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Chemotherapy Benefit for "ER-Positive" Breast Cancer and Contamination of Non-Luminal Subtypes – Waiting for TAILORx and RxPONDER

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Introduction

Adjuvant chemotherapy has been widely used in the treatment of estrogen receptor (ER) and/or progesterone receptor (PR) positive breast cancer. Based on the Early Breast Cancer Trialists Collaborative Group meta-analysis, the addition of adjuvant chemotherapy to tamoxifen reduces the risk of breast cancer relapse and mortality in hormone receptor-positive (HR+) disease by approximately 30% and 20%, respectively, but without considering subgroups[1]. The indication of adjuvant chemotherapy includes women with negative axillary lymph nodes and tumors above 0.5 cm at very low absolute risk of recurrence[2]. Routine use of adjuvant chemotherapy for women with positive axillary lymph node(s) is recommended[3,4].

OncotypeDX Recurrence Score (RS) in NSABP B20 and SWOG 8814

Retrospective analyses of NSABP B20 and SWOG 8814 suggested that not all patients with ER-positive disease benefit from the addition of chemotherapy. Both studies support the use of Oncotype DX 21-gene recurrence score (RS) to define patients requiring multi-agent chemotherapy. In NSABP B 20, a trial for node-negative breast cancer[5], patients with high RS (≥31) tumors had a large benefit in distant recurrence free interval (DRFI) from CMF or MF chemotherapy (Relative Risk [RR]=0.26, 95% CI: 0.13-0.53). Patients with low-RS (<18) tumors did not benefit from chemotherapy (RR=1.31, 95% CI: 0.46-3.78). Patients with intermediate-RS tumors (18-30) did not appear to derive a large benefit from chemotherapy (RR=0.61, 95% CI: 0.24-1.59). In SWOG 8814, a trial for node-positive breast cancer[6], it was found that patients with high RS (≥31) tumors had a significant benefit in disease-free survival (DFS) from chemotherapy (Hazard ratio [HR]=0.59, 95% CI: 0.35-1.01). There was no CAF chemotherapy benefit in patients with low RS (<18) tumors (HR=1.02, 95% CI: 0.54-1.93). Patients with intermediate-RS tumors (18-30) did not have an apparent benefit (HR=0.72, 95% CI: 0.39-1.31).

Intrinsic subtypes of breast cancer

Breast cancer is a biologically heterogeneous disease and molecular characterization studies over the last decade have identified 4 main intrinsic subtypes (i.e. luminal A, luminal B, Her2-enriched and basal-like) with significant differences in terms of their incidence, risk factors, prognosis and response to therapies[7,8]. Among them, non-luminal subtypes (i.e. HER2-enriched

and Basal-like) show consistent higher pathological complete response (pCR) rates following neoadjuvant multi-agent chemotherapy compared to luminal disease (i.e. Luminal A and B)[9,10].

The pathological-based biomarkers (e.g. ER, PR, HER2 and Ki67 or grade) currently used in the clinical setting either alone or in combination, do not fully recapitulate the intrinsic subtypes of breast cancer[11]. This poses the question if the chemotherapy benefit observed in NSABP B20 and SWOG 8814 studies might be due to the presence of the generally chemotherapy sensitive non-luminal subtypes? Indeed, gene expression analyses using the research-based PAM50 50-gene classifier have revealed that, although the luminal subtypes predominate within ER-positive breast cancer, all the intrinsic subtype can be identified in ER-positive disease; for example, a re-analysis of a combined cohort of 337 patients with ER+/HER2-unknown and node-negative early breast cancer treated without adjuvant systemic therapy (i.e. no chemotherapy and no endocrine therapy), showed that 18.7% of tumors were of the non-luminal subtype[12]. In this cohort, intrinsic subtyping and a microarray-based version of OncotypeDX RS were found significantly associated with survival outcome (**Fig. 1A and Fig 1B**). Interestingly, 29.1%, 7.7%, and 0% of the patients in the Oncotype DX RS high, intermediate, and low risk groups had non-luminal breast cancer, respectively (**Fig. 1B**).

Further supporting this hypothesis of subtype heterogeneity within ER+ disease, ER status determination has evolved over time. Of note in the NSABP B20, and in a subset of patients from SWOG 8814, ER status was not determined by the current IHC-based methods but by the ligand-binding assay (LBA). This is important since both methods of determination of ER levels are different, and a 14-22% ER-negativity by IHC has been observed among ER-positive tumors by LBA[13]. Similar discordant rates have been observed between two different IHC-based assays for ER status[14]. For example, a recent ring study of the two central pathology laboratories of the ALTTO phase III trial (BIG 2-06/NCCTG N063D) that used different IHC-based assays for ER determination observed a ~12% discordance rate between local and central, and a 15% discordance rate between the two central laboratories[15]. Finally, NSABP B20 and SWOG 8814 did not take into account HER2 status, and HER2-positivity enriches for the presence of the HER2-enriched subtype[11,16,17]. In fact, recent studies with central pathology determination of ER

and HER2 and gene expression analyses have shown that the non-luminal contamination within ER+/HER2-negative disease represents ~5.0%[18,19].

Overall, these data suggest that NSABP B20 and SWOG 8814 may be contaminated by non-luminal tumors, especially in high-risk RS group, which may have more contamination from non-luminal disease. Thus, how would the NSABP B20 and SWOG8814 results according to Oncotype Dx RS be if non-luminal tumors/patients had been excluded?

Simulation of chemotherapy benefit in ER-positive breast cancer

We evaluated distant relapse-free survival (DRFS) data from a previously published combined cohort of 1,227 patients with ER+ breast cancer treated with adjuvant tamoxifen-only (551 node-negative and 676 node-positive)[20]. To simulate the addition of chemotherapy effect, it was assumed that among patients who had a DRFS event before 5 years, 43% of the basal-like group, 35% of HER2-enriched group, 17% of luminal B group and 3% of luminal A group could be distant relapse-free at 5 years of follow-up if chemotherapy would have been administered; these estimated chemotherapy effects/times were based on the pCR rate of each intrinsic subtype following neoadjuvant anthracycline/taxane-based chemotherapy[9]. Patients who attain pCR following neoadjuvant chemotherapy have outstanding survival outcomes at 5-years[21-23].

Simulations were done separately for the node-negative and node-positive cohorts (**Table 1**). We used 9 different set of assumptions regarding the distribution of the intrinsic subtypes in each ER-positive breast cancer population. The distribution of the intrinsic subtypes within each type of ER-positive population (except the all Luminal A and all Basal-like cases) was based on a cohort of 337 patients with ER+/HER2-unknown with complete Oncotype DX RS and intrinsic subtype information[12]. Under each set of assumptions, first, a random sample set of 1,000 patients was drawn with replacement. Second, a survival advantage as described above was added in 50% of the patients of each random sample set to simulate the results if chemotherapy would have been administered. Finally, the hazard ratio (HR) of tamoxifen plus chemotherapy vs. tamoxifen and the 5-year DRFS percentages for both treatments were calculated. Each random sample set was repeatedly drawn 10,000 times.

Results and Discussion

As shown in our simulations (**Table 1**), as the percentage of patients with non-luminal disease increases, then the apparent effectiveness of chemotherapy also increases. A higher percent of patients with high risk RS tumors presumably have non-luminal disease, thus the contamination of non-luminal patients on the chemotherapy effect could be large in this group of patients. The geometric mean of the hazard ratio of tamoxifen vs. tamoxifen plus chemotherapy increased from 0.78 to 0.89 for node-negative and 0.80 to 0.88 for node-positive cohorts in our simulations, after removing the non-luminal cases. In patients with intermediate risk RS tumor, the percentage of non-luminal cases is lower. There was some contamination of non-luminal cases, but the influence on the chemotherapy effect was small. The geometric mean of the hazard ratio of tamoxifen vs. tamoxifen plus chemotherapy increased from 0.88 to 0.94 for node-negative and 0.90 to 0.93 for node-positive cohorts in our simulations, after removing the non-luminal subtype. For patients with low risk RS tumors, there were no non-luminal cases, and thus no contamination of non-luminal subtype on the chemotherapy effect was analyzed.

TAILORx and RxPONDER are phase III clinical trials using the Oncotype DX assay to identify patients for whom adjuvant endocrine therapy alone is sufficient treatment due to their inherent prognosis with endocrine therapy alone, due to their lack of benefit from chemotherapy, or both[24,25]. TAILORx is designed for node-negative patients and enrolled its final patient in 2010. RxPONDER is designed for patients with 1-3 positive nodes and is still open to accrual.

The primary objective of TAILORx[24] is to determine whether adjuvant hormonal therapy is not inferior to adjuvant chemotherapy plus hormonal therapy for patients with ER-positive/HER2-negative breast cancer with intermediate Oncotype Dx RS values between 11-25. In this study, after the Oncotype DX RS is determined, patients are assigned or randomized based on the RS. Patients with RS 10 or less are assigned to receive hormonal therapy alone. Patients with RS 11-25 are randomized to receive chemotherapy plus hormonal therapy versus hormonal therapy alone. Patients with RS 26 or higher are assigned to receive chemotherapy plus hormonal therapy. The primary endpoint is disease-free survival (DFS). A difference in the 5-year DFS rate from 90% with chemotherapy to 87% or lower on hormonal therapy alone (corresponding to

a HR for hormonal vs. chemotherapy + hormonal of 1.322) would be considered unacceptable. The accrual goal for the randomized cohort is 6,860 patients. The study accrued more than 11,000 patients and was closed in October 2010 and the final analyses are expected in 2015.

The primary objective of RxPONDER[25] is to test whether the difference of chemotherapy compared to no chemotherapy depends directly on RS score in ER-positive/HER2-negative breast cancer with 1-3 positive axillary nodes. If the difference depends on RS, an optimal cut-point for recommending chemotherapy will be determined. In this study, after knowing the results of Oncotype DX RS, patients with RS 25 or less are randomized to receive chemotherapy plus endocrine therapy versus endocrine therapy alone. The primary endpoint is DFS. In the final analysis, the interaction of treatment and RS will be tested first. If it is statistically significant and a point of equivalence can be obtained in the range 0-25 from a Cox regression model with the interaction term, the upper limit of a one-sided 95% confidence interval for the point of equivalence marks the RS for which there is a significant benefit of chemotherapy. If this upper limit is within 0-25, then a clinically significant effect of chemotherapy can be expected for RS values above this cutoff point. Below the cutoff point, there will be no statistically significant chemotherapy benefit. If the interaction of treatment and RS is not statistically significant, the overall effect of chemotherapy will be tested in all patients. The study plans to randomize 4,000 patients via screening more than 9,000 patients. The study is still accruing patients.

In our dataset evaluated here, the percentages of Luminal A, luminal B, HER2-enriched and Basal-like in patients with RS of 11-25, were 85.7%, 12.7%, 1.6% and 0%, respectively. For patients with RS ≤25 tumors, the percentages of luminal A, luminal B, HER2-enriched and Basal-like were 89.6%, 9.4%, 0.9% and 0%, respectively. Thus, the potential contamination of non-luminal subtype on the chemotherapy effect in patients with RS 11-25 or RS ≤ 25 could be small.

Our study has a few limitations. First, our simulations in the dataset of 1,227 patients with ER-positive disease were based on the outcome of all patients, regardless of the RS scores. Thus, our simulations were only looking at the effect of different composites of percentages of the four intrinsic subtypes. Second, the chemotherapy effect was based on pCR as a surrogate marker of "non-relapse" despite that the risk of relapsing in patients with pCR is not exactly zero[21]. In

addition, the pCR rates of each intrinsic subtype were obtained from a cohort of patients with ER-positive and ER-negative tumors; however, less clear are the pCR rates of the intrinsic subtypes within ER-positive/HER2-negative disease. Finally, our simulations only considered the relative percent of patients who were cured with chemotherapy at the 5-year time-point. Thus, we did not consider the potential delay in time to distant recurrence due to chemotherapy.

To conclude, we conducted simulations in order to obtain a better understanding of how the NSABP B20 and SWOG8814 results would have been if non-luminal breast cancer would have been excluded. The results of the simulations suggest that the non-luminal tumors are augmenting the apparent benefit of chemotherapy, but does not appear to be responsible for the entire effect. This finding supports the current St Gallen International Expert Consensus of administering adjuvant multi-agent chemotherapy in addition to endocrine therapy in Luminal B tumors[26]. However, our data suggests that the magnitude of benefit from chemotherapy in this subset of patients with luminal disease is likely lower than the one reported in the Oxford meta-analysis[1]. These simulations could also provide information about the potential influence of contamination by unexpected tumor subtypes on the future results of TAILORx and RxPONDER clinical trials.

Table 1. Simulation results under different scenarios and distribution of the intrinsic subtypes*.

		Node-negative			Node-positive		
			Mean 5-year DRFS %			Mean 5-year DRFS %	
Type of ER-positive Population	**Distribution of subtypes**	**Geometric mean of HR**	**Tamoxifen-only**	**Tam.+Chemo.**	**Geometric mean of HR**	**Tamoxifen-only**	**Tam.+Chemo.**
All LumA	LumA=100%	0.98 (0.62,1.54)	96% (93%,97%)	96% (94%, 98%)	0.98 (0.76,1.27)	85% (81%, 88%)	85% (82%, 88%)
All Basal-like	Basal-like=100%	0.52 (0.42, 0.64)	66% (62%, 70%)	81% (78%, 83%)	0.51 (0.42, 0.63)	63% (58%, 67%)	79% (76%, 81%)
Fan et al.	Basal-like=5.9%, Her2-E=12.8%, LumA=43.9%, LumB=37.4%	0.82 (0.61, 1.11)	88% (85%, 90%)	90% (88%, 93%)	0.85 (0.68, 1.05)	74% (71%, 78%)	79% (76%, 82%)
Fan et al. (with no contamination)	Basal-like=0%, Her2-E=0%, LumA=54%, LumB=46%	0.91 (0.66, 1.26)	91% (88%, 93%)	92% (90%, 94%)	0.91 (0.73, 1.12)	77% (73%, 80%)	80% (76%, 83%)
Low RS	Basal-like=0%, Her2-E=0%, LumA=90.4%, LumB=9.6%	0.96 (0.64, 1.45)	95% (92%, 97%)	95% (93%, 97%)	0.96 (0.75, 1.24)	83% (79%, 86%)	84% (81%, 87%)
Interm. RS (contaminated)	Basal-like=3.1%, Her2-E=4.6%, LumA=67.7%, LumB=24.6%	0.88 (0.63, 1.24)	91% (89%, 94%)	93% (91%, 95%)	0.9 (0.72, 1.13)	79% (75%, 82%)	82% (78%, 85%)
Interm. RS (with no contamination)	Basal-like=0%, Her2-E=0%, LumA=73.3%, LumB=26.7%	0.94 (0.65, 1.34)	93% (90%, 95%)	94% (91%, 95%)	0.93 (0.74, 1.18)	80% (76%, 84%)	82% (79%, 85%)
High RS (contaminated)	Basal-like=9.0%, Her2-E=20.1%, LumA=19.1%, LumB=51.8%	0.78 (0.60, 1.03)	84% (80%, 87%)	88% (85%, 90%)	0.8 (0.66, 0.98)	70% (66%, 74%)	77% (73%, 80%)
High RS (with no contamination)	Basal-like=0%, Her2-E=0%, LumA=26.9%, LumB=73.1%	0.89 (0.67, 1.19)	88% (85%, 90%)	90% (87%, 92%)	0.88 (0.72, 1.07)	72% (68%, 76%)	76% (73%, 80%)

* Simulations were done separately for the node-negative and node-positive cohorts. In each case, 10,000 replicates were analyzed. In each replicate, the sample size was 1000. Summary data of the geometric mean of HR, mean 5-year DRFS rates and their 2.5th and 97.5th percentiles out of the 10,000 replicates were obtained for each case. The distribution of the intrinsic subtypes within each type of ER-positive population was based on a cohort of 337

patients with ER+/HER2-unknown with complete microarray-based Oncotype DX RS and intrinsic subtype information. In 3 scenarios, contaminating non-luminal subtypes (i.e. Basal-like and HER2-enriched) were removed. In the low OncotypeDX RS group, no potential contamination issue was observed in the dataset evaluated. RS, recurrence score; HR, hazard ratio; DRFS, distant relapse-free survival; Her2-E, Her2-enriched.

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Figure Legend

Fig 1. Relapse-free survival of patients with ER-positive/HER2-unknown early breast cancer treated without adjuvant systemic therapy. (**A**) Based on the intrinsic subtypes; (**B**) Based on microarray-based OncotypeDX RS groups.

Figure 1