Table 2

Results of HER2 Immunohistochemistry and FISH

Assay	Patients (*n* [%])
HER2 CB11 score	
Negative	130 (80%)
Positive	32 (20%)
Total	162 (100%)
HER2 HercepTest score	
0	63 (40%)
1	45 (28%)
2	17 (11%)
3	33 (21%)
Total	158 (100%)
HER2 FISH	
HER2/CEP17 ratio <2.0	113 (74%)
HER2/CEP17 ratio ≥2.0	39 (26%)
Total	152 (100%)

FISH, fluorescent *in situ* hybridization.

have class II p53 mutations, which have been postulated to be associated with defects in spindle checkpoint control.

Because not all mutations in p53 lead to IHC evidence of p53 over-expression, we assessed the sensitivity and specificity of IHC for detecting p53 mutations that had been identified by sequencing. We found that IHC had a sensitivity of 63% and a specificity of 73%. Because previous studies have shown that over-expression of p53 is highly correlated with missense mutations [39], we evaluated the level of agreement between IHC evidence of p53 over-expression and the presence of a missense mutation in p53. IHC detected the majority of missense mutations identified by sequence analysis, with a sensitivity of 90%. However, the specificity was 72%, reflecting the fact that not all mutations detected by sequencing were detected by IHC.

Treatment response

The rate of response to paclitaxel did not differ significantly on the basis of HER2 or p53 status (Table 4). The response rate was 23% among women with HER2-positive tumors and 24% among those with HER2-negative tumors ($P = 0.96$); the respective response rates for tumors with and for those without p53 over-expression were 23% and 21% ($P = 0.79$). Lower response rates were observed in patients aged 50 years or less (14% versus 29%) and a HER2 score of 0 to 1+ (18% versus 35%); however, only age was significantly associated with TTF ($P = 0.045$) after adjustment for ER/PR status and HER2 status (Table 5).

Outcomes

There was no meaningful difference in TTF between tumors with and without p53 over-expression (median time: 4.0 months versus 4.4 months; $P = 0.061$); however, OS was significantly reduced in this group of patients (11.5 months versus 14.4 months; $P = 0.002$; Figure 1). There was a trend toward a shorter TTF among patients who were HER2 positive than among those who were HER negative (2.3 months versus 4.2 months; $P = 0.067$), but HER2 status was not associated with statistically significant differences in OS (Table 6).

Although TTF did not differ by ER status ($P = 0.13$) in the biomarker subset, OS was significantly better in this group of ER-positive tumors ($P = 0.003$). This observation was also true of ER status in the entire cohort of patients from CALGB 9342, in which TTF did not differ by ER status ($P = 0.27$), whereas ER positivity was associated with more favorable OS ($P = 0.0003$).

To determine whether particular p53 mutations predicted a worse outcome, we grouped missense mutations into nonoverlapping categories, according to the methodology of Alsner and coworkers [24]. However, the number of patients in each subgroup was too small to make meaningful comparisons (nine with mutations in direct DNA contact or zinc binding residues, 10 with mutations in conserved domains, and 18 with mutations outside conserved domains).

Table 3

Agreement among methods for measuring HER2

Method	Cohen's kappa	Sensitivity	Specificity
FISH versus CB11	83.0% (SE 5.3%)	97%	93%
HercepTest versus FISH			
(0–1 versus 2–3) versus FISH	72.0% (SE 6.2%)	92%	87%
(0–2 versus 3) versus FISH	79.2% (SE 6.0%)	78%	97%
HercepTest versus CB11			
(0–1 versus 2–3) versus CB11	70.0% (SE 6.3%)	100%	85%
(0–2 versus 3) versus CB11	84.2% (SE 5.4%)	90%	96%

FISH, fluorescent *in situ* hybridization; SE, standard error for Cohen's kappa.