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ORIGINAL REPORT

TBCRC 001: Randomized Phase II Study of Cetuximab in Combination With Carboplatin in Stage IV Triple-Negative Breast Cancer

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ABSTRACT

Purpose
Epidermal growth factor receptor (EGFR) is a targetable receptor frequently overexpressed in basal-like breast cancer, which comprises most triple-negative breast cancers (TNBCs), the only subtype without established targeted therapy.

Patients and Methods
In this randomized phase II trial, patients with metastatic TNBC received anti-EGFR antibody cetuximab (400 mg/m^2 load then 250 mg/m^2 per week intravenously [IV]) alone, with carboplatin (area under the curve of 2, once per week IV) added after progression or as concomitant therapy from the beginning. Response rate (RR) was the primary end point; others included time to progression (TTP), overall survival (OS), and toxicity. Embedded correlative studies included molecular subtyping on archival tissue. Fresh tumor tissue before and after 7 to 14 days of therapy was used for microarray analyses exploring EGFR pathway activity and inhibition.

Results
In 102 patients with TNBC, RRs were 6% (two of 31) to cetuximab and 16% (four of 25) to cetuximab plus carboplatin after progression. RR to those treated from the beginning with cetuximab plus carboplatin was 17% (12 of 71); 31% of patients responded or had prolonged disease stabilization. The cetuximab plus carboplatin regimen was well tolerated, but both TTP and OS were short at 2.1 months (95% CI, 1.8 to 5.5 months) and 10.4 months (95% CI, 7.7 to 13.1 months), respectively. Of 73 patients with archival tissue for analysis, 74% had basal-like molecular subtype. Sixteen patients had tumor biopsies before and 1 week after therapy; genomic patterns of the EGFR pathway showed activation in 13 and inhibition by therapy in five.

Conclusion
Despite strong preclinical data, combination cetuximab plus carboplatin in metastatic TNBC produced responses in fewer than 20% of patients. EGFR pathway analysis showed that most TNBCs involved activation. However, cetuximab blocked expression of the EGFR pathway in only a minority, suggesting that most had alternate mechanisms for pathway activation.

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INTRODUCTION

Breast cancer is a heterogeneous disease composed of several biologically distinct subtypes.[1,2] One of these subtypes—basal-like breast cancer—comprises approximately 15% of breast cancers[3] and carries poor prognosis.[4-6] Basal-like breast cancer is of great interest, because it is typically hormone receptor and human epidermal growth factor receptor 2 (HER2) negative and comprises the majority of tumors that are triple negative on clinical assays for estrogen receptor (ER), progesterone receptor (PR), and HER2. Triple-negative breast cancer (TNBC) is the only clinical subset for which we have no known targeted therapy.

The epidermal growth factor receptor (EGFR) is an intriguing target in basal-like breast cancer. It is highly expressed in the basal cluster on cDNA arrays[5]; approximately half of basal-like cancers express EGFR by immunohistochemistry,[7] and basal-like cell lines are dependent on the EGFR pathway for proliferation and are sensitive to EGFR inhibitors.[8] We hypothesized that EGFR inhibition would be successful in basal-like breast cancer selected by use of the triple-negative phenotype.

In this multicenter randomized phase II study performed by the Translational Breast Cancer Research Consortium, an academic medical center collaborative group, we examined response and outcome to the anti-EGFR monoclonal antibody cetuximab alone or with carboplatin in metastatic TNBC. Cetuximab had not been tested in breast cancer, so one arm included single-agent cetuximab with carboplatin added on progression, whereas the other explored combination cetuximab plus carboplatin throughout, a combination with high effectiveness in cell line–based preclinical models.[8] Recognizing that our ability to understand sensitivity and resistance to targeted therapy is limited in clinical trials, this study was designed around, and focused on, several a priori planned analyses of correlative end points from archival specimens as well as fresh tumor samples obtained before and after initiation of therapy in women with accessible metastatic tumor. These analyses included determining the proportion of TNBCs that were basal like and examining EGFR-related signatures in predicting response to therapy.

PATIENTS AND METHODS

Patients

Eligible women had metastatic breast cancer measurable by RECIST criteria and negative for ER, PR, and HER2 (0 or 1+ on immunohistochemistry and/or normal gene copy number by fluorescence in situ hybridization) and were recruited from October 2005 to October 2007. Receptor status was determined by institutional criteria; central review was not required. Participants were allowed up to three previous chemotherapy regimens (adjuvant or metastatic), no previous EGFR inhibitor or platinum for metastatic disease, Eastern Cooperative Oncology Group performance status < 3, no significant organ dysfunction, CNS metastasis if stable for at least 3 months, and life expectancy of at least 6 months. All patients provided written informed consent, and the study was approved by the institutional review board at each site.

Treatment

Patients were randomly assigned (in a ratio of one to one) to receive cetuximab alone with carboplatin added on progression (arm one) or combination cetuximab plus carboplatin (arm two). Cetuximab was administered at 400 mg/m^2 intravenous (IV) load followed by infusions at 250 mg/m^2 once per week, and carboplatin was administered at an AUC of 2 IV on days 1, 8, and 15 of each 28-day cycle. Growth factors were permitted. Baseline and cyclic evaluations included history, physical examination, and serum chemistry and hematology profiles. Patients were restaged using computed tomography or magnetic resonance imaging every 8 weeks and were treated until objective disease progression, unacceptable toxicity, or withdrawal of consent. Treatment was allowed to be delayed for up to 4 weeks beyond planned resumption of the next cycle.

Correlative Studies

Formalin-fixed paraffin-embedded blocks were obtained from archival specimens, and intrinsic subtyping was performed using a quantitative real-time polymerase chain reaction–based method and the PAM50 gene set.[9] When feasible (and with written consent), two tumor core biopsies were obtained before and after 7 to 14 days of treatment. Any recurrent site and any time during that timeframe were permitted. Gene expression profiling was performed using Agilent DNA microarrays (Agilent Technologies, Santa Clara, CA).[8] Microarray data were deposited in the Gene Expression Omnibus (GSE23428). The microarray data and the same PAM50 algorithm applied for the quantitative real-time polymerase chain reaction were used for frozen tissue intrinsic subtyping.[9] A centroid-based predictor was used for claudin-low subtyping.[10]

EGFR pathway status was determined by previously published gene sets representing the whole pathway (defined by genes induced after removal of EGFR inhibition in a basal-like cell line model)[8]; subsequent modeling demonstrated that EGFR cluster 2 was most related to EGFR pathway activation. Activation was defined by a cutoff value of the log2 ratio 0.147, as previously described.[8] Two other signatures were also examined as predictors of clinical benefit using analysis of variance significance testing, including an 11-gene proliferation signature (*BIRC5*, *CCNB1*, *CDC20*, *NUF2*, *CEP55*, *NDC80*, *MKI67*, *PTTG1*, *RRM2*, *TYMS*, *UBE2C*)[11] and a *KRAS*-amplicon signature conserved between human and mouse.[12] Cytoscape schematic diagrams of the EGFR pathway (gene colors representing relative expression) were derived by combining 36 arrays unique to this study with 248 breast tissue sample arrays previously described,[8] and genes were median centered across all 284 samples. Statistical analysis was performed in R Development Core Team 2008 (release 2.5.1; R Foundation for Statistical Computing, Vienna, Austria).

Blood samples for circulating tumor cells (CTCs) were obtained at study entry, after 7 to 14 days of single-agent therapy, after 7 to 14 days of combination therapy, every 4 weeks during therapy, and at progression. CTCs from whole blood were processed and enumerated using a previously described method,[13] with staining for EGFR (immunoglobulin G1, kappa light chain mouse monoclonal produced in house by Veridex [North Raritan, NJ]) measured in the fourth channel. Any CTCs found in 7.5 cm^3 whole blood were considered positive.

Data Analysis and Interpretation

The primary end point of the study was response rate (RR) by RECIST criteria. Tumor response was evaluated every 8 weeks by the investigator and confirmed independently by investigators blinded to arm and not involved with the study. A Simon optimal two-stage design[14] with maximum sample size of 41 allowed early discontinuation of single-agent cetuximab for futility if $\leq$ one of the first 21 patients responded (72% likelihood of early stopping if true RR was null value of 5%; 6% likelihood of early stopping if true RR was alternative value of 20%). For the combination arm, a maximum sample size of 52 allowed futility stopping after 21 patients if $\leq$ two patients responded (37% likelihood of halting if true RR was null value of 15%; 3% likelihood of early stopping if true RR was alternative of 30%). Constrained block randomization (block size 21 plus 21) kept the imbalance between the arms to four at most.[15] Because both arms included combination therapy, accrual overruns were permitted during interim analysis. If arm one was halted, and arm two was not, another futility analysis was planned after 31 more patients were assigned to arm two. If $\leq$ seven of 51 patients responded, the trial would stop. Primary analysis was of the intent-to-treat population; preplanned analyses included evaluable patients (who received 4 weeks and at least 50% of planned treatment; however, clearly progressing patients were evaluable). Complete response (CR), partial response (PR), or disease stabilization for at least 24 weeks was considered clinical benefit (CB). Secondary end points included overall survival (OS; defined from treatment initiation to death as a result of any cause and calculated according to the Kaplan-Meier method), time to disease progression (TTP; defined from treatment initiation to documented progression), and correlative end points. Data cutoff was September 30, 2009. All patients were evaluable for safety. Adverse events were graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. Patient allocation and treatment assignment are detailed in the CONSORT diagram (Fig 1).

RESULTS

Patient demographics and tumor characteristics are listed in Table 1. Of 102 patients enrolled, 97 (95%) had received prior chemotherapy in the (neo)adjuvant or metastatic setting. Of 86 patients with prior (neo)adjuvant chemotherapy, 84 (98%) had received an anthracycline; 65 (76%) had also received a taxane. Most had received chemotherapy for metastatic disease; 55 (54%) were treated in the second- or third-line setting. As expected with TNBC, 78 (76%) had visceral involvement; the liver or lung was the dominant site in 54 patients (53%). They were minimally symptomatic; 94% had Eastern Cooperative Oncology Group performance status of 0 to 1.

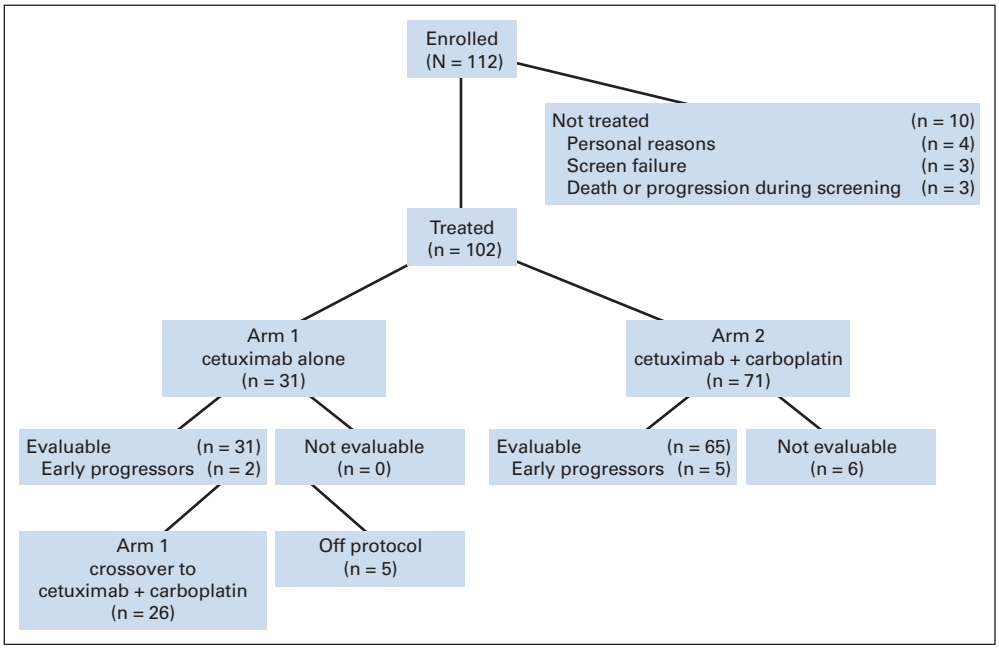

Fig 1. CONSORT diagram.

CTCs

CTCs were assessed in 95 patients; 70 (74%) had at least five CTCs per 7.5 mL, and 82 (86%) had any CTCs. Presence of CTCs in a single 7.5-mL blood sample was associated with progression-free survival (PFS), a trend at baseline ($P = .07$) and significantly so after 7 to 14 days of treatment ($P = .002$). EGFR-positive CTCs were present in 36 patients (44%), which did not predict RR or PFS.

Table 1. Patient Demographics and Tumor Characteristics (N = 102)

Characteristic	Arm 1 (n = 31) No.	Arm 1 (n = 31) %	Arm 2 (n = 71) No.	Arm 2 (n = 71) %
Age, years				
Median	49		52	
Range	33-71		28-83	
Race/ethnicity				
White	19	61	51	72
African American	11	36	17	24
American Indian	1	3	1	1
Asian	0	0	2	3
ECOG PS				
0	17	55	37	52
1	14	45	28	39
2	0	0	4	6
Missing	0	0	2	3
Menopausal status				
Postmenopause	21	68	56	79
Pre- or perimenopause	10	32	15	21
Dominant metastatic site				
Lung	9	29	20	28
Liver	8	26	17	24
LN	4	13	14	20
Locoregional	5	16	10	14
Bone	1	3	4	6
Skin/soft tissue	3	10	5	7
Other	1	3	1	1
Prior chemotherapy	30	97	67	94
Adjuvant/neoadjuvant	26	84	60	85
Metastatic	21	68	34	48

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; LN, lymph node.

Single-Agent Cetuximab

Two of 31 patients experienced PR (6%; 95% CI, 1% to 21%), and one had durable stable disease (SD; CB, 10%; 95% CI, 2% to 26%; Table 2), with a TTP of 1.4 months (95% CI, 1.1 to 1.8). Both patients with responses were in at least the second-line setting. Patients received a median of 6 weeks of therapy (range, 3 to 166 weeks); discontinuation was primarily for disease progression (19 patients; 61%); two patients (6%) discontinued because of toxicity. Those with CB continued receiving single-agent cetuximab for 24, 57, and 166 weeks,

Table 2. Response Within Treatment Arms and to Combined Therapy in Basal-Like Disease

Response	Arm 1 (n = 31) C Only No.	Arm 1 (n = 31) C Only %	Arm 1B (n = 25) C + Cb† No.	Arm 1B (n = 25) C + Cb† %	Arm 2 (n = 71) C + Cb No.	Arm 2 (n = 71) C + Cb %	C + Cb* (n = 51) Basal-Like Tumors‡ No.	C + Cb* (n = 51) Basal-Like Tumors‡ %
CR	0	0	0	0	1	1	1	2
PR	2	6	4	16	11	16	7	14
SD	3	10	7	28	15	21	8	16
> 6 months	1	3	3	12	10	14	7	14
PD	26	84	12	48	38	54	32	63
NE	0	0	2	8	6	8	3	6

Abbreviations: C, cetuximab; Cb, carboplatin; CR, complete response; NE, nonevaluable; PD, progressive disease; PR, partial response; SD, stable disease.

*Combined Arms 1B and 2.

†After progression while receiving C.

‡Limited to those with confirmed basal-like disease by quantitative real-time polymerase chain reaction–based intrinsic subtype assay.

respectively. Arm one was closed after 31 patients, based on early stopping rules for futility.

Cetuximab Plus Carboplatin

Of 71 patients treated in arm two (cetuximab plus carboplatin), one experienced CR and 11 experienced PR, for an overall RR of 17% (95% CI, 9% to 28%; Table 2). Ten had prolonged SD (CB, 31%; 95% CI, 21% to 43%). The patients treated with combination cetuximab plus carboplatin in arm one after progression while receiving single-agent cetuximab had similar results (RR, 16%; CB, 28%). Response was unrelated to line of therapy: five of 37 patients treated in first line responded, and seven of 34 patients treated in second or third line responded. Patients received a median of 8 weeks (range, 1 to 119 weeks) of combination cetuximab plus carboplatin; discontinuation was because of progression in 55 patients (79%) and toxicity in nine (13%).

TTP was 2.1 months (95% CI, 1.8 to 5.5) for those in arm two (combination therapy from the beginning). It was 2.6 months (95% CI, 1.8 to 4.7) for those receiving combination therapy after progression on single agent (Fig 2A).

Heterogeneity in response and outcomes after progression on single-agent cetuximab was substantial. Two of 31 patients in the single-agent arm and five of 71 in the combination arm rapidly progressed within 4 weeks of limiting drug exposure. Interestingly, four of the five patients experiencing rapid progression while receiving combination therapy were first-line patients. Conversely, as also seen in another EGFR inhibitor trial,[16] we saw long-term responses of >1 year in two patients receiving single-agent cetuximab (one ongoing over 3 years) and two receiving combination therapy (one ongoing nearly 2.5 years).

Survival

At a median follow-up of 26 months, 30 of 31 patients in arm one had progressed through single-agent cetuximab administration, and 26 had died. In arm two, 55 of 71 patients had progressed, and 59 had died. Median OS was 7.5 months (95% CI, 5.0 to 11.6) for arm one and 10.4 months (95% CI, 7.7 to 13.1) for arm 2 (Fig 2B).

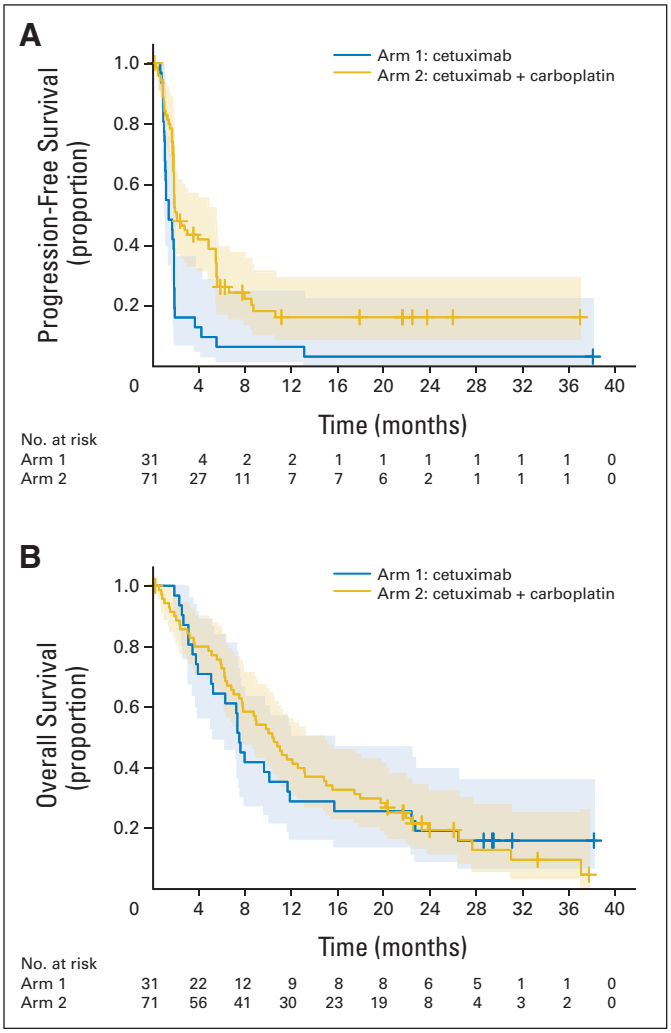

Fig 2. (A) Time to progression (TTP) with cetuximab alone and combination cetuximab plus carboplatin. (B) Overall survival (OS) in patients treated initially with cetuximab alone followed by cetuximab plus carboplatin on progression (blue line) and in patients treated with cetuximab plus carboplatin from the beginning (yellow line). 95% confidence limits shaded; crosses indicate censored data.

Toxicity

Single-agent toxicity was mild and, as expected, included rash, fatigue, and infusion reactions. Adverse effects seen in at least 10% of patients receiving cetuximab plus carboplatin included rash, fatigue, and nausea. Grades 3 to 4 adverse events were uncommon and included rash, fatigue, neutropenia, nausea, and hypersensitivity reactions. A more detailed description of toxicity can be found in Appendix Table A1 (online only). Both patients with grade 4 infusion reactions came from the geographic region previously associated with cetuximab hypersensitivity.[17]

Intrinsic Subtyping and Drug Effects on the EGFR Pathway

Formalin-fixed paraffin-embedded archival tumor was available from 73 patients: 54 patients (74%) had basal-like, five (7%) had HER2-enriched, four (5%) had luminal A, one (1%) had luminal B, and nine (12%) had normal-like tumors. The very long-term (166 weeks) responder to single-agent therapy had luminal A TNBC.

We obtained fresh metastatic tumor from 18 patients. Two were only able to be biopsied pretreatment, but 16 provided serial biopsies before and after treatment for gene expression microarray analysis: 11 (61%) had basal-like, four (22%) had claudin-low (a subtype not represented in the PAM50 assay), one (5%) had HER2-enriched, and two (11%) had normal-like tumors. In 14 of 16 serial biopsies, subtype stayed the same. One basal-like tumor and one normal-like tumor changed to claudin low; this subtype represented six (38%) of the post-treatment samples. In 13 (81%), EGFR was expressed, and the pathway activated, allowing assessment of inhibition with treatment (Figs 3A and 3B). Five (38%) had at least 20% decrease in EGFR signature expression with treatment (Fig 4A; Appendix Table A2, online only): one PR (with $>$ 100% change based on comparison to reference [much higher before and much lower after therapy]), two SD, and two progressive disease. All eight tumors with persistent EGFR pathway activation after treatment had progressive disease.

Although EGFR as a single marker was unassociated with CB, several other markers were associated with PR or SD (Figs 4B and 4C;

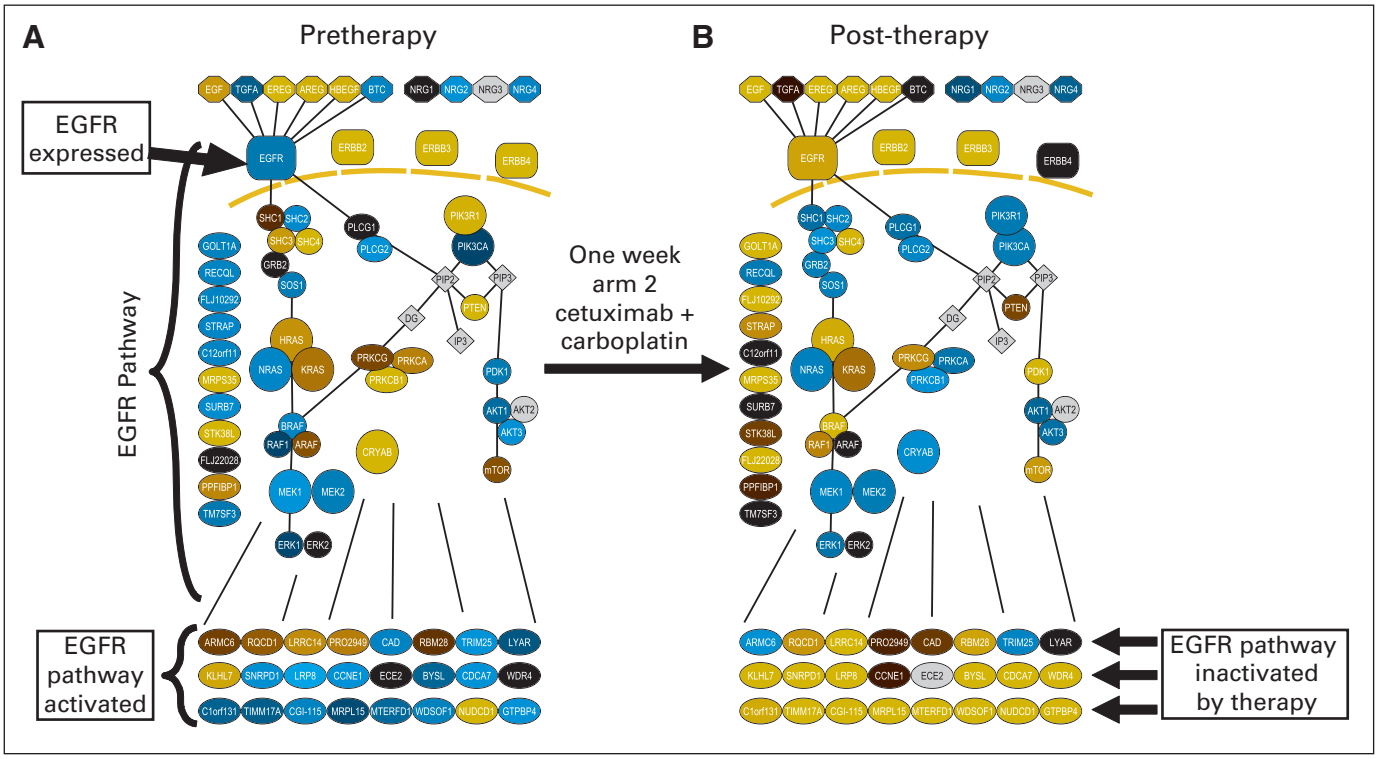

Fig 3. Example of a target lesion biopsied and examined by microarray for epidermal growth factor receptor (EGFR) pathway expression and activation (A) before and (B) after therapy. Each bubble represents the relative gene expression of a different gene/mRNA in the EGFR pathway (blue, above average expression; dark gold, average; gold, below average). Across the bottom are three previously defined EGFR activation signatures[8] demonstrating activation in the pretherapy specimen and inactivation after 1 week of combination cetuximab plus chemotherapy. This patient experienced clinical response.

Figs 5A to 5F), including low expression of the EGFR activation cluster in the samples obtained 7 to 14 days after beginning therapy. Low proliferation signature ($P < .001$) was associated with CB ($P < .001$). *KRAS* expression itself was unrelated; however, low *KRAS*-amplicon expression in post-treatment specimens was associated with response ($P = .04$). Because *KRAS* mutations are uncommon in breast cancer,[8,12] unlike in colon cancer, these variations were likely the result of nonmutational influences. Previous studies have implicated phosphatidylinositol 3-kinase (PI3K) –Akt pathway upregulation in resistance.[8,18,19] We did not see change by gene expression in PIK3CA or other pathway members (Akt, mammalian target of rapamycin) in the 11 nonresponders (data not shown); the largest PIK3CA expression increase was in two patients experiencing SD.

DISCUSSION

This trial, among the largest prospective clinical trials limited to TNBC, allowed us to examine a therapeutic question, define the subtype composition of TNBC, and illustrate the behavior and prognosis of this phenotype. Findings included a high degree of visceral involvement; 2-month TTP, with several patients experiencing rapid progression; and OS < 1 year. Response to cetuximab alone was uncommon, and there was low-level activity (RR, 17%) of combination platinum plus cetuximab.

Many studies in TNBC are based on its overlap with the basal-like molecular subtype. However, concordance between TNBC on clinical assays and basal-like gene expression patterns is debated. We found that basal-like breast cancer comprised approximately 75% of TNBCs; the remaining 25% represented all other subtypes, including the claudin-low subtype.[12,20] Although a moderately effective enrichment tool if one is interested in biology of the basal-like subtype, this molecular heterogeneity must be considered when using triple-negative clinical assay status as an eligibility criterion.

Both breast cancer type 1 (BRCA1) –associated and sporadic basal-like breast cancers have characteristics consistent with aberrant DNA repair and genome-wide instability,[21,22] supporting the use of DNA-damaging agents such as the platinum in this study. Clinical data are controversial. Evidence suggests high responsiveness to cisplatin in BRCA1-associated breast cancer[23]; in non-BRCA1 carriers, neoadjuvant cisplatin results in a pathologic CR rate < 20%.[24] The expected RR to platinum agents in unselected patients ranges from < 10% (pretreated) to 25% (chemotherapy naive).[25] Although TNBC may be more chemosensitive in general,[26-28] in a recently reported trial, RR to cisplatin in first- and second-line treatment of TNBC was only 10%,[29] and the pretreated cohort in this study demonstrated an RR of 17%. CALGB 40603 (Cancer and Leukemia Group B; clinicaltrials.gov identifier NCT00861705), a randomized neoadjuvant study of carboplatin added to taxane-based therapy, includes embedded correlative studies addressing mechanisms of platinum sensitivity and resistance. In the meantime, existing clinical data do not support preferential use of platinum agents in sporadic TNBC.

Given the asymptomatic nature of most of the patients in this study and the short PFS typical in TNBC, both response and prolonged SD may be worthy therapeutic goals; however, these

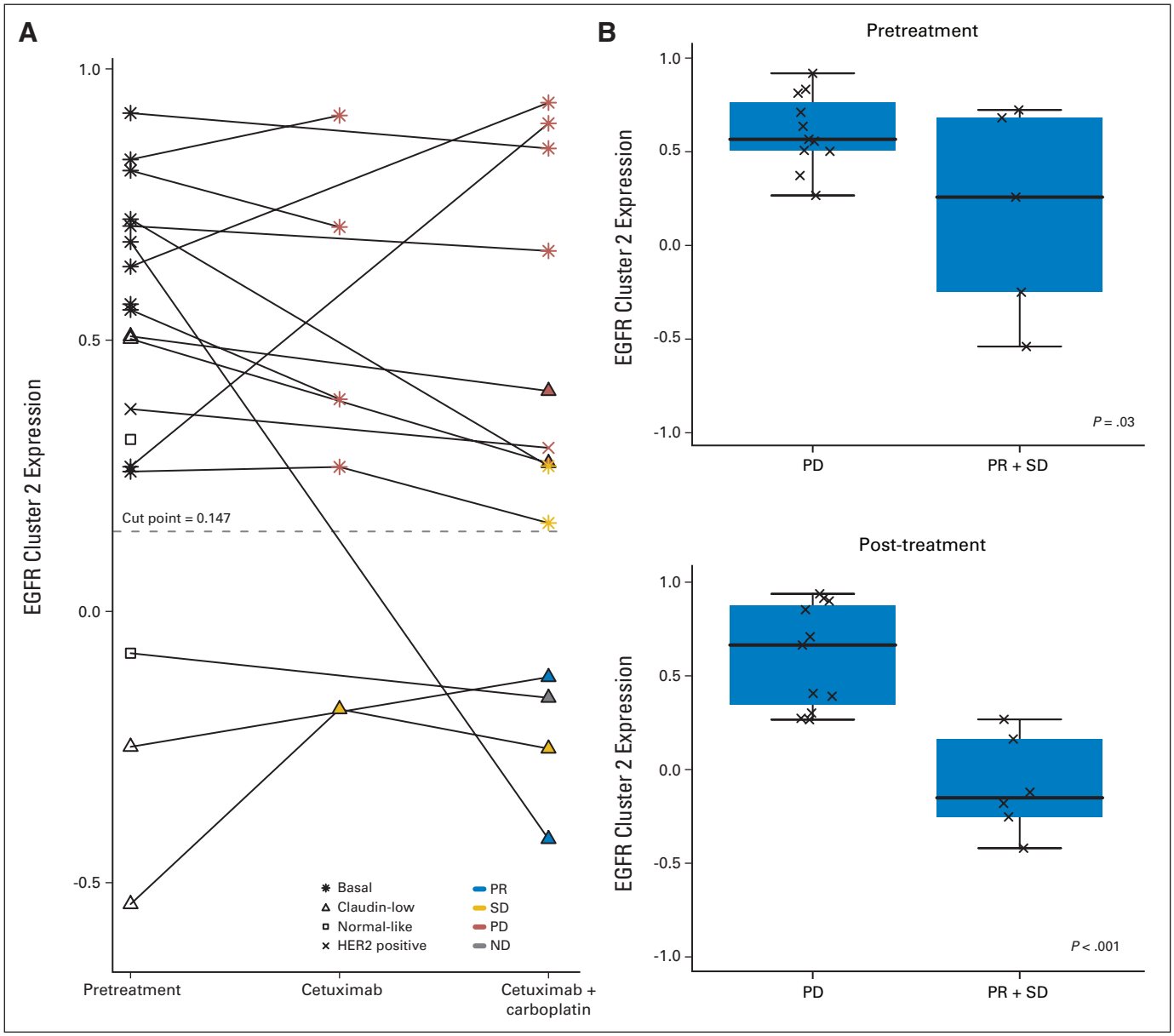

Fig 4. Effect of cetuximab-based therapy on epidermal growth factor receptor (EGFR) pathway expression. (A) Mean expression of the a priori–defined EGFR activation cluster 2[8] in 18 patients, including 16 serial biopsy specimens before and after beginning anti-EGFR therapy, demonstrating that most, but not all, triple-negative breast cancers were basal like, and most had activation of the EGFR pathway (above dashed line). EGFR pathway inhibition was apparent in only a minority of tumors 7 to 14 days after beginning anti-EGFR therapy. Subtype is designated by shape, whereas colors designate clinical response. Dashed line represents the cut point for high or "on" EGFR activation signature. (B) Relationship of EGFR expression signature to response before treatment, demonstrating that high EGFR expression was significantly associated with poor responsiveness to therapy, particularly if EGFR expression remained high after 7 to 14 days of therapy. HER2, human epidermal growth factor receptor 2; ND, not known; PD, progressive disease; PR, partial response; SD, stable disease.

remained minorities. Only 31% of patients had disease control for at least 6 months with combined EGFR inhibitor plus platinum agent in this study, and PFS for the group as a whole was only 2.1 months, suggesting that the majority without CB had a particularly aggressive course. One limitation of this study is that it was not possible to assess the relative contribution of EGFR inhibitor versus chemotherapy. However, in the randomized phase II trial BALI-1 (NCT00463788), cetuximab added to cisplatin increased RR from 10% to 20% and PFS from 1.5 to 3.7 months.[29] The TNBC subset of a randomized study of cetuximab added to irinotecan plus carboplatin also found improved RR, but no improvement in PFS or OS was seen; as in this study, a few patients had sustained long-term responses.[16] In fact, the one patient experiencing PFS > 3 years with single-agent cetuximab had a luminal A metastatic tumor. A broad body of literature implicates EGFR in endocrine resistance; however, EGFR inhibition in ER-positive breast cancer without additional selection criteria has similarly had mixed and generally disappointing results. Although *EGFR* is a key gene in the basal-like gene cluster, and EGFR expression is consistent among basal-like breast cancers, TNBC does not select for EGFR inhibitor sensitivity. Important lessons can be learned from embedded correlative science, especially if serial fresh tissue is obtained. Despite

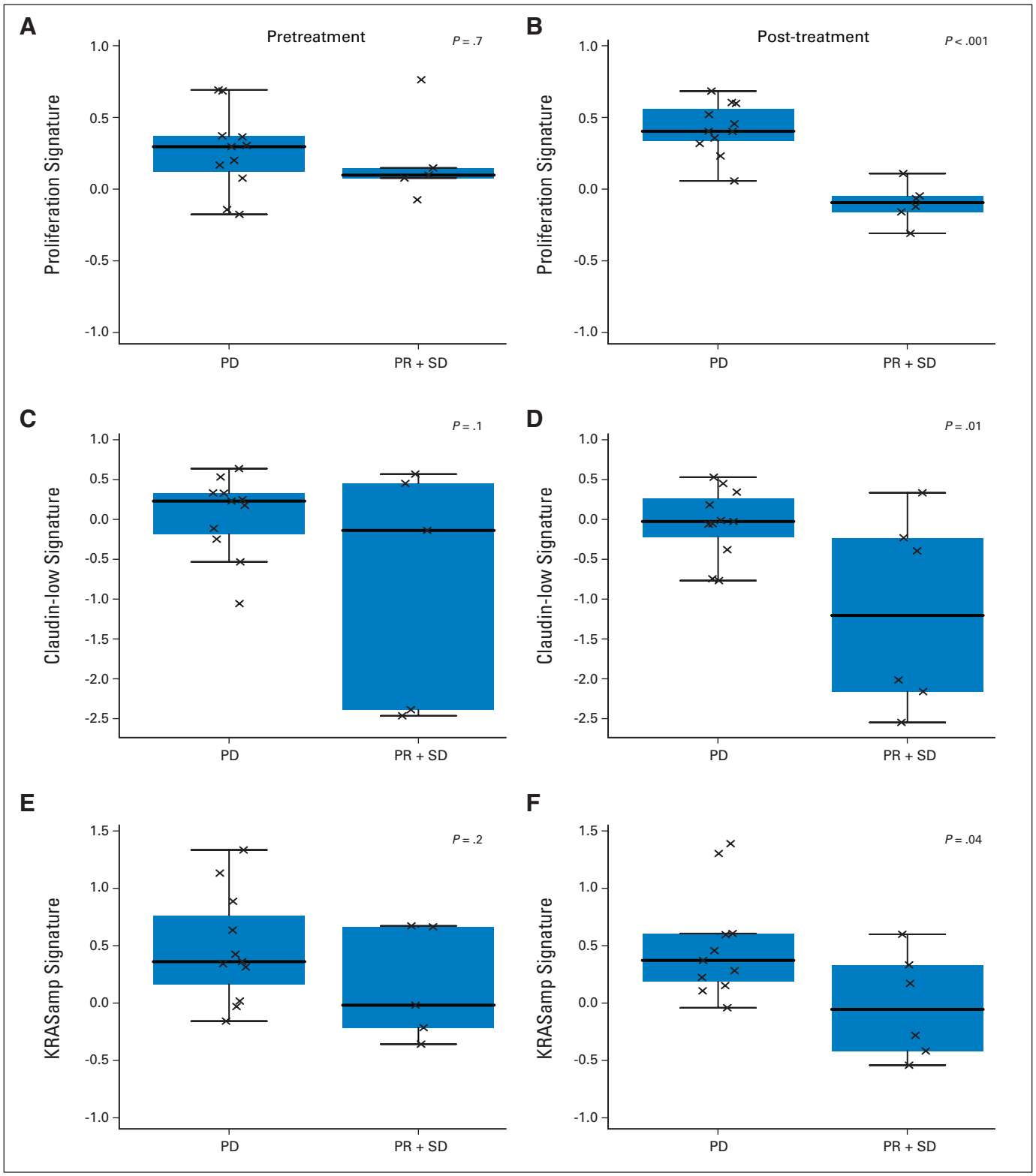

Fig 5. Relationship of signatures (measured pretreatment and after 7 to 14 days) known to affect epidermal growth factor receptor signaling on response to cetuximab-based therapy. Low expression of several signatures measured in pretreatment samples was not associated with response; however, low expression of the same signatures after 7 to 14 days of treatment was significantly associated with clinical benefit: (A, B) 11-gene proliferation signature, (C, D) distance to claudin-low centroid (smaller distance indicates more claudin-low like), and (E, F) *KRAS* amplicon (amp) signature. PD, progressive disease; PR, partial response; SD, stable disease.

interest and expertise among participating institutions, fewer than 20% of patients underwent serial biopsy. Rational therapeutic development in the molecular era requires that we improve this rate. This serial biopsy substudy, which included patients in both single-agent and combination arms, found that most TNBCs had EGFR pathway activation, as assessed by a genomic signature. However, after 1 to 2 weeks of EGFR inhibitor therapy, only a minority demonstrated pathway inhibition. This suggests that either cetuximab is ineffective against this target (unlikely, given activity in other tumor types) or that there are alternate mechanisms that do not depend on ligand-dependent EGFR-mediated activation. In this limited sample, we found biologically plausible candidates such as the EGFR pathway and *KRAS* amplicon. As was seen with the preoperative endocrine prognostic index for neoadjuvant endocrine therapy,[30] we also found that tumor biomarkers may be more informative 1 to 2 weeks after beginning therapy. Preliminary data from a combined data set of TNBC treated in this and another study[16] suggested that certain molecules, such as phosphatase and tensin homolog loss and alpha B-crystallin expression, may affect responsiveness to EGFR inhibitor–based therapy.[31] We believe that many TNBCs may be EGFR pathway dependent, but the constitutive pathway activation in many cases may not be via EGFR but instead by downstream components such as *KRAS* amplification or *CRYAB* expression. From the clinical and correlative studies, it is clear that EGFR inhibition alone is unlikely to provide disease control in most TNBCs; combination strategies targeting other components of the pathway and dedicated tissue–based studies are likely to be necessary.

Metastatic TNBC carries a poor prognosis, and treatment is limited to chemotherapy. This study demonstrated that although most TNBCs were basal like, a breast cancer subtype characterized by higher expression of EGFR, there was little activity seen with an EGFR inhibitor added to a platinum agent. Serial biopsies of the target tumor in a subset of patients before and after therapy revealed that although most had EGFR expression and pathway activation, the EGFR inhibitor seldom inactivated this pathway. Therapy targeting growth factor pathways in this subtype may require a far better understanding of the pathways maintaining EGFR activity, molecular inhibitors of the downstream pathways, and combinatorial strategies. In practical terms, this means that clinical advances in TNBC are unlikely to be successful unless we can seamlessly incorporate tissue-based studies in parallel with clinical trials and do it in 100% of patients.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Perou CM, Sørlie T, Eisen MB, et al: Molecular portraits of human breast tumours. Nature 406:747-752, 2000

2. Sotiriou C, Pusztai L: Gene-expression signatures in breast cancer. N Engl J Med 360:790-800, 2009

3. Carey LA, Perou CM, Livasy CA, et al: Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 295:2492-2502, 2006

4. Sørlie T, Perou CM, Tibshirani R, et al: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 98:10869-10874, 2001

5. Sorlie T, Tibshirani R, Parker J, et al: Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 100:8418-8423, 2003

6. Sotiriou C, Neo SY, McShane LM, et al: Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci U S A 100:10393-10398, 2003

7. Nielsen TO, Hsu FD, Jensen K, et al: Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 10:5367-5374, 2004

8. Hoadley KA, Weigman VJ, Fan C, et al: EGFR associated expression profiles vary with breast tumor subtype. BMC Genomics 8:258, 2007

9. Parker JS, Mullins M, Cheang MC, et al: Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27:1160-1167, 2009

10. Prat A, Parker JS, Karginova O, et al: Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 12:R68, 2010

11. Hu Z, Fan C, Oh DS, et al: The molecular portraits of breast tumors are conserved across microarray platforms. BMC Genomics 7:96, 2006

12. Herschkowitz JI, Simin K, Weigman VJ, et al: Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. Genome Biol 8:R76, 2007

13. Cristofanilli M, Budd GT, Ellis MJ, et al: Circulating tumor cells, disease progression, and survival

in metastatic breast cancer. N Engl J Med 351:781-791, 2004

14. Simon R: Optimal two-stage designs for phase II clinical trials. Control Clin Trials 10:1-10, 1989

15. Berger VW, Ivanova A, Knoll M: Minimizing predictability while retaining balance trough the use of less restrictive randomization procedures. Stat Med 22:3017-3028, 2003

16. O'Shaughnessy J, Weckstein D, Vukelja S, et al: Randomized phase II study of weekly irinotecan/carboplatin with or without cetuximab in patients with metastatic breast cancer. Presented at the 30th Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 13-16, 2007

17. O'Neil BH, Allen R, Spigel DR, et al: High incidence of cetuximab-related infusion reactions in Tennessee and North Carolina and the association with atopic history. J Clin Oncol 25:3644-3648, 2007

18. Hoeflich KP, O'Brien C, Boyd Z, et al: In vivo antitumor activity of MEK and phosphatidylinositol 3-kinase inhibitors in basal-like breast cancer models. Clin Cancer Res 15:4649-4664, 2009

19. Mirzoeva OK, Das D, Heiser LM, et al: Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. Cancer Res 69:565-572, 2009

20. Prat A, Perou CM: Deconstructing the molecular portraits of breast cancer. Mol Oncol 5:5-23, 2011

21. Andre F, Job B, Dessen P, et al: Molecular characterization of breast cancer with high-resolution oligonucleotide comparative genomic hybridization array. Clin Cancer Res 15:441-451, 2009

22. Bergamaschi A, Kim YH, Wang P, et al: Distinct patterns of DNA copy number alteration are associated with different clinicopathological features and gene-expression subtypes of breast cancer. Genes Chromosomes Cancer 45:1033-1040, 2006

23. Byrski T, Gronwald J, Huzarski T, et al: Pathologic complete response rates in young women with BRCA1-positive breast cancers after neoadjuvant chemotherapy. J Clin Oncol 28:375-379, 2010

24. Silver DP, Richardson AL, Eklund AC, et al: Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. J Clin Oncol 28:1145-1153, 2010

25. Decatris MP, Sundar S, O'Byrne KJ: Platinum-based chemotherapy in metastatic breast cancer: Current status. Cancer Treat Rev 30:53-81, 2004

26. 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

27. 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

28. Gradishar W: Management of advanced breast cancer with the epothilone B analog, ixabepilone. Drug Des Devel Ther 3:163-171, 2009

29. Baselga J, Gomez P, Awada A, et al: The addition of cetuximab to cisplatin increases overall response rate and progression-free survival in metastatic triple-negative breast cancer:results of a randomized phase II study (BALI-1). Presented at the 35th European Society of Medical Oncology Congress, Milan, Italy, October 8-12, 2010

30. Ellis M, Tao Y, Luo J, et al: Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. J Natl Cancer Inst 100:1380-1388, 2008

31. Carey LA, O'Shaughnessy J, Hoadley KA, et al: Potential predictive markers of cetuximab benefit in metastatic triple negative breast cancer: An analysis of two randomized phase II trials. Presented at the 32nd Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 9-13, 2009

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