility to use CDK 4/6 inhibitors or other biological agents in Arm B (first line CT or first line ET plus biological agents).

Patients

Pre- and postmenopausal women ER+/HER2- MBC, who had not received previous systemic therapy for advanced disease, were eligible. Patients had either measurable or non-measurable, but evaluable disease, according to Response Evaluation Criteria in Solid Tumors [RECIST], version 1.1[23], an ECOG PS < 2, and life expectancy > 3 months. Prior adjuvant or neo-adjuvant CT and ET were allowed. Patients progressing either during or after adjuvant endocrine therapy were eligible.

Endpoints

Primary objective of this study was to compare the activity of first line ET versus first line CT in MBC patients with ER+/HER2- MBC and ^{18}F-FES SUV <2 at baseline CT/PET scan.

- Primary endpoint: Disease Control Rate (DCR), as defined by the proportion of patients who did not experience disease progression within 3 months of treatment.
- Secondary objectives were: To evaluate DCR with ET in patients with ^{18}F-FES SUV $\geq$ 2; To compare the DCR with ET observed in patients with ^{18}F-FES SUV $\geq$ 2 with that of patients with ^{18}F-FES SUV < 2; To correlate ER expression in the primary tumor and overall ^{18}F-FES-uptake in metastases; To assess Overall Survival (OS) in all patients and by ^{18}F-FES SUV value.

Due to the low number of patients experiencing disease progression or death at 3 months, Progression Free survival (PFS) and Overall Survival (OS) were considered the main outcome measures.

Study procedures

All eligible patients underwent a whole-body ^{18}F-FES PET/CT scan at baseline (before the start of treatment), in addition to conventional diagnostic and staging procedures as clinically indicated. For the ^{18}F-FES PET/CT study, the radiotracer was produced and supplied to the diffent sites by Advanced Accelerator Applications, radiopharmaceutical company – St Genis Pouilly, France. Approximately 200 MBq of tracer were injected via the antecubital vein. To optimize the timing of the imaging, ^{18}F-FES PET/CT and contrast enhanced CT were acquired at the same time using the same Hybrid PET/CT scanner. Fifty minutes after the tracer injection, patients were positioned supine in the PET scanner, and 3-min scans for bed position were acquired in 3D mode (5-6 bed positions) to completely cover the head to mid-thighs.

PET/CT image analysis: For semi-quantitative analysis, SUV is a commonly used PET parameter to measure the uptake of various radiopharmaceuticals; because of metabolic heterogeneity the hottest voxel value (SUVmax) within the region of interest (ROI) was used. Circular ROI with a fixed size in diameter (10 mm) were drawn on the lesions to obtain the local SUV max. Maximum SUVmax measured in the 3 largest lesions evaluated on CT, was used to dichotomize results (endocrine sensitive vs resistant): in semi-quantitative measurement of regional ER binding, a threshold of ^{18}F-FES SUVmax $\geq$ 2 was used as an indicator of endocrine responsiveness[24,25].

The activity concentration was normalized to the injected dose of PET tracer and body weight. The ratio between the maximal uptake in the pathological regions and the reference region (mediastinal blood pool) was recorded for each scan. This parameter, basically independent on technical characteristics of every single PET/CT scanner, was used as a standard of reference for SUV max. SUV is a semiquantitative value that can be influenced by several biological factors, such as the patient's serum glucose, necrosis, infection, medication, the time between tracer injection and image acquisition and reconstruction protocol. However, these items often lead to a variation of 10-20% in SUV, which can be considered insignificant in cancers. To verify the reproducibility of SUV max measurements and to minimize variation in SUV value, a periodic case review across participating centers was carried out.

PET/CT dosimetry: With a scheduled injected activity of 3 MBq/kg of body weight, which was 200 MBq for a typical female body weight, the effective dose was 4.4 mSv, to which should be added the radiation dose from CT scan, which was variable according to the examined field and the acquisition parameters. Overall, the effective dose did not exceed 10 mSv, which was less than the irradiation during a diagnostic contrast enhanced CT scan of the torso and was perfectly admissible in the indication of MBC.

Statistical considerations

Sample size: A total of 220 patients with ER+/HER2- MBC were planned. In the light of previous data showing a 50% overall response rate to ET among all ER+ MBC patients, it was expected that in patients with a low ^{18}F-FES CT/PET SUV, the proportion of patients with response or stable disease at 3 months could be in the range of 30%. Since ^{18}F-FES PET/CT SUV variable has been previously described (8) to be normally distributed with a mean/median value nearly equal to 2, it was expected that approximately 50% of the patients (n=110) were classified as endocrine resistant, with a ^{18}F-FES SUV < 2, computed as the mean of values for up to the three largest tumor sites in the whole body acquisition for each patient, and randomized to first line single agent ET (control arm - A) or CT (experimental arm - B).

With a total 110 endocrine resistant patients randomized (55 per arm), the study had an 85% power to detect an absolute 20% difference in DCR between arms (i.e. 50% in the CT arm vs. 30% in the ET arm) after 3 months of therapy, assuming a 5% two-sided alpha level and a 10% drop-out rate. Sample size calculation was estimated using a Fisher Exact test to compare Disease Control Rate in the 2 treatment arms.

The trial was prematurely closed due to the COVID-19 pandemic; production and delivery of ^{18}F-FES from the manufacturing site of production (Advanced Accelerator Applications, radiopharmaceutical company – St Genis Pouilly, France) was stopped as of December 2020, after an overall enrollment of 147 patients.

Statistical analysis: The distributions of all studied patients were reported with respect to their demographic, clinical, and biologic characteristics and were summarized as frequencies and percentage. Continuous variables were reported as median and range of variation. All primary and secondary efficacy analyses were done on an intention-to-treat basis. The median period of follow-up was calculated for the entire study cohort according to the reverse Kaplan-Meier method. Distributions of time-to-event variables for both progression-free and overall survival were estimated with the Kaplan-Meier product-limit method, and compared with the log-rank test. Ninety-five percent confidence intervals of progression-free survival and overall survival rates were calculated according to the log-log approach. Hazard ratios (HRs) estimates, and appropriate 95% CIs, were obtained by means of the Cox proportional hazard model. All statistical tests were two-sided, and p values of 0.05 or less were deemed significant. No adjustments for multiple comparisons were made. Statistical analyses were done using SAS version 9.4.

RESULTS

Patient Characteristics

From April 25, 2015 to December 20, 2020, a total of 147 patients were enrolled and underwent a ^{18}F-FES CT/PET at baseline. Of these, 117 (79.6%) presented with a mean 18F-FES SUV ≥ 2; 4 of them were not included in the ITT analysis due to ineligibility (3 patients) and consent withdraw (1 patient). Overall, 113 patients, with a mean ^{18}F-FES SUV ≥ 2 were considered for the ITT population. 30 patients (20.4%), presented with a mean 18F-FES SUV < 2 and were randomized to ET (Arm A - 14 patients) or CT/ET + biological agents (Arm B - 16 patients); one of them (Arm A) was not considered for the ITT analysis due to ineligibility. Overall, in the randomized study, 28 patients were available for safety analysis (Figure 1).

The characteristics of the 142 patients included in the ITT analysis are reported in Table 1. Median age was 65 years (range 36 to 90); 21 patients (14.8%) were pre-/peri-menopausal; 113 (79.6%) patients had an ECOG PS = 0; 31 patients (21.8%) had primary metastatic disease. The disease-free

interval (DFI) at baseline was longer than 24 months in 98 patients (69.0%). Visceral disease was reported in 49 patients (34.5%), and 50 (35.2%) had bone-only metastases.

Treatment

At time of analysis, single agent ET is still ongoing in 39 patients with SUV ≥ 2 (35.5%) and in 6 patients (35.3%) in ARM A (SUV < 2). Median duration of single agent ET in patients with SUV ≥ 2 (110 registered, available for safety analysis) was 16.8 months (range 0.1 - 93.9 months) versus 13.2 months (range 0.9 – 68.4 months) in patients with SUV < 2 (15 randomized Arm A). In patients with SUV≥ 2, the most common reason for treatment interruption was disease progression or death in 70 patients, 1 patient refused treatment, and single agent ET is ongoing in 39 patients. In Arm B, 11 patients (81.0%) received first line CT according to local clinical practice, 2 patients, randomized after protocol amendment received ET + biological agents, and 3 patients refused the assigned treatment. Median duration of treatment was 8.13 months (range 2.1 - 46.8 months). Treatment was interrupted due to disease progression in 5 patients and in 5 patients due to toxicity; 1 patient treated with ET + biological agent is still on treatment.

Efficacy

Median duration of follow up was 62.4 months (IQR 36.2 - 68.4 months). Overall, at the cut-off date of 31 December 2022, 104 patients (73.2%) had disease progression and 53 died (37.3%).
Median PFS was 18.0 months (95%CI 11.2 - 23.1) in patients with 18F-FES SUV ≥ 2, treated with single agent ET. In patients with SUV < 2 randomised to ET (Arm A), median PFS was 12.4 months (95%CI 3.1 - 59.6) versus 23.0 months (95%CI 7.7 - 30.0) in those treated with CT (Arm B), (HR = 0.71, 95%CI 0.3 - 1.7). Kaplan-Meier curves of PFS are reported in Figure 2.

At 24 months, the PFS rate was 40.2% (95%CI 31.1 – 49.2) in patients with 18F-FES SUV≥2, 33.3% (95% CI 10.3 – 58.8) in Arm A and 48.6% (95%CI 21.9 – 70.3) in Arm B. Median OS was not reached in patients with SUV ≥ 2 treated with single agent ET and 28.2 months (95%CI 14.2 - NE) in patients with SUV < 2 randomised to ET (Arm A) versus 52.8 months (95%CI 16.2 - NE) in Arm B (CT); (HR 0.97, 95%CI 0.3 – 3.1).
At 60 months, the OS rate was 59.6% in patients with 18F-FES SUV ≥ 2 (95%CI 48.6 – 69.0%), versus 41.6% in Arm A (95%CI 10.4 – 71.1%) and 42.0% in Arm B (95%CI 14.0 – 68.2%). Kaplan-Meier curves of OS are reported in Figure 3.
Among the 110 pts with SUV ≥ 2 treated with ET, 61 (55.5%) received an aromatase inhibitor (AI) and 49 (44.5%) fulvestrant or tamoxifen. Median PFS was 24.4 months (95%CI 14.9 – 31.6) in patients treated with AI versus 11.0 months (95%CI 5.6 – 18.0) in patients treated with fulvestrant or tamoxifen. At 12 months, the PFS rate was 67.1% in patients treated with an AI (95%CI 53.8 – 77.4%) and 45.8% with fulvestrant or tamoxifen (95%CI, 31.4 – 59.1%); at 24 months, the PFS rate was 50.3% (95%CI 37.2 – 62.1%) and 27.1% (95%CI 15.5 – 40.0%), respectively (HR 0.6; 95%CI 0.4 – 0. 9 log-rank test p = 0.026;). Figure 4.
The Kaplan-Meier estimates of OS at 60 months was 72.6% (95%CI 58.5 – 82.6%) if treated with aromatase inhibitors versus 40.6% in case of tamoxifen or fulvestrant (95%CI 24.5 – 56.1%) (HR 0.5; 95% CI 0.2– 0.9 log-rank test p = 0.011). Figure 5.

DISCUSSION

The prospective multicenter ET-FES trial shows that the introduction of 18F-FES CT/PET in the baseline diagnostic workout of ER+/HER2– MBC, allows to identify a subset of patients, classified as endocrine resistant based on a mean SUV max < 2, where the upfront administration of first line CT resulted into an improved outcome compared to first line ET. This benefit was observed in a randomized comparison of first line ET vs. CT in patients evaluated for whole body ER expression at different metastatic sites. However, the small number of patients enrolled does not allow to draw a definite conclusion.

Conversely, in patients selected for endocrine sensitivity based on a mean SUVmax $\geq$ 2, who received first line single agent ET, we observed an exceptionally long overall survival, with 60% of them alive at 5 years. This survival rate is in line with that observed in the MONALEESA-2 trial of first line letrozole plus ribociclib, in a similar patient population with endocrine sensitive disease. However, in the ET-FES trial, this survival rate was achieved by endocrine monotherapy in a patient population selected for overall endocrine responsiveness at the different metastatic sites. According to the results of MONALEESA-2 trial, an even better outcome could be expected for the association of ET and ribociclib in ER+/HER2 – MBC patients selected for endocrine sensitivity by 18F-FES CT/PET. Yet, we cannot exclude that in patients selected for endocrine resistance based on a 18F-FES SUV < 2, addition of a CDK 4/6 inhibitor to first line ET may improve the efficacy of ET.

Our results are in line with recent evidence supporting the predictive role of 18F-FES PET/CT based on SUV value[26–29].

As compared to tissue biopsy on the primary tumor or metastatic lesions, ER assessment through molecular imaging has some advantages. The first one is the non invasivity of the procedure, which can be repeated at different time points in the clinical management. The second and the most important, is the ability to assess ER distribution and viability at the different metastatic sites at the same time. As a consequence, 18F-FES PET/CT may well be considered as a complementary exam to support treatment choice allowing heterogeneity assessment. In this perspective, data from a review by Boers et al., indicate that 18F-FES PET/CT may have an impact on therapeutic decision in 50% of MBC patients with ER+/HER2- disease, compared with conventional diagnostics[26].

An intriguing finding from the ET-FES trial is different response to single agent AI as compared to selective ER modulators (SERM) such as tamoxifen or selective ER downregulators (SERD) like fulvestrant. In particular, the efficacy in terms of PFS and OS related to the use of AI in patients with endocrine sensitive disease, with a mean 18F-FES SUV $\geq$ 2, was significantly higher than fulvestrant or tamoxifen. 18F-FES SUV is considered a biomarker of ER availability at the different metastatic sites, and it has been identified as an interesting tool for response prediction of SERMs/SERDs[4,6,8,15,17], even if sound evidence has not been presented so far. Available evidence is in fact based on limited patient series; moreover, studies were mainly focused on the proof of concept of ER downregulation rather than on treatment effectiveness by 18F-FES SUV value[30]. Conversely, AI have been shown to have a limited impact on ER binding ability as measured by 18F-FES CT/PET. It must be considered that in our study we selected a population of "true" endocrine sensitive patients, based on molecular imaging and other clinical characteristics (first line, long DFI). In this subset of ER positive MBC patients, it may be reasonably assumed that ligand-depleting agents retain a more global effect by lowering estrogen levels both in the plasma and at the tumor site. On the other side, in the ET-FES trial, the choice of ET was left to the treating physician and was possibly influenced by the type of prior ET.

Our trial has several limitations but also strengths. The slow and overall low accrual rate of the ET-FES trial was in part due technical difficulties in the activation of an international, multicenter, academic clinical trial involving the use of an investigational radiotracer[31] in different EU countries; this issue has been previously analyzed[31]. Furthermore, the observed percentage of patients with a mean 18F-FES SUVmax < 2 (i.e. 20.4%) differed substantially from the expected 50% according to available evidence at the time of study planning[25]; nevertheless, a similar cut-off was also chosen in a very recent publication[32]. A major strength is that ET-FES is the first international, multicenter, prospective trial to evaluate the role of 18F-FES CT/PET as a tool to identify endocrine sensitive and endocrine resistant patients, selected by 18F-FES SUV. Furthermore, in this study, patients classified as endocrine resistant based on a mean SUVmax <2, were randomised to ET or first line CT. The 18F-FES radiotracer was produced by the same company (AAA – St Genis Pouilly, FR) for all clinical centers, assuring a homogeneous quality and identical ER binding capacity. Moreover, technical criteria for scan evaluations were prospectively defined by the nuclear medicine physicians involved in the trial.

Finally, some practical aspects need to be discussed, in the light of a possible introduction of 18F-FES PET/CT in everyday clinical practice. These include the high cost of the radiotracer and its availability as well as logistic issues in single institutions. Furthermore, the analysis of 18F-FES PET/CT should be homogeneously shared among the different nuclear medicine facilities, and feasible, as in the case of 18F-FDG PET/CT.

The results of the ET-FES trial should be considered exploratory due to the fact that its primary endpoint was not reached; yet, they support and confirm previous evidence on the ability of this type of molecular imaging in selecting patients with a high probability to achieve a response to ET.

In conclusion, the ET-FES trial has demonstrated for the first time that the population of ER+/HER2 neg MBC patients can be divided at first evidence of relapse in two groups according to overall endocrine sensitivity as measured by 18F-FES CT/PET SUV at the different metastatic sites. This observation is of particular importance today, given the availability of innovative drugs with a significant improvement in OS in luminal-like MBC. Moreover, after the results of the RIGHT Choice trial[33], showing no benefit for CT versus ET plus ribociclib in patients with aggressive visceral disease, a better selection of endocrine resistant patients could be explored, by the introduction of 18F-FES assay. On the other hand, the early identification of true endocrine sensitive patients may prompt an earlier use of non-endocrine directed therapies such as immunoconjugates, trastuzumab-deruxtecan, and other novel agents in patients with a low probability to achieve a response to ET-directed agents. Such a selective use of ET including also biological agents such as SERDs, CDK 4/6-, AKT- and PI3K inhibitors may have a substantially impact on outcome in luminal MBC.

FIGURE LEGENDS

- Table 1: Legend: ER = Estrogen Receptor; DFI = Disease Free Interval; NA = Not Applicable; *Metastatic ab initio; CT = Chemotherapy; ET = Endocrine Therapy.
- Figure 1: Consort Flow Diagram of ET-FES trial
- Figure 2: Kaplan-Meier curves of PFS for patients with SUV≥2 and patients with SUV<2 (Arm A, ET and Arm B, CT/ET+biological agents). CI, confidence interval; PFS, progression-free survival.
- Figure 3: Kaplan-Meier curves of OS for patients with SUV≥2 and patients with SUV<2 (Arm A and Arm B). CI, confidence interval; PFS, progression-free survival; HR, hazard ratio.
- Figure 4 Kaplan-Meier curves of PFS for aromatase inhibitors versus tamoxifene/fulvestrant. CI, confidence interval; PFS, progression-free survival, HR, harzard ratio; p, p value.
- Figure 5 Kaplan-Meier curves of OS for aromatase inhibitors versus tamoxifene/fulvestrant. CI, confidence interval; PFS, progression-free survival, HR, harzard ratio; p, p value .

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DISCLOSURES
The authors report no Conflict of Interest

APPENDIX

COLLABORATORS: ET-FES Collaborative Group*

Bianca Malagutti, Bassam Dib, Carmen Branni, Maggiore University Hospital, Novara, Italy; Mauro D'Amico, Nicoletta Provinciali, Davide Corradengo, Francesco Fiz, Massimiliano Iacozzi, E.O. "Ospedali Galliera, Genova, Italy; Andrea Rocca, University of Trieste, Italy; Tom Degenhardt, Department of Obstetrics and Gynecology and CCC Munich, LMU University Hospital, Munich, Germany; Patrick Vavasseur, Department of Medical Oncology, Institut Curie - Hôpital René Huguenin, Saint-Cloud, France.

REFERENCES

1. Robertson, J. F. R. *et al.* Health-related quality of life from the FALCON phase III randomised trial of fulvestrant 500 mg versus anastrozole for hormone receptor-positive advanced breast cancer. *Eur J Cancer* **94**, 206–215 (2018).
2. Robertson, J. F. R. *et al.* Fulvestrant 500 mg versus anastrozole 1 mg for hormone receptor-positive advanced breast cancer (FALCON): an international, randomised, double-blind, phase 3 trial. *The Lancet* **388**, 2997–3005 (2016).
3. Saatci, O., Huynh-Dam, K. T. & Sahin, O. Endocrine resistance in breast cancer: from molecular mechanisms to therapeutic strategies. *Journal of Molecular Medicine* vol. 99 1691–1710 Preprint at https://doi.org/10.1007/s00109-021-02136-5 (2021).
4. Gennari, A. *et al.* ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer ☆. *Annals of Oncology* **32**, 1475–1495 (2021).
5. D Johnston, S. R. *et al.* Abemaciclib Combined With Endocrine Therapy for the Adjuvant Treatment of HR1, HER22, Node-Positive, High-Risk, Early Breast Cancer (monarchE). *J Clin Oncol* **38**, 3987–3998 (2020).
6. Shiino, S. *et al.* Prognostic Impact of Discordance in Hormone Receptor Status Between Primary and Recurrent Sites in Patients With Recurrent Breast Cancer. *Clin Breast Cancer* **16**, e133–e140 (2016).
7. Metcalfe, C. & Lauchle, J. O. Clinical Translation: Targeting the Estrogen Receptor. in 297–309 (2022). doi:10.1007/978-3-031-11836-4_17.
8. Downton, T., Zhou, F., Segara, D., Jeselsohn, R. & Lim, E. Oral Selective Estrogen Receptor Degraders (SERDs) in Breast Cancer: Advances, Challenges, and Current Status. *Drug Design, Development and Therapy* vol. 16 2933–2948 Preprint at https://doi.org/10.2147/DDDT.S380925 (2022).
9. André, F. *et al.* Alpelisib for PIK3CA -Mutated, Hormone Receptor–Positive Advanced Breast Cancer . *New England Journal of Medicine* **380**, 1929–1940 (2019).
10. Litton, J. K. *et al.* Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation . *New England Journal of Medicine* **379**, 753–763 (2018).
11. Robson, M. *et al.* Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation . *New England Journal of Medicine* **377**, 523–533 (2017).
12. Rugo, H. S. *et al.* Sacituzumab Govitecan in Hormone Receptor-Positive/Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer. *J Clin Oncol* **40**, 3365–3376 (2022).
13. Modi, S. *et al.* Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. *New England Journal of Medicine* **387**, 9–20 (2022).
14. Kurland, B. F. *et al.* Whole-Body Characterization of Estrogen Receptor Status in Metastatic Breast Cancer with 16α-18F-Fluoro-17β-Estradiol Positron Emission Tomography: Meta-Analysis and Recommendations for Integration into Clinical Applications. *Oncologist* **25**, 835–844 (2020).
15. van Kruchten, M. *et al.* Positron emission tomography of tumour [18F]fluoroestradiol uptake in patients with acquired hormone-resistant metastatic breast cancer prior to oestradiol therapy. *Eur J Nucl Med Mol Imaging* **42**, 1674–1681 (2015).
16. Peterson, L. M. *et al.* A phase 2 study of 16α-[18F]-fluoro-17β-estradiol positron emission tomography (FES-PET) as a marker of hormone sensitivity in metastatic breast cancer (MBC). *Mol Imaging Biol* **16**, 431–440 (2014).
17. Chae, S. Y. *et al.* Diagnostic accuracy and safety of 16α-[18 F]fluoro-17β-oestradiol PET-CT for the assessment of oestrogen receptor status in recurrent or metastatic lesions in patients with breast cancer: a prospective cohort study. *Lancet Oncol* **20**, 546–555 (2019).

18. Jager, A. *et al.* A phase 1b study evaluating the effect of elacestrant treatment on estrogen receptor availability and estradiol binding to the estrogen receptor in metastatic breast cancer lesions using 18F-FES PET/CT imaging. *Breast Cancer Research* **22**, (2020).
19. Boers, J. *et al.* Molecular imaging to identify patients with metastatic breast cancer who benefit from endocrine treatment combined with cyclin-dependent kinase inhibition. *Eur J Cancer* **126**, 11–20 (2020).
20. Liu, C. *et al.* Evaluation of tumour heterogeneity by 18F-fluoroestradiol PET as a predictive measure in breast cancer patients receiving palbociclib combined with endocrine treatment. *Breast Cancer Research* **24**, (2022).
21. He, M. *et al.* The Predictive Value of Early Changes in 18F-Fluoroestradiol Positron Emission Tomography/Computed Tomography During Fulvestrant 500 mg Therapy in Patients with Estrogen Receptor-Positive Metastatic Breast Cancer. *Oncologist* **25**, 927–936 (2020).
22. Bardia, A. *et al.* AMEERA-1 phase 1/2 study of amcenestrant, SAR439859, in postmenopausal women with ER-positive/HER2-negative advanced breast cancer. *Nat Commun* **13**, (2022).
23. Eisenhauer, E. A. *et al.* New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* **45**, 228–247 (2009).
24. Dehdashti, F. *et al.* PET-based estradiol challenge as a predictive biomarker of response to endocrine therapy in women with estrogen-receptor-positive breast cancer. *Breast Cancer Res Treat* **113**, 509–517 (2009).
25. Linden, H. M. *et al.* Quantitative fluoroestradiol positron emission tomography imaging predicts response to endocrine treatment in breast cancer. *Journal of Clinical Oncology* **24**, 2793–2799 (2006).
26. Boers, J., de Vries, E. F. J., Glaudemans, A. W. J. M., Hospers, G. A. P. & Schröder, C. P. Application of PET Tracers in Molecular Imaging for Breast Cancer. *Current Oncology Reports* vol. 22 Preprint at https://doi.org/10.1007/s11912-020-00940-9 (2020).
27. Van Geel, J. J. L. *et al. Clinical Validity of 16a-[18 F] Fluoro-17b-Estradiol Positron Emission Tomography/Computed Tomography to Assess Estrogen Receptor Status in Newly Diagnosed Metastatic Breast Cancer*. *J Clin Oncol* vol. 40 https://doi.org/10. (2022).
28. van Kruchten, M. *et al. Review PET imaging of oestrogen receptors in patients with breast cancer*. *www.thelancet.com/oncology* www.thelancet.com/oncology (2013).
29. Ulaner, G. A. *et al.* Summary: Appropriate Use Criteria for Estrogen Receptor–Targeted PET Imaging with 16a-18F-Fluoro-17b-Fluoroestradiol. *Journal of Nuclear Medicine* **64**, 351–354 (2023).
30. Linden, H. M. *et al.* Fluoroestradiol positron emission tomography reveals differences in pharmacodynamics of aromatase inhibitors, tamoxifen, and fulvestrant in patients with metastatic breast cancer. *Clinical Cancer Research* **17**, 4799–4805 (2011).
31. Monti, M. *et al.* ERANET JTC 2011: Submission and Activation of an International Academic Translational Project in Advanced Breast Cancer. Experience From the ET-FES Study. *Front Med (Lausanne)* **8**, (2022).
32. Iqbal, R. *et al.* [18F]FDG and [18F]FES PET/CT Imaging as a Biomarker for Therapy Effect in Patients with Metastatic ER+ Breast Cancer Undergoing Treatment with Rintodestrant. *Clinical Cancer Research* OF1–OF10 (2023) doi:10.1158/1078-0432.ccr-22-2720.
33. Lynda Williams, S. medwireNews R. RIGHT Choice Results: Ribociclib–Endocrine Therapy Offers Alternative To Combination Chemotherapy. (2022).

Table 1. Characteristics of the patients at baseline

Patients characteristics	Registered (n=113) n - %	ARM A (n=13) n - %	ARM B (n=16) n - %	Total (n=142) n - %
Median age (range) – yrs	66 (36-90)	60 (38-79)	62 (38-87)	65 (36-90)
Menopausal status				
Pre/peri-menopausal	14 (12.4)	2 (15.4)	5 (31.3)	21 (14.8)
Post-menopausal	99 (86.6)	11 (84.6)	11 (68.7)	121 (85.2)
ECOG Performance Status				
0	89 (77.9)	10 (76.9)	14 (87.5)	113 (79.6)
1	24 (22.1)	3 (23.1)	2 (12.5)	29 (20.4)
Histology				
Ductal	84 (74.3)	12 (92.3)	11 (68.8)	107 (75.4)
Lobular	19 (16.8)	1 (7.7)	4 (25.0)	24 (16.9)
Other	10 (8.8)	-	1 (6.2)	11 (7.7)
Hormone Receptor Status	113 (100.0)	13 (100.0)	16 (100.0)	142 (100.0)
Positive (> 1%)	100 (88.5)	13(100.0)	15 (93.7)	128 (90.1)
ER >50%	10 (8.9)	-	1 (6.3)	11 (7.8)
ER ≤ 50%	3 (2.6)	-	-	3 (2.1)
Missing				
Disease-Free Interval				
DFI ≤ 24 mos	11 (9.7)	1 (7.7)	1 (6.3)	13 (9.2)
DFI > 24 mos	75 (66.4)	9 (69.2)	14 (87.5)	98 (69.0)
NA*	27 (23.9)	3 (23.1)	1 (6.2)	31 (21.8)
Median (range) - mos	98.8 (0.3-360.3)	64.9 (4.2-196.3)	141.6 (2.8-272.3)	89.0 (0.3-360.3)
Metastatic ab initio	27 (23.9)	3 (23.1)	1 (6.2)	31 (21.8)
Prior Treatment				
Prior Neo/Adiuvant CT	68 (60.2)	9 (69.2)	11 (68.8)	88 (62.0)
Prior Adjuvant ET	78 (69.0)	8 (61.5)	13 (81.3)	99 (69.7)
Site of metastases				
Bone Only	41 (36.3)	4 (30.8)	5 (31.3)	50 (35.2)
Bone + Other	31 (27.4)	3 (23.1)	-	34 (23.9)
Visceral Any	38 (33.6)	5 (38.5)	6 (37.5)	49 (34.5)
Soft Tissue Any	37 (32.7)	5 (38.5)	6 (37.5)	48 (33.8)
Other	8 (7.1)	1 (7.7)	1 (6.3)	10 (7.0)

Legend: ER = Estrogen Receptor; DFI = Disease Free Interval; NA = Not Applicable; *Metastatic ab initio; CT = Chemotherapy; ET = Endocrine Therapy.

Figure 1: Consort Flow Diagram of ET-FES trial

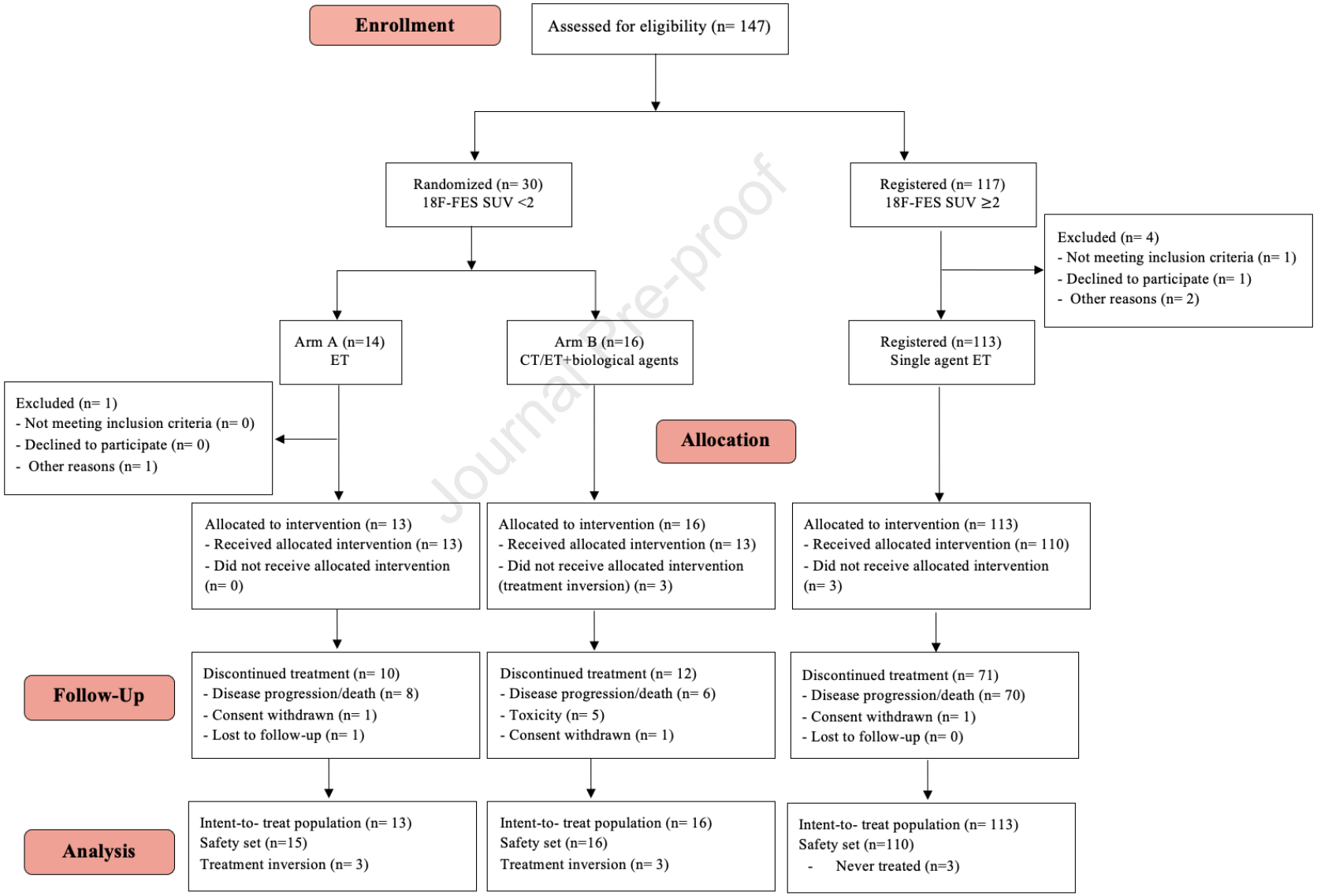
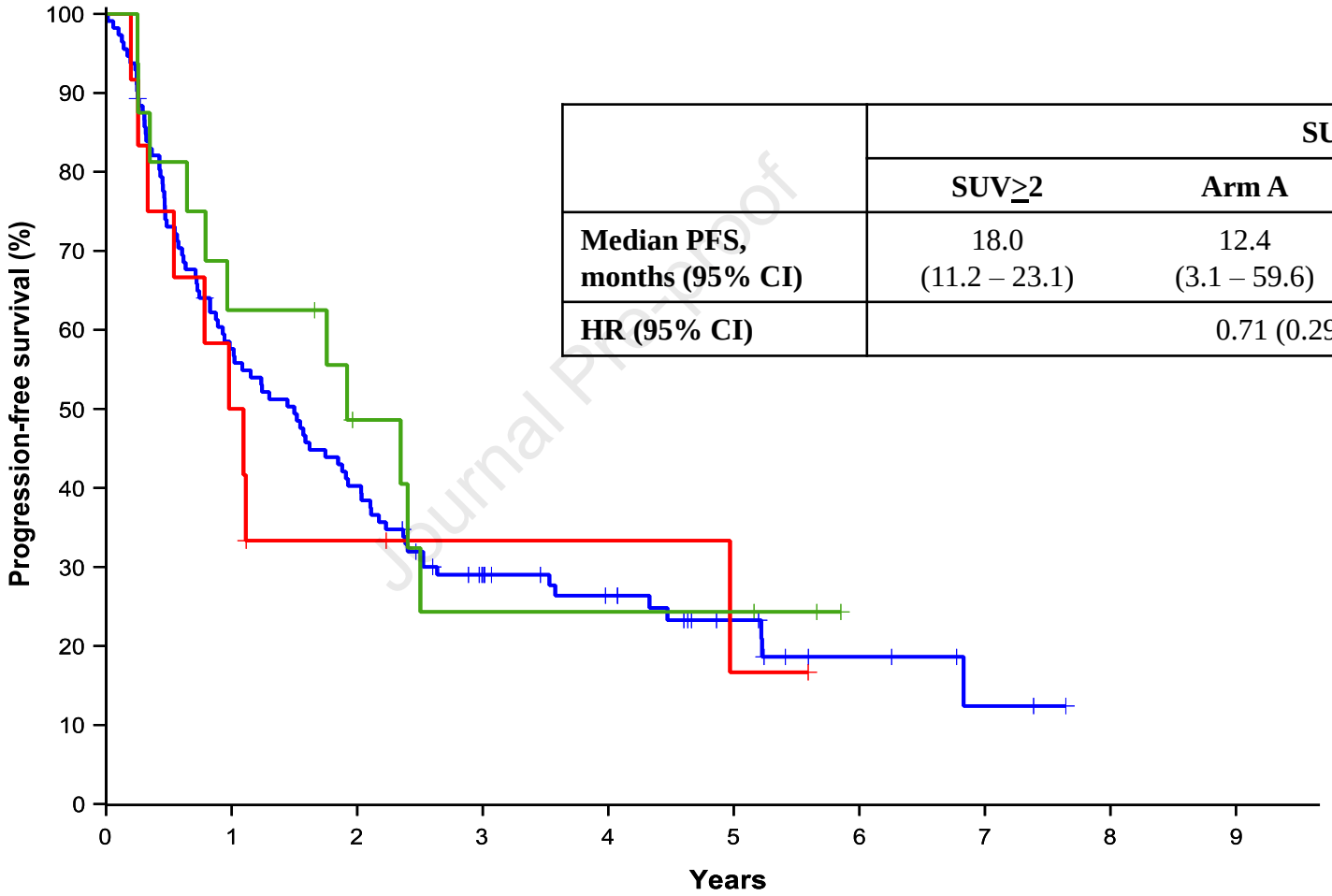
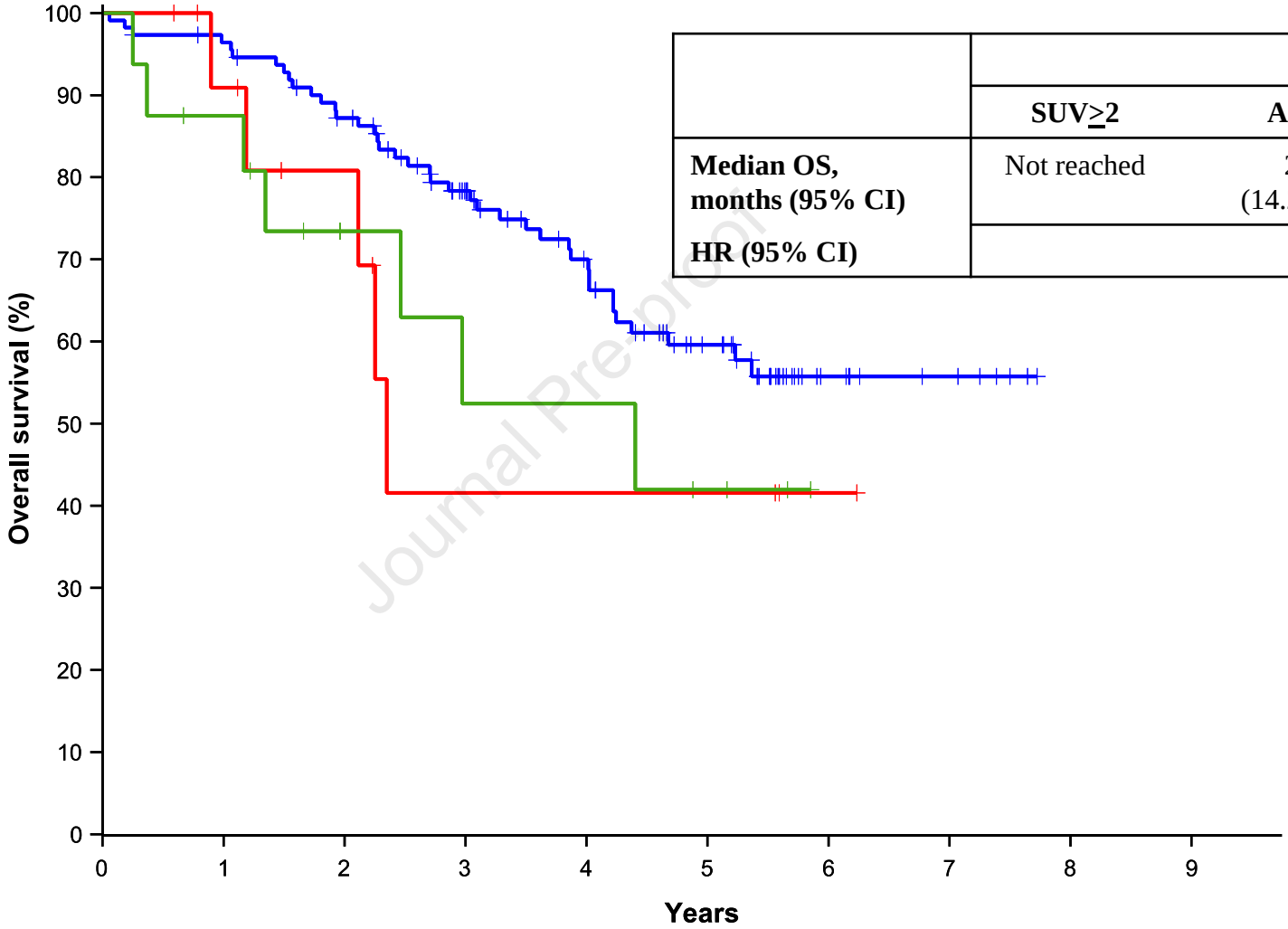


Figure 2: Kaplan-Meier curves of PFS for patients with S[redacted]+biological agents). CI, confidence interval; PFS, progression-free survival.



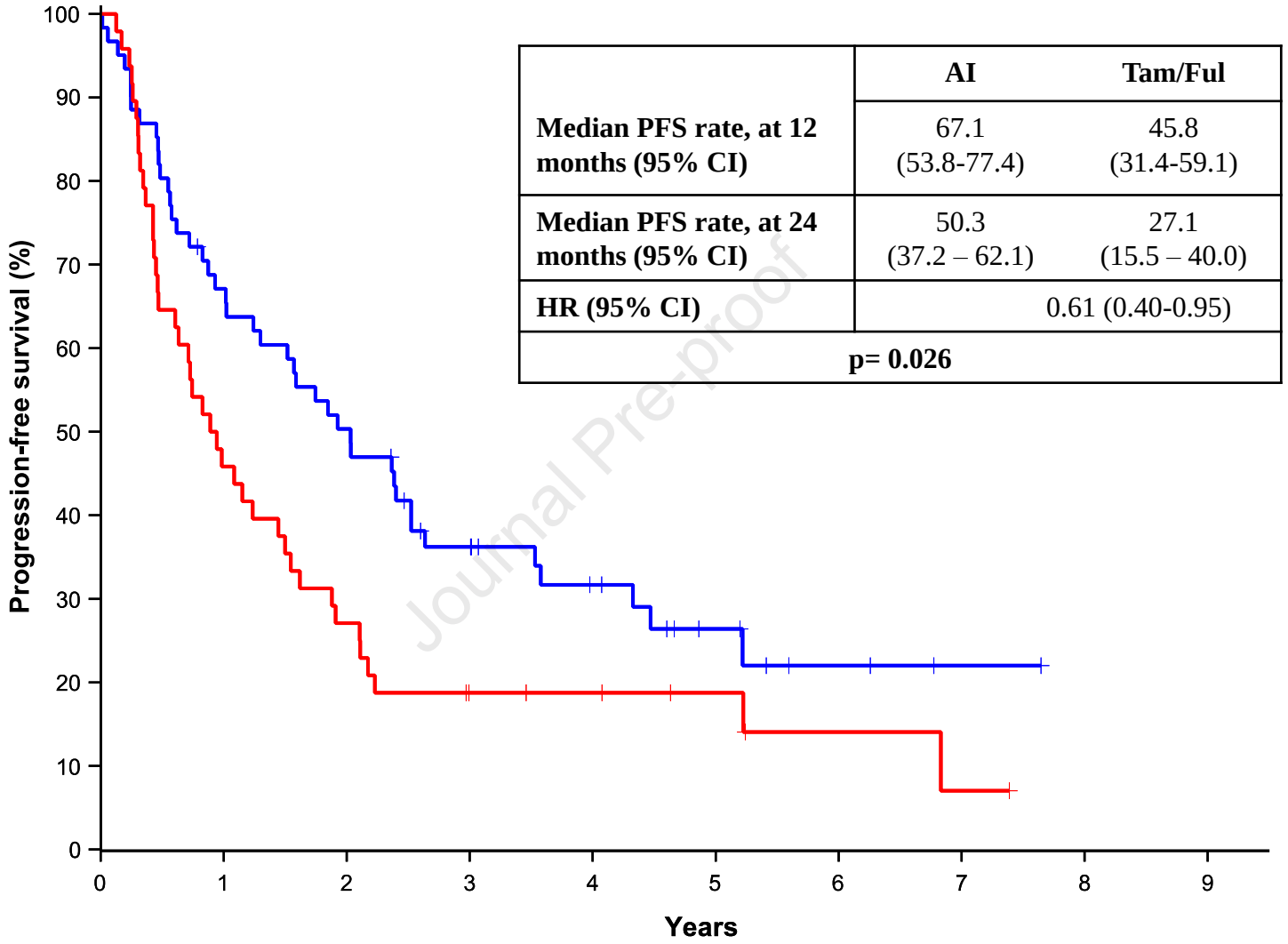
	SUV<2	
	SUV≥2	Arm A	Arm B
Median PFS, months (95% CI)	18.0 (11.2 – 23.1)	12.4 (3.1 – 59.6)	23.0 (7.7 – 30.0)
HR (95% CI)	0.71 (0.29 – 1.72)		

Figure 3: Kaplan-Meier curves of OS for patients with SUV<2 and patients with SUV≥2 (Arm A and Arm B). CI, confidence interval; PFS, progression-free survival; HR, hazard ratio.



	SUV<2	
	SUV≥2	Arm A	Arm B
Median OS, months (95% CI)	Not reached	28.2 (14.2 – NE)	52.8 (16.2 – NE)
HR (95% CI)	0.97 (0.31 – 3.09)	

Figure 4 Kaplan-Meier curves of PFS for aromatase inhibitors versus tamoxifene/fulvestrant. CI, confidence interval; PFS, progression-free survival, HR, harzard ratio; p, p value

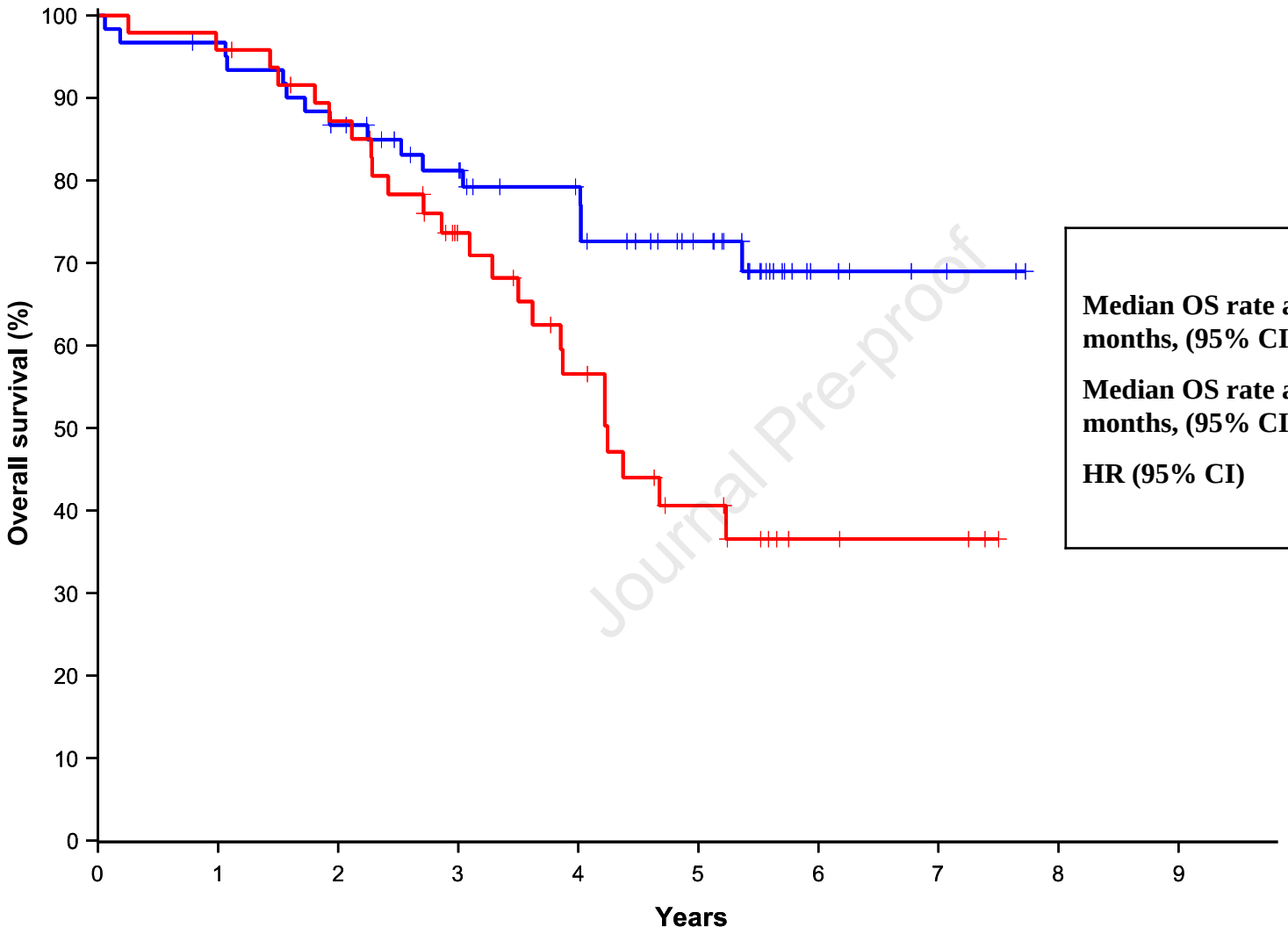


	AI	Tam/Ful
Median PFS rate, at 12 months (95% CI)	67.1 (53.8-77.4)	45.8 (31.4-59.1)
Median PFS rate, at 24 months (95% CI)	50.3 (37.2 – 62.1)	27.1 (15.5 – 40.0)
HR (95% CI)		0.61 (0.40-0.95)
p= 0.026		

No. at risk

Aromatase Inhibitors	61	40	30	19	13	7	3	1	0
Tamoxifene/Fulvestrant	49	22	13	7	6	4	2	1	0

Figure 5 Kaplan-Meier curves of OS for aromatase inhibitors versus tamoxifene/fulvestrant. CI, confidence interval; PFS, progression-free survival, HR, harzard ratio; p, p value



	AI	Tam/Ful
Median OS rate at 48 months, (95% CI)	79.2 (66.2 – 87.7)	56.6 (39.7 – 70.3)
Median OS rate at 60 months, (95% CI)	72.6 (58.5– 82.6)	40.6 (24.5 – 56.1)
HR (95% CI)		0.45 (0.24 – 0.85)
	p= 0.0011	

No. at risk

Aromatase Inhibitors	61	58	51	43	36	25	6	3	0
Tamoxifene/Fulvestrant	49	46	40	27	19	11	4	3	0