Cohen's kappa statistic was used to measure the level of agreement between HER2 assay methods. We used a test of proportions to compare patient characteristics that were dichotomous. For patient characteristics collected as continuous variables, we compared median values, using the Wilcoxon rank-sum test. Response rates were compared on the basis of categorization of clinical variables and HER2 and p53 status, with the use of a test of proportions. Time to treatment failure (TTF) was measured as the time from study entry to the first locoregional recurrence, first distant metastasis, or death due to any cause; data were censored for patients without events at the last follow-up date. OS was measured as the time from study entry to death, with data censored at the last follow up. Time to event and Kaplan-Meier curves were compared using the log-rank test. Hazard ratios and their 95% confidence intervals (CIs) were calculated for multivariate Cox proportional hazards modeling. The significance of variables in multivariate Cox models was determined using the χ^2 test. All statistical tests were two sided.

Results

Baseline and follow-up characteristics of the patients

Paraffin-embedded tissue blocks were available for 201 of the 474 patients enrolled in CALGB 9342. Blocks from 175 patients passed quality control. A total of 165 patients with at least one biomarker measurement are included in this companion study. The median follow-up time was 8.3 years among patients with at least one biomarker measurement and 8.5 years for the remaining study population (Table 1). The only significant difference between the two groups was the shorter median disease-free interval among patients with biomarker measurements (19 versus 31 months; $P = 0.0003$). Because blocks are often discarded after 10 years, patients with available blocks would, by definition, have a shorter interval between diagnosis and metastasis.

Hormone receptor, HER2, and p53 status

Information on hormone receptor status was available for 148 of the 201 patients from whom tissue blocks were collected. Of these 148 patients, 74 (50%) had tumors that were reported to be hormone receptor positive.

The CB11 assay of HER2 was informative in 162 cases, the HercepTest in 158 cases, and FISH in 152 cases. The results of these assays are summarized in Table 2 and their sensitivity and specificity in Table 3.

The D07 antibody assay for p53 protein was informative in 150 cases; 57 tumors (38%) exhibited evidence of p53 protein over-expression by this method.

p53 Mutations

Sequencing of exons 4 to 11 of the p53 gene was informative in the 152 cases in which we were able to extract adequate DNA from the tumor specimen; 51 mutations were found in 44 patients (29%). Tumors from six patients contained more than one mutation in p53; in the remainder a single mutation was identified. Approximately half were missense mutations, and 31% were categorized as 'other' (in intron sequences). The remaining mutations were classified as splice mutations (9%) or as silent, nonsense, or frameshift mutations (4% each). The largest proportion of mutations was found in either intron 7 (27%) or exon 5 (22%). Mutations in exons 7 and exon 8 accounted for 12% and 18% of the identified mutations, respectively. Mutations in exons 4 and 10 were rare, and there were no mutations in exon 9. Only two tumors were found to

Table 1

Patient characteristics

Characteristic	Patients with biomarker measurements	Patients without biomarker measurements	*P* value
ER positive and/or PR positive (*n* [%])	85 (58%)	159 (62%)	0.45[a]
Prior adjuvant chemotherapy (*n* [%])	98 (60%)	161 (56%)	0.31[a]
Objective response (complete or partial; *n* [%])	38 (24%)	66 (23%)	0.88[a]
Median age (years)	54.9	57.2	0.25[b]
Median follow up (years)	8.3	8.5	0.77[b]
Median number of metastatic sites (*n*)	1	1	0.14[b]
Median disease-free survival (months)	19	31	0.0003[b]
Median time to progression (months)	4.1	4.3	0.62[c]
Median overall survival (months)	12.6	12.3	0.81[c]

Note that data regarding hormone receptor status and prior adjuvant chemotherapy were not available for all patients enrolled in CALGB 9342, and the total number is therefore less than 469 for some of the comparisons. [a]*P* value is based on the test for comparing two proportions. [b]*P* value is based on the Wilcoxon rank-sum test for comparing median values. [c]*P* value is based on a comparison of Kaplan-Meier curves with the log-rank test.