

## Oncotype Dx Results in Multiple Primary Breast Cancers

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### ABSTRACT

**PURPOSE:** To determine whether multiple primary breast cancers have similar genetic profiles, specifically Oncotype Dx Recurrence Scores, and whether obtaining Oncotype Dx on each primary breast cancer affects chemotherapy recommendations.

**METHODS:** A database of patients with hormone receptor-positive, lymph node-negative, breast cancer was created for those tumors that were sent for Oncotype Dx testing from the University of Michigan Health System from 1/24/2005 to 2/25/2013.

Retrospective chart review abstracted details of tumor location, histopathology, distance between tumors, Oncotype Dx results, and chemotherapy recommendations.

**RESULTS:** Six hundred and sixty-six patients for whom Oncotype Dx testing was sent were identified, with 22 patients having multiple breast tumor specimens sent. Of the 22 patients who had multiple samples sent for analysis, chemotherapy recommendations were changed in 6 of 22 patients (27%) based on significant differences in Oncotype Dx Recurrence Scores. Qualitatively, there seems to be a greater difference in genetic profile in tumors appearing simultaneously on different breasts when compared to multiple tumors on the same breast. There was no association between distance between tumors and difference in Oncotype Dx scores for tumors on the same breast.

**CONCLUSIONS:** Oncotype Dx testing on multiple primary breast cancers altered management in regards to chemotherapy recommendations and should be considered for multiple primary breast cancers.

**KEYWORDS:** breast cancer, oncotype dx, recurrence score

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### Introduction

Oncotype Dx is a polymerase chain reaction (PCR) assay of 21 genes that is frequently performed on tumors from women with lymph node-negative, estrogen receptor-positive breast cancer. It is used to calculate a recurrence score to estimate the risk of distant recurrence.<sup>1</sup> The 21 genes analyzed include markers of proliferation, invasion, Her2, Estrogen, and reference markers.<sup>2</sup> Given that more than 230,000 women are diagnosed with breast cancer each year, with nearly half of these women with node-negative, hormone receptor-positive disease, Oncotype Dx is applicable to a significant portion of breast cancer patients.<sup>3</sup>

Since the introduction of Oncotype Dx in 2004, there has been significant evidence that gene expression, as reflected

by recurrence score, affects the recommendation regarding chemotherapy for node-negative, estrogen receptor-positive breast cancer.<sup>4,5</sup> There have been numerous studies showing that for this population, Oncotype Dx affects clinical decision making, reduces unnecessary chemotherapy use,<sup>6–8</sup> and is a cost-effective assay.<sup>9,10</sup> In recent years, research has expanded to better understand the utility of Oncotype Dx in populations other than node-negative, estrogen receptor-positive breast cancer. Specifically, Trans Arimidex, Tamoxifen, Alone or in Combination (ATAC) showed that Oncotype Dx can be used as a predictive tool for recurrence in both node-negative and node-positive patients,<sup>11</sup> and SWOG 8814 showed that Oncotype Dx predicts benefit of chemotherapy in patients



with node-positive, estrogen receptor-positive disease with a high recurrence score.<sup>12</sup>

While breast cancer often occurs as a single tumor, studies using sectioning of mastectomy specimens identify additional, separate tumors in approximately 30% of patients with breast cancer.<sup>13,14</sup> In these mastectomy specimens, there may be a range of invasive to non-invasive cancers. While there has been significant investigation into the role of Oncotype Dx in the treatment of node-negative, estrogen receptor-positive breast cancer, there has been extremely sparse data on the role of Oncotype Dx in the treatment of multiple primary node-negative, estrogen receptor-positive breast cancers. Specifically, it is not known for multiple primary breast cancers whether the histopathology, receptor status, and genetic profiles as analyzed by Oncotype Dx are significantly different to warrant analyzing multiple synchronous tumors and furthermore, whether this analysis alters management. Our goal was to determine whether obtaining Oncotype Dx on multiple primary node-negative, estrogen receptor-positive breast cancers may lead to different chemotherapy recommendations.

## Methods

Institutional Review Board approval was obtained to review records for all patients older than age 18 with one or more samples sent for Oncotype Dx testing to Genomic Health from the University of Michigan Health System from 3/1/2003 to 3/1/2013, with the original patient list from Genomic Health Online. This included 666 patients from 1/24/2005 to 2/25/2013. Of the 666 patients, 16 were excluded because of inability to obtain medical record number (MRN). To qualify as having multiple primary breast cancers, masses had to appear within one month of each other on imaging and/or upon pathologic specimen processing. Of the remaining 650 patients, 522 patients had a single tumor focus; therefore, 128 patients had multiple primary tumors. Of these 128 patients, 102 patients only had a single Oncotype Dx sent, 2 patients had multiple Oncotype Dx sent but had insufficient tissue for analysis to be completed, 1 patient had multiple Oncotype Dx sent but these were separated in time by greater than one month, and 1 patient had pathology returned HER2 positive (HER+). Twenty-two patients with multiple primary tumors, multiple Oncotype Dx sent with sufficient tissue, and with adjuvant systemic therapy recommendations made up the analysis group.

Data extraction sheets were created prior to chart review, and included tumor size, location, histopathology, grade, receptor status, Oncotype Dx, distance between tumors, difference of recurrence scores between tumors, and whether the difference in recurrence score led to different management, specifically recommendation of administration of chemotherapy. Distance was determined using anatomic pathology when available, and radiographic imaging including mammography and ultrasound when distance between tumors was not available in the pathology report. To determine

whether the difference in recurrence score led to difference in chemotherapy recommendation, each set of tumors was analyzed in terms of Oncotype Dx risk stratification (low, intermediate, or high risk). Tumor sets that had one tumor that was low risk and another tumor that was either intermediate or high risk and chemotherapy was recommended, were considered have multiple primary tumors in which Oncotype Dx led to a difference in chemotherapy recommendations. Differences in tumor characteristics for each individual patient and between subgroups of those with similar versus different Oncotype Dx scores were analyzed using Fisher's Exact test for categorical variables and Wilcoxon rank sum test for continuous variables. The distance between two tumors on the same breast was correlated to the difference in Oncotype Dx scores using Spearman's correlation.

## Results

All study patients had hormone receptor-positive and lymph node-negative breast cancer as per their clinical pathology reports. The overall study group of 650 patients for whom Oncotype Dx was used as part of their clinical treatment planning included 641 females and 9 males, with ages ranging from 27 to 85, with an average age of 55.8. The 522 patients with non-multifocal tumors included 513 females and 9 males, with ages ranging from 27 to 85 with an average age of 56.0, and the 106 patients with multifocal tumors with only one tumor sent for Oncotype Dx were all female, with ages ranging from 30 to 77 with an average age of 54.5.

The analysis group included a total of 22 patients, with patient characteristics, tumor size, histopathology, receptor status, and Oncotype Dx score as seen in Table 1. All tumors were estrogen receptor-positive and HER2-negative. In regards to the analysis group, all 22 patients were female, and had ages ranging from 40 to 77 with an average age of 55.5 years. Of the patients for whom multiple Oncotype Dx specimens were sent, 18 patients had multiple primary tumors in the same breast and 4 patients had their multiple primary tumors in different breasts. The average distance between tumors for the 18 patients with multiple primary tumors in the same breast was 2.8 cm. Of the 45 tumors analyzed (one patient had 3 primary tumors analyzed), 62.2% were ductal carcinomas, 26.7% were lobular carcinomas, and 11.1% were mixed with ductal and lobular features.

**Bilateral breast cancers (N = 4).** Of the four patients with multiple primary tumors in different breasts, three (75%) patients had different histology between their tumors. Additionally, two of the four cases (50%) had different histologic grades, and one case of the four (25%) had differing progesterone receptor status. The average difference in Oncotype Dx recurrence score was 13.0. This data, along with the data for multiple primary tumors in the same breast, is summarized in Table 2.

**Multicentric/multifocal breast cancers (N=18).** Of the 18 patients with multiple primary tumors in the same breast,



Table 1. Summary of patient information.

#	AGE	T SIZE	LOCATION	T1-T2	T HISTO	T GRADE	PR	ONCDX	RISK	%REC	DIFF	REC CH?	
1	49	2.8 × 2.0 × 1.4	Right UO	0.6	Ductal	Grade 2	POS	14	Low	9%	0	No	
		2.4 × 2.0 × 1.3	Right UO		Ductal	Grade 2	POS	14	Low	9%			
2	60	1.8 × 1.2 × 1.2	Right LI	6.0	Ductal	Grade 3	POS	32	High	21%	0	No	
		2.3 × 2.1 × 1.3	Right UI		Ductal	Grade 3	NEG	32	High	21%			
3	45	0.9 × 0.8 × 0.5	Left UO	3.0	Ductal	Grade 2	POS	14	Low	9%	1	No	
		0.6 × 0.6 × 0.5	Left UO		Ductal	Grade 2	POS	13	Low	8%			
4	40	1.4 × 1.2 × 1.0	Right UI	4.0	Ductal	Grade 3	POS	10	Low	7%	2	No	
		1.8 × 1.5 × 1.2	Right UO		×	Ductal	Grade 3	POS	9	Low			7%
		1.6 × 1.5 × 1.4	Right Mid-O		4.0	Ductal	Grade 3	POS	11	Low			7%
5	48	2.1 × 1.9 × 1.4	Left LI	7.5	Ductal	Grade 2	POS	2	Low	4%	2	No	
		1.1 × 1.0 × 0.5	Left LO		Ductal	Grade 2	POS	4	Low	5%			
6	46	1.4 × 0.9 × 0.5	Right UI	1.8	Lobular	Grade 2	POS	9	Low	7%	2	No	
		0.9 × 0.5 × 0.4	Right UO		Lobular	Grade 2	POS	7	Low	6%			
7	45	1.5 × 1.3 × 1.3	Left UO	7.3	Ductal	Grade 3	POS	12	Low	8%	2	No	
		1.2 × 1.2 × 0.5	Left UO		Lobular	Grade 1	POS	14	Low	9%			
8	60	1.1 × 1.1 × 1.0	Right UO	0.6	Mixed	Grade 2	POS	17	Low	11%	2	No	
		1.5 × 1.3 × 1.3	Right UO		Mixed	Grade 2	POS	15	Low	10%			
9	50	1.9 × 1.5 × 1.5	Right Mid-I	0.8	Ductal	Grade 3	POS	20	Int.	13%	2	No	
		0.7 × 0.5 × 0.5	Right LI		Ductal	Grade 3	POS	22	Int.	14%			
10	77	1.5 × 1.0 × 0.5	Right UO	2.0	Mixed	Grade 2	POS	24	Int.	15%	2	No	
		2.3 × 1.0 × 1.0	Right UO		Mixed	Grade 2	NEG	22	Int.	14%			
11	43	2.5 × 2.0 × 2.0	Left UO	2.5	Ductal	Grade 3	POS	10	Low	7%	3	No	
		1.3 × 1.0 × 0.8	Left UO		Ductal	Grade 3	POS	7	Low	6%			
12	62	3.7 × 2.2 × 2.3	Right UI	0.1	Lobular	Grade 1	POS	9	Low	6%	3	No	
		2.7 × 2.5 × 2.5	Right UI		Lobular	Grade 1	POS	12	Low	8%			
13	60	4.8 × 3.2 × 2.7	Right UO	5.5	Lobular	Grade 2	NEG	19	Int.	12%	3	Yes	
		4.2 × 3.0 × 2.3	Right LI		Lobular	Grade 2	POS	16	Low	10%			
14	41	0.9 × 0.8 × Unk	Left UO	0.4	Ductal	Grade 2	POS	19	Int.	12%	4	Yes	
		0.9 × 0.8 × Unk	Left UO		Ductal	Grade 1	POS	15	Low	10%			
15	64	1.6 × 1.5 × 1.0	Left UO	1.0	Lobular	Grade 2	NEG	17	Low	11%	5	No	
		1.0 × 0.9 × 0.9	Left UO		Ductal	Grade 2	NEG	22	Int.	14%			
16	70	2.0 × 1.5 × 1.0	Left UI	4.4	Ductal	Grade 3	POS	25	Int.	16%	10	No	
		2.0 × 1.5 × 1.5	Left UI		Ductal	Grade 3	POS	35	High	24%			
17	64	1.0 × 0.4 × 0.4	Left UO	0.3	Ductal	Grade 1	POS	8	Low	6%	11	Yes	
		1.0 × 0.6 × 0.6	Left UO		Lobular	Grade 1	POS	19	Int.	12%			
18	63	1.4 × 1.2 × 0.8	Left UO	2.0	Lobular	Grade 2	NEG	44	High	30%	30	Yes	
		0.6 × 0.5 × 0.6	Left UO		Ductal	Grade 2	POS	14	Low	9%			
19	62	2.2 × 1.5 × 1.0	Right UO	Diff Breasts	Ductal	Grade 1	POS	6	Low	5%	0	No	
		1.8 × 1.5 × 1.5	Left UO		Lobular	Grade 1	POS	6	Low	5%			
20	44	1.3 × 0.8 × 0.5	Right LI	Diff Breasts	Ductal	Grade 2	NEG	15	Low	10%	8	No	
		1.0 × 0.8 × 0.1	Left UO		Ductal	Grade 2	POS	7	Low	6%			
21	71	2.0 × 1.3 × 0.6	Right LO	Diff Breasts	Ductal	Grade 2	POS	27	Int.	18%	17	Yes	
		1.0 × 0.8 × 0.6	Left Mid-I		Lobular	Grade 1	POS	10	Low	7%			
22	58	2.5 × 2.5 × 2.0	Right LI	Diff Breasts	Mixed	Grade 1	POS	17	Low	11%	27	Yes	
		1.8 × 1.0 × 0.8	Left LO		Ductal	Grade 2	POS	44	High	30%			

**Abbreviations:** T Size, Tumor Size (in centimeters); T1-T2, Distance between Tumor 1 and Tumor 2 (in centimeters); UO, Upper Outer; UI, Upper Inner; LI, Lower Inner; LO, Lower Outer; T Histo, Tumor Histology; T Grade, Tumor Grade; PR, Progesterone Receptor; Onc Dx, Oncotype Dx; Risk, Oncotype Dx Risk; %Rec, Oncotype Dx% Recurrence, Diff, Difference in Oncotype Dx Score; Rec Ch?, Chemotherapy recommendation change.



**Table 2.** Differences in characteristics by tumors of the same vs. different breasts.

DIFFERENCES IN CHARACTERISTICS	SAME BREAST	DIFFERENT BREAST	P-VALUE
Quadrant	33.3%	75.0%	0.26
Histology	22.2%	75.0%	0.077
Tumor grade	11.1%	50.0%	0.14
Progesterone receptor status	22.2%	25.0%	1.0
Oncotype Dx risk group	22.2%	50.0%	0.29
Oncotype Dx score	4.6	13.0	0.24

4 of the 18 (22%) of patients had different histology between their tumors. Of the 4 patients with differing histology, the average distance between tumors was 2.66 cm, whereas the 14 patients with similar histology had an average distance between tumors of 2.80 cm ( $P = 0.83$ ). The mean difference in Oncotype Dx score was 2.5 (median = 2.0) for those of the same histology and 12.0 (median = 8.0) for those with different histology, significant with a  $P$ -value of 0.039 by Wilcoxon test. Additionally, in the 18 patients with multiple primary tumors in the same breast, they had differing histologic grades in 2 of the 18 (11%) cases and differing progesterone receptor status in 4 of the 18 (22%) cases. Correlation between the distance between tumors and difference in Oncotype Dx for primary tumors in the same breast was  $-0.28$  with a  $P$ -value of 0.26 (see Figure 1). The average difference in Oncotype Dx recurrence score for patients with multiple primary tumors in the same breast was 4.6 (median = 2.0).

**Alterations in chemotherapy recommendations (N = 6).** Obtaining multiple Oncotype Dx led to different recommendations in chemotherapy in 2 of the 4 (50%) patients with multiple primary tumors in different breasts and 4 of the 18 (22%) patients with multiple primary tumors in the same breast.

Overall, 8 of the 22 (38%) patients had different Oncotype Dx recurrence score stratification (low, intermediate, high) between their multiple primary tumors. The difference in Oncotype Dx recurrence score stratification was prominent enough in 6 of the 22 (27%) patients to change chemotherapy recommendations. In the two patients whose management was not altered, one patient had intermediate and high risk tumors, and chemotherapy was recommended regardless, and another patient had low and intermediate risk tumors, with chemotherapy not administered.

A comparison of the subset of 6 patients for whom obtaining multiple Oncotype Dx samples altered chemotherapy recommendations versus the subset of 16 patients for whom obtaining multiple Oncotype Dx samples did not alter chemotherapy recommendations is summarized in Table 3. As shown in Table 3, in the 16 patients where obtaining multiple Oncotype Dx did not alter management, the tumors were in

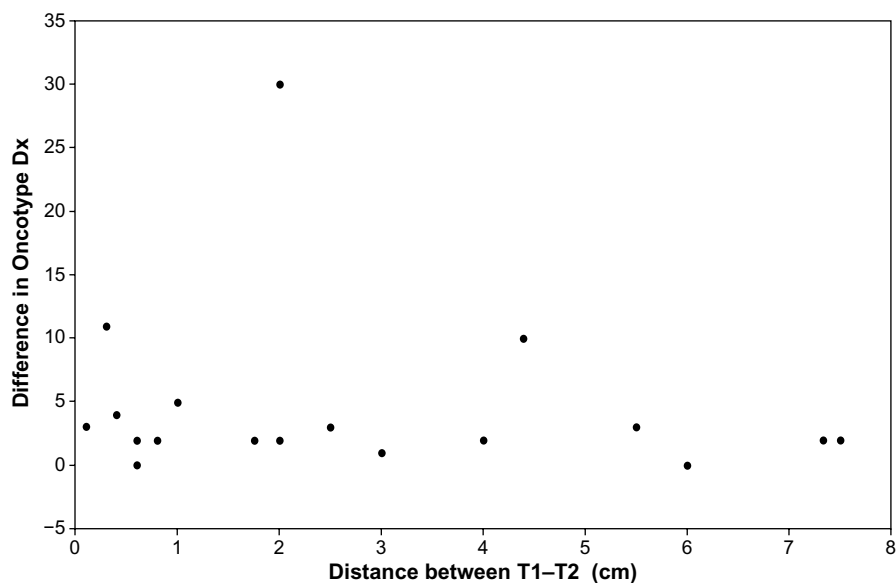
**Table 3.** Differences in characteristic by tumors of similar vs. different (those altering chemotherapy recommendations) Oncotype Dx scores.

DIFFERENCES IN CHARACTERISTICS	SIMILAR ONCDX	DIFFERENT ONCDX	P-VALUE
Quadrant	37.5%	50.0%	0.66
Breast	12.5%	33.3%	0.29
Histology	18.8%	66.7%	0.049
Tumor grade	6.3%	50.0%	0.044
Progesterone receptor status	18.8%	33.3%	0.59
Oncotype Dx score	2.7	15.3	0.003
Average distance between tumors	3.0	2.1	0.40

different breasts in 12.5% of the patients and different quadrants in 37.5% for patients with multiple tumors in the same breast. Regarding histopathologic and genetic profile, the tumor histology was different in 18.8% of patients, the grade was different in 6.3% of patients, the progesterone receptor status was different in 18.8% of patients, and the Oncotype Dx averaged a difference of 2.7 between the tumors in patients where obtaining multiple Oncotype Dx did not alter management. The average distance between tumors in this subset was 3.0 cm. In comparison, for the 6 patients for whom obtaining multiple Oncotype Dx did alter management, the tumor histology was different in 67% of patients, the grade was different in 50% of patients, the progesterone receptor status was different in 33% of patients, and the Oncotype Dx averaged a difference of 15.3 between the tumors. Analysis between the subset of 6 patients for whom obtaining multiple Oncotype Dx samples altered chemotherapy recommendations versus the subset of 16 patients for whom obtaining multiple Oncotype Dx samples did not alter chemotherapy recommendations showed statistically significant differences for histology ( $P = 0.049$ ) and tumor grade ( $P = 0.044$ ).

## Discussion

While multiple primary tumors are typically analyzed separately in terms of histology, pathology, and receptor status, the question arises: should multiple primaries undergo separate Oncotype Dx analysis? In this retrospective study, Oncotype Dx results in multiple primary tumors altered chemotherapy recommendations for both unilateral and bilateral synchronous breast cancers, in 27% of the patients. This supports the possibility that each primary tumor is a distinct tumor, thus portraying a different risk profile for recurrence and progression. It is already well established that breast cancer is a heterogeneous disease, with differing DNA, RNA, and protein expression.<sup>15,16</sup> Furthermore, more recent evidence indicates that even within tumors, there exists significant heterogeneity.<sup>17</sup>



**Figure 1.** Difference in Oncotype Dx scores versus distance between tumors.

While the different Oncotype Dx scores suggest heterogeneity in the tumors, the majority of primary tumors had minimal differences in categorical Oncotype Dx results. Seven out of twenty-two (32%) of patients in our analysis had a difference in recurrence score greater than 4. Oncotype Dx results have a standard deviation of 2 recurrence scale on a 100-unit scale, indicating that the wide variance of recurrence score noted in the synchronous samples should not be attributed to the Oncotype Dx test, but rather requires an alternative explanation.<sup>18</sup> One possible explanation is that the tumors are independent primary tumors, but they have a similar genetic profile as reflected by Oncotype Dx, possibly due to the same underlying DNA of the patient and additionally the same environmental exposures.<sup>19,20</sup> This is in line with the data showing a difference in histology, pathologic grade, and receptor status at a higher percentage in patients with differing Oncotype Dx than in patients with similar Oncotype Dx. The possibility exists that the multiple primaries are actually a single primary with extension that is not able to be seen either radiographically or pathologically.<sup>20–22</sup> If this were the case, one would expect to see correlation between distance between tumors and similarity of Oncotype Dx score, which was not the case in our analysis.

While 2/4 (50%) of patients with multiple primary tumors in different breasts had sufficiently different Oncotype Dx scores to influence management, there still existed 4/18 (22%) patients with multiple primary tumors in the same breast having sufficiently different Oncotype Dx scores to influence management. This leads to the conclusion that while patients with multiple primaries in different breasts are more likely to be further informed from multiple Oncotype Dx assessments, approximately two in ten patients with multiple primaries in the same breast that would also gain useful information from multiple Oncotype Dx assessments. Furthermore, this

data correlates with the possibility that genetic dissimilarity is more likely in patients with primaries on different breasts, as indicated by greater difference in Oncotype Dx scores, albeit on a small sample size. Regarding multiple primaries on the same breast, there appeared to be no correlation between difference in Oncotype Dx score and distance between primary tumors, which would argue against the theory of a single connected primary.

There was a statistically significant difference in regards to tumor histology and tumor grade when comparing the subset of patients for whom analyzing both tumors led to alterations in chemotherapy recommendations versus the subset of patients for whom analyzing both tumors did not lead to alterations in chemotherapy recommendations. This intuitively makes sense as patients with tumors of different grades and histology are more likely have significant genetic differences in regards to Oncotype Dx score, and therefore benefit from analyzing each tumor by Oncotype Dx. However, it is possible that the clinician, after seeing differences in tumor histology and tumor grade for a patient, preferentially sent both tumors for Oncotype Dx testing, thus introducing clinician selection bias. Alternatively, a clinician may have decided to only evaluate the lesion with more aggressive characteristics, such as higher tumor grade. Of note, this data demonstrates that despite having the same tumor grade and tumor histology between multiple primary tumors there exists the possibility to obtain significantly different Oncotype Dx scores that in turn alter chemotherapy recommendations.

**Limitations.** The primary limitations of this study are the small sample size and the retrospective design. Given that there are more than 230,000 new breast cancer cases each year, of which approximately 30% are multiple primary tumors, and of which slightly more than half are hormone receptor-positive and lymph node-negative, one would expect



a significantly higher sample size of multiple primaries from the original set of 666 patients. In our analysis 128 of the 650 (19.7%) obtainable records had multiple primaries, with only 17.2% of the tumors with multiple primaries having Oncotype Dx testing completed on each tumor. The lower percentage of patients with multiple primaries when compared to the previously cited 30% may be explained by many study patients undergoing lumpectomy as opposed to mastectomy (data not shown). Hence, there is less breast tissue for the pathologist to review for occult tumors. The variance between the number of multiple primary tumors and the number of patients with multiple primary tumors sent for Oncotype Dx can be explained by a number of factors. The first and most prominent is clinician bias, where it was assumed, influenced by the proximity of tumors, the pathologic appearance and staining, or due to other factors influencing the decision, that sending multiple samples for Oncotype Dx was unnecessary. These biases may be present across disciplines of surgery, pathology and medical oncology. There did not appear to be an individual physician bias in sending multiple specimens, with each provider sending 3–5 cases of the final analysis group (data not shown). Additionally, there may have been limitations from a financial or insurance standpoint regarding sending multiple samples for Oncotype Dx testing, as well as patient-related factors impacting the use of Oncotype Dx.

**Summary.** This retrospective study of women with hormone receptor-positive, lymph node-negative breast cancer suggests that Oncotype Dx results from synchronous breast tumors influence treatment decisions. From a practical standpoint, it is difficult to determine which patients with multiple breast tumors would benefit from having multiple Oncotype Dx analyses. Further studies, with prospective design and larger sample sizes, could be undertaken in order to determine which patients with multiple synchronous tumors would benefit from multiple Oncotype Dx testing.

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### Author Contributions

Conceived and designed the experiments: CVP. Analyzed the data: MJT, KMK, CVP. Wrote the first draft of the manuscript: MJT. Contributed to the writing of the manuscript: MJT, CVP. Agree with manuscript results and conclusions: MJT, KMK, CVP. Jointly developed the structure and arguments for the paper: MJT, CVP. Made critical revisions and approved final version: MJT, KMK, CVP. All authors reviewed and approved of the final manuscript.

### DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance

with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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