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Preliminary results of centralized HER2 testing in ductal carcinoma in situ (DCIS): NSABP B-43

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Abstract

NSABP B-43 is the first prospective, randomized phase III multi-institution clinical trial targeting high-risk, HER2-positive DCIS. It compares whole breast irradiation alone with WBI given concurrently with trastuzumab in women with HER2-positive DCIS treated by lumpectomy. The primary aim is to determine if trastuzumab plus radiation will reduce in-breast tumor recurrence. HER2-positive DCIS was previously estimated at >50 %, occurring primarily in ER-negative, comedo-type DCIS of high nuclear grade. There has been no documented centralized multi-

institutional HER2 analysis of DCIS. NSABP B-43 provides a unique opportunity to evaluate this in a large cohort of DCIS patients. Patients undergoing lumpectomy for DCIS without evidence of an invasive component are eligible. A central review of each patient's pure DCIS lesion is carried out by immunohistochemistry analysis. If the lesion is 2+, FISH analysis is performed. Patients whose tumors are HER2 3+ or FISH-positive are randomly assigned to receive two doses of trastuzumab during WBI or WBI alone. NSABP B-43 opened 11/9/08. As of 7/31/2013, 5,861 patients have had specimens received centrally, and 5,645 of those had analyzable blocks; 1,969 (34.9 %) were HER2 positive. A total of 1,428 patients have been accrued, 1,137 (79.6 %) of whom have follow-up information. The average follow-up time for the 1,137 patients is 23.3 months. No grade 4 or 5 toxicity has been observed. In NSABP B-43 the HER2-positive rate for pure DCIS among patients undergoing breast-preserving surgery is 34.9 %, lower than the previously reported rate. No trastuzumab-related safety signals have been observed. Interest in this trial has been robust.

Keywords

DCIS; Non-invasive breast cancer; HER2; Clinical trials

Introduction

Ductal carcinoma in situ (DCIS) of the breast, once a rare lesion, now represents 15–30 % of all newly diagnosed breast cancer cases. DCIS comprises 30–40 % of breast cancers detected mammographically. In 2013, approximately 235,000 new cases of invasive breast cancer (IBC) are expected in the US, with an additional 63,640 new cases of in situ breast cancer (85 %) being DCIS. Since 2004, in situ breast cancer incidence rates have been stable in white women and increasing in African American women by 2.0 % annually [1, 2].

Because DCIS is considered a precursor to the majority of IBCs [3], intense interest has developed to understand the molecular pathways driving progression. This is leading to new treatment strategies that intercept progression. Similar to IBC, DCIS consists of many different subtypes of diverse biologic potential [4, 5]. In contrast to IBC, little progress has been made toward identifying targeted treatments for DCIS subtypes outside of the established use of tamoxifen for estrogen receptor (ER)-positive DCIS [6–8]. Tailored treatments are lacking for ER-negative DCIS, a subtype of DCIS that often overexpresses the HER2/neu proto-oncogene.

To explore the potential of a novel targeted approach, the National Surgical Adjuvant Breast and Bowel Project (NSABP) launched NSABP B-43 to investigate the use of trastuzumab as a targeted agent in the treatment of HER2/ neu-positive DCIS. B-43 is a prospective, randomized phase III adjuvant clinical study comparing trastuzumab given concurrently with radiation therapy (RT) versus RT alone for women with HER2-positive DCIS following breast-conserving surgery. We hypothesized that the addition of trastuzumab in this group of patients would lower ipsilateral breast cancer recurrence, ipsilateral skin cancer recurrence, and ipsilateral DCIS. If successful, this treatment may decrease the incidence of IBTR and

increase the use of breast-conserving therapy for high-risk women with HER2-positive DCIS through the use of molecular targeting.

Rationale

Targeting HER2 is a promising strategy for HER2-over-expressing DCIS and addresses the lack of targeted therapy in this subgroup, many of whom lack ER and will not benefit from tamoxifen. Trastuzumab, a monoclonal antibody that targets the HER2 protein [9, 10], was FDA-approved in 1998 for use in metastatic HER2-positive disease and in 2006 for use in the adjuvant setting. It is widely used for patients with HER2-positive breast cancer in the metastatic and adjuvant settings in combination with chemotherapy.

Preclinical studies have shown that trastuzumab enhances radiosensitivity of HER2-expressing cancer cells in xenograft models and cell lines with no detrimental effect on HER2-normal cells [11, 12]. The evaluation of molecular markers in trastuzumab-treated patients shows that apoptosis occurs within 1 week of beginning single-agent trastuzumab, suggesting that shorter rather than longer treatment duration with trastuzumab may be effective [13].

Ongoing adjuvant trials using trastuzumab during breast irradiation provide ample evidence of safety. Trastuzumab may also play a role as a chemopreventive agent, interrupting the progression of HER2-positive DCIS to invasive carcinoma. In a recent study, the immunological effects of even a single dose of trastuzumab were reported in HER2-positive DCIS patients; with this brief exposure to trastuzumab, therapeutic concentrations of the drug were detected in nipple aspirates. This is an indication that trastuzumab crosses the basement membrane and makes it available for cellular activity [14]. Unlike lapatinib, trastuzumab induces antibody-dependent cell-mediated cytotoxicity activity.

Because targeted anti-HER2 therapies are not standard treatments for DCIS, HER2 testing is not a routine part of pathologic evaluation of DCIS. HER2 positivity is not well documented, and there are no prospective data describing its incidence. This analysis presents positivity incidence in DCIS submitted for eligibility evaluation in NSABP B-43. This report represents about 71 % of the patients who will be entered into this currently accruing trial. This population represents the largest cohort of DCIS patients in the literature with detailed analysis of HER2 overexpression. This is an international trial and the largest to date. Results presented here also help reconcile the long-standing discrepancy of the perceived higher percentage of DCIS lesions overexpressing HER2 compared with the reported lower percentage of HER2 overexpression in invasive breast lesions.

Eligibility

Eligible patients must be female, ≥ 18 years, with a documented ECOG performance status of 0 or 1, and histologic confirmation of DCIS. All DCIS must have been resected by lumpectomy with pathologic-free tumor margins. If axillary staging is performed, nodal staging must be pN0, pN0(i-), or pN0(i+). Patients with mixed DCIS and lobular carcinoma in situ are eligible. ER and/or progesterone receptor (PR) status must be determined before assignment. Final eligibility requires HER2-positive DCIS by central testing.

Schema

Eligible HER2-positive patients are assigned to either RT alone or two doses of trastuzumab given concurrently with RT during weeks 1 and 4. RT must be either WBI (with or without a boost) over 5–6 weeks or, at investigator discretion, accelerated fractionation (3 weeks) may be used. For patients who have hormone receptor-positive DCIS, hormonal therapy should be given for a minimum of 5 years by investigator discretion (Fig. 1, CONSORT; Fig. 2, Schema).

Methods

This trial was approved by local human investigations committees or institutional review boards in accordance with assurances filed with and approved by the Department of Health and Human Services. Written informed consent is required for participation.

Central testing for HER2 status

Currently, routine HER-2 testing for DCIS is not performed, thus centralized testing is required by the study. Centralized testing to determine HER2 status is performed at Rush University Medical Center (RUMC). HER2 status is first evaluated by immunohistochemistry (IHC) using HercepTest™ (Dako, Carpinteria, CA), for all cases and is reflexed to fluorescence in situ hybridization (FISH) (PathVysion,™ HER2 DNA probe kit, by Vysis, Inc, Downers Grove, IL) for those cases with a 1+ or 2+ IHC reading. A 0 or 3+ IHC reading is considered final.

HercepTest™ is interpreted as negative for HER2 protein overexpression at 0 and 1 + staining intensity, weakly positive at 2+, and strongly positive at 3+. HER2 is considered overexpressed if 30 % of the tumor cells show complete membranous staining for HER2, as per current ASCO/CAP guidelines [15].

After initial evaluation, all 1+ and 2+ IHC cases are reflexed to FISH evaluation. After observing an exceedingly low rate of HER2 amplification by FISH of the HER2 1+ cases, the B-43 protocol was amended to require reflex FISH testing of only the IHC HER2 2+ cases.

HER2 is considered amplified in a specimen if a ratio of 2.2 is calculated, as per current CAP/ASCO guidelines [15].

Analysis of B-43 specimens is performed in real-time. Blocks of eligible cases are shipped to RUMC and properly accessioned. After HER2 status is analyzed by IHC and, if appropriate, by FISH as above, results are promptly communicated to the sites, initially by fax, followed by a mailed hard copy, to the patient's original institution's pathology department. Documentation is also provided to the NSABP Biostatistical Center.

Endpoints—The primary aim of NSABP B-43 is to determine the value of trastuzumab given during RT compared to RT alone in preventing the occurrence of ipsilateral breast cancer recurrence, ipsilateral skin cancer recurrence, or ipsilateral DCIS in women with HER2-positive DCIS resected by lumpectomy.

The secondary aims are to determine the value of trastuzumab given during RT compared to RT alone in improving invasive or DCIS DFS, as follows:

- Prolonging in-breast tumor recurrence-free interval,
- Increasing invasive or DCIS recurrence-free interval,
- Improving regional or distant recurrence,
- Improving the incidence of contralateral invasive or DCIS breast cancer, and
- Improving overall survival.

Statistical considerations—More than 8,000 patients are expected to be tested centrally for HER2 expression.

The study design requires 2,000 patients. Definitive analysis of the primary endpoint will be performed upon 163 IBC events. This number of events results in a power of 80 % to detect a hazard reduction of 36 %. The 36 % observed reduction in the hazard of IBC event rates on the trastuzumab arm is based on a projection of 40 % hazard reduction if compliance were perfect but conservatively assumes that a 10 % noncompliance rate will occur. Power calculations take into account a 1 % loss to follow-up in each study arm.

Results

Patient entry and progress—NSABP B-43 opened for accrual on November 9, 2008, with the first patient accrued on December 10, 2008. As of July 31, 2013, 1,428 (71.4 %) patients have been accrued, and post-random assignment follow-up information has been received for 1,137 (79.6 %). Among these 1,137, mean follow-up was 23.3 months.

As of July 31, 2013, of 5,861 patients for whom specimens were submitted to RUMC for central testing for HER expression, 1,969 (33.6 %) were HER2 positive and eligible. There were 489 (24.8 %) who were beyond the eligibility time line. Additionally, one patient was randomly assigned after the analysis cutoff date of July 31, 2013, and another 51 whose paperwork was in progress. (Fig. 1, CONSORT).

Of the 5,645 patients for whom central testing results for HER2 were available, 1,907 (33.8 %) were negative for HER2 at score 0, 1,424 (25.2 %) were negative for HER2 at score 1+, 437 (7.7 %) were indeterminate for HER2 at score 2+, and 1,877 (33.3 %) overexpressed HER2 at score 3+. Cases with HER2 1+ or 2+ were subsequently tested for HER2 amplification by FISH, as per the protocol. Of the 1,424 cases with 1+, only 1 (0.1 %) was amplified by FISH. Of the 437 cases with 2+, 91 (20.8 %) were amplified by FISH. Overall, 1,969 cases (of 5,645, 34.9 %) were HER2 positive (either by IHC or by FISH). Site HER-2 testing has been too infrequent to analyze.

Patient characteristics—As of July 31, 2013, 1,428 patients were randomly assigned, 715 to the RT arm and 713 to the RT-plus-trastuzumab arm. Patient characteristics are reasonably balanced across arms (Table 1). 22.95.6 % were 49 years in the RT-alone arm versus 22.63.5 % in the RT-plus-trastuzumab arm. Similarly, 38.69.7 % in the RT-alone,

versus 41.70.3 % in the RT-plus-trastuzumab arm, were 50–59 years. Finally, 38.54.7 % in the RT-alone, versus 35.86.1 % in the RT-plus-trastuzumab arm, were 60 years.

The majority of patients had high-grade DCIS: 82.2 % in the RT-alone arm versus 83.5 % in the RT-plus-trastuzumab arm, and 17.1 % in the RT-alone, versus 15.1 % in the RT-plus-trastuzumab arm had intermediate-grade DCIS. Finally, only 0.7 % in the RT-alone, versus 1.4 % in the RT-plus-trastuzumab arm, had low-grade DCIS.

Hormonal status is closely balanced between each arm. Specifically, 57.2 % are ER positive in the RT-alone arm versus 59.3 % in the RT-plus-trastuzumab arm, and 42.5 % are ER negative in the RT-alone arm versus 40.7 % in the RT-plus-trastuzumab arm (Table 1).

Toxicity report—As of July 31, 2013, grade 3 toxicities were reasonably balanced at 4 and 5 % in RT-alone and RT-plus trastuzumab arms, respectively ($p = 0.41$; NS). No grade 4 or 5 toxicities (deaths) were reported, and no other trastuzumab safety signals have been detected.

Discussion

DCIS currently represents 15–20 % of all newly diagnosed breast cancer cases and up to 30–40 % of mammographically detected breast cancer cases [1, 2]. Historically, DCIS was treated with mastectomy, which was therapeutic in most cases. Based on results of NSABP B-17 [6], lumpectomy-followed-by-RT is a standard treatment option for appropriately selected patients with DCIS. In NSABP B-24, the addition of tamoxifen to lumpectomy plus RT regimen was shown to further reduce risk of recurrence (both ipsilateral and contralateral) in patients with DCIS, similar to the already documented effect of tamoxifen on IBC [7].

Currently, the use of tamoxifen for ER-positive DCIS cases is the only example of targeted therapy for DCIS lesions. Similar to IBC, DCIS encompasses a heterogeneous group of lesions with unique biologic characteristics, a propensity for progression to invasive carcinoma, and variable response to treatment. DCIS lesions are subdivided into comedo and non-comedo subtypes [16, 17]. Reporting of DCIS nuclear grade is an improvement in further subcategorizing DCIS lesions [18–20]. Luminal A, luminal B, basal-like, and HER2 subtypes of DCIS comparable to those that exist for IBC have been recently reported [4, 5]. High nuclear grade DCIS lesions are usually negative for ER and frequently overexpress HER2 [21–37]. Among tumors from 255 women presenting to the Yale-New Haven Hospital with DCIS, HER2 expression was inversely related to ER status: 60 % of HER2-positive tumors were ER negative [28]. There is a considerable variability in HER2 expression reported in DCIS lesions, ranging from 28 to 65 % [21–37]. Most of these investigators used IHC methods to measure HER2 expression. However, the numbers of DCIS patients in these studies are limited, ranging from 37 to 255 patients.

This analysis is the first large-scale national DCIS study reporting on the HER2 status of the first 5,861 DCIS patients from a large number of institutions. In our patient population, the percentage of HER2-positive DCIS cases is lower than what has been reported previously, namely 34.9 % [37]. This lower percentage is much better correlated with the percentage of

HER2-positive IBC, reported to be between 15 and 20 % [10, 25]. Our results may support the recent progression model of breast cancer suggesting that high-grade DCIS evolves to high-grade invasive carcinomas, and low-grade DCIS precipitates to low-grade invasive carcinomas, a concept supported by recent genetic studies [38]. This will require further investigation as defined by study outcomes.

Although our results are preliminary, the series in this trial represents by far the largest and most recent analysis of HER2 testing on DCIS. In addition, we employed ASCO/CAP guidelines, unlike many of the older series, which did not.

These guidelines were recently updated and are modified from the original guidelines of 2007 [39], the main revision of which is an interpretation of what constitutes a positive result (amplification) of HER2. In the 2007 guidelines, a FISH ratio of >2.2 was required to call a case HER2 positive, while ratios of 1.8–2.2 put a case into the equivocal category. The updated guidelines consider cases with ratios of ≥ 2 to be positive for HER2 amplification. This will ultimately change rates of HER2 positivity overall. In our study, the HER2 status of all 5,645 patients with analyzable blocks was evaluated based on the 2007 guidelines as established at the beginning of the trial, which opened on 11/9/2008. To date, 71.4 % of the patients have been accrued based on those criteria. For trial consistency, no changes are planned in the HER2 definition of positivity for the duration of this trial.

Because accrual to this trial is still ongoing and the event rate for primary endpoint analysis has yet to be reached, we are not permitted by our data monitoring committee to release any data beyond what are reported here.

A limitation of our study is that it tested only the subset of DCIS cases with negative margins that could be treated by lumpectomy. Therefore, the incidence of HER2 positivity may under-represent the incidence of HER2 expression among all DCIS cases. It is possible that more DCIS patients requiring mastectomy are HER2-positive than those who are candidates for lumpectomy. This may also explain the higher-than-expected rate of ER-positivity in our population. Analysis of HER2 status in a study sample of over 1,428 DCIS cases reported here provides for the first time a more accurate estimate of incidence of HER2 positivity in DCIS lesions, which are being image detected and are of high grade (Table 1).

HER2 overexpression is seen in a small percentage of ER-positive DCIS. In the Yale-New Haven study, 19 % of ER-positive DCIS lesions also expressed HER2 [28]. Collins and Schnitt [25] recently calculated that because 20 % of newly diagnosed breast cancer cases annually in the US are DCIS cases (a total of ~45,000) and ~80 % are ER positive (resulting in 36,000 such cases), if 10–20 % of these ER-positive DCIS cases also overexpress HER2, then between 3,600 and 7,200 DCIS cases/year in the US will be of the ER-positive/HER2-positive phenotype. Knowledge of HER2-overexpression status may have potential clinical implications about tamoxifen usage in this subset of DCIS patients, especially in light of experimental data and data on IBC suggesting that the simultaneous presence of HER2 overexpression in ER-positive DCIS lesions may interfere with the beneficial effects of tamoxifen in these lesions [36–38, 40–44]. The high incidence of high-grade DCIS (>80 %)

in this analysis does not reflect the incidence of high-grade lesions among DCIS patients in the general population. This high rate could also reflect selection bias of clinicians among whom there is an assumption that high-grade lesions are more likely to be HER2 positive than lower grade lesions, a desire by patients with high-grade lesions to consider participation in this trial, or the biology of HER2-positive DCIS. Upon completion, an analysis of relationships between tumor size, tumor grade, ER status, and HER2 amplification will be made.

Our results demonstrate that in a highly selected patient population, with primarily high-grade DCIS, the incidence of HER2 amplification documented through standard measures in a central laboratory was 34.9 %.

In DCIS, the underlying molecular pathways and newer treatments continue to challenge our preexisting concepts and understanding of this disease and are expected to lead to improved patient outcomes.

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Fig. 1.
CONSORT: NSABP B-43 *IHC* immunohistochemistry, *FISH* fluorescence in situ hybridization

Fig. 2.
Schema NSABP B-43

Table 1

Patient characteristics: NSABP B-43

Characteristics ^a	RTx <i>N</i> = 715		RTx + T <i>N</i> = 713	
	Number	%	Number	%
Mean follow-up time (mos) ^b	<u>23.4^c</u>		<u>23.2^d</u>	
Age				
49	164	22.9	161	22.6
50–59	276	38.6	297	41.7
60	275	38.5	255	35.8
Nuclear grade				
Low	5	0.7	10	1.4
Intermediate	122	17.1	108	15.1
High	588	82.2	595	83.5
Estrogen receptor status				
Positive	409	57.2	423	59.3
Negative	304	42.5	290	40.7
Unknown	2	0.3	0	0.0

RTx Radiation therapy, *T* Traztuzumab^a Numbers and percents are based on all randomly assigned patients unless specified otherwise^b As of July 31, 2013^c Based on 567 patients with follow-up information^d Based on 570 patients with follow-up information