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ORIGINAL REPORT

Pathologic Complete Response Predicts Recurrence-Free Survival More Effectively by Cancer Subset: Results From the I-SPY 1 TRIAL—CALGB 150007/150012, ACRIN 6657

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ABSTRACT

Purpose
Neoadjuvant chemotherapy for breast cancer provides critical information about tumor response; how best to leverage this for predicting recurrence-free survival (RFS) is not established. The I-SPY 1 TRIAL (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis) was a multicenter breast cancer study integrating clinical, imaging, and genomic data to evaluate pathologic response, RFS, and their relationship and predictability based on tumor biomarkers.

Patients and Methods
Eligible patients had tumors ≥ 3 cm and received neoadjuvant chemotherapy. We determined associations between pathologic complete response (pCR; defined as the absence of invasive cancer in breast and nodes) and RFS, overall and within receptor subsets.

Results
In 221 evaluable patients (median tumor size, 6.0 cm; median age, 49 years; 91% classified as poor risk on the basis of the 70-gene prognosis profile), 41% were hormone receptor (HR) negative, and 31% were human epidermal growth factor receptor 2 (HER2) positive. For 190 patients treated without neoadjuvant trastuzumab, pCR was highest for HR-negative/HER2-positive patients (45%) and lowest for HR-positive/HER2-negative patients (9%). Achieving pCR predicted favorable RFS. For 172 patients treated without trastuzumab, the hazard ratio for RFS of pCR versus no pCR was 0.29 (95% CI, 0.07 to 0.82). pCR was more predictive of RFS by multivariate analysis when subtype was taken into account, and point estimates of hazard ratios within the HR-positive/HER2-negative (hazard ratio, 0.00; 95% CI, 0.00 to 0.93), HR-negative/HER2-negative (hazard ratio, 0.25; 95% CI, 0.04 to 0.97), and HER2-positive (hazard ratio, 0.14; 95% CI, 0.01 to 1.0) subtypes are lower. Ki67 further improved the prediction of pCR within subsets.

Conclusion
In this biologically high-risk group, pCR differs by receptor subset. pCR is more highly predictive of RFS within every established receptor subset than overall, demonstrating that the extent of outcome advantage conferred by pCR is specific to tumor biology.

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INTRODUCTION

Advances in adjuvant therapy as well as screening have helped reduce breast cancer mortality,[1] but approximately 20% of patients with breast cancer in the United States still die of their disease.[2] Mortality is highest among women who present with larger, palpable tumors[3] and in whom the absolute incidence has not decreased much.[4] Thus, better treatments are needed.

Breast cancer is a heterogeneous disease that varies widely in outcomes and response to standard therapies.[5,6] Neoadjuvant or preoperative chemotherapy yields outcomes equivalent to adjuvant therapy[7,8] but has the benefit of downstaging tumors and increasing breast conservation rates,[9] and it permits assessment of individual tumor response to treatment.[7-10]

The I-SPY 1 TRIAL (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis) is a multicenter neoadjuvant breast cancer study designed to establish standards for collecting molecular and imaging data over the course of care. Primary objectives were

to evaluate whether response to therapy—as measured by imaging (magnetic resonance imaging [MRI] volume) response and pathologic complete response (pCR)—would predict recurrence-free survival (RFS), overall and within biologic and imaging subsets. Secondary objectives were to develop a resource of clinical, molecular, genetic, and imaging biomarker data and a multicenter network to support high-quality real-time biomarker evaluation for future trials of tailored therapy. This first report describes the ability of short-term response to therapy, as measured by pCR, to predict RFS, both overall and within receptor subsets.

PATIENTS AND METHODS

The I-SPY 1 TRIAL was a collaboration of the American College of Radiology Imaging Network (ACRIN), Cancer and Leukemia Group B (CALGB), and the National Cancer Institute (NCI)'s Specialized Programs of Research Excellence (SPORE). It consisted of two protocols developed to identify markers of response to conventional neoadjuvant chemotherapy: CALGB 150007 (molecular marker component) and ACRIN 6657/CALGB 150012 (imaging component). The protocol (schema is shown in Fig 1) was approved by institutional review boards at all participating institutions. Patients signed one combined informed consent form before joining the study, which allowed them to simultaneously enroll onto the CALGB and ACRIN protocols. Details of accrual have been published previously.[10a]

The primary end point for the trial was RFS according to the STEEP (Standardization of Events and End Points) criteria.[11] RFS was calculated from the date of chemotherapy initiation. An estimated target sample size of 244 patients with 15% drop rate was needed to be able to detect (with 90% power and 0.05 type I error) a hazard ratio of 0.5 between two biomarker-defined groups (eg, MRI volume change in response to neoadjuvant chemotherapy or risk groups defined by molecular signatures). Defining the relationship of biomarkers to RFS were secondary aims of the trial. All sites tested for estrogen receptor (ER) status, progesterone receptor (PR) status, and human epidermal growth factor receptor 2 (HER2) overexpression and assessed pCR. pCR was defined as the absence of invasive tumor in both breast and axillary lymph nodes after neoadjuvant therapy.

Patient Eligibility

Eligible patients had histologically confirmed invasive breast cancers measuring at least 3 cm by clinical examination or imaging, with no evidence of distant metastatic disease, and were candidates for neoadjuvant chemotherapy with an anthracycline-based regimen. Patients with T4 or inflammatory disease were eligible. Patients were considered evaluable if they completed neoadjuvant chemotherapy.

Study Treatment and Procedures

After four cycles of anthracycline-based therapy, patients could either undergo surgical excision or receive a taxane before surgery. Treatment after surgery, including chemotherapy, radiation, and hormone therapy, was at the physician's discretion.

Biopsies and imaging studies were conducted at four time points during neoadjuvant chemotherapy (Fig 1). Details of the imaging component (ACRIN 6657/CALGB 150012) are described elsewhere.[12] Trastuzumab treatment was not dictated by the protocol and was not used as neoadjuvant therapy until April 2005.

Standard Clinical Biomarkers

Hormone receptor (HR) status (ER positive or PR positive) and HER2 overexpression were measured from diagnostic samples obtained by a standard method in Clinical Laboratory Improvement Amendments (CLIA) –approved laboratories at local sites on formalin-fixed, paraffin-embedded core biopsies obtained at diagnosis.

Both ER and PR status were determined by immunohistochemistry (IHC) and were considered positive if the Allred score was ≥ 3[13]; HER2 status was determined by IHC and/or fluorescent in situ hybridization assays locally and centrally at the University of North Carolina.[14] HER2 status was regarded as positive if there was 3+ staining and/or fluorescent in situ hybridization positivity (positive defined as HER2/CEP17 ratio ≥ 2.2) locally or centrally.

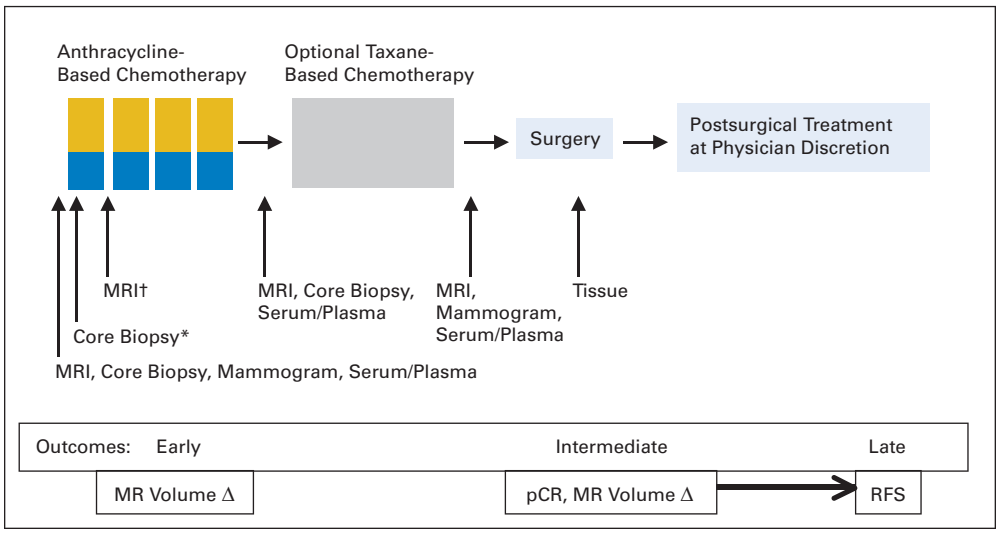

Fig 1. I-SPY 1 TRIAL (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis) schema. Sixteen-gauge core-needle biopsies were performed at four time points: T1, before treatment; T2, between 24 and 96 hours after starting treatment; T3, after completing the regimen that contained doxorubicin (if a taxane was to be given); and T4, at the time of surgical resection. Blood was drawn to obtain serum and plasma before chemotherapy, between anthracycline and taxane regimens, if applicable, and before surgery. Magnetic resonance (MR) images were obtained before chemotherapy, approximately 2 weeks after the first dose of anthracycline, at the end of anthracycline treatment (if the patient went on to receive a taxane), and again before surgical resection. The goal of the trial was to relate early and late outcomes. Early outcome measures included MR volume change and rates of pathologic complete response (pCR). Late outcomes were measured by recurrence-free survival (RFS), which was measured as the proportion of patients who did not experience an invasive breast cancer recurrence in the ipsilateral breast or regional nodes, distant organ sites, or death from any cause for the specified time period. MRI, magnetic resonance imaging. (*) Twenty-four to 96 hours after the start of anthracycline. (†) Two weeks after the start of anthracycline.

Ki67 IHC staining was performed centrally at the University of North Carolina by using standard avidin-biotin complex technique.[15] Tumors were categorized as low, intermediate, or high proliferation index for less than 10%, 10% to 25%, and more than 25% of tumor cell nuclei staining positive, respectively, and were interpreted by a single pathologist (C.L.). Molecular assays and analyses are described in detail elsewhere.[10a]

Data Collection and Platform Integration for Analysis

The NCI Center for Bioinformatics developed a Web-based system, caINTEGRATOR, to support centralized reporting of results across disparate sources and platforms[17] and provided a common platform for accessing data (https://caintegrator-stage.nci.nih.gov/ispy/index2.jsp). I-SPY 1 data dated February 2011 was used for the analyses in this article.

Statistical Analysis

Rates of pCR were calculated for each combination of HR and HER2 status. Kaplan-Meier survival curves were generated for patients who achieved pCR versus those who did not, overall and within each receptor subset. The RFS hazard ratios and *P* values for comparisons of patients who achieved pCR versus those who did not were based on multivariate Cox proportional hazards models, adjusting for age and clinical stage. These analyses were conducted by using JMP Version 8.0.1 (SAS Institute, Cary, NC).

RESULTS

Patients

Between May 2002 and March 2006, 237 patients were enrolled. Figure 1 shows the protocol schema; Figure 2 shows the number of patients accrued and reasons for exclusions. The remaining 221 patients received neoadjuvant chemotherapy and were evaluable. Of these, 215 (97%) underwent surgical resection and had pCR information available (Fig 2). HER2 and HR information was available for 210 of the 215 patients (HER2 status was missing for four, and HR information was missing for one).

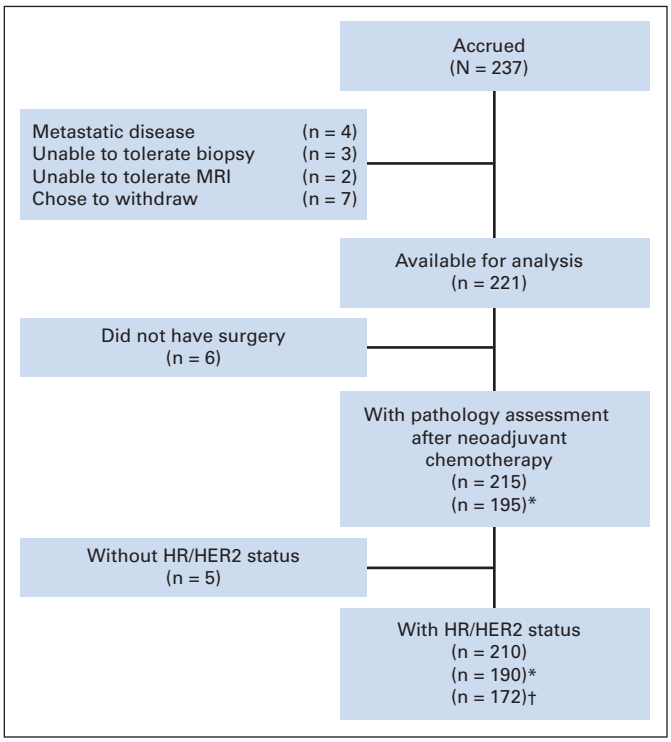

Fig 2. I-SPY 1 (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis) CONSORT diagram. Of the 237 patients enrolled, 221 patients were evaluable, 215 had pathology results, and 210 had receptor data available. HER2, human epidermal growth factor receptor; HR, hormone receptor. (*) No. of patients who did not receive neoadjuvant trastuzumab. Trastuzumab was approved for use during the last year of study enrollment and was available to HER2-positive patients after that time. (†) No. of patients who did not receive any trastuzumab.

The characteristics of patients who participated in the trial are provided in Table 1. Average baseline tumor size was 6.0 cm (range, 0 to 25 cm) clinically and 6.8 cm (range, 0 to 18.4 cm) by MRI. Most patients (65%) had clinically or pathologically confirmed axillary lymph node involvement at diagnosis; 90% had tumors of intermediate or high histologic grade. Overall, 59% of patients were HR positive (n = 131), and 31% were HER2 positive (n = 67); 29% (n = 55) were scored as Ki67 low, 33% (n = 63) as Ki67 medium, and 38% (n = 71) as Ki67 high.

All patients received doxorubicin plus cyclophosphamide as initial chemotherapy (54% on a dose-dense [every 2 weeks] schedule, 34% on a standard [every 3 weeks] schedule, and 12% on a modified schedule), and 95% subsequently received a taxane (55%, every 2 weeks; 26%, every 3 weeks; and 19%, once per week). Among the 66 patients with HER2-positive tumors, 46 (69%) were treated before trastuzumab was approved for adjuvant breast cancer in 2005 and therefore did not receive concurrent neoadjuvant trastuzumab. Of the 46 HER2-positive patients who did not receive neoadjuvant trastuzumab, 17 HER2-positive patients (36%) received adjuvant trastuzumab (plus one HER2-negative patient). A total of 172 received the same chemotherapy regimen without any trastuzumab (Fig 2). The median follow-up time was 3.9 years (range 3.0 to 7.5 years).

Tumor Receptors and Rates of pCR

Of the 210 patients with receptor status and surgery results, 56 (27%) experienced a pCR. Of the 190 patients who did not receive neoadjuvant trastuzumab, 44 (23%) experienced a pCR. The pCR rate was lowest (9%) in the HR-positive/HER2-negative receptor subset and highest (45%) in the HR-negative/HER2-positive receptor subset. The incremental effect of being HR negative versus HR positive resulted in an estimated 24% increase in the rate of pCR; the incremental increase in the likelihood of pCR in HER2-positive versus HER2-negative patients was 21% (Table 2).

The impact of Ki67 is best appreciated within the receptor subsets. Ki67 staining was available for 166 of the 190 patients who did not receive neoadjuvant trastuzumab (Table 3). The pCR rate was significantly higher within the high Ki67 group (34% *v* 11% in the low/medium group) overall, and Ki67 remained a significant predictor of pCR when HR and HER2 status were taken into account (multivariate logistic regression $P = .02$). When the impact of Ki67 on pCR was assessed within receptor subsets (Table 3), high Ki67 was significantly associated with increased responsiveness only in the HER2-negative subsets. In addition, the incremental impact of HER2 positivity on pCR rate was observed only within the low/medium Ki67 (+31%) but not the high Ki67 (−7%) subset. These findings suggest that adding Ki67 to standard receptor subtyping may further improve the prediction of pCR.

Relationship of pCR to RFS

RFS for all patients (excluding those treated with trastuzumab) and the receptor subsets are shown in Figure 3, stratified by whether a pCR was achieved (solid line) or not (dotted line). Table 4 details the

Table 1. Characteristics of Patients in the I-SPY 1 TRIAL

Characteristic	I-SPY 1 Trial Evaluable* (n = 221) No.	%	Patients Without Trastuzumab Treatment† (n = 172) No.	%
Age, years				
Median	49		48	
Range	26-68		27-68	
Premenopausal	106	48	82	48
Race				
White	165	75	133	77
African American	42	19	28	16
Asian	9	4	6	3
Other	5	2	5	3
Clinical tumor size, cm				
Median	6.0		6.0	
Range	0-25		0-18	
Tumor longest diameter on baseline MRI, cm				
Median	6.8		6.8	
Range	0-18.4		2.0-16.6	
Clinically node positive at diagnosis	143	65	107	62
Histologic grade (baseline)				
Low	18	8	15	9
Intermediate	96	43	76	44
High	103	47	78	45
Indeterminate	4	2	3	2
Clinical stage (baseline)				
I‡	3	1	3	2
IIA	43	19	39	23
IIB	61	28	46	27
IIIA	78	35	59	35
IIIB	11	5	11	6
IIIC	7	3	4	2
Inflammatory	17	8	9	5
Indeterminate	1	< 1	1	< 1
Hormone receptors (baseline)				
ER positive	125	57	104	60
PR positive	104	47	88	51
HR positive (ER or PR)	131	59	109	63
HER2 positive (baseline)	67	30	29	17
HR negative/HER2 negative (baseline; triple negative)	53	24	52	30
Neoadjuvant treatment				
Anthracycline only	11	5	11	6
Anthracycline + taxane	187	85	159	92
Anthracycline + taxane + trastuzumab	20	9	0	0
Anthracycline + taxane+ other	3	1	2	1
Surgery type				
Mastectomy	123	56	96	56
Lumpectomy	92	41	77	45
No surgery	6	3	0	0
Postoperative adjuvant therapy				
Any hormonal therapy	128	58	108	63
Tamoxifen	75	34	67	39
Aromatase inhibitor	95	43	79	46
Ovarian suppression	20	9	15	9
Ovarian ablation	7	3	6	3
Trastuzumab	35	16	0	0
Ki67				
Negative	8	4	8	5
Low	47	21	37	22
Intermediate	63	29	48	28
High	71	32	57	33
Indeterminate	32	14	22	13

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; I-SPY 1 TRIAL, Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis; MRI, magnetic resonance imaging; PR, progesterone receptor.

*Of the 221 evaluable patients, 215 had surgical excision, as shown in the CONSORT diagram (Fig 2).

†Patient subset with pathology assessment, known receptor status, and without any trastuzumab treatment.

‡After the data lock for this analysis, it was determined that two of the three patients designated as stage I had clinical stage II disease.

hazard ratios, confidence intervals, and absolute difference between 3- and 5-year survival on the basis of achieving pCR versus not achieving pCR. For the 172 patients treated without neoadjuvant or adjuvant trastuzumab, the hazard ratio for RFS of pCR versus no pCR was 0.29 (95% CI, 0.07 to 0.82; $P = .02$). pCR is more highly predictive of RFS when the three established receptor categories—HR positive/HER2 negative, triple negative, and HER2 positive—are added to the multivariate model, with a hazard ratio of 0.18 (95% CI, 0.04 to 0.53; $P < .001$). Given the potential confounding of results by nonrandomized use of trastuzumab, Figure 3 shows patients with HER2-positive tumors who did not receive trastuzumab as neoadjuvant (n = 20) or as adjuvant (n = 17) therapy. Since the total sample size was small, the HER2-positive patients are shown as a combination of the entire HER2-positive subset, both HR positive and HR negative (Fig 3). The RFS curves by pCR in Figure 3 (right side) show that when partitioning a population into the three biomarker subsets, each of the three

Table 2. Rates of pCR by Receptor Subset for Patients Who Did Not Receive Neoadjuvant Trastuzumab (n = 190)*

Receptor Subset	HR Positive No. pCR/No.	%	HR Negative No. pCR/No.	%	Overall No. pCR/No.	%
HER2 status						
Positive*	8/24	33	10/22	45	**18/46**	**39**
Negative	8/93	9	18/51	35	**26/144**	**18**
Overall	**16/117**	**14**	**28/73**	**38**	**44/190**	**23**

NOTE. Rates of pCR are shown for the receptor subsets and include patients for whom pathologic evaluation and receptor status were available and exclude the 20 patients who received trastuzumab as neoadjuvant therapy. The Overall (%) column shows that being HR negative *v* HR positive results in a 24% higher probability of achieving a pCR ($P < .01$). The bottom row (Overall) shows that being HER2 positive *v* HER2 negative results in a 21% higher probability of achieving a pCR ($P < .01$). Overall data appear in bold.

Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hormone receptor; pCR, pathologic complete response.

*For the 20 patients who received neoadjuvant trastuzumab, the pCR rate was 60%. These 20 patients are not included in the table.

Table 3. Rates of pCR Within Ki67 Classes Stratified by Receptor Status

Receptor Subset	Ki67 Low/Medium (n = 105)						Ki67 High (n = 61)					
	HR Positive		HR Negative		Overall		HR Positive		HR Negative		Overall	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
HER2 status												
Positive	4/12	33	4/10	40	**8/22**	**36**	1/7	14	4/10	40	**5/17**	**29**
Negative	2/67	3	2/16	13	**4/83**	**5**	3/15	20	13/29	45	**16/44**	**36**
Overall	**6/79**	**8**	**6/26**	**23**	**12/105**	**11**	**4/22**	**18**	**17/39**	**44**	**21/61**	**34**

NOTE. Rates of pCR are shown for the receptor subsets, which were further stratified by Ki67 dichotomized as low/medium *v* high. High Ki67 includes patients with > 25% of tumor cells staining positive for Ki67. For the low/medium Ki67 group, the Overall (%) column shows that the incremental effect of HR negative over HR positive is 15%, and the incremental effect of HER2 positive over HER2 negative is 31% (χ^2 test $P < .05$ for both). For the high Ki67 group, the Overall (%) column shows that the incremental effect of HR negative over HR positive is 26% (χ^2 test $P < .05$), and the incremental effect of HER2 positive over HER2 negative is 7%. Overall data appear in bold.

Abbreviations: HER2, human epidermal growth factor receptor 2; HR, hormone receptor; pCR, pathologic complete response.

estimated hazard ratios is smaller than the overall hazard ratio (eg, 0.00, 0.25, 0.14 are all less than 0.29). This is an instance of Simpson's paradox.[18] However, the parameters of which these are estimates are not precisely known and so they may not have the same relationships.

DISCUSSION

The I-SPY 1 population had clinical and biologic high-risk features: median age 49 years, 41% HR negative, 90% intermediate or high grade, and 91% high risk by the 70-gene profile.[10a] For patients with biologically high-risk invasive breast cancers, 3-year RFS is better for those who experience a pCR after neoadjuvant chemotherapy than for those who do not.

The observation of Simpson's paradox based on the estimated hazard ratios in Figure 3 is associated with well-known characteristics of breast cancer. Of the three tumor subtypes in Figure 3, the tumors that are least sensitive to either adjuvant or neoadjuvant chemotherapy are those that are HR positive/HER2 negative.[5,19,20] However, these tumors have the best prognosis in the absence of chemotherapy. Despite the fact that these tumors have the lowest rate of pCR (9% *v* 36% and 41%), patients with these tumors tend to have a better RFS than patients with triple-negative or HER2-positive tumors, both overall and within pCR and no pCR categories. So although our relatively small study is not sufficient by itself to conclude that the population parameters have the same relationship as their estimates, this observation is consistent with what we understand about the biology of breast cancer and is to be expected. Other researchers should categorize tumors by molecular and receptor characteristics in relating pCR and longer-term end points such as RFS and overall survival.

These results provide additional insights into previous neoadjuvant trials that have examined the relationship between pCR and RFS.[21] For example, although most neoadjuvant chemotherapy trials have shown that pCR is associated with favorable outcome, this was not seen in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-27 neoadjuvant trial. In that trial, although pCR improved significantly when paclitaxel was added to a doxorubicin-based chemotherapy regimen, the improvement was not of the same magnitude as that for RFS or overall survival.[7] The possibility of a Simpson's paradox relationship suggests that analyzing only overall pCR and survival rates may have underestimated the true predictive effect of pCR in NSABP B-27.

The rate of pCR in the HR-positive/HER2-positive subset was lower than in the HR-negative/HER2-positive subset in the I-SPY TRIAL (in the absence of neoadjuvant trastuzumab). The sample sizes in our study were small, but this effect has also been observed consistently in three large randomized phase III neoadjuvant trials: NeoALTTO (Neoadjuvant Lapatinib or Trastuzumab Optimization Study),[22] GeparQuinto,[23] and NEOSPHERE (Neoadjuvant Study of Pertuzumab and Herceptin in an Early Regimen Evaluation)[24]; the effect was less than that mentioned in a personal communication with G. von Minckwitz on August 19, 2011, and more than that mentioned in the CHER-LOB (Preoperative Chemotherapy Plus Trastuzumab, Lapatinib or Both in HER2-Positive Operable Breast Cancer) phase II trial.[25] In the nonrandomized MD Anderson Cancer Center neoadjuvant series,[26] pCR rates were also lower in the HR-positive/HER2-positive subset than in the HR-negative/HER2-positive subset, and the subsequent RFS rates were also lower in this group. Our trial results are limited by the small number of patients who received neoadjuvant trastuzumab. Ongoing and future trials will help us better understand whether different treatment approaches will be needed to improve outcome for HER2-positive disease on the basis of HR status.

High rates of proliferation, as measured by Ki67, increased the likelihood of pCR. However, Ki67 is highly correlated with receptor subsets and appears to improve the ability to predict response within all subsets except HER2 positive.

Clinical trials for breast cancer have historically contained a mix of receptor subsets. Given our findings and those of others that tumor biology is different for these subsets, comparisons should be anchored within molecular subsets rather than across whole trial populations. Sufficiently powered analyses of subsets on the basis of receptor status or molecular profiles (which are highly correlated with receptor status) should be a planned feature of future trials if we hope to extract the maximal value from them.[10a] Molecular profiles at baseline may provide the opportunity to identify, beyond HR positivity alone, patients likely to have a good survival outcome as shown by I-SPY 1 molecular analyses.[10a]

Getting a drug approved for marketing is estimated to take more than 10 years and $1 billion.[27,28] To shorten the period of time for drug development (what has been referred to as knowledge turns[29]), a new approach is needed. The neoadjuvant approach gives us the opportunity to use response to therapy as an early evaluation end point. Fortunately, whether chemotherapy is

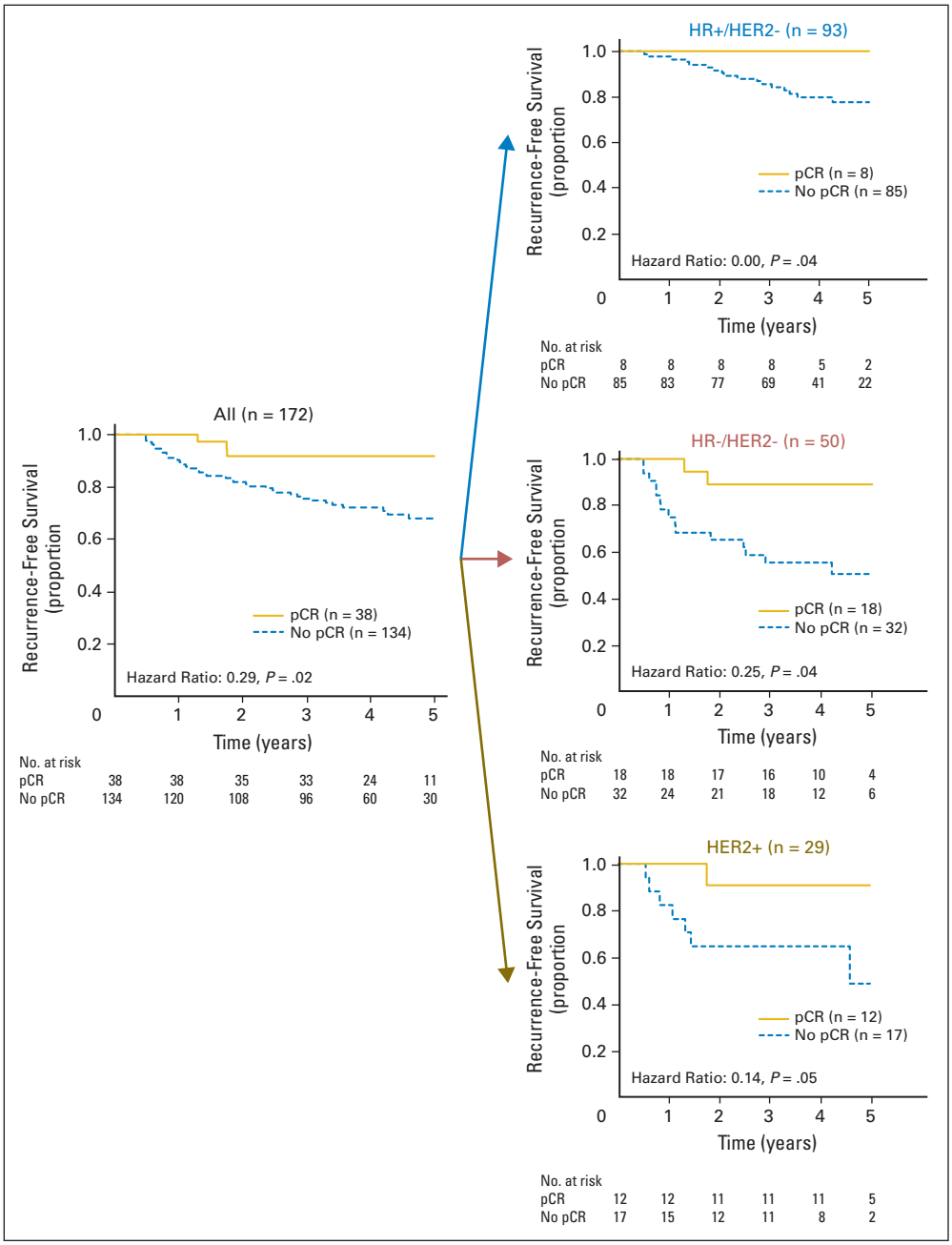

Fig 3. Recurrence-free survival stratified by pathologic complete response (pCR) for the overall population (left side) of patients (excluding all patients treated with trastuzumab; n = 38) and (right side) by hormone receptor (HR) –positive/human epidermal growth factor receptor 2 (HER2) –negative, HR-negative/HER2-negative (triple negative), and HER2-positive subsets.

given before surgery or after does not influence RFS and overall survival,[30] but giving chemotherapy before surgery offers an opportunity both to evaluate response to therapy and to alter subsequent treatment if the tumor is downstaged.[9] More importantly, it informs us about the success of standard and experimental therapies. For example, in the MD Anderson Cancer Center neoadjuvant trial, in which HER2-positive patients were randomly assigned to receive paclitaxel followed by fluorouracil, epirubicin, and cyclophosphamide plus or minus trastuzumab, the significant increase in pCR in the trastuzumab arm[31] (65.2% *v* 26% for patients who did not receive trastuzumab) in just 34 patients foreshadowed the results of the adjuvant trastuzumab trials, which accrued more than 9,000 patients and were completed 1 year later.[32,33] Several trials are now evaluating novel agents in the neoadjuvant setting, including poly (ADP-ribose) polymerase inhibitors, CALGB 40601, NEOSPHERE, and GeparQuinto. Our results support this approach and further emphasize that evaluation should be performed by receptor or molecular subset.

Despite optimal therapy, many patients still remain at substantial risk for disease progression. To make more rapid progress in finding agents that may reduce risk for these women, we have incorporated the observations from I-SPY 1 TRIAL into the design of the I-SPY 2 TRIAL. That trial uses pCR as an end point and focuses on women with biologically higher risk disease—in the context of either standard marker subsets or molecular subsets of breast cancer—and uses an adaptive design to screen phase II agents in combination with the taxane portion of chemotherapy.[34] The goal is to test these novel agents for their ability to improve pCR rates for 10 subsets of breast cancer defined by both standard and molecular markers.[35] By

Table 4. pCR Paradox

Population	RFS Hazard Ratio	95% CI	*P*	Absolute Difference in RFS at 3 Years (%)*	Absolute Difference in RFS at 5 Years (%)*
Overall (n = 172)	0.29	0.07 to 0.82	.02	16	23
HR positive/HER2 negative (n = 93)	0.00	0.00 to 0.94	.04	14	22
HR negative/HER2 negative (triple negative; n = 50)	0.25	0.04 to 0.97	.04	34	39
HER2 positive (n = 29)	0.14	0.01 to 1.0	.05	26	42

NOTE. The hazard ratios for pCR are shown for the 172 patients who had both pathologic information and receptor status available. Patients who received neoadjuvant (n = 20) or adjuvant (n = 18) trastuzumab were excluded (n = 38).

Abbreviation: HR, hormone receptor; pCR, pathologic complete response; RFS, recurrence-free survival.

*Based on whether pCR was achieved or not.

focusing on high-risk patients and analyzing data by subsets, the neoadjuvant setting can provide a unique opportunity to accelerate learning.

In an effort to develop a noninvasive measure of pCR, I-SPY 1 also included multiple imaging parameters[12] and demonstrated that change in MR volume is the imaging marker that best predicts pCR, has the potential to serve as a noninvasive measure of response to therapy, and may further accelerate evaluation of novel agents in combination with standard treatments. In I-SPY 2, MR volume is being used to provide information about response to chemotherapy between regimens, information that cannot otherwise be obtained without surgical resection.

The conclusions of our study are limited by the short follow-up time. It is well known that the HR-positive patients remain at risk for recurrence for many years, and early recurrence data may not reflect the overall outcome. However, the Oxford Overview Analysis[36] of early breast cancer trials strongly suggests that the benefit of chemotherapy is reflected by the distant disease-free survival at 5 years; that is, the survival curves diverge during the first 5 years, but after year 5, they are parallel, suggesting that the survival benefit from chemotherapy is likely to be manifest in the first 5 years. With the median follow-up for the I-SPY 1 TRIAL currently at 3.9 years, current rates of RFS are likely to reflect primarily the benefit from chemotherapy.

The outcome for patients in I-SPY 1 is also affected by hormonal therapy given adjuvantly, although molecular predictors of sensitivity to endocrine therapy have been described and will likely further refine our ability to focus on patients most at risk for disease progression at the time of diagnosis.[37,38]

In conclusion, the I-SPY 1 TRIAL shows that the ability of pCR to predict outcome is substantially improved when analyzed within tumor subsets. This predictive power is sufficiently strong that pCR can be used as an early indicator of RFS. Indeed, pCR is the primary end point of the next generation study, the I-SPY 2 TRIAL, which is designed to identify agents early in the drug development cycle that improve the rates of pCR and for which molecular subsets this is so.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Berry DA, Cronin KA, Plevritis SK, et al: Effect of screening and adjuvant therapy on mortality from breast cancer. N Engl J Med 353:1784-1792, 2005
2. Hser YI: Self-reported drug use: Results of selected empirical investigations of validity. NIDA Res Monogr 167:320-343, 1997
3. Valero VV, Buzdar AU, Hortobagyi GN: Locally advanced breast cancer. Oncologist 1:8-17, 1996
4. Esserman LJ, van 't Veer LJ, Perou C, et al: Biology of breast cancers that present as interval cancers and at young age should inform how we approach early detection and prevention. Cancer Res 69, 2009 (suppl 2; abstr 6034)
5. Carey LA, Dees EC, Sawyer L, et al: The triple negative paradox: Primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 13:2329-2334, 2007
6. Rouzier R, Perou CM, Symmans WR, et al: Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 11:5678-5685, 2005
7. Bear HD, Anderson S, Smith RE, et al: Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer: National Surgical Adjuvant Breast and Bowel Project Protocol B-27. J Clin Oncol 24:2019-2027, 2006
8. Fisher B, Brown A, Mamounas E, et al: Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: Findings from

National Surgical Adjuvant Breast and Bowel Project B-18. J Clin Oncol 15:2483-2493, 1997

9. Buchholz TA, Lehman CD, Harris JR, et al: Statement of the science concerning locoregional treatments after preoperative chemotherapy for breast cancer: A National Cancer Institute conference. J Clin Oncol 26:791-797, 2008

10. Symmans WF, Peintinger F, Hatzis C, et al: Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 25:4414-4422, 2007

10a. Esserman LJ, Berry DA, Cheang MC, et al: Chemotherapy response and recurrence-free survival in neoadjuvant breast cancer depends on biomarker profiles: Results from the I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657). Breast Cancer Res Treat [epub ahead of print on December 25, 2011]

11. Hudis CA, Barlow WE, Costantino JP, et al: Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: The STEEP system. J Clin Oncol 25:2127-2132, 2007

12. Hylton NM, Blume J, Bernreuter W, et al: MRI prediction of response to neoadjuvant chemotherapy for women with locally-advanced breast cancer: Results from the ACRIN 6657/I-SPY Trial. Radiology (in press)

13. Allred DC, Harvey JM, Berardo M, et al: Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 11:155-168, 1998

14. Livasy C, Carey L, DiMichelle A, et al: Biomarkers associated with pathologic complete response to neoadjuvant chemotherapy in women with locally advanced breast cancer: Results from the I-SPY TRIAL (CALGB 150007/150012 & ACRIN 6657). Cancer Res 69, 2009 (suppl 2; abstr 5102)

15. Spyratos F, Ferrero-Poüs M, Trassard M, et al: Correlation between MIB-1 and other proliferation markers: Clinical implications of the MIB-1 cutoff value. Cancer 94:2151-2159, 2002

16. Reference deleted

17. Buxton MB, Esserman LJ: The Challenge of Integrating Information and Improving Care for Breast Cancer: The I SPY Trial Informatics Effort. American Society of Clinical Oncology Educational Book 2005, pp 503-509

18. Simpson EH: The interpretation of interaction in contingency tables. J R Stat Soc Series B (Methodological) 13:238-241, 1951

19. Berry DA, Cirrincione C, Henderson IC, et al: Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. JAMA 295:1658-1667, 2006

20. Hayes DF, Thor AD, Dressler LG, et al: HER2 and response to paclitaxel in node-positive breast cancer. N Engl J Med 357:1496-1506, 2007

21. Dawood S, Broglio K, Kau SW, et al: Prognostic value of initial clinical disease stage after achieving pathological complete response. Oncologist 13: 6-15, 2008

22. Baselga J, Bradbury I, Eidtmann H, et al: First results of the NeoALTTO Trial (BIG 01-06/EGF 106903): A phase III, randomized, open label, neoadjuvant study of lapatinib, trastuzumab, and their combination plus paclitaxel in women with HER2-positive primary breast cancer. Cancer Res 70, 2010 (suppl 2; abstr S3-3)

23. von Minckwitz G, Eidtmann H, Loibl S, et al: Integrating bevacizumab, everolimus, and lapatinib into current neoadjuvant chemotherapy regimen for primary breast cancer. Safety results of the GeparQuinto trial. Ann Oncol 22:301-306, 2011

24. Gianni L, Pienkowski T, Im Y-H, et al: Neoadjuvant pertuzumab (P) and trastuzumab (H): Antitumor and safety analysis of a randomized phase II study ('NeoSphere'). Cancer Res 70, 2010 (suppl 2; abstr S3-2)

25. Guarneri V, Frassoldati A, Bottini A, et al: Final results of a phase II randomized trial of neoadjuvant anthracycline-taxane chemotherapy plus lapatinib, trastuzumab, or both in HER2-positive breast cancer (CHER-LOB trial). J Clin Oncol 29:46s, 2011 (suppl; abstr 507)

26. Peintinger F, Buzdar AU, Kuerer HM, et al: Hormone receptor status and pathologic response of HER2-positive breast cancer treated with neoadjuvant chemotherapy and trastuzumab. Ann Oncol 19:2020-2025, 2008

27. Loi S, Buyse M, Sotiriou C, et al: Challenges in breast cancer clinical trial design in the postgenomic era. Curr Opin Oncol 16:536-541, 2004

28. Newman LA, Mamounas EP: Review of breast cancer clinical trials conducted by the National Surgical Adjuvant Breast Project. Surg Clin North Am 87:279-305, 2007

29. Grove AS: Efficiency in the health care industries: A view from the outside. JAMA 294:490-492, 2005

30. Gralow JR, Burstein HJ, Wood W, et al: Preoperative therapy in invasive breast cancer: Pathologic assessment and systemic therapy issues in operable disease. J Clin Oncol 26:814-819, 2008

31. Buzdar AU, Ibrahim NK, Francis D, et al: Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: Results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 23:3676-3685, 2005

32. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al: Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 353:1659-1672, 2005

33. Romond EH, Perez EA, Bryant J, et al: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 353: 1673-1684, 2005

34. Popovici V, Chen W, Gallas BG, et al: Effect of training-sample size and classification difficulty on the accuracy of genomic predictors. Breast Cancer Res 12:R5, 2010

35. Barker AD, Sigman CC, Kelloff GJ, et al: I-SPY 2: An adaptive breast cancer trial design in the setting of neoadjuvant chemotherapy. Clin Pharmacol Ther 86:97-100, 2009

36. Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: An overview of the randomised trials. Lancet 365:1687-1717, 2005

37. Caudle AS, Gonzalez-Angulo AM, Hunt KK, et al: Predictors of tumor progression during neoadjuvant chemotherapy in breast cancer. J Clin Oncol 28:1821-1828, 2010

38. Hatzis C, Pusztai L, Valero V, et al: A genomic predictor of response and survival following taxane-anthracycline chemotherapy for invasive breast cancer. JAMA 305:1873-1881 2011

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