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Imaging Androgen Receptors in Breast Cancer with ^{18}F-fluoro-5α-dihydrotestosterone-PET: A Pilot Study

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ABSTRACT

Most breast cancers express androgen receptors (AR). This prospective imaging sub-study explored imaging AR with ^{18}F-fluoro-5α-dihydrotestosterone (FDHT)-PET in patients with metastatic breast cancer (MBC) receiving selective AR modulation (SARM) therapy (GTx-024).

Methods: 11 post-menopausal women with estrogen receptor positive MBC underwent FDHT-PET/CT at baseline, 6, and 12 weeks after starting SARM therapy. Abnormal tumor FDHT uptake was quantified using maximum SUV (SUVmax). AR status was determined from tumor biopsy specimens. FDHT-SUVmax percent change between scans was calculated. Best overall response was categorized as clinical benefit (CB: non-progressive disease [PD]), or PD using RECIST 1.1).

Results: Median baseline FDHT-SUVmax was 4.1 (range 1.4-5.9) for AR+ tumors versus 2.3 (range 1.5-3.2) for AR- tumors (p=0.22). Quantitative AR expression and baseline FDHT uptake were weakly correlated (Pearson rho=0.39, p=0.30). Seven participants with CB at 12 weeks tended to have larger declines in FDHT uptake compared to those with PD at both 6 (median decline, range: -26.8%, -42.9 to -14.1% vs. -3.7%, -31% to +29%, respectively, p=0.11) and 12 weeks (median decline, range: -35.7%, -69.5 to -7.7% vs. -20.1%, -26.6% to +56.5%, respectively, p=0.17) after starting GTx-024.

Conclusion: This hypothesis-generating data suggests that FDHT-PET/CT is worth further study as an imaging biomarker for evaluating response of MBC to SARM therapy and reiterates the feasibility of including molecular imaging in multidisciplinary therapeutic trials.

Key Words: FDHT, androgen receptor, AR, PET, positron emission tomography, breast cancer

INTRODUCTION

The androgen receptor (AR), the most abundantly expressed steroid hormone receptor in breast cancer, is co-expressed in 75-95% of estrogen receptor positive (ER+) and 10-35% of triple negative (ER negative [-]/PR-/HER2-) tumors (*1*). Steroidal androgens, notably dihydrotestosterone (DHT) and fluoxymesterone, were widely used in the 1970s to treat metastatic breast cancer (MBC), but virilizing side effects, concern for aromatization to estrogen, and the survival benefit found with tamoxifen led to their disfavor (*2,3*). Recently, AR has re-emerged as a therapeutic target in MBC due to elucidation of the complex relationship between the AR-axis and breast cancer growth and the development of selective androgen receptor modulators (SARMs).

In ER+ breast cancer, AR primarily inhibits tumor proliferation (*4,5*). GTx-024 is a novel oral non-steroidal SARM which specifically binds AR promoting agonist activity. GTx-024 does not bind other steroidal receptors and cannot be aromatized to estrogen (*6*). GTx-024 slowed tumor growth in preclinical models of ER+ breast cancer and was well-tolerated without virilizing effects (*6*).

Derived from DHT, ^{18}F-fluoro-5α-dihydrotestosterone (FDHT) was developed for imaging AR with positron emission tomography (PET) (*7,8*). In prostate cancer, FDHT-PET can quantitate relative levels of AR and be used as a pharmacodynamic imaging biomarker after anti-androgen therapy to provide information about drug-targeting, dose-optimization, and response (*9*).

Overmoyer et al. conducted a prospective phase II clinical trial of GTx-024 in postmenopausal women with ER+ MBC (*10*). As part of this phase II clinical trial, we performed a prospective imaging sub-study designed to (1) determine the feasibility of FDHT-PET/CT to

non-invasively image AR expression in ER+ MBC, and (2) explore the potential of FDHT-PET as an imaging biomarker for evaluating response to SARM therapy.

MATERIALS AND METHODS

Study Design and Participants

This single-site prospective imaging sub-study was performed as part of a larger open-label, multicenter, international, randomized, parallel design phase II trial exploring the clinical benefit (CB) of GTx-024 (G200802, GTx, Inc, NCT02463032). Participants were randomized 1:1 to receive 9 or 18 mg of GTx-024 orally per day. The trial followed Declaration of Helsinki principles and Good Clinical Practice and was approved by our institutional review board. All participants gave informed written consent.

Major eligibility criteria for both the parent therapeutic trial and the imaging sub-study were postmenopausal women with ER+/HER2-, metastatic or locally recurrent advanced breast cancer, radiological or clinical disease recurrence or progression within 30 days of randomization onto the therapeutic trial, ≥ 1 prior hormonal treatment but ≤ 1 course of chemotherapy for metastatic disease, available biopsy or archival tumor tissue , bone-only non-measurable or measurable disease by RECIST 1.1, adequate organ function, and Eastern Cooperative Oncology Group performance status ≤ 1. Participants only received the study drug (GTx-024) and no other hormonal treatment for breast cancer while on study.

FDHT-PET/CT Scan Acquisition and Image Interpretation

FDHT-PET/CT scans were performed at baseline, 6, and 12 weeks after starting GTx-024 (Figure 1). Brigham and Women's Nuclear Medicine/Biomedical Imaging Research Core

manufactured FDHT under IND 122,852 per previously published methods (*11,12*). The final formulated FDHT passed all quality control tests required for clinical use and had radiochemical purity >99% and specific activity>18.5 GBq/μmol for all batches.

No specific preparation was given for FDHT-PET/CT scans. Forty-five minutes after intravenous FDHT administration (333 MBq; 9 mCi), PET scans were obtained in 3D mode from skull vertex to mid-thighs using 3-5 min per bed position (Discovery ST or Discovery MI, GE Healthcare). Images were reconstructed using iterative methods. Non-contrast low-dose CT imaging (3.75-5 mm axial slice thickness) was performed over the same range without breath-hold for anatomic correlation and attenuation correction. For 7 participants, all FDHT-PET/CT scans were obtained on the same scanner. A scanner upgrade during the study period necessitated performance of the 12-week scan for 4 participants on a different scanner.

Lesions on FDHT-PET/CT scan were determined by comparison to the diagnostic contrast-enhanced chest, abdomen and pelvis CT scans used to determine eligibility for the parent therapeutic trial. FDHT uptake was quantitated in measurable lesions >1 cm in one dimension and in non-measurable bone lesions which were allowed on this trial. The following semi-quantitative parameters of FDHT uptake in tumor were recorded at all imaging time points: maximum standardized uptake value corrected for body weight (SUVmax) and corrected for lean body mass (SULmax), SUVpeak (average SUV in 1-cm^3 volume of interest at the tumor's hottest part), SULpeak, and SUVmean in a 70% iso-contour around SUVmax.

Response Assessments

Objective disease response was determined according to RECIST 1.1 in the parent therapeutic trial using contrast-enhanced CT/MRI and bone scans per standard institutional protocols at

baseline, week 12 after starting GTx-024, and every 12 weeks until progressive disease (PD) or study drug discontinuation. CB was defined as complete or partial response or stable disease per RECIST 1.1. No CB was defined as PD. Best overall response (BOR) was defined as best tumor response achieved from treatment start until treatment end. Because FDG-PET/CT is not considered standard of care for assessing treatment response of MBC per National Comprehensive Cancer Network guidelines (*13*) and it was not available at all international sites participating in the parent therapeutic trial, FDG-PET/CT was not included for baseline and disease response assessments.

Pathology and Laboratory Correlates

Tumor tissue from biopsy or archival tissue was reviewed for AR status using standard immunohistochemical techniques with a monoclonal antibody specific for human AR by a central laboratory (QualTek, Goleta, CA). AR was reported qualitatively as positive (i.e., >1% percentage of positive nuclei) or negative and quantitatively as percentage of positive nuclei. The local laboratory evaluated serum for estradiol, testosterone, and sex-hormone binding globulin (SHBG) levels at baseline and serum prostate specific antigen (PSA) levels at baseline, 6-weeks, and 12-weeks after therapy.

Statistical Analyses

The parent therapeutic trial's primary endpoint was CB at 24 weeks after starting GTx-024. Therefore, participants in the imaging sub-study were followed until the 24-week assessment. The lesion with the highest FDHT uptake at baseline was correlated with AR status. Percent change in FDHT uptake using the single-hottest lesion and sum of all measured lesions was

calculated between baseline (S0), week 6 (S1) and week 12 (S2) scans: $\left(\frac{S1\ or\ S2-S0}{S0}\right) * 100\%$. We also explored the correlation of percent change in FDHT uptake between the single-hottest lesions at baseline and follow up to BOR (PERCIST-like criteria) (*14*).

Descriptive statistics summarized baseline and percent change in FDHT uptake. The Mann-Whitney U test compared baseline and percent change in FDHT uptake between BOR groups. Pearson correlations were used for continuous data correlating FDHT uptake versus AR status. At each time point, to account for non-independence among multiple lesions per patient, the referred as repeated measures correlation (rmcorr) was used to assess the common intra-patient association for the various paired quantitative PET parameters to each other (*15*). All P values are two-sided, and all confidence intervals are at the 95% level, with statistical significance defined as $P \leq 0.05$.

RESULTS

Participants and Lesions

Eleven women (median age 59 years, range 47-73) enrolled in the FDHT-PET/CT sub-study (Table 1) and were scanned between March 2017 and February 2018. Ten were randomized to receive 9 mg of GTx-024 and one to receive 18 mg. Nine women completed baseline, 6-week, and 12-week FDHT-PET/CT scans. Two were taken off-study prior to the 12-week scan: one at week 6 due to toxicity and one at week 7 for PD (Figure 1). BOR was CB for 7 participants and no CB for 4 participants.

Table 2 shows all participants' results regarding AR status, FDHT uptake at all time points and outcomes. FDHT uptake was measured in 40 lesions (median 4 per participant, range 1-8). Although all lesions were >1 cm in one dimension, a 2D region of interest was used for

SUV/SUL)max and mean for 13 tumors because either a 1 cm^3 sphere could not be placed within the tumor's anatomic boundaries or due to low uptake, SUV(/SUL)peak was technically unable to be determined. At all imaging time points, high correlations were observed between all PET parameters measured (Supplemental Figure 1). Therefore, the primary analyses are presented with SUVmax.

AR Status vs. Baseline FDHT Uptake

AR status was assessed in 9 of 11 women; 2 from primary tumor and 7 from metastases. Seven tumors were AR+ and 2 were AR- (both from metastatic disease). Two women had inadequate archival tissue available to determine AR status. Median baseline FDHT-SUVmax was 4.1 (range 1.4-5.9) for AR+ tumor and 2.3 (range 1.5-3.2) for AR- tumor (p=0.22, Figure 2A-C). A weak, not significant, correlation was found for baseline FDHT-SUVmax versus quantitative AR expression levels (Pearson rho=0.39, p=0.30, Figure 3). SUVmean had similar results (Supplemental Figure 2).

Baseline FDHT Uptake and Change in FDHT Uptake versus Best Overall Response

Baseline FDHT-SUVmax of the hottest lesion per participant was similar for the 7 participants with CB at 12 weeks after therapy (median 4.1, range 1.4-5.9) compared to the 4 with PD (3.3, 1.5-5.1, p=0.53, Figure 4A). Results were similar for hottest lesion SUVmean and summed SUVmax and summed SUVmean of all lesions (Supplemental Figure 3).

Participants with CB at 12 weeks tended to have larger declines in FDHT uptake at 6 weeks (median decline 26.8%, range -42.9 to -14.1%) after starting GTx-024 compared to those with PD (median decline 3.7%, range -31% to +29%, p=0.11). A similar trend was observed at the 12-

week FDHT-PET/CT scan with a median decline of 35.7% (range -69.5 to -7.7%) for those with CB compared to a median decline of 20.1% (-26.6% to +56.5%, p=0.17) for those with PD (Figures 4B-C, 5). Similar trends were observed for hottest lesion FDHT-SUVmean at 6 and 12 weeks after starting GTx-024 and for summed SUVmax at 6 weeks (Supplemental Figures 4, 5). 6-week summed FDHT-SUVmean declines were larger for those with versus without CB (p=0.04, Supplemental Figure 6). Percent decrease in FDHT-SUVmax between the single hottest lesions at baseline and follow up (i.e., PERCIST-like criteria) was significantly larger for those with CB (percent decline 21.4%, range -42.9% to -14.1%) versus without CB (percent increase 7.6%, range -17.1% to +29.9%, p=0.01) at week 6, but not at week 12 (p>0.5, Supplemental Figure 7).

Five of seven participants with CB at week 12 after starting GTx-024 progressed by week 24. The two participants with continued CB at 24 weeks had the largest declines in FDHT uptake at week 6 and were among the top 3 for largest decline in FDHT uptake at week 12.

FDHT Uptake vs. Hormone and PSA Levels

No correlations were found between baseline FDHT uptake, estradiol, and testosterone levels (Supplemental Figure 8). There tended to be higher baseline FDHT uptake with lower baseline SHBG (Supplemental Figure 9). No correlations were found at baseline or during treatment comparing SUVmax/SHBG to AR status and CB (Supplemental Figure 10). There were no correlations between baseline FDHT-SUVmax and PSA levels. Although the participant with the largest decline in FDHT-SUVmax also had categorical declines in PSA levels at 6 and 12 weeks after starting GTx-024 and CB, no correlations between changes in PSA, FDHT-SUVmax, or BOR were observed (Supplemental Figure 11).

DISCUSSION

Despite targeted therapy shifting therapeutic paradigms in many cancers, tumor heterogeneity, inability to biopsy every lesion, and target conversion within tumor remain challenges to predict who will benefit from specific therapeutic agents. Non-invasive, whole-body molecular imaging evaluates the entire tumor burden providing one potential solution but remains a globally underutilized tool for optimizing therapeutic strategies in large clinical trials (*16,17*). Our data supplements prior work by demonstrating the feasibility of using FDHT-PET/CT for evaluating response to SARM therapy in a large therapeutic clinical trial.

In 13 patients with ER+ MBC who underwent FDHT-PET/CT and metastatic tumor biopsy within 8 weeks, Venema et al. found a correlation between FDHT uptake and AR expression (r^2=0.47, p=0.01) using a semi-quantitative assessment of >10% nuclear staining as positive for AR (*18*). Although not statistically significant, all but one AR+ tumor in our dataset had a baseline FDHT-SUVmax higher than the findings in AR- tumors suggesting a trend for higher baseline FDHT uptake in AR+ tumors. One AR- tumor had baseline FDHT uptake greater than the optimal SUVmax cutoff of 1.9 suggested for differentiating AR+ vs. AR- tumor by FDHT-PET/CT (*18*). Small sample size, tumor heterogeneity, and non-paired lesions for AR status and FDHT uptake may drive the lack of significance in our dataset. Our results as well as those of Venema et al. support further investigation of FDHT-PET/CT as an imaging biomarker of AR expression (*18*).

AR expression is heterogeneous between primary breast cancer and metastases with discordance rates up to 33% (*19*), and in metastases over the natural disease history (*20*). Venema et al. reported AR+ primary tumor with AR- metastatic disease in 2 of 13 patients and

and substantial intra-patient FDHT heterogeneity (*18*), with patients having both FDHT positive and negative lesions and FDHT-SUVs ranging from 0.8-6.5.

The therapeutic trial inclusion criteria mandated objective evidence of progression within 30 days of randomization, but standard imaging modalities, i.e., CT and bone scan, for this purpose often fail to differentiate active disease versus disease that previously responded to treatment. This likely contributed to tumor heterogeneity in our imaging sub-study. FDG-PET/CT may be useful to supplement or replace standard imaging to better identify metabolically active tumor burden and guide interpretation of FDHT-PET/CT. Such a strategy was employed with FES-PET/CT imaging for breast cancer bone metastases (*21*).

Testosterone, DHT, and FDHT competitively bind AR (*7*), and SHBG binds sex hormones, including DHT. Categorically low sex hormones levels in this postmenopausal population likely limited our ability to identify any correlations between baseline FDHT uptake, testosterone, estrogen or PSA levels. Further, the trend we observed for an inverse relationship between FDHT uptake and serum SHBG may be explained by SHBG binding decreasing FDHT availability for tumor binding given the low levels of estrogen and testosterone in our participants. Kramer et al. used a simplified method to correct $SUV_{\text{body weight}}$ for serum SHBG and found an improved correlation with Patlak *Ki* derived from dynamic images (*22*). We did not find any statistically significant correlations at baseline or during treatment between SUVmax/SHBG and AR expression or BOR. Future studies of androgen modulation should be designed considering hormonal status of the study population.

To our knowledge, this is the first study assessing changes in FDHT uptake on PET in patients with MBC treated with SARM therapy. Although CB was not associated with baseline FDHT uptake, those with CB within the first 12 weeks of treatment tended to have larger percent

declines in FDHT uptake after 6 and 12 weeks of SARM therapy for most of the semi-quantitative parameters we explored. All but 2 participants progressed by 24 weeks after starting therapy, but 2 of the 3 participants with the largest FDHT declines at 6 and 12 weeks after starting GTx-024 continued to have CB at 24 weeks. Larger studies are needed to determine the percent decline in SUV that correlates with a clinical disease response.

Boers et al. recently evaluated FDHT-PET/CT for assessing changes in AR availability in 21 patients with AR+ MBC receiving bicalutamide, a pure AR antagonist (*23*). Like our findings with GTx-024, baseline FDHT uptake did not predict clinical benefit to bicalutamide. Decreases in FDHT uptake after 4-6 weeks of bicalutamide also did not predict clinical benefit in the total study population which included both ER+ and ER- tumors, contrasting our findings. In a subgroup analysis of 13 patients with ER+ tumor, the authors reported a trend for larger FDHT uptake declines in 5 patients with clinical benefit from bicalutamide versus 8 with PD (n=8) (*23*). Our study only included participants with ER+ breast cancer and our results support the sub-group analysis trend. The different pharmacology between GTx-024 and bicalutamide is also noted and is important to understand when evaluating imaging biomarkers in specific breast cancer sub-types.

Early imaging time points would be most advantageous for limiting use of ineffective therapy and unnecessary toxicities. The PERCIST-like criteria at 6 weeks after starting therapy best separated those with and without clinical benefit in our cohort. The optimal parameter still needs to be determined in larger studies.

This study had several limitations, notably the small number of participants enrolled. Prospectively including an imaging study in a larger therapeutic trial, however, ensured standardization of FDHT-PET/CT imaging and response assessments using RECIST 1.1. Lesions

were not paired for AR status and FDHT uptake assessment because the parent therapeutic trial allowed archival tissue specimens. Although metastases represented most archival tissue specimens (n=7), they still may not have been from the same site or organ. Additional limitations are the use of different PET/CT scanners and randomization of one participant to the higher GTx-024 dose level. Finally, not including FDG-PET/CT, in addition to, or instead of standard imaging (i.e., CT and bone scan), to identify metabolically active tumor burden to follow for disease response on a new therapeutic trial remains a challenge. We believe that the inclusion of noninvasive whole-body functional imaging combining a metabolic tracer such as FDG, and a specific hormonal targeting agent such as FDHT should be encouraged in this patient population since it could improve tumor characterization, assess tumor heterogeneity, guide biopsy, help with decision making and evaluation of therapeutic response, while also helping validate the investigational specific radiotracer.

CONCLUSION

Our data suggest that FDHT-PET/CT may be a useful imaging biomarker for evaluating response of MBC to SARM therapy and other AR-expressing malignancies and reiterates the feasibility of including molecular imaging in multidisciplinary therapeutic trials. Establishing repeatability and reproducibility of quantitating FDHT uptake in breast cancer and thresholds for predicting response are required next steps to establish FDHT-PET/CT as a non-invasive molecular imaging biomarker. This may be challenging given the underlying tumor heterogeneity seen in hormonally-driven breast cancer.

DISCLOSURE

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KEY POINTS

QUESTION: Does FDHT uptake on PET/CT in metastatic breast cancer correlate with tumor androgen receptor (AR) status and predict response to selective androgen modulation (SARM) therapy?

PERTINENT FINDINGS: In a prospective imaging sub-study of 11 women with metastatic breast cancer receiving SARM therapy, we showed trends associating larger declines in FDHT uptake after starting therapy with clinical benefit.

IMPLICATIONS FOR PATIENT CARE: With further validation in well-designed clinical trials, FDHT-PET/CT could be a valuable tool to characterize tumors and direct strategies modulating AR signaling in breast cancer and other AR positive malignancies.

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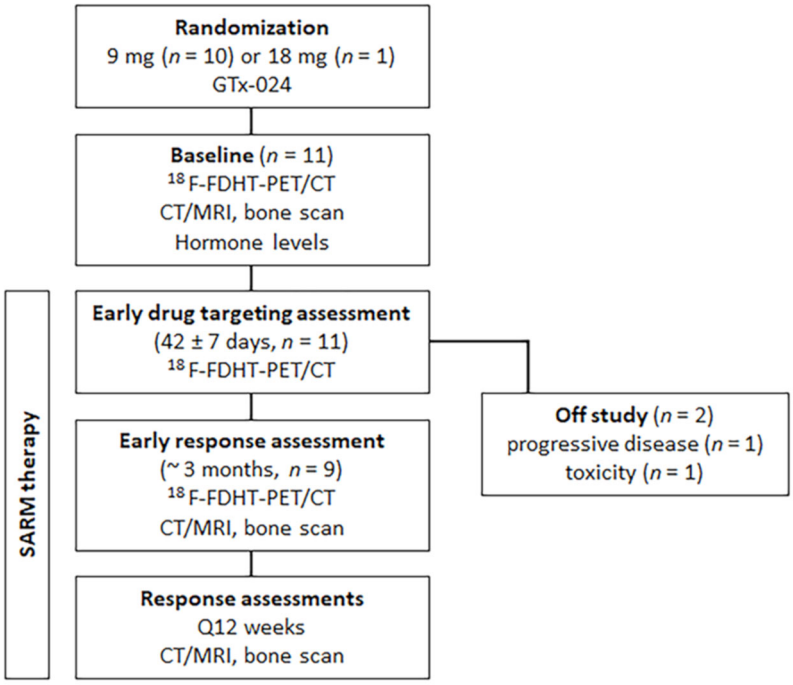


FIGURE 1. Study Schema.

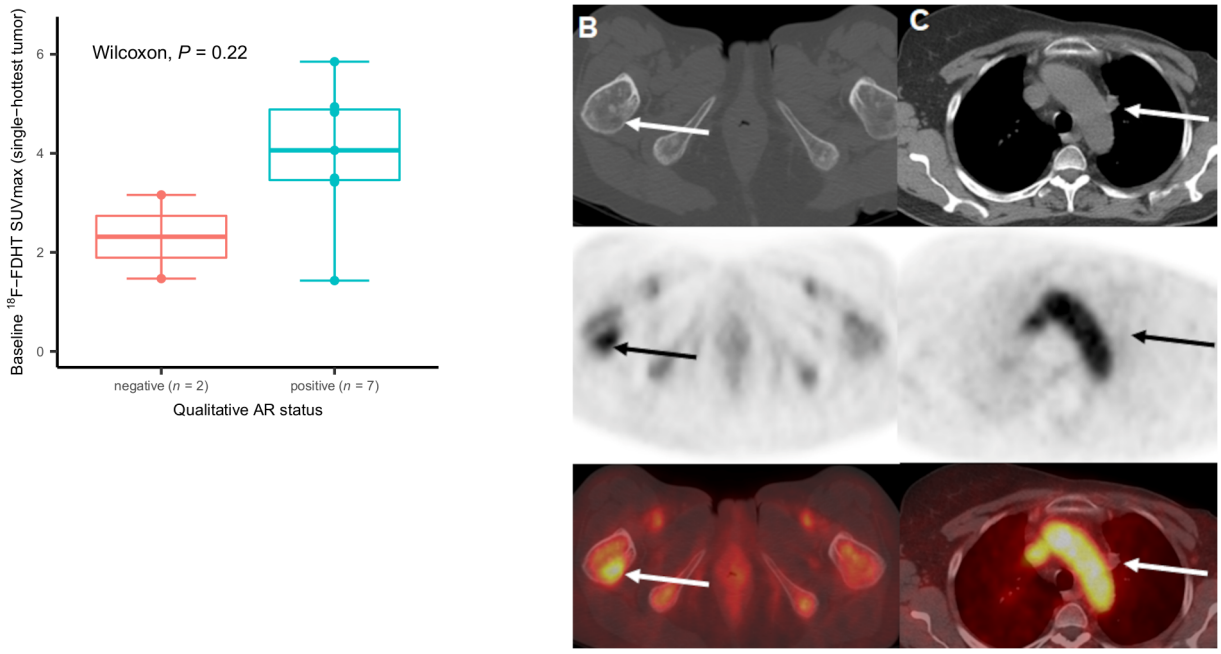


FIGURE 2. Baseline FDHT Uptake and Qualitative Androgen Receptor Status. A) For 9 participants with archival tissue, median baseline FDHT SUVmax was 4.1 (range 1.4 – 5.9) for 7 participants with AR+ tumor and 2.3 (range 1.5 – 3.2) for 2 with AR- tumor (p=0.22). Individual dots on the boxplot represent individual participant's data. **B)** Participant 6 with AR+ tumor and FDHT uptake in a right femur metastasis (arrows, SUVmax 4.9). **C)** Participant 8 with AR- tumor and no FDHT uptake in a prevascular lymph node (arrows, SUVmax 1.5).

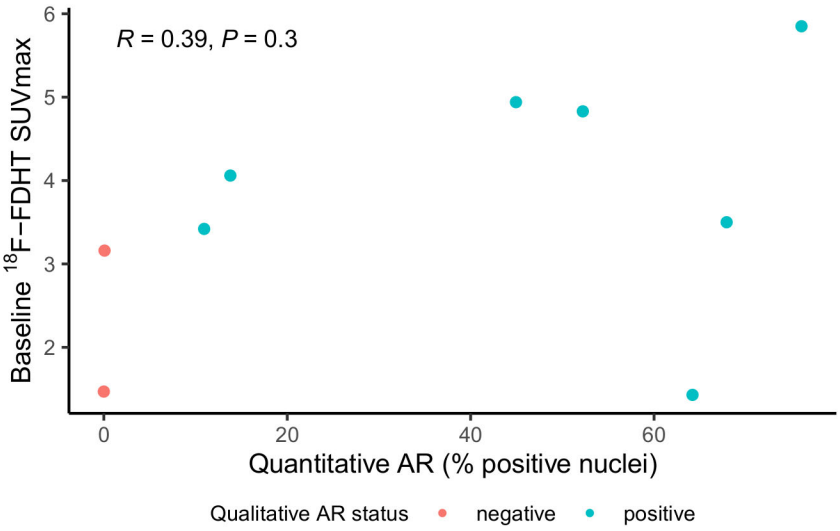


FIGURE 3. Baseline FDHT Uptake and Quantitative Androgen Receptor Status. A weak, but not statistically significant, correlation was observed between quantitative AR expression levels and baseline FDHT uptake (Pearson rho=0.39, p=0.30). (Blue dots: AR+ tumors; red dots: AR-tumors).

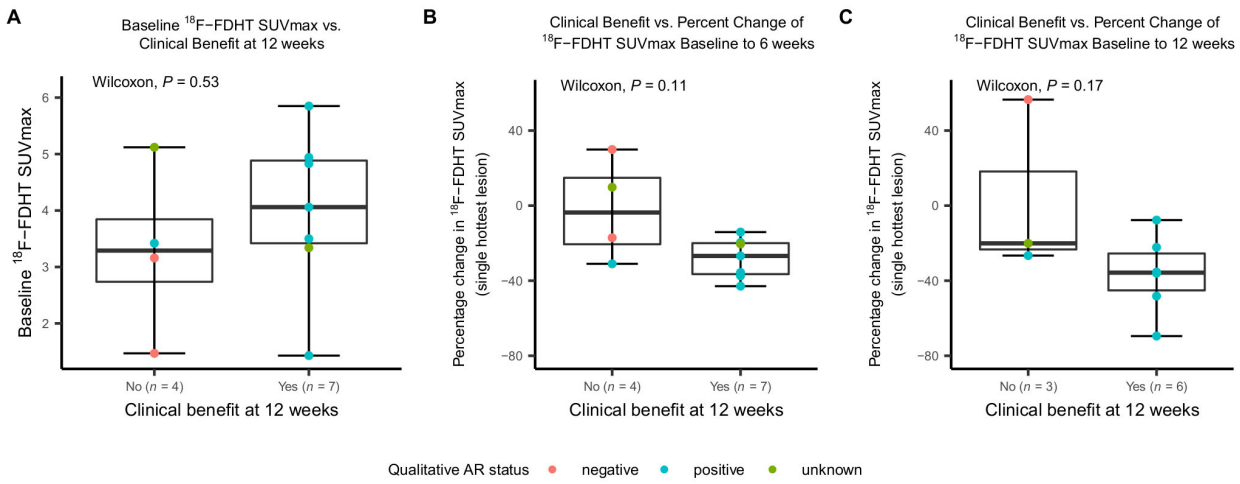


FIGURE 4. Clinical Benefit at 12 Weeks after Starting Therapy vs. Baseline and Change in FDHT Uptake. A) For 7 participants with clinical benefit, median baseline FDHT SUVmax was 4.1 (1.4-5.9) compared to 3.3 (1.5-5.1) for 4 participants with disease progression, p=0.53. Individual dots on the scatterplot represent individual participant's data.

Participants with clinical benefit at 12 weeks tended to have larger declines in FDHT uptake **B)** at 6 weeks (median decline 26.8%, range -42.9 to -14.1%) after starting GTx-024 compared to those with disease progression (median decline 3.7%, range -31% to +29%, p=0.11) and **C)** at 12 weeks (median decline 35.7%, range -69.5 to -7.7%) after starting GTx-024 compared to those with disease progression (median decline 20.1%, range -26.6% to +56.5%, p=0.17).

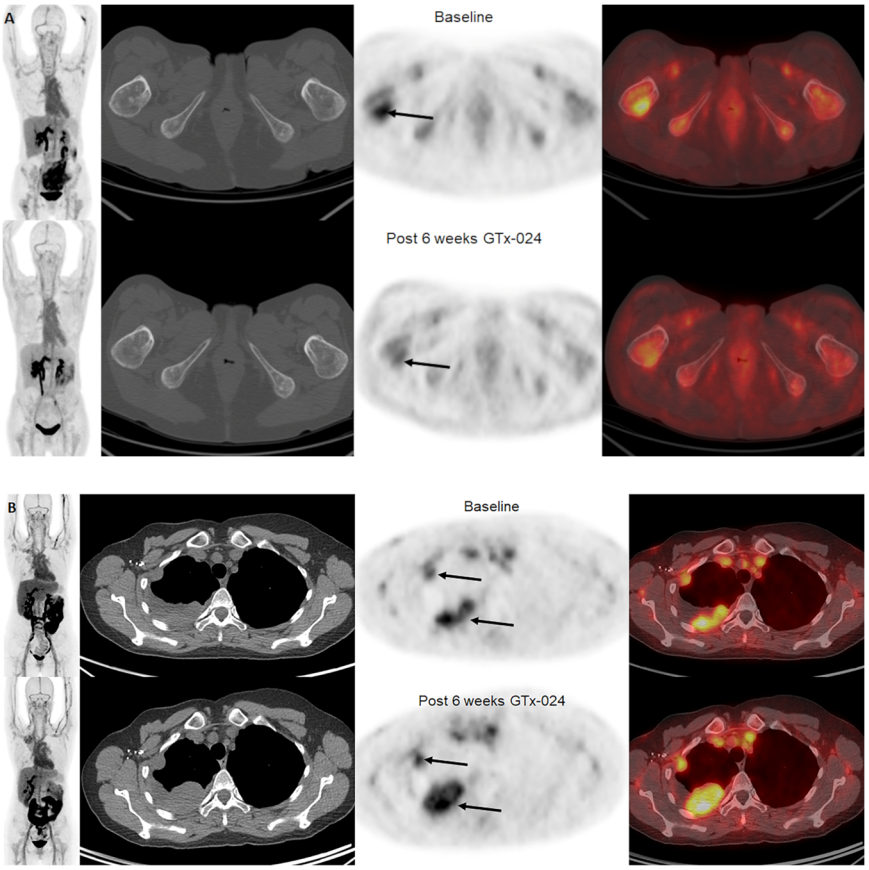
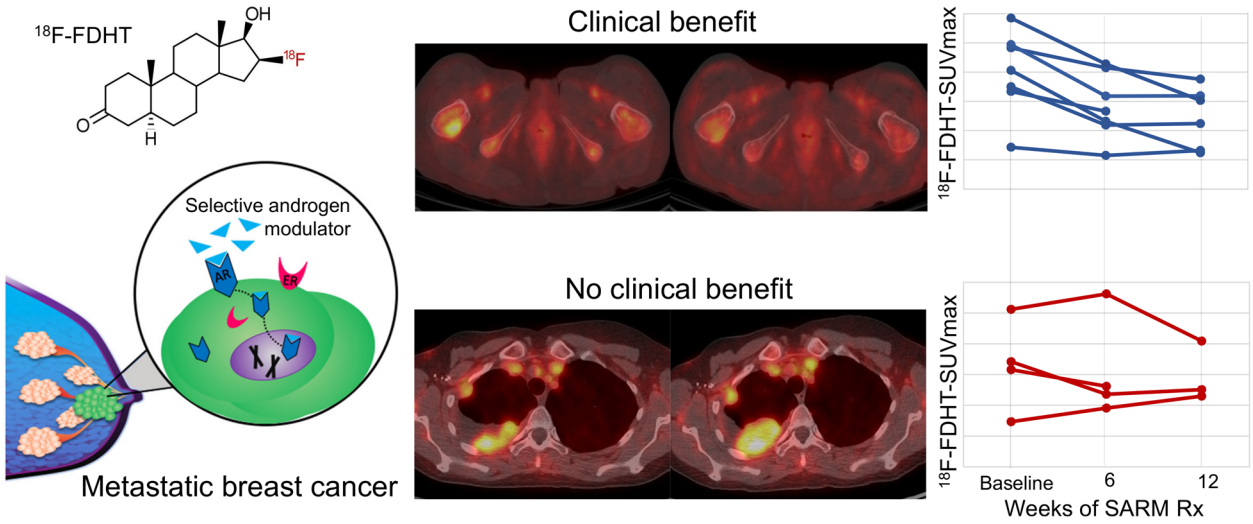


FIGURE 5. A) FDHT-avid AR+ tumor at baseline (top row, arrow SUVmax 4.9) and decline in FDHT uptake 6 weeks after starting GTx-024 (bottom row, arrow). Best overall response was stable disease 12 weeks after starting therapy. **B)** FDHT-avid AR+ tumor at baseline (top row, arrow SUVmax 5.1), no decline in FDHT uptake and increased tumor size 6 weeks after starting GTx-024 (bottom row, arrows). Best overall response was progressive disease 12 weeks after starting therapy.

Graphical Abstract



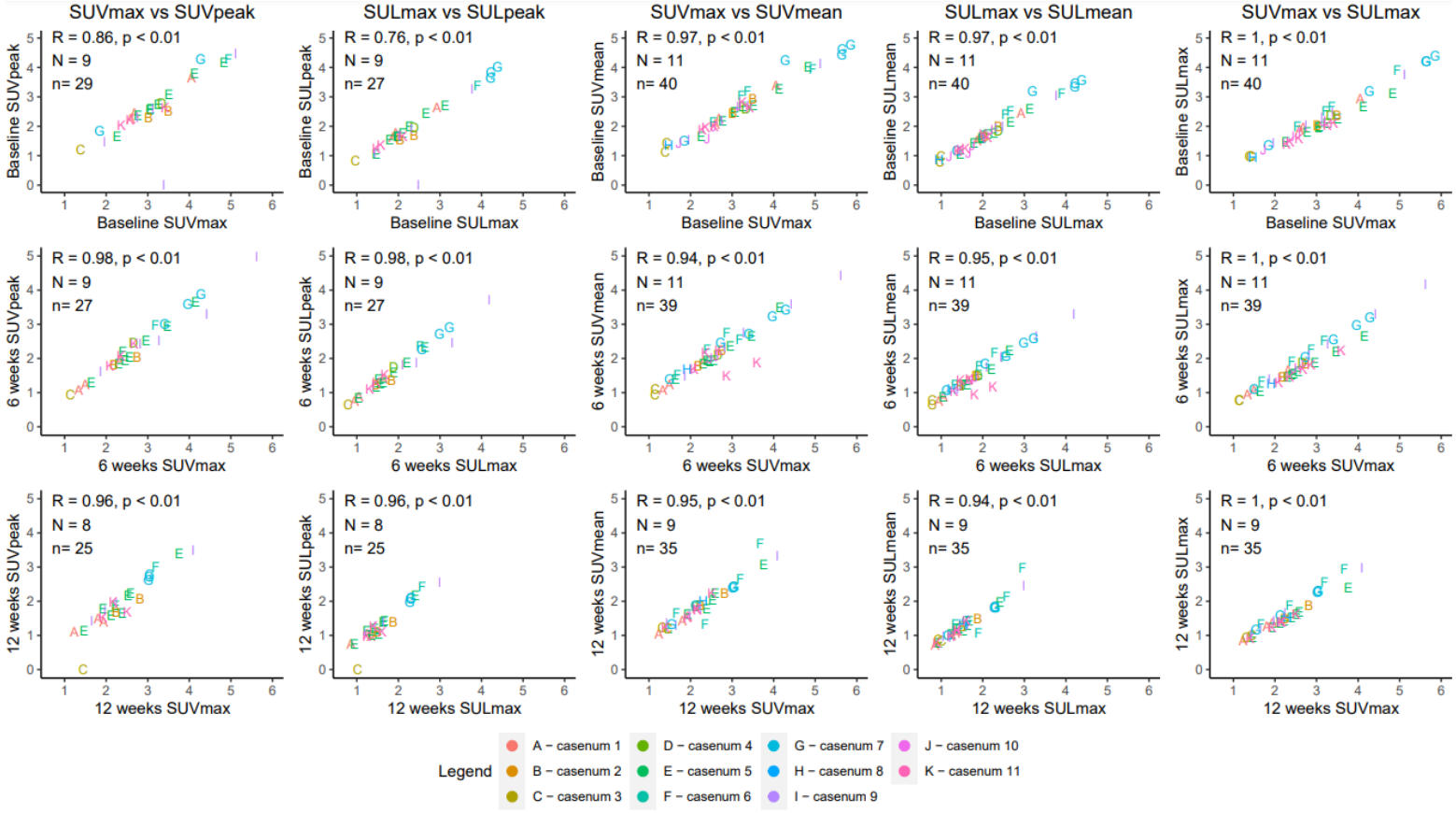
TABLES

Table 1. Participant and Breast Cancer Tumor Characteristics (N=11)		
Age (median, range)	59 years (49-73 years)	
Histology	**N**	
Invasive ductal carcinoma	9	
Invasive lobular carcinoma	2	
Receptor status		
$ER^+/PR^+/HER2-$	6	
$ER^+/PR^-/HER2-$	5	
Metastases at Diagnosis		
Yes	4	
No	7	
Treatment prior to enrollment (median no. lines, range)	**Metastases at Diagnosis (n=4)**	**No Metastases at Diagnosis (n=7)**
Disease-free interval*	not applicable	7 years (3-19 years)
Adjuvant chemotherapy	not applicable	1 (0-1)
Adjuvant endocrine therapy	not applicable	1 (0-2)
Chemotherapy for metastatic disease	1 (0-1)**	0 (0-1)
Endocrine therapy for metastatic disease	2 (1-4)	2 (1-6)
CDK4/6 inhibitor	2	6
mTOR inhibitor	1	2
Dual PI3Kinase mTOR inhibitor	0	1
Radiation therapy to metastatic disease	1 (bone)	2 (bone)
Median number of metastatic sites at enrollment (range)	2 (1-4)	
Location of metastatic sites at enrollment Bone (bone-only) Visceral (liver, vaginal cuff) Pleura Serosa/peritoneal Lymph node	 8 (5) 4 5 2 2	
*disease-free interval: time from adjuvant therapy start to first diagnosis of recurrence/metastatic disease **n=1 with high-dose chemotherapy and stem cell transplant		

Table 2. Androgen Receptor (AR) Tumor Status, FDHT-PET/CT, and Clinical Outcomes								
				% Change FDHT-SUVmax Baseline to		Outcomes		
Participant	**No. of Lesions**	**AR Status/Archival Tissue Location**	**Baseline FDHT-SUVmax Hottest Lesion**	**Week 6**	**Week 12**	**Best Overall Response**	**Time of Best Overall Response Week:**	**Week 24 Response**
1*	3	positive/primary	4.1	-43%	-70%	nonCR/nonPD[§]	12	Clinical benefit
2	2	positive/metastasis	3.5	-37%	-36%	nonCR/nonPD[§]	12	Clinical benefit
3	2	positive/metastasis	1.4	-20%	-8%	nonCR/nonPD[§]	12	No clinical benefit
4	1	not assessed	3.3	-20%	Off-study[†]	nonCR/nonPD[§]	6	No clinical benefit
5	8	positive/metastasis	4.8	-14%	-22%	PR	12	No clinical benefit
6	4	positive/metastasis	4.9	-36%	-35%	SD	12	No clinical benefit
7	5	positive/primary	5.9	-27%	-48%	SD	12	No clinical benefit
8	1	negative/metastasis	1.5	+30%	+56%	PD	12	No clinical benefit
9	5	not assessed	5.1	+10%	-20%	PD	12	No clinical benefit
10	4	negative/metastasis	3.2	-17%	Off-study[†]	PD	7	No clinical benefit
11	5	positive/metastasis	3.4	-31%	-27%	PD	12	No clinical benefit
		*Received 18 mg GTx-024; all others received 9 mg GTx-024 [†]Baseline and week 6 scan only; #4 off study week 6 due to toxicity, #10 off study week 7 due to progression [§]nonCR/nonPD denotes incomplete response but no disease progression for participants with non-measurable disease by RECIST 1.1 Response by RECIST 1.1: CR- complete response; PR: partial response ; SD: stable disease ; PD: progressive disease						

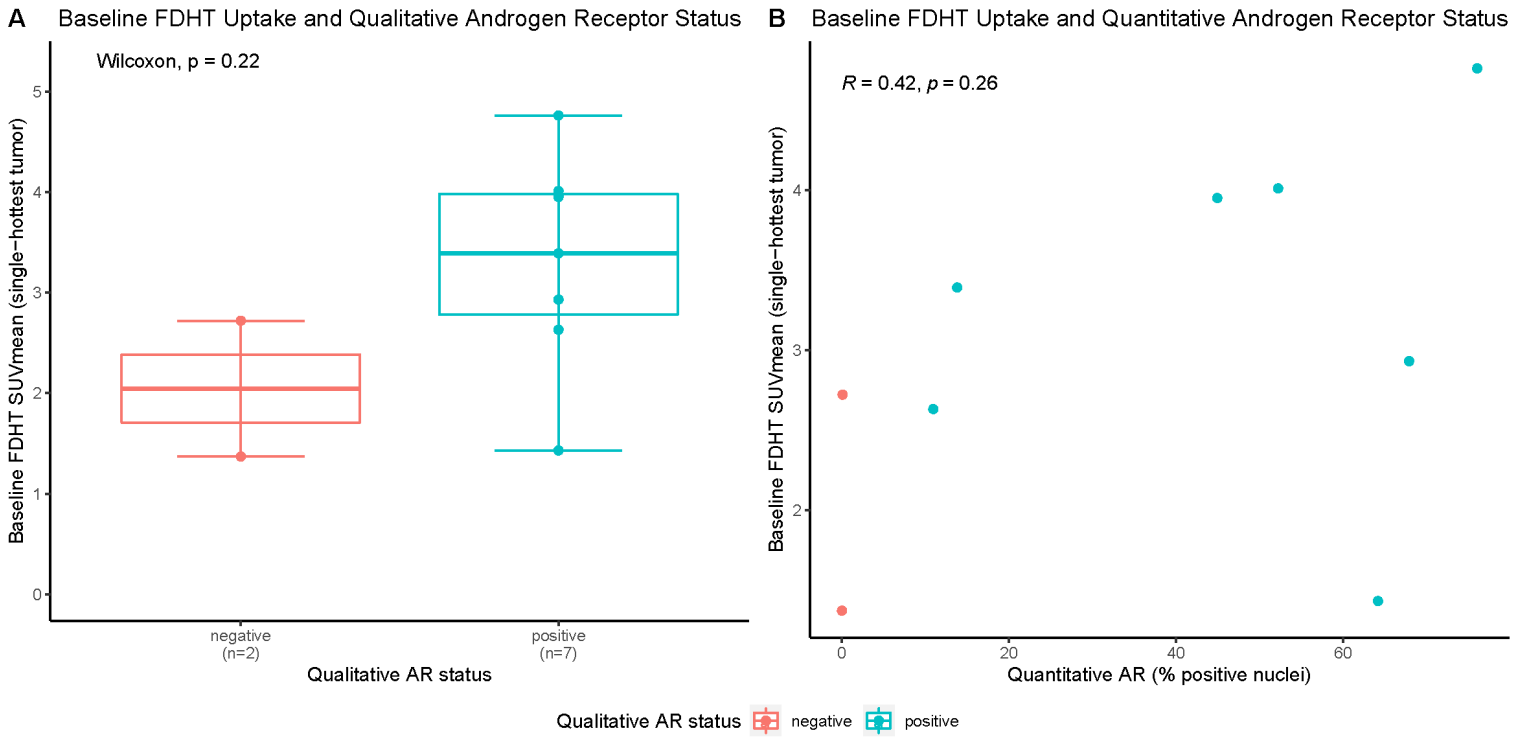
SUPPLEMENTAL DATA

Supplemental Figure 1. Correlations of Semiquantitative Parameters of FDHT Uptake. SUVmax, SUVpeak and SUVmean were highly correlated.



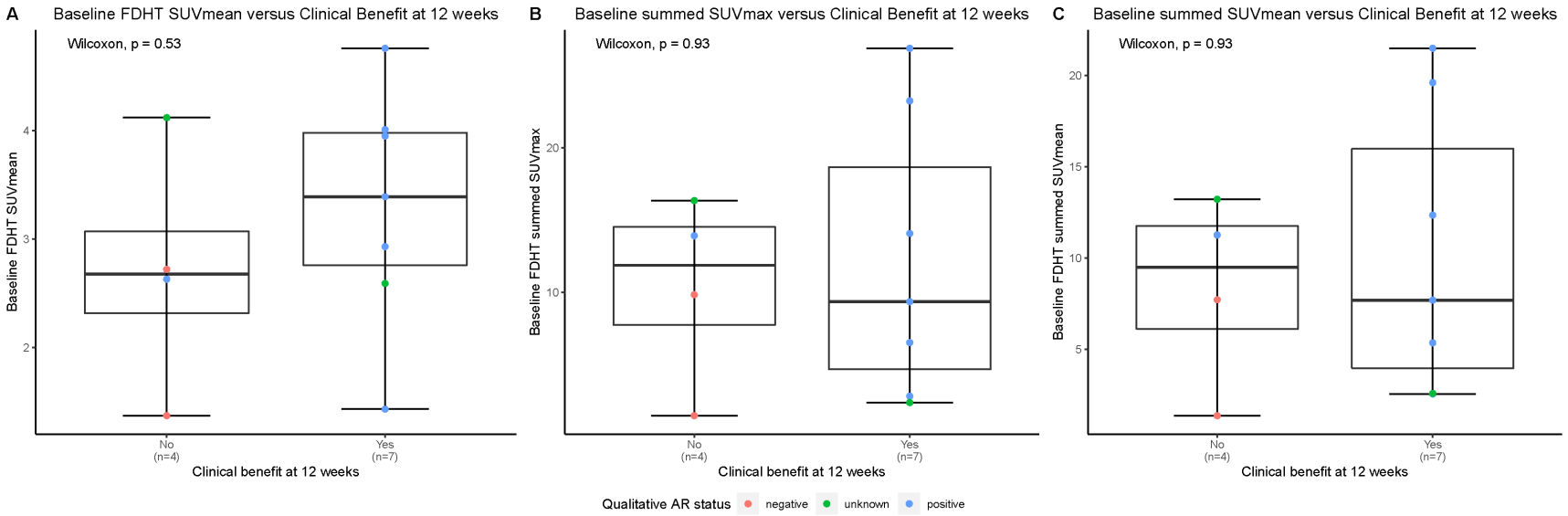
R: repeated measures correlation;
N: number of patients;
n: number of lesions;
Legend: letters corresponding to each patient

Supplemental Figure 2. Correlation of Baseline FDHT SUVmean and Qualitative and Quantitative AR Status.



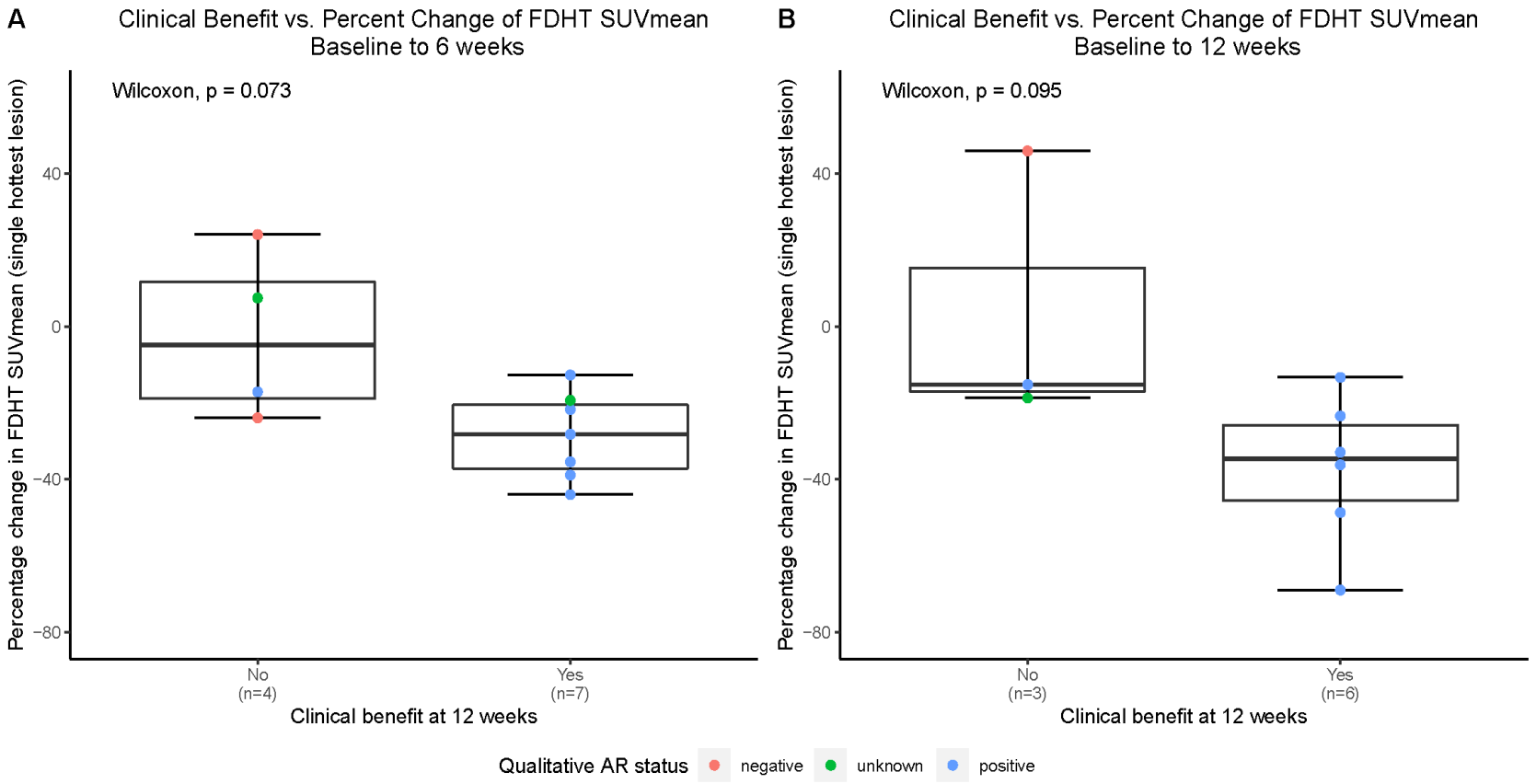
A) For 9 participants with archival tissue assessed for AR status, median baseline FDHT SUVmean was 3.4 (1.4-4.8) for 7 participants with AR positive tumor and 2.0 (1.4-2.7) for 2 with AR negative tumor (p=0.22). The individual dots on the scatterplot represent individual participant's data. **B)** There was a weak correlation between quantitative AR expression levels and baseline FDHT uptake, but this was not statistically significant (Pearson rho=0.42, p=0.26). Blue dots represent participants with AR positive tumor. Red dots represent participants with AR negative tumor.

Supplemental Figure 3. Clinical Benefit at 12 Weeks after Starting Therapy vs. Baseline FDHT SUVmean, summed SUVmax, and summed SUVmean.



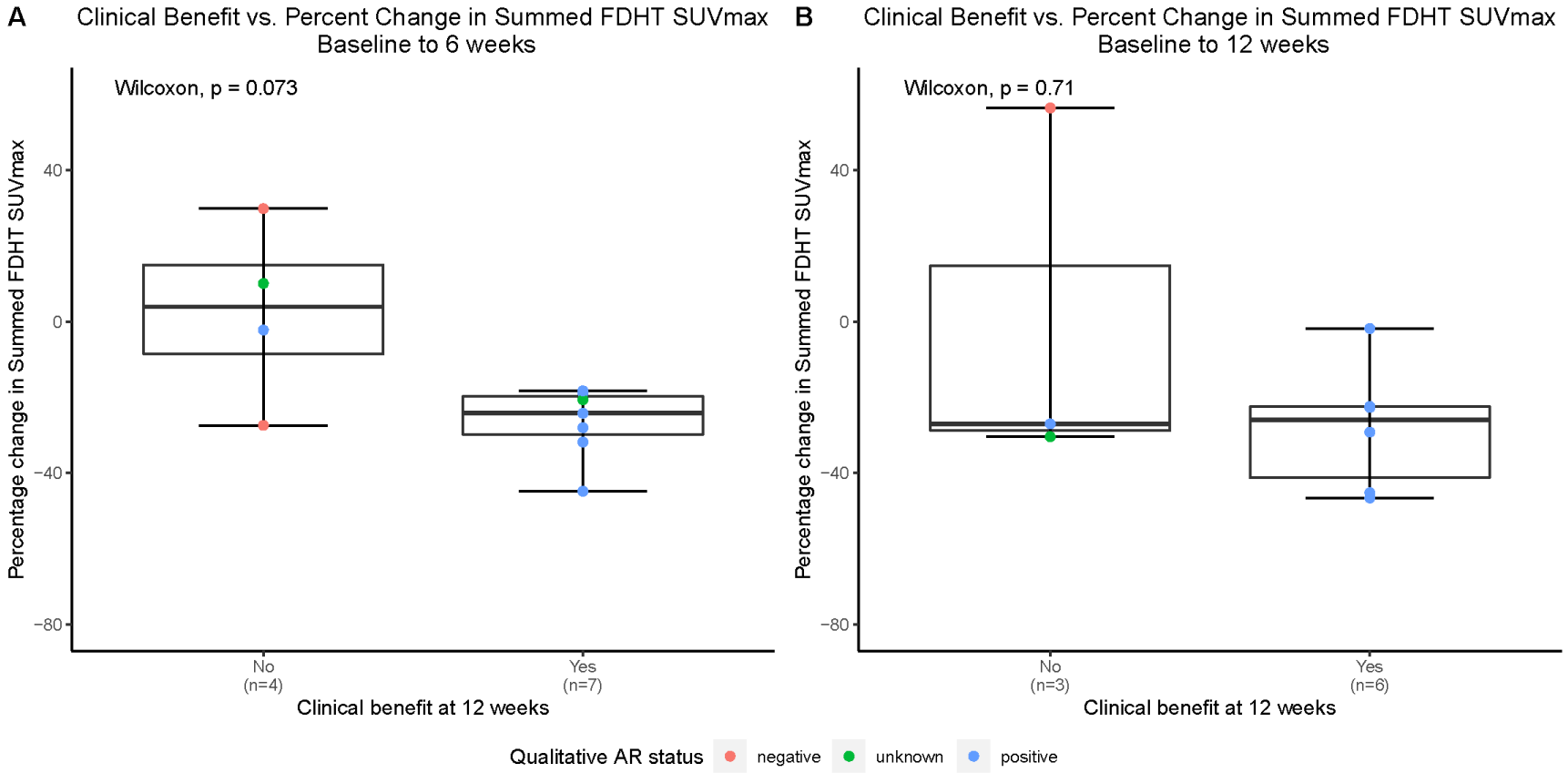
There were no significant differences between **A)** baseline FDHT SUVmean, **B)** baseline FDHT summed SUVmax, or **C)** baseline FDHT summed SUVmean and clinical benefit at 12 weeks after starting treatment with GTx-024. The individual dots on the scatterplot represent the individual participant's data.

Supplemental Figure 4. Clinical Benefit at 12 Weeks after Starting Therapy vs. Change in FDHT Uptake: SUVmean



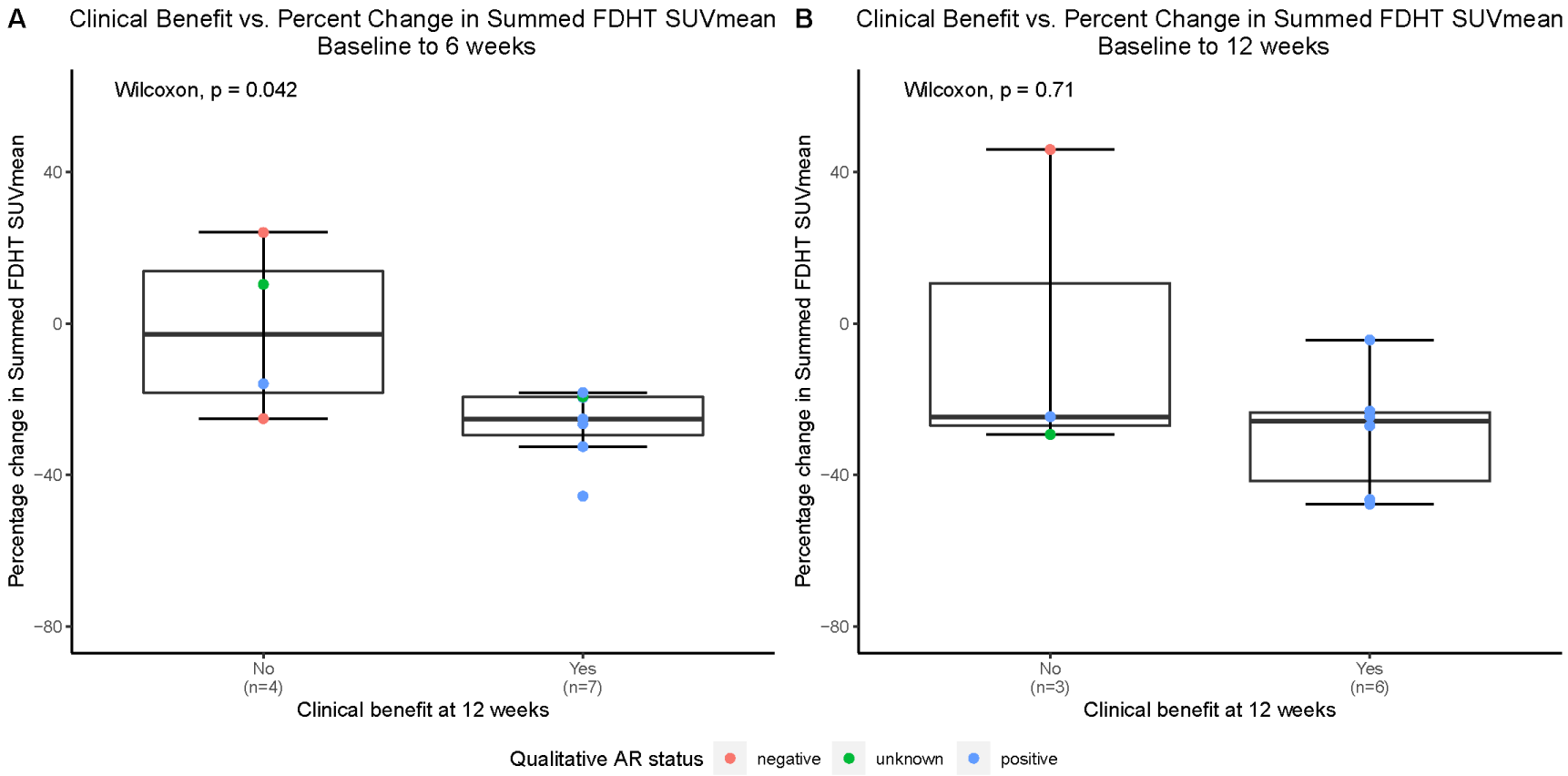
Participants with clinical benefit at 12 weeks tended to have larger declines in FDHT uptake for SUVmean (hottest lesion) at **(A)** 6 weeks after starting GTx-024 and **(B)** 12 weeks after starting GTx-024.

Supplemental Figure 5. Clinical Benefit at 12 Weeks after Starting Therapy vs. Change in FDHT Uptake: summed FDHT SUVmax



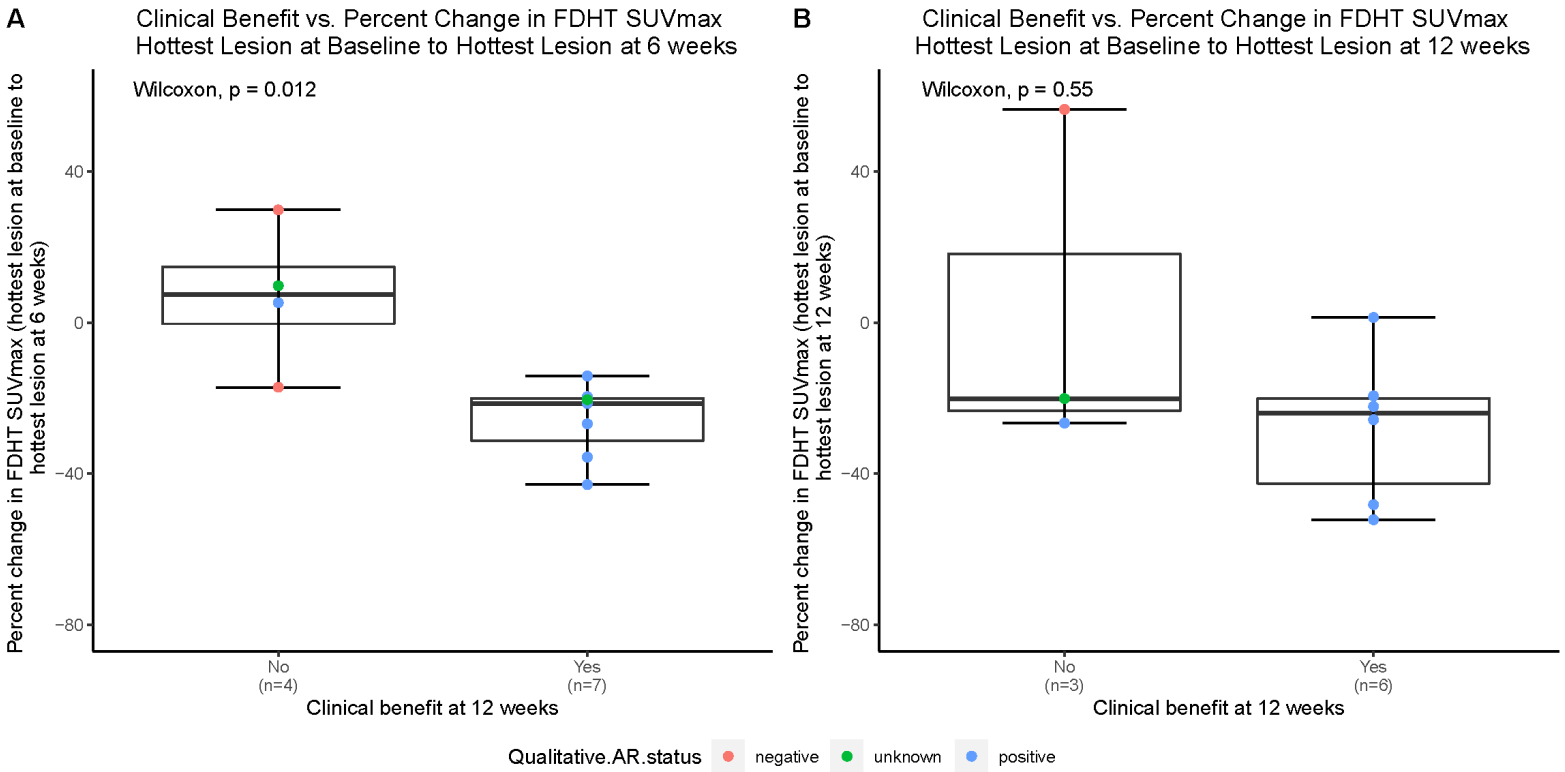
Participants with clinical benefit at 12 weeks tended to have larger declines in FDHT uptake for summed SUVmax at **(A)** 6 weeks after starting GTx-024, but not at **(B)** 12 weeks after starting GTx-024.

Supplemental Figure 6. Clinical Benefit at 12 Weeks after Starting Therapy vs. Change in FDHT Uptake: summed FDHT SUVmean



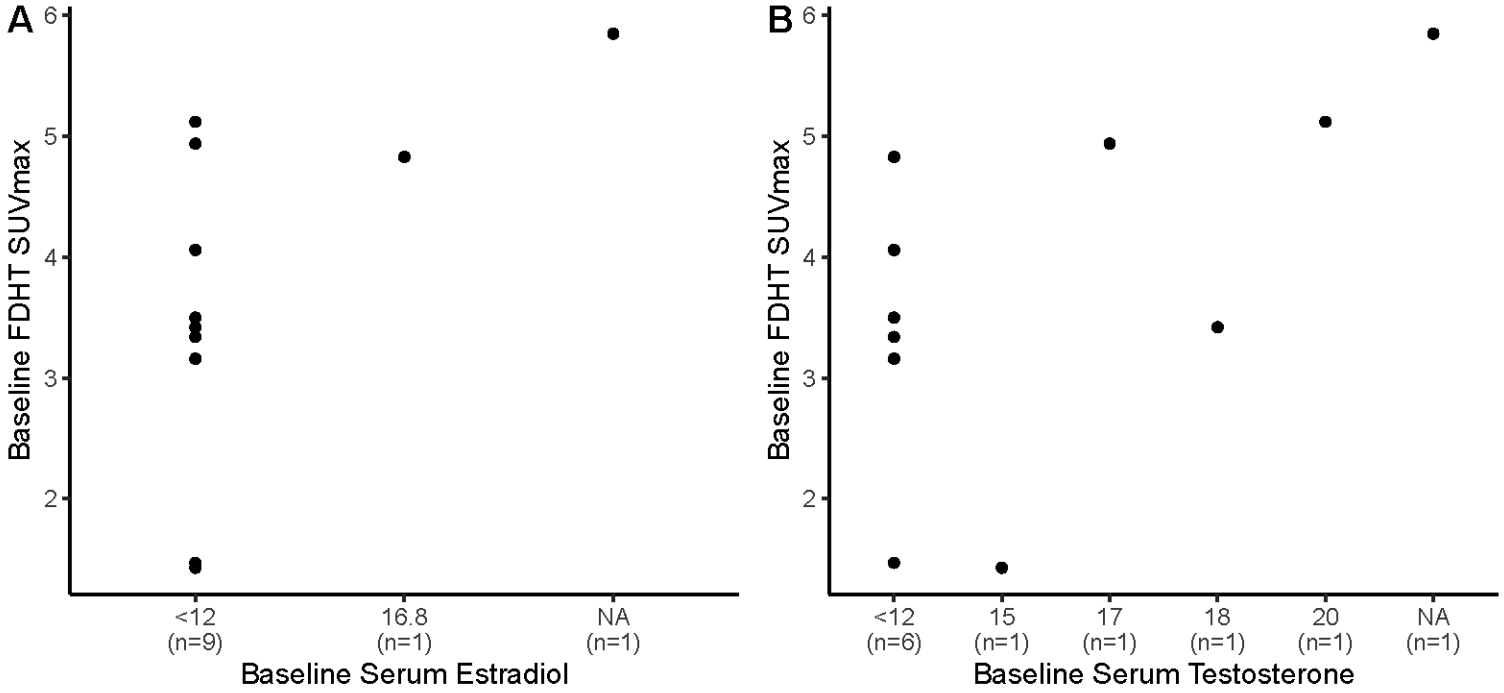
Participants with clinical benefit at 12 weeks tended to have larger declines in FDHT uptake for summed SUVmean at **(A)** 6 weeks after starting GTx-024, but not at **(B)** 12 weeks after starting GTx-024.

Supplemental Figure 7. Clinical Benefit at 12 Weeks after Starting Therapy vs. Change in FDHT Uptake Using PERCIST-like Criteria.

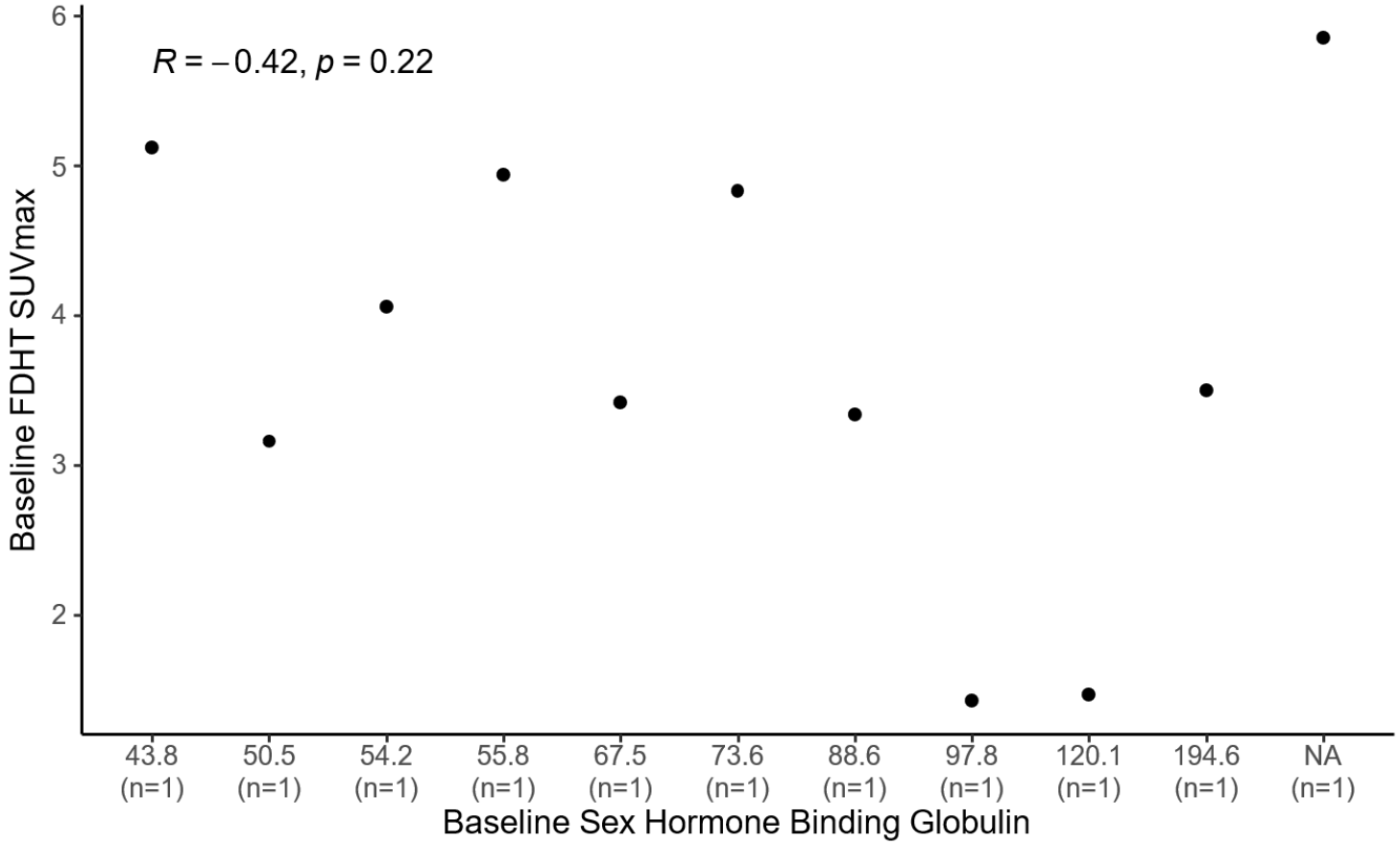


A) Participants with clinical benefit at 12 weeks had larger declines in FDHT SUVmax comparing the hottest lesion at baseline to the hottest lesion at week 6 (median decline 21.4%, range -42.9 to -14.1%) after starting GTx-024 compared to those with disease progression (6 weeks: increase 7.6%, range -17.1%to +29.9%, p=0.012). **B)** No significant differences were seen comparing the change in FDHT SUVmax of the hottest lesion at baseline to the hottest lesion at week 12 after starting GTx-024 between those with and without clinical benefit (p>0.5).

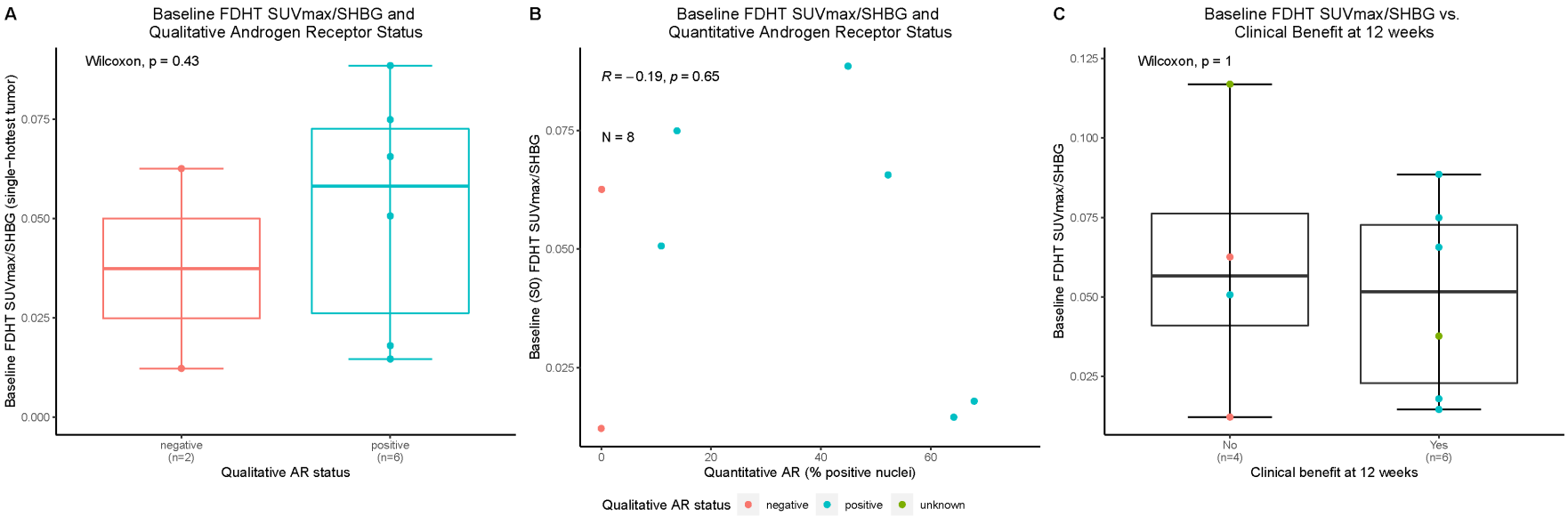
Supplemental Figure 8. Baseline FDHT uptake in tumor vs. baseline estradiol and testosterone levels. No correlations were observed.



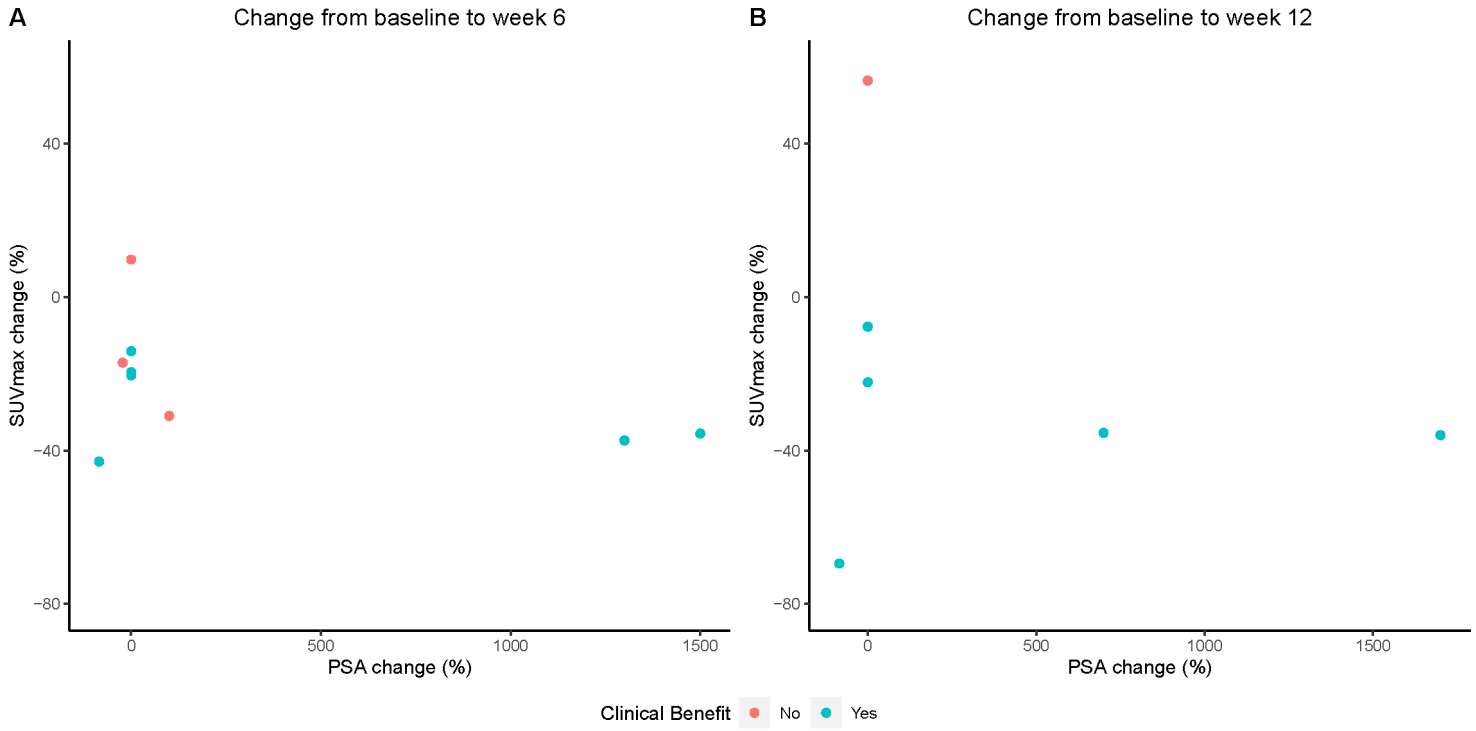
Supplemental Figure 9. Baseline FDHT uptake in tumor vs. baseline sex-hormone binding globulin levels. A trend towards higher baseline FDHT uptake with lower baseline sex-hormone binding globulin levels was observed.



Supplemental Figure 10. FDHT SUVmax/SHBG versus AR Status at Baseline and Clinical Benefit at 12 weeks: No correlations were observed.



Supplemental Figure 11. Change in PSA levels, FDHT uptake and best overall response.



No correlations were observed. At baseline, 10 participants had PSA levels assessed. At 6 weeks after starting GTx-024, 2 participants did not have PSA assessable for change: 1 with clinical benefit and 1 without clinical benefit. At 12 weeks after starting GTx-024, 5 participants did not have PSA assessable for change: 2 with clinical benefit and 3 without clinical benefit.