High-Risk Breast Cancer

18 U.S.C. Section 1734 solely to indicate this fact.

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tumors. The presence of either G/G genotype or an ER-negative tumor who were carriers of any C-allele (G/C or C/C) and had ER-positive allele at a mean follow-up of 55 months. ER status modulated the effect of the G/G genotype had a 2.1-fold increase in the rate of failure and a 2.6-fold increase in the rate of death compared with carriers of any C allele at a mean follow-up of 55 months. ER status modulated the effect of IL-6 polymorphism: we hypothesized that polymorphisms in IL-6 (−174 G>C) or TNF-α (G-238 or G-308) might be associated with prognosis in a subset of patients with high-risk breast cancer.

Genotyping was performed on DNA from stored stem cells in 80 breast cancer patients diagnosed with at least four positive axillary lymph nodes at diagnosis who underwent anthracycline-based adjuvant chemotherapy followed by high-dose multigagent chemotherapy with stem cell rescue. Cox proportional hazards models were used to estimate the effect of genotype and other known prognostic factors on disease-free and overall survival (DFS and OS, respectively).

The presence of at least one C allele in the IL-6 promoter at position −174 was significantly associated with both DFS and OS compared with G/G homozygotes. After adjustment for estrogen receptor (ER) status, number of involved lymph nodes, and tumor size, those patients carrying the G/G genotype had a 2.1-fold increase in the rate of failure and a 2.6-fold increase in the rate of death compared with carriers of any C allele at a mean follow-up of 55 months. ER status modulated the effect of IL-6 polymorphism: both DFS and OS were most favorable in patients who were carriers of any C-allele (G/C or C/C) and had ER-positive tumors. The presence of either G/G genotype or an ER-negative tumor increased the hazard of failure [hazard ratio (HR), 2.6 and 3.2, respectively] and death (HR, 2.0 and 2.2, respectively). The combination of both G/G genotype and ER-negative tumor resulted in an additional increase in the hazard of failure (HR, 5.4; four-group comparison, P = 0.003) and death (HR, 6.2; four-group comparison, P = 0.001). TNF-α −308 and −238 polymorphisms were not associated with variation in DFS or OS in this cohort.

The IL-6−174 promoter polymorphism is associated with clinical outcome in this cohort of node-positive breast cancer patients who received high-dose adjuvant therapy. IL-6 genotype modulated the effect of ER status on outcome. These results support the hypothesis that IL-6 may play an important role in the control of micrometastatic disease in breast cancer. Additional studies are needed to confirm these results and elucidate the mechanisms responsible for these differences.

INTRODUCTION

Metastatic breast cancer is currently incurable. Patients presenting with localized breast cancer involving multiple ipsilateral axillary lymph nodes have a high risk of recurrence and subsequent death from the disease. Standard adjuvant systemic therapy has had limited success, reducing recurrence risks by ~33% overall. These approaches typically involve the use of chemotherapeutic and/or hormonal agents. More recently, high-dose chemotherapy with peripheral stem cell rescue has been used in high-risk patients, but current data suggest that this approach, whereas feasible, does not confer significant advantages in DFS or OS in breast cancer patients. Moreover, systemic adjuvant chemotherapy can result in substantial short- and long-term toxicities that may adversely affect quality of life for breast cancer survivors. There is a need for biomarkers that can reliably identify patients who: (a) will clearly benefit from current adjuvant systemic therapy such that the benefits outweigh potential risks; (b) have been cured with surgical resection and therefore do not need adjuvant therapy; or (c) will recur despite standard therapies and are, thus, candidates for investigational approaches.

Currently available clinical and molecular prognostic tools provide some information on the risk of recurrence among poor-prognosis breast cancer patients. Work in this area has primarily focused on molecular and genetic tumor alterations that influence response to treatment and ultimate clinical outcome. Hormone receptors (estrogen and progesterone receptors) as well as HER-2/ neu overexpression are well-validated tumor markers that can provide important prognostic information as well as predict response to therapies such as tamoxifen or Herceptin. Many other tumor markers, including proteins involved in cell cycling and angiogenesis, are being validated currently in this setting.

We hypothesized that host (patient) factors may also play an important role in determining risk of recurrence by influencing the milieu in which microscopic residual tumor cells are able to proliferate. We have approached the search for new prognostic markers by examining associations between clinical outcome and host genetic variants that might subsequently affect the response of the body to microscopic residual cancer.

Immune surveillance is one such host process that may influence recurrence, because several cytokines have been implicated in mediating this process. IL-6 is a pleiotropic, proinflammatory cytokine that may be involved in the host response to cancer. In vitro data suggest that cytokines such as IL-6 may provide a growth stimulus for breast cancer cells through as-yet unidentified mechanisms. In metastatic breast cancer, high serum IL-6 levels have been associated with a greater number of metastatic sites (1), poorer clinical outcome (1),

ABSTRACT

Axillary lymph node involvement in breast cancer is a marker of recurrence risk. Despite aggressive adjuvant therapy, recurrence in patients with four or more involved lymph nodes approaches 50% at 5 years from diagnosis. Markers that can distinguish those likely to relapse from those likely to be cured are needed to tailor therapy and provide accurate prognostic information to patients. Although most work in this area has focused on tumor characteristics, we hypothesized that the host environment might also play a role in determining risk of relapse. We hypothesized that host inflammatory response, mediated in part by production of interleukin-6 (IL-6), might play a role in the elimination of microscopic residual tumor. Polymorphisms in the IL-6 promoter region appear to modulate serum levels of the cytokine via regulation of gene transcription. A single nucleotide polymorphism involving substitution of cytosine for guanine at position −174 has been associated with reduced transcription and improved outcome in a variety of nonmalignant diseases, including coronary artery disease and several autoimmune conditions. Tumor necrosis factor (TNF) α is a proinflammatory cytokine that also plays a role in regulating IL-6 transcription. We hypothesized that polymorphisms in IL-6 (−174 G>C) or TNF-α (G-238 or G-308) might be associated with prognosis in a subset of patients with high-risk breast cancer.

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Immune surveillance is one such host process that may influence recurrence, because several cytokines have been implicated in mediating this process. IL-6 is a pleiotropic, proinflammatory cytokine that may be involved in the host response to cancer. In vitro data suggest that cytokines such as IL-6 may provide a growth stimulus for breast cancer cells through as-yet unidentified mechanisms. In metastatic breast cancer, high serum IL-6 levels have been associated with a greater number of metastatic sites (1), poorer clinical outcome (1),
lack of response to therapy (2), resistance to chemotherapy (3), and resistance to hormonal therapy (4).

Polymorphic variants in the promoter region of the IL-6 gene may be responsible for variations in transcription that subsequently affect serum levels of the cytokine. The best characterized of these polymorphisms is a SNP at position −174, upstream of the transcription start site, involving substitution of cytosine for guanine (henceforth referred to as IL-6−174 G/C). Presence of the “G” allele has been associated with poor outcome in a variety of diseases, including coronary artery disease (5), graft versus host disease after allogeneic bone marrow transplant (6), more severe manifestations of Sjogren syndrome and systemic lupus erythematosus (7), and increased risk of juvenile rheumatoid arthritis (8).

We hypothesized that IL-6 might play a role in the control of residual microscopic disease among women with high-risk breast cancer and, thus, impact natural history of the disease among aggressively treated patients. We investigated associations between clinical outcome and the IL-6−174 G/C polymorphism in patients who had been diagnosed with node-positive, poor-prognosis breast carcinoma. Because IL-6 production is in part controlled by TNF-α, we also examined two functional polymorphisms in the TNF-α promoter, −238G>A and −308G>A. The primary aim of the current study was to determine whether there were associations between these polymorphisms and either DFS or OS in a population of women with high-risk breast cancer who received a uniform treatment regimen consisting of standard anthracycline-containing chemotherapy followed by high dose chemotherapy. A secondary aim of the study was to evaluate whether the impact of genotype on outcome differed by ER expression in the tumor.

MATERIALS AND METHODS

After Institutional Review Board approval, we evaluated patients with high-risk, node-positive breast cancer (≥4 positive nodes) enrolled previously on one of two clinical trials at the University of Pennsylvania Cancer Center from June 1992 through December 1997. Subjects consenting for these protocols had concurrently consented to the use of their stem cell materials for clinical research. All of the patients in the current study received conventional adjuvant chemotherapy with an anthracycline-containing regimen followed by bone marrow and/or peripheral blood stem cell harvest. After harvest, bone marrow or stem cells were resuspended in a combination of 10% DMSO and autologous plasma before storage at −140°C. The high-dose chemotherapy regimen consisted of concurrent cyclophosphamide 1500 mg/m² as a continuous infusion daily for 4 days and thiopeta 200 mg/m² as a continuous infusion daily for 4 days. Thawed bone marrow or stem cells were reinjected through a central venous catheter at least 48 h after completion of chemotherapy. Patients with positive estrogen and/or progesterone receptors were treated with tamoxifen, 20 mg/day, after hematopoietic recovery. Patients received radiation treatment to the breast or chest wall, and to ipsilateral axillary and supraclavicular lymphatics either before high-dose therapy or after hematopoietic recovery. Patients were followed for recurrence and death.

Genomic DNA was extracted from residual stem cell or bone marrow samples available from the University of Pennsylvania Department of Pathology. Subject samples were identified by sample identification number only, and clinical information was assigned a study identification number. Thus, clinical outcome data were not available to laboratory personnel, and no patient identifiers were linked to clinical data. DNA isolation was performed using the Purgene DNA Isolation kit (Genta, Inc.) according to the manufacturer’s guidelines with minor modifications. The DNA was then stored at 4°C for short-term storage, or between −20°C and −80°C for long-term storage. PCR reactions were used to amplify the regions containing the polymorphisms of interest. The primers 5'-ATGCCAATGTCGTCGCTCACTA-3' (in the forward direction) and 5'-TCGAGGAGATGTCGGCTC-3' (in the reverse direction) were used to amplify the region containing the TNF-α variant. PCR amplification was performed in a final volume of 20 μl containing 80 ng of DNA, 1.5 mM of MgCl₂, 10 mM Tris- HCl (pH 8.3), 50 mM KCl, 0.2 mM each of dCTP, dATP, dGTP, dTTP (Amersham Pharmacia Biotech), each primer at 1.0 μM, and 1.0 unit of Taq polymerase (Boehringer Mannheim) in a 9700 Perkin-Elmer/Cetus Thermocycler.

For the IL-6 G-174C polymorphism, 10 μl of the PCR product was digested with 2 units of NlaIII using the manufacturer’s recommended protocol. PCR products were visualized on 3% agarose gels with 10% ethidium bromide. PCR products for the IL-6 polymorphism were analyzed by RFLP analysis (Fig. 1) and subsequent confirmation of variants by direct sequencing. PCR products for the TNF-α polymorphism were analyzed by direct sequencing.

Descriptive statistics of patient and tumor characteristics, including medians and ranges for continuous measures and frequencies, and percentages for categorical measures were performed. The method of Kaplan and Meier was used to estimate DFS and OS for the entire patient cohort and by each polymorphism. DFS was defined as days from stem cell reinfusion to breast cancer recurrence, death due to any cause, or last patient contact. OS was defined as days from reinfusion to death due to any cause or last patient contact. Subjects were censored for recurrence if a new primary breast cancer (invasive or noninvasive) was diagnosed (1 patient).

Associations between a polymorphism and DFS or OS were assessed by the log rank test. Cox regression analysis (9) was used to estimate the HR for the IL-6 polymorphism, adjusting for other prognostic variables, as well as to test for interaction between IL-6 polymorphism and ER status. All tests of significance are two-sided. Analyses were performed in SPSS (SPSS Inc., Chicago, IL).

RESULTS

A total of 124 patients was identified who had received high-dose adjuvant chemotherapy for node-positive breast cancer at the University of Pennsylvania between July 1992 and December 1997. The majority of subjects identified (n = 119) were enrolled on an in-house protocol, the results of which have been published previously (10). Five additional patients were treated on the high-dose therapy arm of an Eastern Cooperative Oncology Group protocol (E2190) through the University of Pennsylvania Cancer Center during the same time period, on which eligibility criteria and treatment were identical. Stored stem cell aliquots from 92 of the 124 eligible patients (74%) were available for the current study. The 32 patients without stem cell specimens available were similar with respect to patient and disease characteristics; there were no significant differences in median age, median tumor size, or number of positive lymph nodes compared with study subjects with stem cell aliquots available. Of the 92 patients with specimens, 5 specimens did not yield DNA, 3 subjects expired in
transplant, and 4 subjects were lost to follow-up, for a final study cohort of 80 patients with DNA specimens.

Clinical characteristics of the 80 patients with analyzable DNA samples are summarized in Table 1. The median age was 43 (range, 24–60). Median tumor size was 3 cm (range, 0.8–9.4 cm). Eighty-one percent of the cohort had at least 10 positive lymph nodes \((n = 65)\), with the remainder \((n = 15)\) having between 4 and 9 positive lymph nodes. The median number of positive lymph nodes was 16 (range, 4–42). More than half were ER positive (59%) and/or progesterone receptor positive (48%). DFS and OS curves for the entire study cohort \((n = 80)\) are shown in Fig. 2. Median follow-up time for all 80 of the patients was 55 months; median follow-up time for those 53 patients alive at last follow-up is 50 months. As of May 2002, 32 of the patients was 55 months; median follow-up time for those 53 patients is 42 months. Ten patients (13%) had a recurrence of breast cancer; 44 patients (55%) remained disease-free. Disease status before death was unknown on 4 patients (5%). Fifty-three patients (66%) remain alive, and 27 patients remained disease-free. Disease status before death was unknown on 4 patients (5%). Fifty-three patients (66%) remain alive, and 27 patients were confirmed as deceased. Median DFS for the cohort studied was 68 months. Median OS time has not yet been reached.

Allele frequencies were calculated for the IL-6 –174G>C polymorphism and the two TNF-α polymorphisms, –238G>A and –308G>A (Table 2). Variants in the IL-6 gene were most common (64% total; 49% heterozygous G/C and 15% homozygous C/C) with proportions similar to published distributions in the general population (8). TNF-α polymorphisms were less frequent, with 9% having a –238 A variant and 35% having a –308 A variant. For most analyses of the IL-6 polymorphism, carriers of homozygous and heterozygous variants were pooled (due to small numbers of patients with the C/C genotype) and compared with WT G/G homozygotes.

In the univariate analysis for DFS (Table 3), patients without at least one C allele in the IL-6 –174 promoter region had a 4-year DFS of 45% (-10%) compared with 65% (-7%) among patients with at least one C allele \((P = 0.02)\). Among the 29 patients homozygous for the G allele at the IL-6 promoter locus, median DFS was 39 months. Median DFS for G/G plus C/C carriers has not yet been reached. Patients homozygous for the G allele \((G/G)\) had a significantly increased risk of recurrence compared with those with the IL-6 polymorphism \((G/C\) or C/C; HR, 2.2; 95% CI, 1.1–4.1; \(P = 0.02\); Fig. 3). DFS was not significantly different between carriers of either TNF-α variant compared with those with the WT genotype (Table 3).

In the univariate analysis for OS (Table 3), patients homozygous for...
There were no significant associations between either lymph node and DFS, OS, and the IL-6 polymorphism were tested (Table 4). As patients with 10 or more positive lymph nodes. The IL-6 polymorphism in addition to known prognostic factors: ER, lymph node group, and tumor size. The inclusion of other prognostic factors in the model did not impact on the relationship between IL-6 polymorphism and outcome (as demonstrated by similar unadjusted and adjusted HRs for IL-6 polymorphism); patients with the G/G genotype had a 2.1-fold increase in the hazard of developing a recurrence over the study period (95% CI, 1.05–4.1; P = 0.035) and a 2.6-fold increase in the hazard of death (95% CI, 1.2–5.8; P = 0.02) after adjusting for other factors. Of the additional variables considered, only ER status was significantly associated with DFS and/or OS, those with ER-negative tumors had a 3.2-fold increase in hazard of developing a recurrence (95% CI, 1.5–6.8; P = 0.002) and a 3.4-fold increase in the hazard of death (95% CI, 1.4–8.1; P = 0.005) after adjustment for other factors, including IL-6 genotype.

Because of the observed significant association between ER and outcome, as well as our a priori hypothesis that IL-6 may be exerting its effect through hormonally mediated pathways, we examined more closely the interaction between ER status and IL-6 polymorphism on both DFS and OS (Table 5). For DFS (Fig. 5), the most favorable outcome was seen in patients whose tumors were ER positive and who were carriers of at least one C-allele (G/C or C/C). Having either G/G genotype or absence of tumor ER increased the hazard for failure (HR, 2.6 and HR, 3.2, respectively), and the combination of both these factors resulted in an additional increase in the HR (HR = 5.4; four-group comparison, P = 0.003). Similarly, for OS (Fig. 6), having either G/G genotype or absence tumor ER increased the hazard for death (HR, 2.0 and HR, 2.2, respectively), and the combination of both these factors resulted in an additional increase in the HR (HR, 6.2; four-group comparison P = 0.001). Thus, the ER status and IL-6 promoter polymorphism clearly had a significant combined effect on both DFS and OS, although tests of interaction were not significant (P = 0.5 and P = 0.65, respectively), possibly due to the small sample size.

### Table 4: Unadjusted and adjusted HRs for DFS and OS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>Unadjusted HR (95% CI)</th>
<th>P</th>
<th>Adjusted HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>G/G vs. any C</td>
<td>2.2 (1.1–4.1)</td>
<td>0.02</td>
<td>2.1 (1.1–4.1)</td>
<td>0.035</td>
</tr>
<tr>
<td>ER</td>
<td>Neg vs. pos</td>
<td>2.7 (1.4–5.3)</td>
<td>0.004</td>
<td>3.2 (1.5–6.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>Lymph node</td>
<td>&gt;10 vs. 4–9</td>
<td>0.5 (0.2–1.1)</td>
<td>0.07</td>
<td>0.7 (0.3–1.6)</td>
<td>0.40</td>
</tr>
<tr>
<td>T size</td>
<td>&lt;2 vs. ≥2</td>
<td>0.8 (0.4–2.0)</td>
<td>0.73</td>
<td>1.1 (0.5–2.7)</td>
<td>0.22</td>
</tr>
<tr>
<td>OS</td>
<td>&gt;5 vs. ≤5</td>
<td>0.7 (0.3–1.8)</td>
<td>0.5</td>
<td>0.5 (0.2–1.5)</td>
<td>0.5</td>
</tr>
<tr>
<td>IL-6</td>
<td>G/G vs. any C</td>
<td>2.6 (1.2–5.6)</td>
<td>0.01</td>
<td>2.6 (1.2–5.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>ER</td>
<td>Neg vs. pos</td>
<td>2.8 (1.3–6.1)</td>
<td>0.009</td>
<td>3.4 (1.4–8.1)</td>
<td>0.005</td>
</tr>
<tr>
<td>Lymph node</td>
<td>&gt;10 vs. 4–9</td>
<td>0.7 (0.3–1.6)</td>
<td>0.36</td>
<td>1.0 (0.4–2.7)</td>
<td>0.99</td>
</tr>
<tr>
<td>T size</td>
<td>&lt;2 vs. ≥2</td>
<td>1.9 (0.5–6.4)</td>
<td>0.61</td>
<td>2.3 (0.7–7.8)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* Patients with 4–9 positive lymph nodes (n = 15) relapsed more frequently than with 10 or more positive lymph nodes.

### Table 5: Outcome by ER status and IL-6 polymorphism

<table>
<thead>
<tr>
<th>ER Genotype</th>
<th># Patients</th>
<th>4-Year Rate ± SE</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFS ER–</td>
<td>G/G</td>
<td>14</td>
<td>26% ± 12%</td>
<td>5.4 (2.1–14.4)</td>
</tr>
<tr>
<td>Any C</td>
<td>19</td>
<td>47% ± 11%</td>
<td>3.2 (1.3–8.1)</td>
<td></td>
</tr>
<tr>
<td>ER+ G/G</td>
<td>15</td>
<td>64% ± 13%</td>
<td>2.6 (1.0–7.0)</td>
<td></td>
</tr>
<tr>
<td>Any C</td>
<td>32</td>
<td>76% ± 8%</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>OS ER–</td>
<td>G/G</td>
<td>14</td>
<td>31% ± 14%</td>
<td>6.2 (2.2–17.4)</td>
</tr>
<tr>
<td>Any C</td>
<td>19</td>
<td>68% ± 11%</td>
<td>2.2 (0.7–6.8)</td>
<td></td>
</tr>
<tr>
<td>ER+ G/G</td>
<td>15</td>
<td>70% ± 12%</td>
<td>2.0 (0.6–6.5)</td>
<td></td>
</tr>
<tr>
<td>Any C</td>
<td>32</td>
<td>83% ± 7%</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
</tbody>
</table>

*Fig. 5. DFS for groups defined by ER status and IL-6 polymorphism. HRs for failure are 5.4 (95% CI, 2.1–14.4), 3.2 (95% CI, 1.3–8.1), and 2.6 (95% CI, 1.0–7.0) for the ER-negative and G/G, ER-negative and any C, and ER-positive and G/G groups, respectively, compared with the ER-positive and any C group.*
tively, compared with the ER-positive and any C group.

in 269 subjects age 70 or over in Italy. Differences in serum levels
C/C, plasma IL-6. Hulkkonen et al. studied plasma levels of IL-6 in 102 healthy subjects, and the C
174G/C genotype to serum levels, both in normal subjects, and in
product in the serum. Several studies have attempted to link IL-6
gene expression and subsequent activity or quantity of the gene
progression.

important information linking IL-6 to breast cancer growth and pro-
linking a polymorphism in the gene encoding IL-6 provides additional
kines and subsequent serum levels, which can confound the relation-
medications (8). Third, other factors, such as age and nutritional
status, may impact IL-6 levels. These issues beg the question, can
differences in cumulative IL-6 exposure be reliably detected by mea-
urement of serum level in isolated points in time? We conclude that
the available in vivo data demonstrating lower IL-6 levels in carriers
of the IL-6–174 G/C SNP are strongly suggestive, and that more robust data may only come from additional in vitro study of the
impact of the SNP on transcriptional activity. Unfortunately, serum
was not available in our study cohort, and, thus, IL-6 measurements
could not be performed in the current study.

It is thought that circulating levels of IL-6 are largely regulated at
the level of expression due to the rapid plasma clearance of this
cytokine (14). Results of several studies support the notion that the
IL-6–174G/C SNP is a functional alteration that affects gene tran-
scription and subsequent serum levels of IL-6 cytokine. Recent data
from Belluco et al. (15) demonstrates that circulating IL-6 levels are
significantly higher in IL-6–174 G/G homozygotes with colon cancer
compared with carriers of a C allele, and correlated significantly with
the presence of hepatic metastases. Fishman et al. (8) reported the first
population-based study examining IL-6–174G/C genotype, plasma
IL-6 level, and clinical outcome in juvenile chronic arthritis in the
United Kingdom. They found that when comparing constructs of the
5’ flanking region in a luciferase reporter vector transiently trans-
ferred in HeLA cells, the −174C construct showed 0.15-fold lower
expression than the t174G construct. After stimulation with lipopolysaccharide or IL-1, expression from the −174C construct did not
significantly change after 24 h (increase of 0.10-fold), whereas ex-
pression from the 174G construct increased significantly by 0.26-fold
(P = 0.0001). Terry et al. (16) confirmed these findings in an elegant
study examining the functional effects of four separate SNPs in the
IL-6 promoter (including −174G/C) transfected into both HeLa cells and the ECV304 cell line. This study showed that more than one of the
polymporphic sites was functional, but when the −174G/C polymor-
phism was considered alone, variants containing a C allele showed
lower expression than the G/G genotype. Taken together, these studies
provide evidence of an enhancement in transcription of IL-6 associ-
ated with the G-allele, additionally supporting the serum findings
described above, and providing a basis for the use of genotype as a
surrogate marker of cumulative IL-6 exposure in the current study.

Our finding of an additive effect of ER status in the tumor and IL-6
polymorphism in the host provides additional support for the hypoth-
esis that IL-6 exerts its effect on breast cancer cells at least in part
through hormonal pathways. Cytokines, such as IL-6 and TNF-α,
have an important role in regulating estrogen synthesis in peripheral
tissues, including normal and malignant breast tissues (17). In vitro,
the activities of aromatase, estradiol, 17-β-hydroxysteroid dehydro-
genase, and estrone sulfatase are all increased by IL-6 and TNF-α.
This mechanism could in part explain the worse prognosis among
ER-positive patients with the G/G genotype in our study, and warrants
additional research.

These findings, whereas important, are preliminary. The small
sample size requires that these findings be viewed as exploratory and
interpreted in context. We did not have prospectively documented
information on race in the study database, and, thus, could not assess
whether the associations between IL-6 genotype and clinical outcome
(60–80, 81–99, and >99), men without at least one C allele had
higher IL-6 serum levels than male C allele carriers. Whereas these
studies support the hypothesis that IL-6 serum exposure may vary by
genotype, two additional studies have failed to find this association
(12, 13). It is possible that these studies failed to find the association
between genotype and spot serum IL-6 for several reasons. First, there
is tremendous intradividual variation in IL-6 level, even in non-
pathological circumstances. Second, IL-6 levels may be influenced by
medications (8). Third, other factors, such as age and nutritional
status, may impact IL-6 levels. These issues beg the question, can
differences in cumulative IL-6 exposure be reliably detected by mea-
urement of serum level in isolated points in time? We conclude that
the available in vivo data demonstrating lower IL-6 levels in carriers
of the IL-6–174 G/C SNP are strongly suggestive, and that more robust data may only come from additional in vitro study of the
impact of the SNP on transcriptional activity. Unfortunately, serum
was not available in our study cohort, and, thus, IL-6 measurements
could not be performed in the current study.

It is thought that circulating levels of IL-6 are largely regulated at
the level of expression due to the rapid plasma clearance of this
cytokine (14). Results of several studies support the notion that the
IL-6–174G/C SNP is a functional alteration that affects gene tran-
scription and subsequent serum levels of IL-6 cytokine. Recent data
from Belluco et al. (15) demonstrates that circulating IL-6 levels are
significantly higher in IL-6–174 G/G homozygotes with colon cancer
compared with carriers of a C allele, and correlated significantly with
the presence of hepatic metastases. Fishman et al. (8) reported the first
population-based study examining IL-6–174G/C genotype, plasma
IL-6 level, and clinical outcome in juvenile chronic arthritis in the
United Kingdom. They found that when comparing constructs of the
5’ flanking region in a luciferase reporter vector transiently trans-
ferred in HeLA cells, the −174C construct showed 0.15-fold lower
expression than the t174G construct. After stimulation with lipopolysaccharide or IL-1, expression from the −174C construct did not
significantly change after 24 h (increase of 0.10-fold), whereas ex-
pression from the 174G construct increased significantly by 0.26-fold
(P = 0.0001). Terry et al. (16) confirmed these findings in an elegant
study examining the functional effects of four separate SNPs in the
IL-6 promoter (including −174G/C) transfected into both HeLa cells and the ECV304 cell line. This study showed that more than one of the
polymporphic sites was functional, but when the −174G/C polymor-
phism was considered alone, variants containing a C allele showed
lower expression than the G/G genotype. Taken together, these studies
provide evidence of an enhancement in transcription of IL-6 associ-
ated with the G-allele, additionally supporting the serum findings
described above, and providing a basis for the use of genotype as a
surrogate marker of cumulative IL-6 exposure in the current study.

Our finding of an additive effect of ER status in the tumor and IL-6
polymorphism in the host provides additional support for the hypoth-
esis that IL-6 exerts its effect on breast cancer cells at least in part
through hormonal pathways. Cytokines, such as IL-6 and TNF-α,
have an important role in regulating estrogen synthesis in peripheral
tissues, including normal and malignant breast tissues (17). In vitro,
the activities of aromatase, estradiol, 17-β-hydroxysteroid dehydro-
genase, and estrone sulfatase are all increased by IL-6 and TNF-α.
This mechanism could in part explain the worse prognosis among
ER-positive patients with the G/G genotype in our study, and warrants
additional research.

These findings, whereas important, are preliminary. The small
sample size requires that these findings be viewed as exploratory and
interpreted in context. We did not have prospectively documented
information on race in the study database, and, thus, could not assess
whether the associations between IL-6 genotype and clinical outcome

DISCUSSION

This study adds important information to our understanding of IL-6
in breast cancer. The finding that a germ-line SNP is significantly
associated with recurrence and death from breast cancer provides
strong evidence of a mechanistic link between host environment and
tumor growth potential in this disease. To our knowledge, this is the
first study to demonstrate a significant association between host
immune response polymorphisms and clinical outcome from breast
cancer.

Several studies have found a link between IL-6 levels and breast
cancer outcome, although none to date have demonstrated that genetic
polymorphisms in the IL-6 promoter region correlate with prognosis.
Zhang and Adachi (1) demonstrated that serum IL-6 levels are higher
in patients with more numerous metastatic sites and poorer clinical
outcome. Several other studies have found associations between higher
serum IL-6 levels and poor response to breast cancer therapy
(2), including resistance to both chemotherapy (3) and hormonal
therapies (4). However, many factors influence production of cyto-
kines and subsequent serum levels, which can confound the relation-
ship between these substances and outcomes. Our study, significantly
linking a polymorphism in the gene encoding IL-6 provides additional
important information linking IL-6 to breast cancer growth and pro-
gression.

The presence of a SNP in the promoter of IL-6 is only likely to
influence clinical outcome if the presence or absence of the SNP alters
gene expression and subsequent activity or quantity of the gene
product in the serum. Several studies have attempted to link IL-6–174G/C genotype to serum levels, both in normal subjects, and in
those suffering either chronic disease or acute illness. Fishman et al.
(8) studied plasma levels of IL-6 in 102 healthy subjects, and the C
allele was found to be associated with significantly lower levels of
plasma IL-6. Hulkkonen et al. (7), studying 111 patients with Sjogrens Syndrome, found that IL-6 plasma levels were higher in the patients
with the G/G genotype (5.35 + 3.01 pg/ml) compared with those
carrying G/C (3.96 ± 2.71 pg/ml) or C/C (3.52 ± 2.40 pg/ml; G/G
versus C/C, P < 0.05). Bonafe et al. (11) found a similar association
in 269 subjects age 70 or over in Italy. Differences in serum levels
were analyzed by age group and gender. In all three of the age groups

0.12 0.24 0.36 0.48 0.60 0.72 0.84 0.96 1.08 1.20 1.32
8055

Fig. 6. OS for groups defined by ER status and IL-6 polymorphism. HRs for death are
6.2 (95% CI, 2.2–17.4), 2.2 (95% CI, 0.7–6.8), and 2.0 (95% CI, 0.6–6.5) for the
ER-negative and G/G, ER-negative and any C, and ER-positive and G/G groups respec-
tively, compared with the ER-positive and any C group.
remain after adjustment for race. Moreover, the distribution of race/ethnicity in the breast cancer population of the study site consists of 86% Caucasian, 9% African American, and 5% other. Thus, if the study population reflected this overall patient population, our small sample size would likely have precluded any formal analyses by race or ethnicity.

Three patients in our study died during the immediate post-transplant period due to complications of treatment and were, thus, excluded from the disease-related outcomes of interest. All 3 of the patients were homozygous for the “poor prognosis” (G/G) IL-6–174 polymorphism, thus inclusion of these patients would likely have strengthened the association between genotype and outcome. Future studies should consider including toxicity as a disease outcome that might be associated with genotype, as IL-6-mediated inflammation may play a role in the development of treatment-related morbidity.

Characteristics of the breast tumors of our study patients might have shed additional light on the mechanisms by which an association between IL-6 production and microscopic tumor growth might operate. In vitro data suggest that inflammatory cytokines provide signals for tumor cells, operating through specific cell membrane receptors to trigger intracellular signaling pathways rather than simply as regulators of the inflammatory response (18,19). IL-6 signaling occurs through both IL-6 receptors (20) and via crosstalk between the IL-6 gp130 signal transducing subunit and other membrane receptors, including the HER family of receptor tyrosine kinases (18,20). A soluble IL-6R has been identified that has paracrine activity (21), also signaling through gp130. These interactions are thought to be important for modulation and amplification of cytokine signaling in tumor-specific settings. Unfortunately, tumor samples were not available on this retrospective cohort. Future prospective studies are needed to evaluate whether tumor cells themselves contain increased IL-6 receptor or cytokine, whether tumor-infiltrating lymphocytes express IL-6 mRNA, or whether IL-6 genotype is associated with alterations in cell signaling pathways in breast tumors, resulting in detrimental physiological effects on residual microscopic breast cancer cells in node-positive patients.

Finally, as with any observational study of this type, our findings of an association between IL-6 genotype and breast cancer outcomes may have resulted from the linkage of this polymorphism with another susceptibility locus rather than from a direct causal effect. A single observational study is not sufficient to determine a causal link, despite a biologically plausible hypothesis and strong, independent association. Findings of an association between the IL-6–174 promoter polymorphism and outcome in a variety of other diseases, including coronary artery disease (22) and autoimmune diseases (7), suggest that this SNP may have functional consequences, but additional work is clearly necessary to both confirm our findings in breast cancer and to elucidate the functional consequences of IL-6–174 promoter polymorphisms in this patient population.

REFERENCES