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^{18}F-Fluoroestradiol (^{18}F-FES)-PET imaging in a Phase II trial of vorinostat to restore endocrine sensitivity in ER+/HER2- metastatic breast cancer

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ABSTRACT

Rationale: Histone deacetylase inhibitors (HDACi) may overcome endocrine resistance in estrogen receptor positive (ER+) metastatic breast cancer. We tested whether ^{18}F-Fluoroestradiol (^{18}F-FES)-PET imaging would elucidate pharmacodynamics of combination HDACi and endocrine therapy.

Methods: Patients with ER+/HER2- metastatic breast cancer with prior clinical benefit from endocrine therapy but later progression on aromatase inhibitor (AI) therapy were given vorinostat (400mg daily) sequentially or simultaneously with AI. ^{18}F-FES PET and ^{18}F-Fluorodeoxyglucose (^{18}F-FDG) PET scans were performed at baseline, week 2, and week 8.

Results: Eight patients were treated sequentially, then 15 simultaneously. Eight patients had stable disease at week 8 and six of these eight patients had >6 months of stable disease. Higher baseline ^{18}F-FES uptake was associated with longer progression-free survival (PFS). ^{18}F-FES uptake did not systematically increase with vorinostat exposure, indicating no change in regional ER estradiol binding, and ^{18}F-FDG uptake did not show significant decrease, as would have been expected with tumor regression.

Conclusion: Simultaneous HDACi and AI dosing in patients with cancers resistant to AI alone showed clinical benefit (6+ months without progression) in 4 of 10 evaluable patients. Higher ^{18}F-FES-PET uptake identified patients likely to benefit from combination therapy, but vorinostat did not change ER expression at the level of detection of ^{18}F-FES-PET.

Key Words
FES, vorinostat, ER+ breast cancer, metastatic breast cancer, estrogren receptors

INTRODUCTION

Nearly two thirds of invasive breast carcinomas express the estrogen receptor (ER) (*1*). Endocrine therapy is the mainstay of treatment for these tumors, due to favorable toxicity profile and efficacy. For post-menopausal women with advanced or metastatic hormone receptor positive (HR+) disease, whose disease is considered treatable but not curable, the initial standard of care treatment is aromatase inhibitors (AIs), with or without CDK (cyclin-dependent kinase) 4/6 inhibition (*2*). Upon progression, salvage endocrine therapy with molecularly targeted agents, or chemotherapy is indicated (*3*). Recent Phase III trials combining later-line endocrine therapy with a targeted agent, such as palbociclib, alpelisib or everolimus, have demonstrated considerable improvement in outcome (*4-6*) over endocrine therapy alone.

Epigenetic modulation by histone deacetylase inhibitors (HDACi) has been proposed as a mechanism to reverse endocrine resistance (*7*). The transcription of estrogen receptors (ERs) is regulated by epigenetic modifications including HDACs, and HDACi reverse resistance to antiestrogen therapies in vitro (*8-12*). HDACi activity has been shown to increase breast cancer drug sensitivity in vitro (*13, 14*) and cell lines engineered for endocrine resistance demonstrated restored endocrine sensitivity after treatment with an HDACi (*7, 15*).

Clinical studies have shown promising results when combining endocrine therapy with HDACi, including exemestane with entinostat (*16*), tamoxifen with vorinostat (*17*), and a randomized phase III study (NCI-E2112; ClinicalTrials.gov #NCT02115282) of endocrine therapy plus entinostat/placebo in patients with hormone receptor-positive advanced breast cancer (*18*).

^{18}F-FES-PET measures ER status (*19, 20*), and ^{18}F-FES uptake predicts response to endocrine therapy (*21-24*). ^{18}F-FDG PET measures tumor glycolytic activity; a decrease in ^{18}F-FDG-PET has been shown to be a robust measure of early response of breast cancer to endocrine therapy and chemotherapy (*25, 26*), and is prognostic in metastatic breast cancer (*27-29*). We hypothesized that serial ^{18}F-FES and ^{18}F-FDG PET imaging could assess restored endocrine sensitivity in patients with ER+ tumors with prior clinical benefit from endocrine therapy but later progression on an AI, and could be used to predict treatment response.

Vorinostat is a potent HDACi targeting class 1 and 2 HDACs, with anti-tumor activity seen in Phase I trials (*30*). However, a Phase II trial to determine response rate of single agent vorinostat (200 mg orally twice daily, administered for the first 14 days of each 21 day cycle) in 14 patients with Stage IV metastatic breast cancer failed to reach its primary endpoint (*31*) suggesting that ER targeting in addition to HDACi may be essential. Vorinostat combined with endocrine therapy has shown promise (*16, 17*), suggesting that HDACi might be combined with AIs to effectively target ER+ tumors and potentially overcome resistance in patients whose tumors may have endocrine sensitivity. The combination of HDACi in synergy with AI may result in re-sensitization to endocrine therapy.

We used correlative molecular imaging (FES-PET and FDG-PET) in our study of combined vorinostat and AI therapy, to examine the impact of HDACi on tumor ER expression and metabolism.

METHODS

Patients

Eligible patients had metastatic breast cancer and were required to have had documentation of prior clinical benefit from endocrine therapy and subsequent progression while on an AI. Prior chemotherapy was allowed. Patients agreed to a study of AI therapy with vorinostat, imaging with ^{18}F-FES-PET and ^{18}F-FDG-PET, and clinical follow-up of up to 5 years. The institutional review board (IRB) approved this study and all subjects signed a written informed consent. Additional eligibility criteria are shown in **Supplemental Table 1.**

Study Design and Treatment Plan

An open label Phase II clinical trial was conducted in two cohorts. Initially, patients were given vorinostat 400mg orally daily for 2 weeks, followed by an AI daily for 6 weeks. As emerging data demonstrated the safety of concurrent vorinostat with endocrine therapy (*17*), the study protocol was modified to simultaneous administration: 400mg vorinostat daily for five consecutive days in 3 weeks with 4th week off in two 28-day cycles and given concomitantly with the daily AI, as illustrated in **Figure 1**.

Paired ^{18}F-FES-PET and ^{18}F-FDG-PET were performed at baseline, 2, and 8 weeks of treatment as shown in **Figure 1**. Conventional imaging (CT, bone scan) was performed at baseline and at week 8, and tumor response assessed by RECIST criteria in patients with measurable disease or clinical signs of progression (*32*). Patients were also followed for progression-free survival. Patients with response or stable disease were offered continuation of study treatment on the same schedule until disease progression, unacceptable toxicity, or study withdrawal.

^{18}F-FES was synthesized at the University of Washington according to requirements of IND #101203, as previously described (*32*). ^{18}F-FDG was purchased commercially from Cardinal Health (Seattle, WA). All doses underwent quality control testing prior to injection.

^{18}F-FES-PET imaging was performed as previously described (*32*). ^{18}F-FDG-PET imaging was performed according to clinical protocol. All imaging was done on a whole body PET scanner (GE Advance) or PET/CT (GE Discovery STE) scanner. Torso surveys covering five adjacent 15 cm axial fields-of-view (FOVs) beginning approximately 60 minutes after isotope injection were used for analysis in this study.

Image analysis

^{18}F-FES-PET scans were qualitatively and quantitatively analyzed. For each site of active disease, two trained observers blinded to the clinical data, but with access to ^{18}F-FDG-PET and other correlative imaging studies, qualitatively determined if ^{18}F-FES uptake above background levels was present at known sites of disease. Any differences between observers were resolved by consensus, with only one value recorded. Analysis of FES and FDG-PET images was based on prior experience using combined imaging to predict endocrine responsiveness (*23*). Uptake was quantified using lean body mass adjusted (*33*) mean SUV (SULmean) for ^{18}F-FES and the maximum standardized uptake value (SUVmax) for ^{18}F-FDG. Regions-of-interest (ROIs) were generated around the SUVmax for ^{18}F-FDG studies. ROIs of ~1.5 cm diameter were drawn on three adjacent planes using PMOD software (Zurich, Switzerland) on the ^{18}F-FES images over the same lesions as in the ^{18}F-FDG images. Up to ten lesion sites

on the static torso survey were quantified. Pre-defined patient-level summaries were selected based on the results of a prior study in which patient-level ^{18}F-FES uptake summary (SULmean) of <0.85 predicted inferior PFS on endocrine monotherapy for patients with ^{18}F-FDG SUVmax values of 2.2 or greater (*23*). These patient-level summaries were the geometric mean for up to 3 lesions with highest ^{18}F-FDG SUVmax:

Equation 1:

patient-level ^{18}F-FES uptake summary = $\mathrm{antilog}\left[\left(\sum_1^{n_i}\log(^{18}\text{F-FES SULmean})\right)/n_i\right]$

Equation 2:

patient-level ^{18}F-FDG uptake summary = $\mathrm{antilog}\left[\left(\sum_1^{n_i}\log(^{18}\text{F-FDG SUVmax})\right)/n_i\right]$

Percentage change from baseline in uptake between ^{18}F-FES and ^{18}F-FDG-PET scans was computed at the lesion level and for patient-level summaries.

Statistical Methods

The primary objective was to estimate the extent of clinical benefit defined as freedom from progressive disease for 6 months after start of therapy. In the original protocol (sequential cohort), a clinical benefit in 3 or more of 20 patients would indicate a promising treatment. The amended protocol (simultaneous cohort) updated the criteria to clinical benefit in 2 or more of 14 patients (so that the lower bound of a 90% score confidence interval would exceed the null rate of 5%). Secondary objectives included assessment of safety, PFS and overall survival (OS) from start of study therapy, restoration of endocrine sensitivity (by ^{18}F-FES-PET) and tumor metabolic response (by ^{18}F-FDG-PET). Restoration of endocrine sensitivity (i.e. an increase in ER expression measured by radioligand

binding) could be indicated by qualitative ^{18}F-FES uptake above background levels at a post-baseline scan for a lesion that was qualitatively ^{18}F-FES-negative at baseline, or by passing a (arbitrary) threshold of 20% increase in ^{18}F-FES SULmean. Lesion-level analysis of time trends in ^{18}F-FES and ^{18}F-FDG uptake (log-transformed) and relationships with clinical benefit used linear mixed effects models with patient- and lesion-level random intercepts.

RESULTS

Eight patients enrolled in the sequential cohort; sixteen patients enrolled in the simultaneous cohort, including 1 patient later identified as a screen failure who never received study therapy. **Table 1** describes patient and disease characteristics of treated patients in each cohort. All patients were female; most had extensive prior exposure to both endocrine therapy (range = 2-6 lines for all patients) and cytotoxic chemotherapy (range = 1-10 lines for all patients). The number of lesions ranged from 1-10 for all patients in both cohorts, and location of metastases was not reason for exclusion. Individual patient imaging and efficacy data are shown in **Supplemental Tables 2 and 3**.

Efficacy analysis

In the sequential cohort (n=8), 2 patients withdrew during cycle 1 due to vorinostat toxicity (grade 3 fatigue) and were not evaluable for week 8 response. Four patients had progressive disease at week 8, and 2 patients had stable disease at week 8 (33%, 90% CI 12%-65%), with eventual progression at 4 and 7 months from start of therapy.

In the simultaneous cohort (n=15), 5 patients were not evaluable for week 8 response: Two had rapidly progressing disease during cycle 1, and 3 chose to withdraw from study treatment due to adverse events including grade 3 hyperglycemia, grade 3 dizziness, and grade 2 rigor/chills. Four of the remaining 10 patients had progressive disease at week 8, so the proportion of evaluable patients with stable disease at week 8 was 60% (90% CI 35%-81%). Four patients in the simultaneous cohort experienced clinical benefit of at least 6 months on study therapy without progressive disease.

PFS and OS are reported for each patient in **Figure 2**. The median PFS for the 8 patients in the sequential cohort was 3 months (range 2-13), and the median OS was 29 months (range 16-54). In the simultaneous cohort, the median PFS was 2 months (range 0-21); OS includes one patient still alive 55 months after starting study therapy; a Kaplan-Meier estimate of median OS is 19 months (range 1-55).

Toxicity

Twenty-five adverse events were recorded in 12 of the 23 patients. Grade 3 and 4 adverse events (AEs) are listed in **Table 2** with the full list presented in **Supplemental Table 4**. Most adverse events, including Grade 3 fatigue and Grade 2 nausea/vomiting likely related to vorinostat, occurred during the first month and were self-limiting with supportive care. Side effects at later cycles were uncommon; renal insufficiency led to a vorinostat dose reduction at 161 days, and another patient had muscle cramps also at 161 days (for which vorinostat was held then reduced), followed by an unrelated fracture at 496 days. No AEs occurred as a result of ^{18}F-FES imaging.

Imaging

Both ^{18}F-FES and ^{18}F-FDG-PET imaging were completed pre-therapy, after week 2, and after week 8, unless patients had already gone off study therapy (2 patients in sequential, 5 in simultaneous cohort), or when ^{18}F-FES-PET (2 sequential, 4 simultaneous) or ^{18}F-FDG-PET (1 in simultaneous cohort) was not performed because of scheduling or other difficulties. Patient-level geometric means for ^{18}F-FES SULmean and ^{18}F-FDG SUVmax for up to 3 lesions with highest baseline ^{18}F-FDG SUVmax are shown in **Figure 3**. Lesion-level data are displayed in **Supplemental Figure 1**. Median value of the geometric means for baseline ^{18}F-FDG SUVmax for the 3 most ^{18}F-FDG-avid lesions was 4.9 (range 2.7-12.8). All these baseline ^{18}F-FDG uptake summaries were above our previously determined threshold of 2.2(*23*), suggesting glycolytically active, relatively aggressive disease. The median value for ^{18}F-FES SULmean geometric mean (3 most ^{18}F-FDG-avid lesions) was 1.3 (range 0.4-4.0). Most patients (18/23, 78%) had baseline average ^{18}F-FES SULmean ≥0.85 (*23*). Patients with baseline average ^{18}F-FES (SULmean ≥0.85) had higher average PFS (median PFS 2.9 months, 95% CI 1.9-6.7) than patients with baseline ^{18}F-FES SULmean <0.85 (median 1.7 months, 95% CI 0.8-5.9) (log-rank p=0.036) (**Supplemental Figure 2**). In qualitative assessments, baseline ^{18}F-FES uptake was at or below background for all lesions in 4 of the 5 scans with geometric mean ^{18}F-FES SULmean <0.85 (**Supplemental Table 2**). In the fifth scan, one lung lesion (quantitative SULmean = 0.94) was above background and one (quantitative SULmean =0.43) was not. Single qualitatively ^{18}F-FES-

negative lesions in patients with 3+ ^{18}F-FES-positive lesions occurred in 2 other cases (**Supplemental Table 2**).

Vorinostat did not systematically increase ^{18}F-FES uptake for patients in either cohort. For example, of 5 patients with FES-negative (geometric mean ^{18}F-FES SUL <0.85) imaging at baseline, none had average ^{18}F-FES SULmean ≥0.85 at any subsequent scan (**Supplemental Table 2**). Three patients had a >20% increase in geometric mean ^{18}F-FES SUL from baseline to 2 weeks, but none maintained this increase at 8-weeks. One patient that did not have an increase at the 2-week scan had a >20% increase in geometric mean ^{18}F-FES SUL at 8-weeks. Representative ^{18}F-FES and ^{18}F-FDG image examples are shown for a patient with progressive disease (**Figure 4**) and with clinical response (**Figure 5**).

Associations between imaging measures and the primary endpoint (clinical benefit, PFS ≥6 months) were explored further in the simultaneous cohort patients (the primary efficacy analysis cohort). Analysis of 86 lesions in 15 patients corroborates observations from the patient-level descriptive analysis. Baseline/pretreatment ^{18}F-FES SULmean was estimated to be 171% higher (p=0.03, 95% CI 11%-565%) for patients with clinical benefit (baseline fitted average 2.7, 95% CI 1.2-6.0) than without (baseline fitted average 1.0, 95% CI 0.7-1.5). Average baseline ^{18}F-FDG SUVmax did not differ between patients with or without 6-month clinical benefit (Wald test p=0.84). Clinical benefit was also not associated with decrease in ^{18}F-FDG SUVmax (**Supplemental Figure 1**).

DISCUSSION

In this study ^{18}F-FES and ^{18}F-FDG measures were stable over 8 weeks of therapy; ^{18}F-FES or ^{18}F-FDG uptake changes were not a marker of clinical benefit. This may be expected, since stable disease rather than tumor regression (*26*) was the criterion for treatment benefit, and many of the lesions were in bone where progression is often slower than in visceral metastases (*29*).

The combination of HDACi (vorinostat) and AI is an active and durable treatment regimen for ER+/HER2- metastatic breast cancer. Despite prior disease progression on prior endocrine therapy, approximately half of evaluable patients had stable disease at 8 weeks, with 40 percent of patients in the simultaneous cohort remaining on treatment for more than 6 months. Two patients had extended benefit of 16 and 21 months until progression. These results are consistent with other phase II studies combining HDACi and endocrine therapy (*17*). The combination of vorinostat and AI was relatively well tolerated, and AIs were recycled; thus the observed benefit is likely from the activity of vorinostat or synergy with the AI.

There are limitations to this study. Although several lesions were available per patient for evaluation, the total number of patients evaluated was small (n=23), and not all patients completed the study (some due to vorinostat toxicity). In addition, contemporaneous tissue biopsy of each lesion can not be available as a biomarker to predict efficacy. Restoration of endocrine sensitivity was defined both qualitatively and quantitatively, but it is necessary to note that ^{18}F-FES measures the functional ability of ER to bind and concentrate ligand, and ER is not, by itself, a marker of sensitivity. It is also important to note that rigorous protocols are needed to ensure measurement precision. Our centers implement a qualification process using NIST-traceable reference sources for scanners and dose

calibrators, regular calibration, and common patient and imaging protocols yielding highly reproducible SUV measurements (*34, 35*).

Study inclusion criteria selected patients who had benefitted from endocrine therapy before developing resistance. It is expected that we would see ER expression, but the question remains as to whether this predicts response to endocrine (recycled) therapy plus a molecularly targeted agent like vorinostat. We suspect that persistent ER binding measured by ^{18}F-FES shows likelihood of endocrine clinical benefit. A challenge of managing these patients is that multiple pathways may override the endocrine sensitivity, which would explain persistent ER function measured by ^{18}F-FES in the face of progressive disease.

This study validates prior observations that baseline high ^{18}F-FES predicts PFS on later-line AI therapy (here in combination with vorinostat), with the qualitative status of “most lesions” as an accurate patient-level summary of ^{18}F-FES uptake (*36*).

Serial ^{18}F-FES and ^{18}F-FDG PET imaging can be used to monitor the effect of the combination of HDACi (vorinostat) and an AI on ER expression and tumor glycolytic rate in metastatic breast cancer.

CONCLUSIONS

We found that ^{18}F-FES-PET predicts response to HDACi/AI therapy, and that ^{18}F-FES uptake remains stable during the initial 8 weeks of treatment. This study also suggests that the addition of vorinostat to AI in patients with ER+ breast cancer results in tumor response or stable disease in about half of evaluable patients who had progressed on prior endocrine therapies. Our results support further study of serial molecular imaging along with combined HDACi

and AI therapy (such as ECOG-ACRIN study E2112), to further delineate the role of HDACi and potential biomarkers in AI-refractory ER+ advanced breast cancer.

DISCLOSURES The authors declare no potential conflict of interest.

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KEY POINTS

QUESTION:

Can molecular imaging (FES- and FDG-PET) be used to image the potential re-sensitization of estrogen receptors in ER+ metastatic breast cancer patients that received an HDACi and AI therapy?

PERTINENT FINDINGS:

- Higher ^{18}F-FES-PET uptake at baseline predicts response to HDACi/AI therapy.
- The addition of vorinostat to AI in patients with ER+ breast cancer resulted in tumor response or stable disease in about half of evaluable patients who had progressed on prior endocrine therapies.

IMPLICATIONS FOR PATIENT CARE:

Serial molecular imaging along with combined HDACi and AI therapy may further delineate the role of HDACi and potential biomarkers in AI-refractory ER+ advanced breast cancer.

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Table 1. Patient characteristics at enrollment

Sequential Cohort (n=8) Characteristics	**Median (n)**	**Range (%)**
Age (years)	55	44-74
Duration of metastatic disease (years)	5	2-12
Prior chemotherapy regimens (neoadjuvant, adjuvant, metastatic)	3.5	1-9
Prior endocrine regimens	4.5	2-6
Number of lesions	5	2-9
Sites of disease		
Soft tissue and/or bone	6	75%
Includes visceral disease (lung and/or liver lesions)	2	25%
Average ^{18}F-FDG SUVmax*	4.7	3.6-9.9
Average ^{18}F-FES SULmean*	1.3	0.6-4.0
Average ^{18}F-FES SUVmax	3.3	1.9-7.6

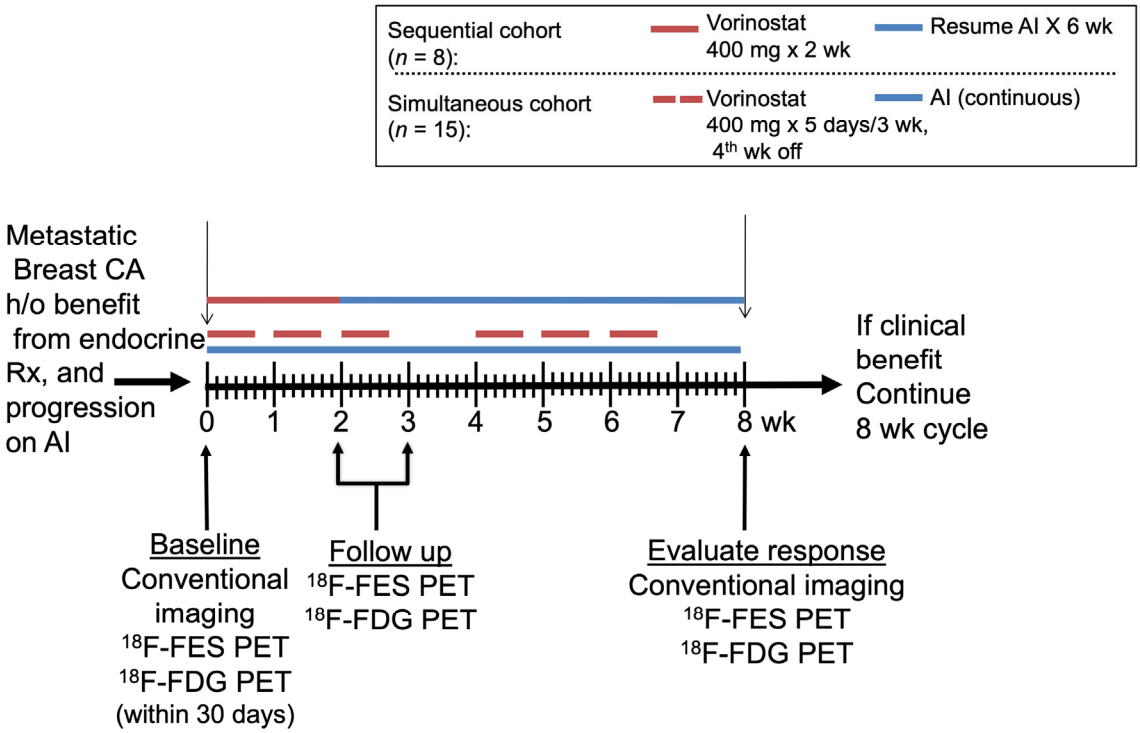
Simultaneous Cohort (n=15) Characteristics	**Median (n)**	**Range (%)**
Age (years)	65	32-76
Duration of metastatic disease (years)	4	0.5-10
Prior chemotherapy regimens (neoadjuvant, adjuvant, metastatic)	4	2-10
Prior endocrine regimens	3	2-5
Number of lesions	7	1-10
Sites of disease		
Soft tissue only	2	13%
Soft tissue and/or bone	6	40%
Includes visceral disease (lung and/or liver lesions)	7	47%
Average ^{18}F-FDG SUVmax*	5.2	2.7-12.8
Average ^{18}F-FES SULmean*	1.2	0.4-3.9
Average ^{18}F-FES SUVmax	3.2	0.9-10.1

*geometric mean of up to 3 lesions with highest ^{18}F-FDG SUVmax

Table 2. Grade 3+ Toxicity Summary

Patient identifier	Toxicity	Grade	SAE?	Days on vorinostat	Relation to vorinostat	Relation to AI
Sequential Cohort						
03	fatigue	3	No	3	very likely	not related
06	flu-like syndrome	3	Yes	24	not related	not related
08	fatigue	3	No	10	very likely	not related
Simultaneous Cohort						
14	dizziness	3	No	4	possible	doubtful
15	liver dysfunction/failure	4	Yes	4	not related	not related
23	hypermagnesemia	3	No	19	doubtful	not related
23	neutrophils	3	No	19	very likely	not related
24	diarrhea	3	No	1	very likely	not related
24	hyperglycemia	3	No	7	possible	not related

SAE = serious adverse event
AI = aromatase inhibitor



- Clinical follow up every 3 months for the first two years, and then every 6 months.
- Laboratory safety tests prior to each cycle for the first 2 cycles and then every 3 cycles thereafter.
- Adverse Events (AEs) assessed each cycle for the first 4 months, and then every 3 months until study treatment discontinuation.

Figure 1. Study schema

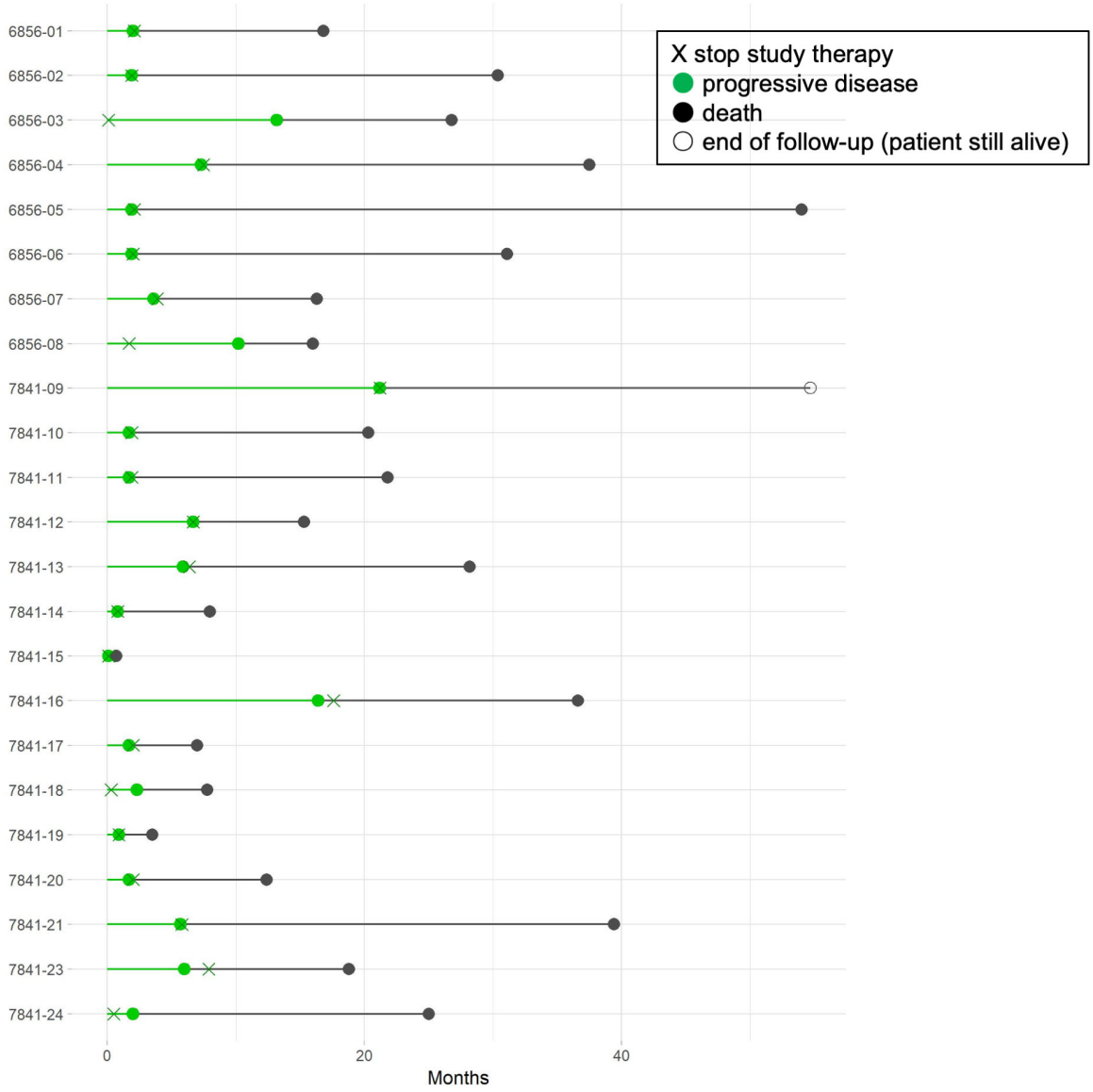


Figure 2: Progression-free survival (PFS) and overall survival (OS, months) per patient. (6856 = sequential cohort; 7841 = simultaneous cohort).

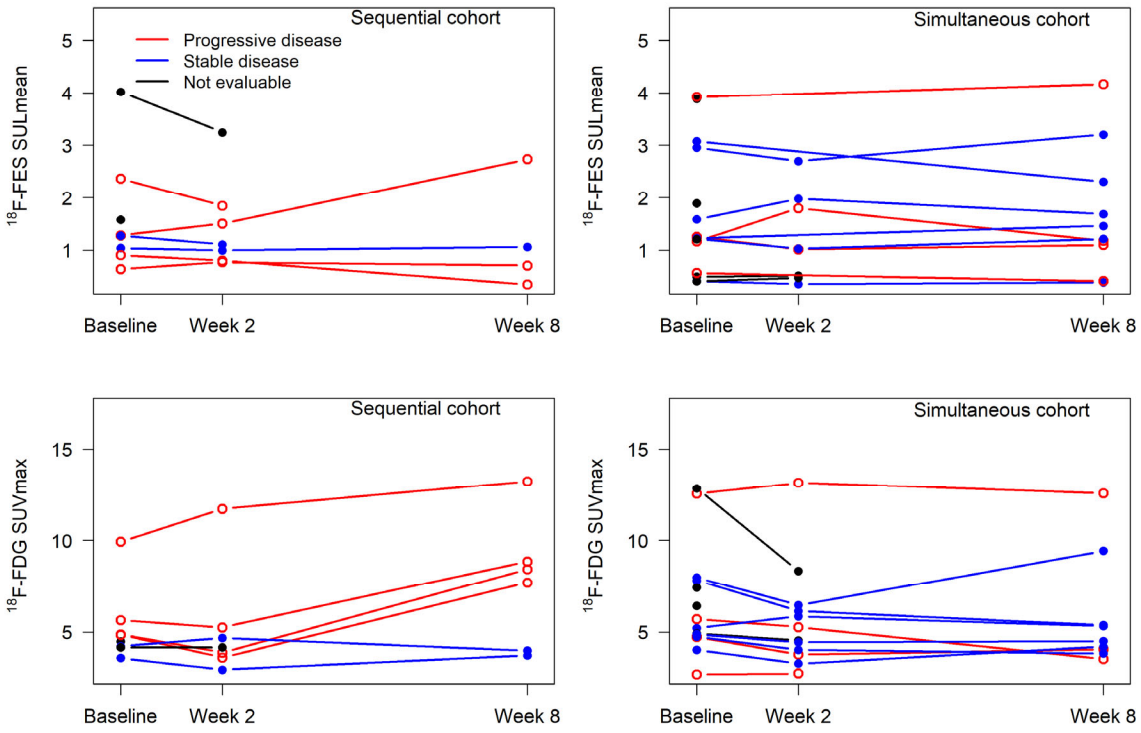


Figure 3. Geometric mean ^{18}F-FES SULmean (top row) and ^{18}F-FDG SUVmax (bottom row) for up to 3 lesions per patient (highest baseline ^{18}F-FDG SUVmax). Sequential cohort (left column) and Simultaneous cohort (right column). Colors indicate 8-week response.

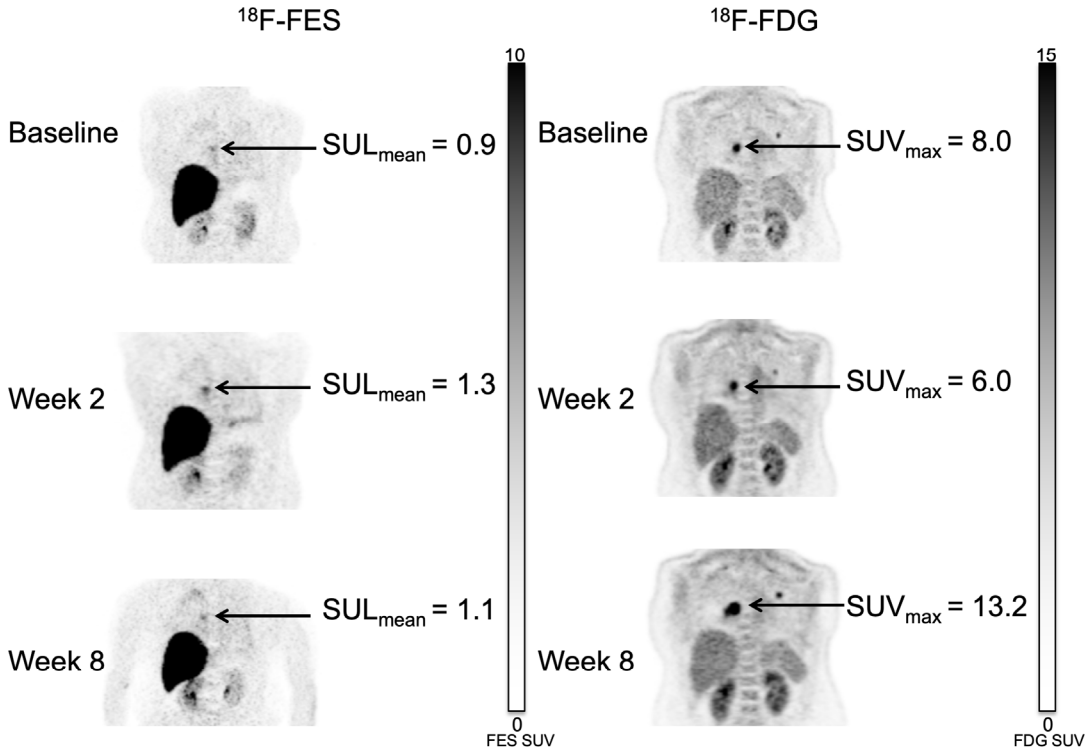


Figure 4. 47-year old, female with 2 invasive ductal carcinoma metastases to the lung treated in the sequential cohort (02). Primary lesion was ER and PR positive/HER2 negative. Although ^{18}F-FES SULmean rose slightly and ^{18}F-FDG SUVmax decreased after 2 weeks of therapy, the lesion size appeared stable. At the 8-week time point, with more than doubling of the ^{18}F-FDG SUVmax from the second scan, the RECIST measure showed 37% increase in lesion size, indicating progressive disease.

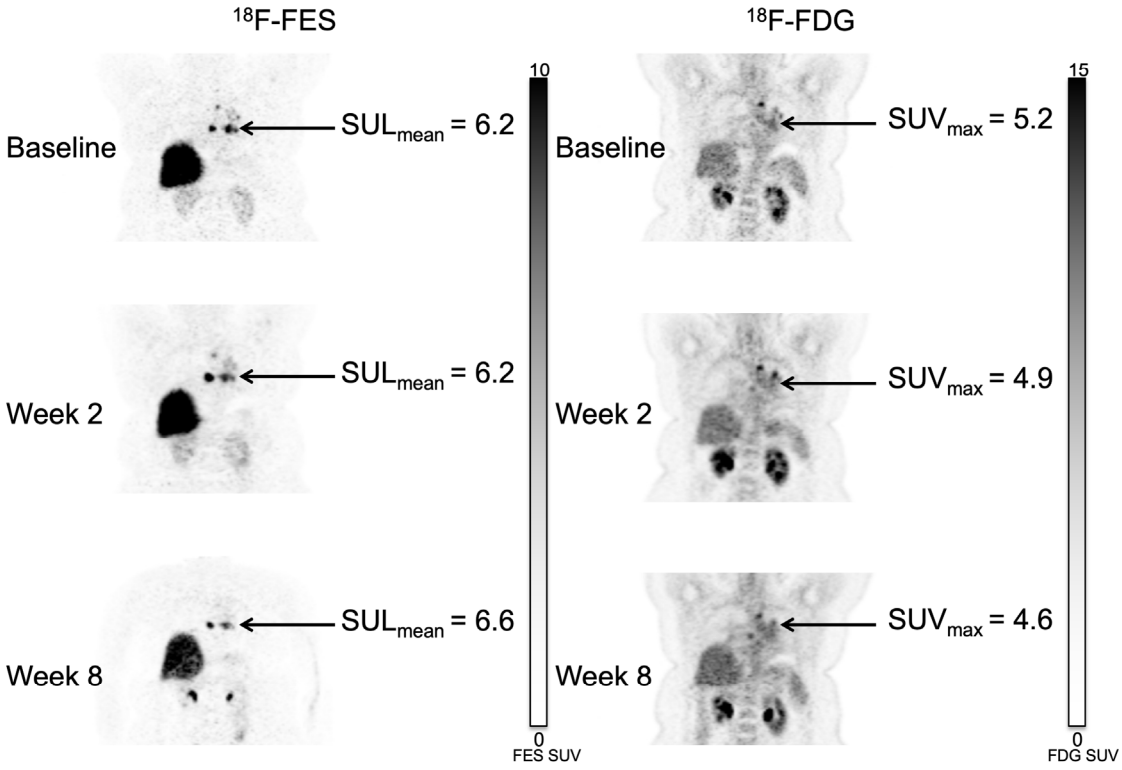


Figure 5. Mediastinal lymph node lesions in a 53-year old female with history of ER and PR positive/HER2 negative right breast invasive ductal carcinoma (patient 12, simultaneous cohort). Uptake in both ^{18}F-FES and ^{18}F-FDG imaging remained stable through all 3 time-points. RECIST measures also showed stable disease. She remained on study therapy for 6.7 months until disease progression.

SUPPLEMENTARY MATERIAL

^{18}F-Fluoroestradiol (^{18}F-FES)-PET imaging in a Phase II trial of vorinostat to restore endocrine sensitivity in ER+/HER2- metastatic breast cancer

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Supplemental Table 1:

Inclusion Criteria

1. Histologically or cytologically proven diagnosis of breast cancer.
2. Stage IV disease.
3. Patient has previously derived clinical benefit from endocrine therapy, but is no longer deriving benefit to endocrine therapy in the opinion of the treating investigator.
4. At least one site of measurable disease, as defined by the modified RECIST criteria
5. ECOG performance status 0-2.
6. Female patient is post menopausal as defined by one of the following; free from menses for > 2 years, surgically sterilized, FSH and Estradiol in post menopausal range AND surgical absence of uterus OR chemotherapy induced amenorrhea lasting > 1 year OR currently on ovarian suppression.
7. Female patient of childbearing potential has a negative urine or serum (β-hCG) pregnancy test within 14 days prior to receiving the first dose of vorinostat.
8. Male patient agrees to use two barrier methods of contraception or abstain from intercourse for the duration of the study.
9. Patient must have adequate organ function as indicated by the following laboratory values:

Absolute neutrophil count (ANC) ≥1,500 /mcL
Platelets ≥ 50,000 / mcL
Hemoglobin ≥ 9 g/dL
Coagulation Prothrombin Time or INR ≤1.5x upper limit of normal (ULN) unless receiving therapeutic anticoagulation
Partial thromboplastin time (PTT) ≤1.2 times the ULN unless the patient is receiving therapeutic anticoagulation.
K levels Normal limits
Mg levels Normal limits
Renal Calculated creatinine clearance a ≥30 mL/min
Serum total bilirubin ≤ 1.5 X ULN
AST (SGOT) and ALT (SGPT) ≤ 2.5 X ULN
Alkaline Phosphatase ≤ 2.5 X ULN

10. Patient, or the patient's legal representative, has voluntarily agreed to participate by giving written informed consent.
11. Patient is ≥18 years of age on day of signing informed consent.
12. Patient has a life expectancy of at least 12 weeks in the opinion of the treating investigator.
13. Patient is willing to continue on same AI therapy.
14. Patient agrees to participate in imaging Protocol 7184 and is separately consented.

Exclusion Criteria Based on Prior or Concomitant Therapy

1. Patient has not derived clinical benefit from prior endocrine therapy.
2. Patient is currently participating or has participated in a study with an investigational compound or device within 30 days of initial dosing with study drug(s) other than the imaging protocol 7184.
3. Patient has received an ER blocking therapy (selective estrogen receptor modulating or downregulating SERM or SERD i.e. tamoxifen or fulvestrant) within the past 6 weeks.
4. Patient had prior treatment with an HDAC inhibitor (e.g., romidespin (Depsipeptide), NSC-630176, MS 275, LAQ-824, belinostat (PXD-101), LBH589, MGCD0103, CRA024781, etc). Patients who have received compounds with HDAC inhibitor-like activity, such as valproic acid, as anti-tumor therapy should not enroll in this study. Patients who have received such compounds for other indications, e.g. valproic acid for epilepsy, may enroll after a 30-day washout period.
5. Patient is on any systemic steroids that have not been stabilized to the equivalent of ≤10mg/day prednisone during the 30 days prior to the start of the study drugs.

Exclusion Criteria Based on Medical History or Current Medical Status

6. Patient has known hypersensitivity to the components of study drug or its analogs.
7. Patients with uncontrolled brain metastases.
8. NYHA Class III or IV congestive heart failure, myocardial infarction within the previous 6 months, QTc>0.47 seconds, or uncontrolled arrhythmia.
9. Type I Diabetes Mellitus. Patients with Type II Diabetes Mellitus will be included as long as their glucose can be controlled to under 200 mg/dL.
10. Patient is pregnant or breast feeding, or expecting to conceive or father children within the projected duration of the study.
11. Patient with a "currently active" second malignancy, other than non-melanoma skin cancer and carcinoma in situ of the cervix, should not be enrolled.

    Patients are not considered to have a "currently active" malignancy if they have completed therapy for a prior malignancy,are disease free from prior malignancies for >5 years or are considered by their physician to be at less than 30% risk of relapse.
12. Patients with known active viral hepatitis.
13. Patient has a history or current evidence of any condition, therapy, or lab abnormality that might confound the results of the study, interfere with the patient's participation for the full duration of the study or is not in the best interest of the patient to participate.

Supplemental Table 2: Patient imaging data listing, sequential (n=8) and simultaneous (n=15) therapy cohorts

id	# of lesions analyzed	Qualitative # FES-neg lesions	Qualitative 1=FES-neg 0=FES-pos	*baseline FES SULmean	*2 wk FES SULmean	*8 wk FES SULmean	*baseline FES SUVmax	*2 wk FES SUVmax	*8 wk FES SUVmax	*baseline FDG SUVmax	*2 wk FDG SUVmax	*8 wk FDG SUVmax
01	6	0	0	2.4	1.8	.	5.9	4.6	.	5.7	5.2	8.9
02	2	1	0	0.6	0.8	0.7	1.9	2.2	2.3	4.9	3.9	8.4
03	9	0	0	1.6	.	.	4.5	.	.	4.5	.	.
04	4	0	0	1.0	1.0	1.1	2.5	2.8	2.6	3.6	3.0	3.7
05	4	1	0	0.9	0.8	0.3	2.1	2.0	1.2	4.9	3.6	7.7
06	5	0	0	1.3	1.5	2.7	3.2	3.3	5.1	9.9	11.7	13.2
07	5	0	1	1.3	1.1	.	3.3	3.1	.	4.2	4.7	4.0
08	5	0	0	4.0	3.2	.	7.6	5.8	.	4.2	4.2	.
09	1	0	0	1.6	2.0	1.7	4.2	5.4	4.7	8.0	6.5	9.4
10	10	1	0	1.3	1.0	1.1	2.5	2.1	2.2	5.7	5.3	3.5
11	1	0	0	1.2	1.8	1.2	3.2	5.5	2.7	4.7	3.8	4.1
12	5	0	0	3.0	2.7	3.2	10.1	8.8	10.1	5.2	5.9	5.3
13	7	7	0	0.4	0.4	0.4	1.0	0.7	1.2	7.8	6.1	5.4
14	4	4	0	0.5	0.5	.	1.2	1.5	.	4.9	4.5	.
15	7	0	0	3.9	.	.	7.0	.	.	4.8	.	.
16	6	0	0	3.1	.	2.3	5.7	.	4.4	4.9	4.4	4.5
17	7	0	0	3.9	.	4.2	7.0	.	7.0	12.5	13.1	12.6
18	7	0	0	1.9	.	.	7.4	.	.	7.4	.	.
19	7	7	0	0.4	0.5	.	1.6	1.4	.	12.8	8.3	.
20	4	4	0	0.6	.	0.4	0.9	.	0.9	2.7	2.8	.
21	7	0	0	1.2	.	1.5	2.2	.	2.5	4.0	3.3	4.2
23	6	0	0	1.2	1.0	1.2	2.5	2.2	2.5	4.7	4.0	3.8
24	7	0	0	1.2	.	.	4.5	.	.	6.4	.	.

neg=negative; pos=positive; *geometric mean of up to 3 lesions with highest FDG SUVmax; Shading indicates discordance between 0.85 FES SULmean threshold and 1.5 FES SUVmax threshold (both applied to geometric mean of 3 lesions)

Supplemental Table 3: Patient efficacy data listing, sequential (n=8) and simultaneous (n=15) therapy cohorts

id	# of lesions analyzed	# soft tissue/LN lesions	# of bone lesions	# of visceral lesions	Liver Lesions by RECIST	Target lesions size (mm) Baseline	Target lesions size (mm) 8 wk	8 wk response	Circumstances at progression	mo on study tx	PFS (mo)	OS (mo)
01	6	3	3	0	Yes	117.3	165.5	PD	Radiographic progression - new sites	2.1	2.0	16.8
02	2	0	0	2	No	30	41.3	PD	Radiographic progression - existing sites	1.9	1.9	30.4
03	9	0	9	0				NE		0.1	13.2	26.8
04	4	0	4	0	Yes	61.7	71	SD		7.5	7.3	37.5
05	4	0	4	0	Yes	24.4		PD	Radiographic progression - new sites Tumor marker progression	2.1	1.9	54.0
06	5	0	5	0	No			PD	Radiographic progression - new sites	2.0	1.9	31.1
07	5	0	4	0	No			SD		3.9	3.6	16.3
08	5	0	5	0				NE		1.7	10.2	16.0
09	1	1	0	0	No	37.5	33.2	SD		21.2	21.2	54.7+
10	10	2	7	1	Yes	18.6	39.9	PD	Clinical progression Bone disease progression Tumor marker progression Radiographic progression - new sites Radiographic progression - existing sites	1.9	1.7	20.3
11	1	0	1	0	Yes	46.6	66.8	PD	Radiographic progression - existing sites Radiographic progression - new sites Tumor marker progression	1.9	1.7	21.8
12	5	3	1	1	Yes	76.6	72.1	SD	Radiographic progression - new sites Radiographic progression - existing sites	6.7	6.7	15.3

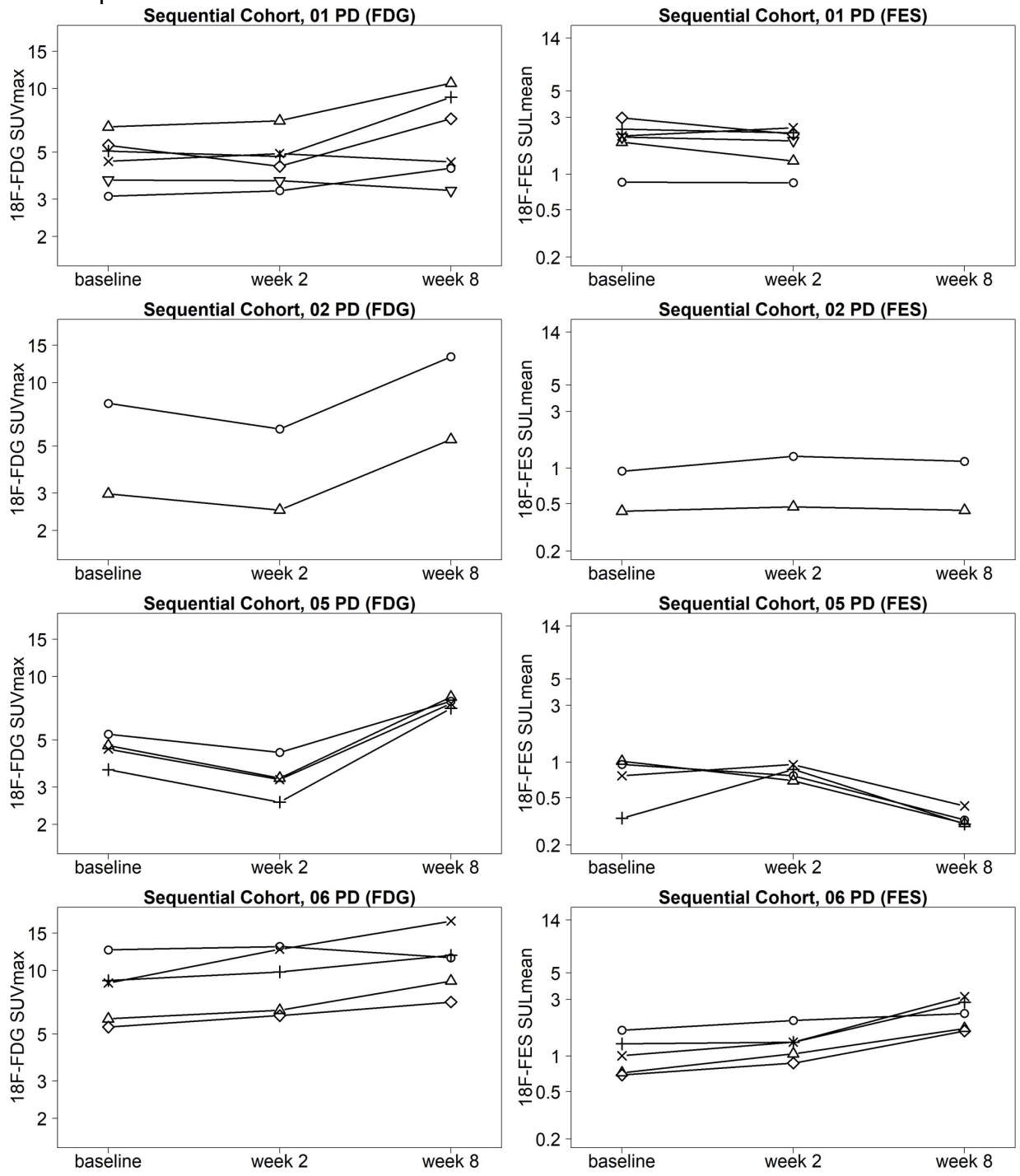
id	# of lesions analyzed	# soft tissue/LN lesions	# of bone lesions	# of visceral lesions	Liver Lesions by RECIST	Target lesions size (mm) Baseline	Target lesions size (mm) 8 wk	8 wk response	Circumstances at progression	mo on study tx	PFS (mo)	OS (mo)
13	7	0	7	0	No	20.1	19.4	SD	Bone disease progression Radiographic progression - existing sites Radiographic progression - new sites	6.4	5.9	28.2
14	4	0	4	0				NE	Radiographic progression - existing sites Tumor marker progression	0.8	0.8	8.0
15	7	3	4	0				NE	Clinical progression	0.1	0.1	0.7
16	6	5	0	1	No	42	44.7	SD		17.6	16.4	36.6
17	7	3	4	0	Yes	101.3	113.4	PD	Clinical progression Radiographic progression - existing sites	2.0	1.7	7.0
18	7	0	7	0				NE	Clinical progression Bone disease progression Radiographic progression - new sites	0.3	2.3	7.8
19	7	7	0	0				NE	Clinical progression Tumor marker progression	0.9	0.9	3.5
20	4	0	4	0	Yes	38.8	42.6	PD	Clinical progression Radiographic progression - existing sites Radiographic progression - new sites Tumor marker progression	2.0	1.7	12.4
21	7	0	7	0	No	17.1	16.5	SD	Bone disease progression Radiographic progression - existing sites Radiographic progression - new sites Tumor marker progression	5.8	5.7	39.4
23	6	1	5		No	21	19.7	SD	Tumor marker progression	7.9	6.0	18.8
24	7	0	4	3				NE		0.5	2.0	25.0

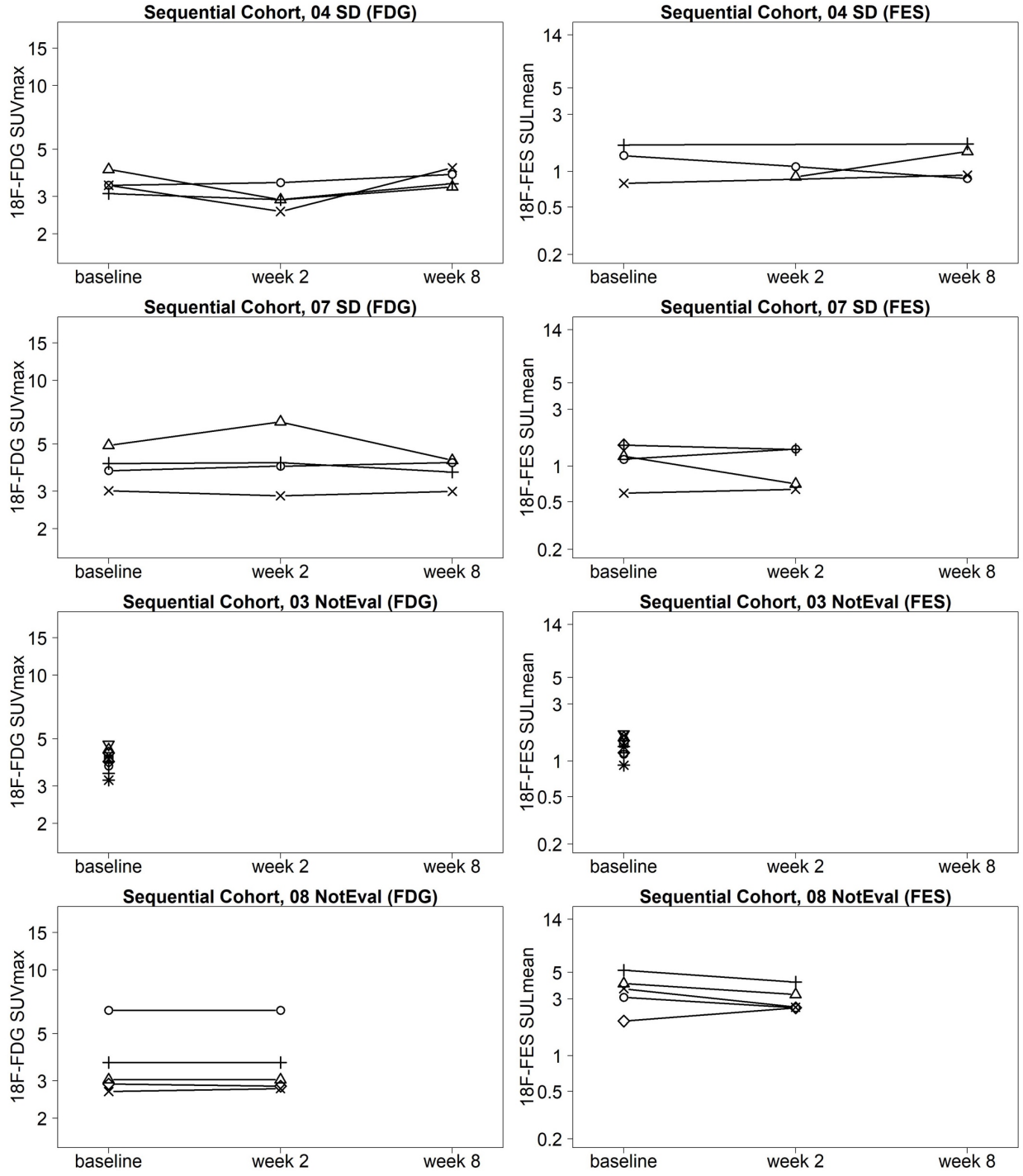
PFS = progression-free survival from start of study therapy; OS = overall survival from start of study therapy, PD=progressive disease, NE = not evaluable, SD = stable disease; +Patient remains alive

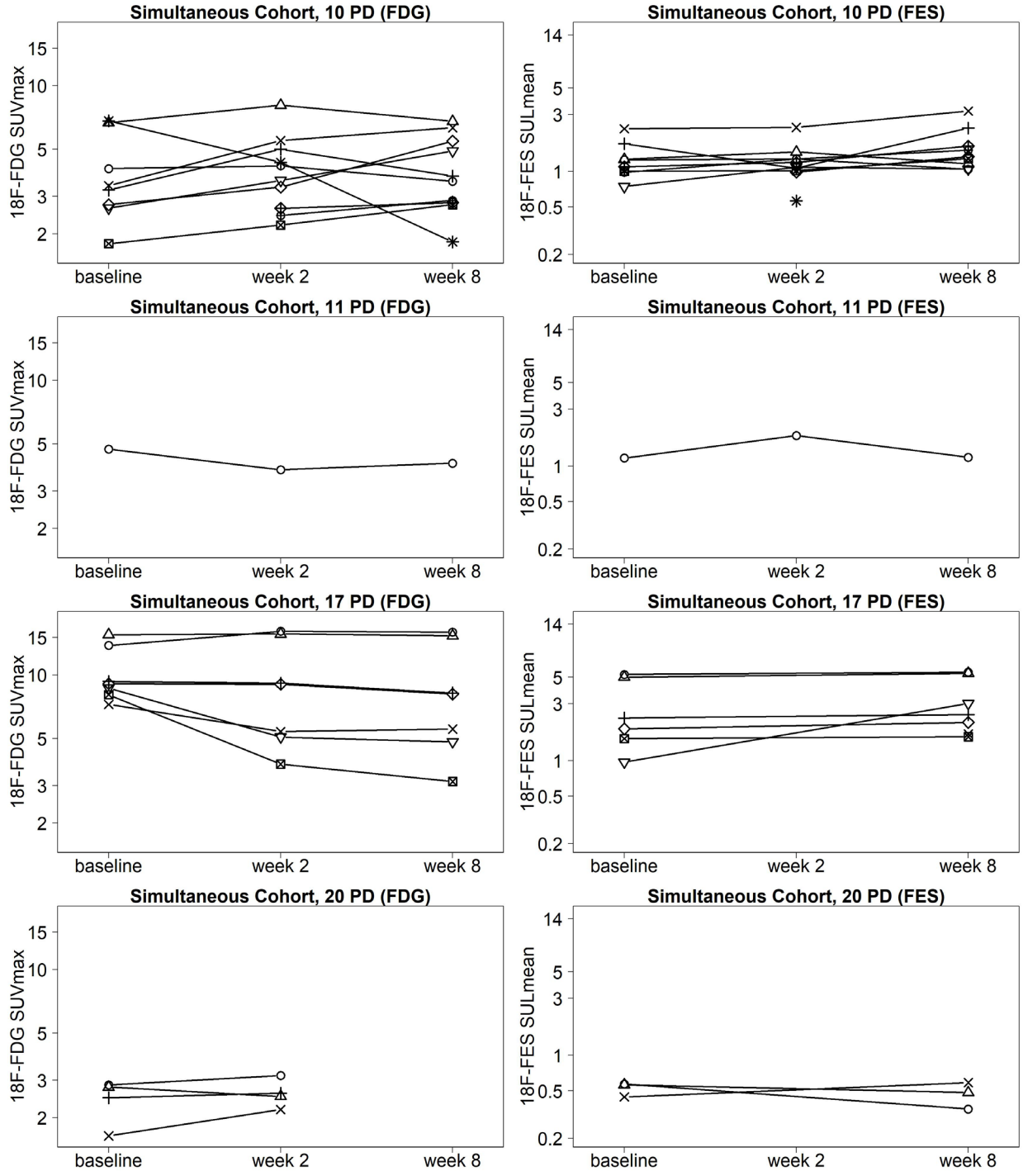
Supplemental Table 4: All AE toxicity summary

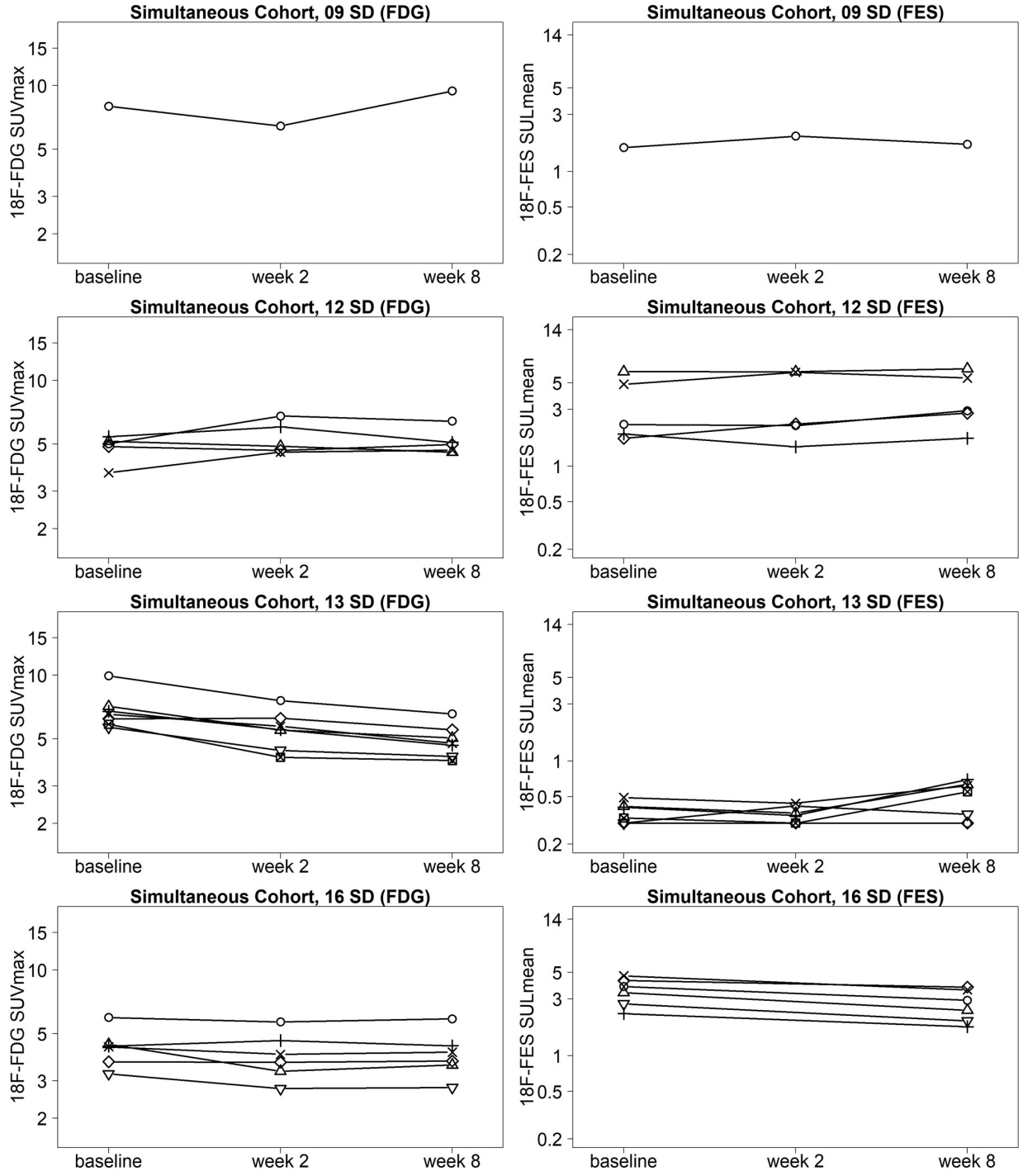
Pt. ID	Toxicity	Grade	SAE?	Days on vorinostat	Relation to vorinostat	Relation to AI
Sequential Cohort						
03	fatigue	3	No	3	very likely	not related
06	flu-like syndrome	3	Yes	24	not related	not related
08	pancreatitis	2	Yes	43	not related	not related
08	fatigue	3	No	10	very likely	not related
Simultaneous Cohort						
09	creatinine increase	1	No	161	very likely	not related
10	mucositis	2	No	-5	not related	not related
14	dizziness	3	No	4	possible	doubtful
15	liver dysfunction/failure	4	Yes	4	not related	not related
16	vomiting	2	No	157	possible	not related
16	muscle cramps	2	No	161	probable	doubtful
16	fracture	2	No	496	not related	not related
18	rigors/chills	2	No	5	probable	not related
18	infection (normal ANC)	2	Yes	1	not related	not related
21	anorexia	2	No	7	probable	not related
21	platelets	2	No	20	very likely	not related
21	creatinine increase	2	No	26	possible	not related
21	decrease in glom filtration	1	No	26	possible	not related
23	hypermagnesemia	3	No	19	doubtful	not related
23	neutrophils	3	No	19	very likely	not related
23	platelets	2	No	19	very likely	not related
24	diarrhea	3	No	1	very likely	not related
24	nausea	2	No	10	very likely	not related
24	vomiting	2	No	10	very likely	not related
24	fatigue	2	No	12	probable	not related
24	hyperglycemia	3	No	7	possible	not related

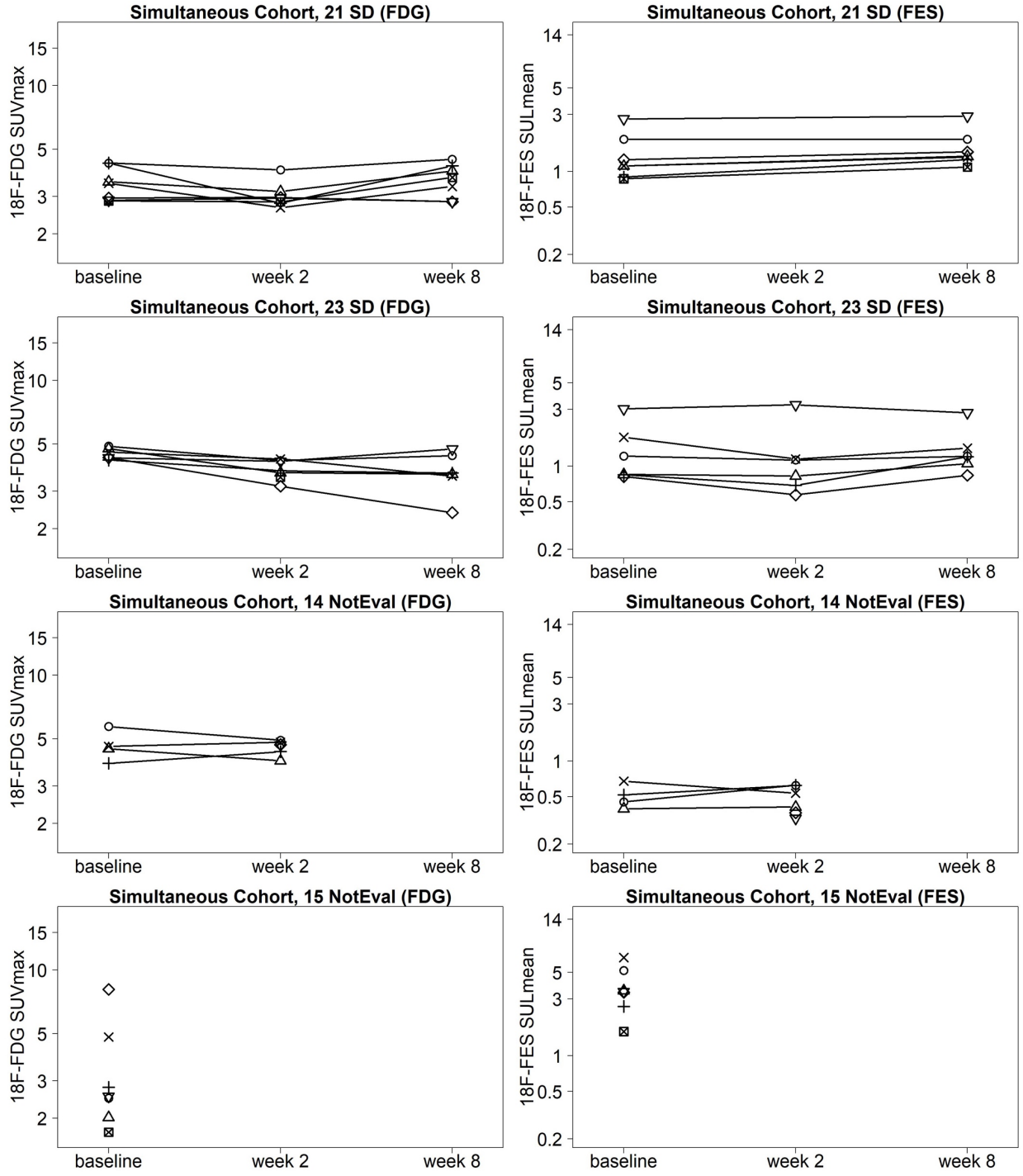
Supplemental Figure 1: Lesion-level summaries of ^{18}F-FDG SUVmax (left column) and ^{18}F-FES SULmean (right column) for individual patients, sorted by cohort and 8-week response status.

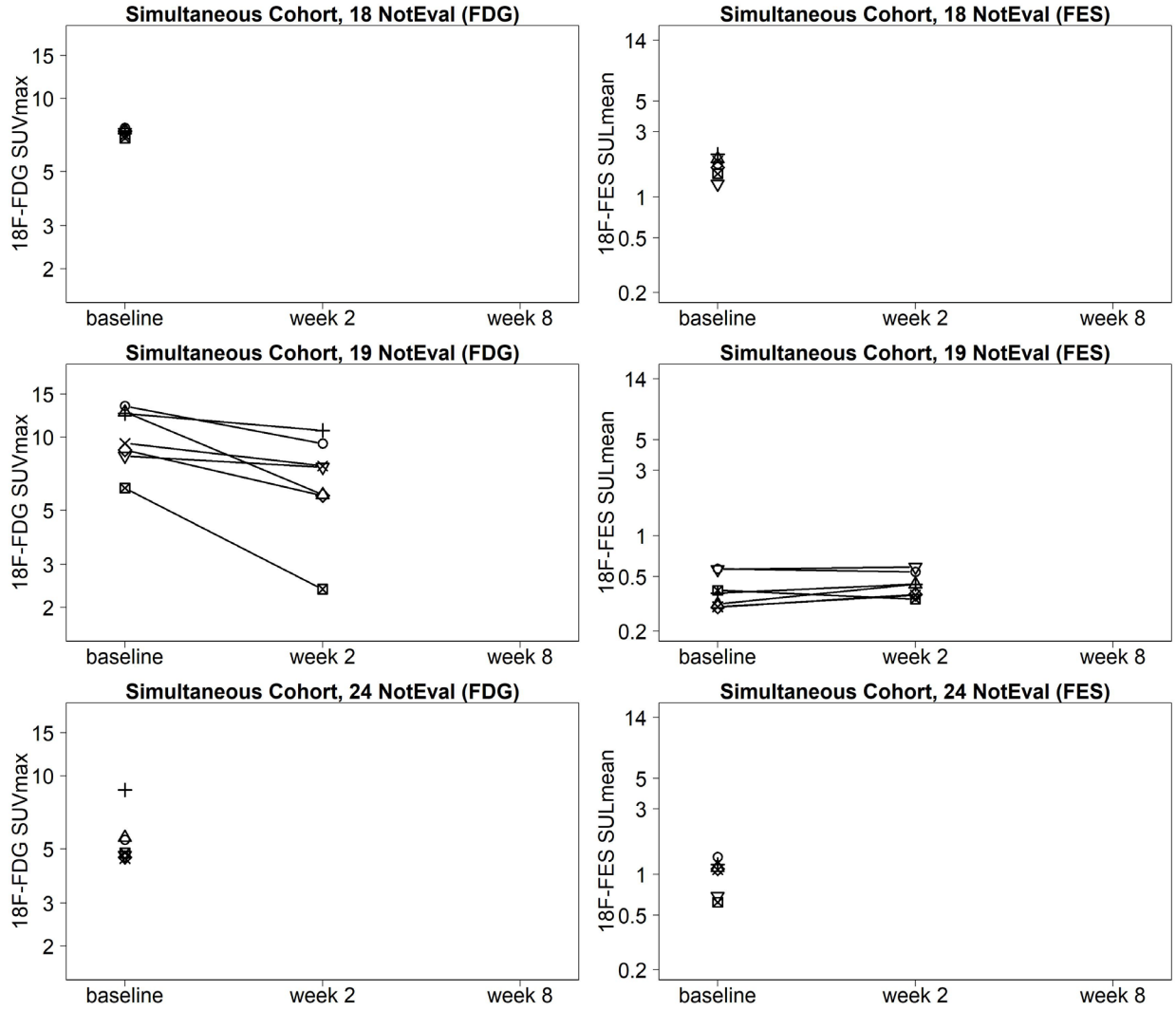



Sequential Cohort, 04 SD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Sequential Cohort, 04 SD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Sequential Cohort, 07 SD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Sequential Cohort, 07 SD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Sequential Cohort, 03 NotEval (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Sequential Cohort, 03 NotEval (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Sequential Cohort, 08 NotEval (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Sequential Cohort, 08 NotEval (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8


Simultaneous Cohort, 10 PD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 10 PD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 11 PD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 11 PD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 17 PD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 17 PD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 20 PD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 20 PD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8


Simultaneous Cohort, 09 SD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 09 SD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 12 SD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 12 SD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 13 SD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 13 SD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 16 SD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 16 SD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8


Simultaneous Cohort, 21 SD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 21 SD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 23 SD (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 23 SD (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 14 NotEval (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 14 NotEval (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 15 NotEval (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 15 NotEval (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8


Simultaneous Cohort, 18 NotEval (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 18 NotEval (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 19 NotEval (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 19 NotEval (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8
Simultaneous Cohort, 24 NotEval (FDG)
18F-FDG SUVmax
15
10
5
3
2
baseline
week 2
week 8
Simultaneous Cohort, 24 NotEval (FES)
18F-FES SULmean
14
5
3
1
0.5
0.2
baseline
week 2
week 8

Supplemental Figure 2: Progression-free survival (PFS) by baseline FES PET, with log-rank test p-value. Cutpoint of 0.85 for FES SULmean (geometric mean of up to 3 lesions with highest FDG SUVmax) defined from study in non-overlapping cohort.