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Telomerase Reverse Transcriptase Promoter Alterations Across Cancer Types as Detected by Next-Generation Sequencing: A Clinical and Molecular Analysis of 423 Patients

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Abstract

BACKGROUND—Telomerase reverse transcriptase (TERT) promoter mutations that may affect telomerase activity have recently been described in human malignancies. The purpose of this study was to investigate the clinical correlates of TERT promoter abnormalities in a large cohort of patients with diverse cancers.

METHODS—This study analyzed TERT promoter alterations and clinical characteristics of 423 consecutive patients for whom molecular testing by next-generation sequencing was performed between August 2014 and July 2015.

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AUTHOR CONTRIBUTIONS
Maria Schwaederle: Conception and design, acquisition and statistical analysis of data, data interpretation and initial manuscript writing, critical revision/editing of the manuscript, and approval of the final manuscript. Nithya Krishnamurthy: Data interpretation and initial manuscript writing, critical revision/editing of the manuscript, and approval of the final manuscript. Gregory A. Daniels: Provision of study materials, critical revision/editing of the manuscript, and approval of the final manuscript. David E. Piccioni: Provision of study materials, critical revision/editing of the manuscript, and approval of the final manuscript. Santosh Kesari: Provision of study materials, critical revision/editing of the manuscript, and approval of the final manuscript. Paul T. Fanta: Provision of study materials, critical revision/editing of the manuscript, and approval of the final manuscript. Richard B. Schwab: Provision of study materials, critical revision/editing of the manuscript, and approval of the final manuscript. Sandip P. Patel: Provision of study materials, critical revision/editing of the manuscript, and approval of the final manuscript. Barbara A. Parker: Provision of study materials, critical revision/editing of the manuscript, and approval of the final manuscript. Razelle Kurzrock: Conception and design, data interpretation and initial manuscript writing, critical revision/editing of the manuscript, and approval of the final manuscript.

CONFLICT OF INTEREST DISCLOSURES
Richard B. Schwab reports an ownership interest in Orimedix LLC. Sandip P. Patel reports research funding from MedImmune, Genentech, Pfizer, Agenon, Xcovery, Lilly, and Bristol-Myers Squibb and speaking fees from Boehringer Ingelheim and Merck. Barbara A. Parker reports research funding from GlaxoSmithKline and Genentech and stock ownership in Merck. Razelle Kurzrock reports research funding from Genentech, Merck Seroso, Pfizer, Sequenom, Foundation Medicine, and Guardant Health as well as consulting fees from XBiotech and Actuate Therapeutics and an ownership interest in Novena, Inc, and Curematch, Inc.
RESULTS—Of the 423 patients, 61 (14.4%) had TERT promoter mutations, and this placed TERT promoter alterations among the most prevalent aberrations after tumor protein 53 (TP53; 39%) and KRAS and cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B) alterations (15% each) in this population. TERT promoter alterations were more frequent in men (P=.031) and were associated with brain cancers (P=.001), skin cancers/melanoma (P=.001), and a higher number of aberrations (P=.0001). A co-alteration analysis found that TERT promoter alterations were significantly correlated with CDKN2A/B (P=.001) and BRAF abnormalities (P=.0003). Patients harboring TERT promoter alterations or TP53 or CDKN2A/B alterations and those with 4 or more alterations demonstrated shorter survival (hazard ratio for normal TERT promoters vs aberrant ones, 0.44; P=.017). However, only a higher number of alterations remained significant in the multivariate analysis.

CONCLUSIONS—Overall, TERT promoter alterations were among the most prevalent aberrations in this population, with very high rates in brain cancers (48% of patients) and melanomas (56% of patients). These aberrations frequently coexist with a high number of other aberrations, with the latter feature also significantly associated with poorer overall survival. Therapeutic options for targeting tumors with TERT promoter mutations are currently limited, although a variety of novel approaches are under development.

Keywords
BRAF; glioblastoma; melanomas; next-generation sequencing; survival; telomerase reverse transcriptase (TERT) promoter mutations

INTRODUCTION
Cancer is driven by molecular aberrations allowing oncogenic cells to thrive by growing and eventually metastasizing. Research studies investigating oncogenic mechanisms have highlighted the strategies that cancer cells can develop to survive by manipulating pathways conferring a selective growth advantage to the tumor. Examples of such acquired mechanisms include sustaining proliferative signaling, evading growth suppressors, resisting cell death, inducing angiogenesis, activating invasion and metastasis, and enabling replicative immortality.1 Indeed, in addition to the accumulation of mutations conferring a selective growth advantage, malignant cells can acquire aberrations leading to immortality.

In 1995, Chadeneau et al2 demonstrated that telomerase, the enzyme that elongates telomeric DNA, was present in human cells immortalized in vitro and in metastatic ovarian and colorectal carcinomas but not in normal tissue. Telomeres are present at the ends of eukaryotic chromosomes and are composed of simple, repetitive G-rich sequences. Telomerase reverse transcriptase (TERT) is a catalytic subunit of the telomerase enzyme responsible for catalyzing the addition of nucleotides to the end of a chromosome’s telomeres.3 In normal cells, the shortening of telomeres has the ability to activate the senescence pathway, or the loss of a cell’s power of division and growth.4,5 In parallel, it has been demonstrated that telomere length stabilization by telomerase would allow unlimited proliferation.3 It has, therefore, been hypothesized that telomeres hold an important key to both aging and cancer.6
Reactivation or re-expression of telomerase is believed to be a widespread feature of human cancers, although its genetic basis remains poorly understood. Although it appears that somatic mutations in the coding region of TERT are rather infrequent in cancer, somatic mutations in the TERT promoter region have been described in several specific types of human cancers (eg, glioblastoma, bladder cancer, thyroid cancer, and skin cancer), and they lead to increased telomerase expression. Mutations within the promoter region of TERT that confer enhanced TERT promoter activity have been reported in 2 major hotspots, which are located at −124 and −146 base pairs upstream of the transcriptional start site (also designated C228T and C250T, respectively). Interestingly, mutations in the TERT promoter region, as opposed to the coding region, allow the creation of additional binding sites for transcription factors and may represent a novel mechanism of oncogenic activation in cancer.

Our study objectives were to investigate the frequency of TERT promoter mutations in our population of patients with diverse cancer types and to delineate correlations with other clinical parameters.

**MATERIALS AND METHODS**

**Patients**

We retrospectively reviewed the characteristics and clinical outcomes of 423 consecutive patients for whom molecular testing had been performed between August 2014 and July 2015 and who had been seen at the Moores Cancer Center (University of California San Diego). This study was performed and consent was obtained in accordance with the institutional review board guidelines of the University of California San Diego.

**Next-Generation Sequencing**

Next-generation sequencing was performed with FoundationOne (Foundation Medicine, Cambridge, Massachusetts), which is a Clinical Laboratory Improvement Amendments–approved clinical-grade next-generation sequencing test that interrogates 315 cancer-related genes plus introns from 28 genes often rearranged or altered in cancer to a typical median depth of coverage greater than 500 × (the full list is available at http://www.foundationone.com/learn.php#2). This test can detect base substitutions, insertions and deletions, copy number alterations, and rearrangements from a routine tissue sample (including core or fine-needle biopsies).

**Statistical Analysis**

Patients’ baseline characteristics were presented with descriptive statistics. Associations between categorical variables were evaluated with Fisher’s exact test, whereas association testing for continuous dependent variables used the Mann-Whitney test. Multiple logistic regressions (multivariate analysis) were fit to analyze the association between TERT promoter mutations and other patient characteristics. Overall survival was defined as the time from diagnosis to death or the last follow-up date for patients who were alive. Patients still alive at the last follow-up were censored at that date. Estimations for overall survival were performed with a Kaplan-Meier analysis and were compared among subgroups by the
log-rank test. The Cox regression model was fit to assess the association between overall survival and multiple other patient characteristics (covariates). Unless otherwise specified, only variables with \( P \) values ≤ 0.05 were included in the multivariate models. All statistical analyses were performed by one of the authors (Maria Schwaederle) with SPSS version 22.0.

**RESULTS**

**Patient Characteristics**

The medical records of 423 consecutive patients who were seen at the Moores Cancer Center (University of California San Diego) and had comprehensive molecular testing performed were reviewed and analyzed. There was a slight preponderance of women over men (54% vs 46%). The median age at diagnosis was 57.2 years (95% confidence interval, 55.1–58.5 years). The majority of our patients were white (69%); the next most common ethnicity was Asian (10.4%). The most common primary tumor sites were gastrointestinal (30.3%); they were followed by hematologic malignancies (11.6%), breast cancer (10.9%), brain cancer (10.4%), lung cancer (10.2%), and skin cancer/melanoma (8%). The median number of alterations per patient was 4.0 (range, 0–22; Table 1).

**TERT Promoter Alterations and Correlation Analysis**

In the overall population, 61 patients (14.4%) had a *TERT* promoter mutation, and this placed *TERT* promoter alterations among the most prevalent aberrations after tumor protein 53 alterations (TP53; 39%) and KRAS and cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B) alterations (15% each) in our population including diverse cancer types (Fig. 1A). Forty-three of 61 patients (70.5%) carried *TERT* promoter –124 C>T alterations, 14 patients (23%) carried 146 C>T alterations, and 4 patients (6.6%) carried 124–125 CC>TT or 138–139 CC>TT alterations (2 patients each).

In a univariate analysis, *TERT* promoter alterations were found more often in men (21.5%) than women (8.3%), and they were associated with brain \( (P < .0001) \), skin/melanoma \( (P < .0001) \), and head and neck tumors \( (P = .045) \). On the other hand, *TERT* promoter alterations were significantly less commonly observed in gastrointestinal, hematologic, breast, and lung cancers. Interestingly, *TERT* promoter alterations were significantly associated with an increased median number of alterations (5 vs 3; \( P < .0001 \); Table 1). We also observed a trend toward an association with an older median age at diagnosis (59.1 vs 56.7 years; \( P = .060 \)).

To consider potential confounders, we consecutively performed a multivariate analysis, which confirmed that *TERT* promoter alterations correlated with men \( (P = .031) \), brain cancers \( (P = .001) \), skin cancer/melanoma \( (P = .001) \), and a higher number of aberrations \( (P = .0001 \); Table 2). Indeed, *TERT* promoter alterations were the most frequent alterations detected in patients with brain cancers (48% of whom harbored these alterations), and they were followed by TP53 alterations (34%) and phosphatase and tensin homolog (PTEN) abnormalities (30%; Fig. 1B). Similarly, 56% of patients with a skin/melanoma malignancy carried a *TERT* promoter mutation, and this made the gene the most frequently altered, with
TP53 (38%) and CDKN2A/B alterations being in the second and third positions, respectively (Fig. 1C). Even though it was just a trend in the multivariate analysis ($P = .184$), it is worth mentioning that 29% of the patients with head and neck cancers harbored a TERT promoter mutation (Fig. 1D).

For 2 patients with ependymoma, TERT promoter mutations were observed as single alterations, and the patients were still alive after being diagnosed in 2001 and 2003, respectively.

**Co-Alteration Analysis**

We next investigated the possible associations of TERT promoter alterations with other alterations, and we found that TERT promoter alterations were significantly associated with CDKN2A/B, PTEN, neurofibromin 1 (NFI), and BRAF alterations in a univariate analysis (all $P$ values ≤ .004; Table 3). Once adjustments were made for potential confounding variables in a multivariate analysis including brain and skin/melanoma primary tumor sites, only CDKN2A/B ($P = .001$) and BRAF alterations ($P = .0003$) remained independently associated with TERT promoter alterations.

When we focused only on patients with brain tumors ($n = 44$), TERT promoter alterations were associated with epidermal growth factor receptor (EGFR) alterations (33% vs 4.3%; $P = .019$), CDKN2A/B alterations (43% vs 4.3%; $P = .003$), and PTEN alterations (48% vs 13%; $P = .020$). Although it was not statistically significant, the co-occurrence of TERT promoter alterations was less frequent in patients with TP53 alterations (19% vs 34%; $P = .060$). However, none of these associations remained statistically significant in the multiple logistic regression model including the alterations with $P < .1$ in the univariate analysis.

In patients with skin/melanoma tumors ($n = 34$), we could detect an association between TERT promoter alterations and BRAF alterations (37% vs 7%) in the multivariate model including the alterations with $P < .1$ in the univariate analysis.

**Overall Survival**

A log-rank test (univariate) highlighted significantly shorter overall survival for patients harboring TERT promoter alterations in the overall population ($P = .01$) as well as TP53 or CDKN2A/B alterations. In addition, patients with 4 or more alterations (4 alterations being the median in the overall population) also demonstrated significantly shorter overall survival. The median overall survival from diagnosis was still not reached at the time of our analysis (median follow-up, 27.3 months). In the Cox regression model (multivariate analysis), only 4 or more alterations remained an independent prognostic factor associated with shorter survival (Table 4). Interestingly, subanalyses of the 3 tumor types with the highest prevalence of TERT alterations demonstrated consistently shorter survival (or a trend toward shorter survival) for patients with altered TERT promoters in brain tumors ($n = 44$; $P = .037$), head and neck cancers ($n = 28$; $P = .2$), or melanoma/skin tumors ($n = 34$; $P = .15$).
DISCUSSION

The *TERT* gene encodes the reverse transcriptase component of the telomerase complex, which is necessary for telomere stabilization and cell immortalization. Recently, *TERT* promoter mutations have been reported in human malignancies; they create de novo ETS1-binding motifs upregulating *TERT* messenger RNA and telomerase activity in malignant cells.\(^8,10,13,14\)

In our study population, 61 patients (14.4%) had a *TERT* promoter mutation, and this placed *TERT* promoter alterations among the most prevalent aberrations after *TP53* (39%). In the multivariate analysis, *TERT* promoter alterations were more frequent in men (21.5% of men and 8.3% of women had an alteration; \(P = .031\)) and were associated with brain tumors (48% of patients; \(P = .001\)) and skin cancer/melanoma (56% of patients; \(P = .001\); Table 2). In previous studies, *TERT* promoter mutations were found to be the most common point mutations in several tumor types, including glioblastoma (83%),\(^15\) melanoma (71%),\(^10,11\) bladder cancer (66%),\(^16\) and hepatocellular carcinoma (47%).\(^17\) Interestingly, in a recent study investigating the mutational landscape of metastatic cancer in an extensive cohort (10,000 patients), Zehir et al.\(^18\) found a very similar frequency of *TERT* alterations in their population covering different tumor types (approximately 15%). In our study, there was also a trend toward an association with older patients in the univariate analysis but not in the multivariate analysis. The latter is consistent with other studies in which there has been an association between *TERT* promoter alterations and increased age.\(^8,15,19\)

In univariate analyses, survival was significantly shorter for patients harboring *TERT* promoter alterations in the overall population (\(P = .017\)) and also for patients with brain tumors (\(P = .037\); Fig. 2A). Although it did not reach statistical significance, perhaps because of the limited number of patients, we also observed a trend toward shorter survival in individuals with melanoma and head and neck tumors (Fig. 2B,C). Similarly, Zehir et al.\(^18\) described poorer survival with several tumor types for patients harboring *TERT* promoter alterations (cutaneous melanoma, papillary thyroid cancer, and bladder urothelial carcinoma); however, it was statistically significant in the univariate analysis only for bladder urothelial carcinoma. In addition, the presence of *TERT* promoter mutations was previously associated with decreased overall survival in several other studies examining thyroid cancer,\(^20\) urogenital cancer,\(^21\) melanoma,\(^22\) laryngeal tumors,\(^23\) and glioblastomas.\(^19,24\) However, in our study, only a higher number of alterations was retained as a significant independent variable correlating with survival in the multivariate analysis. Finally, *TERT* promoter mutations were associated with alterations in *CDKN2A*, and the latter anomalies have also been associated with a poor prognosis.\(^25,26\) *TERT* promoter alterations were significantly associated with an increased median number of alterations (5 vs 3; \(P < .0001\)) in our population. These results may be of importance because a larger total number of aberrations is of prognostic value in several tumor types, with more aberrations predicting shorter progression-free survival.\(^27–29\)

In our study, 48% of the patients with brain tumors had the *TERT* promoter mutation, and patients with these alterations had shorter survival (Fig. 2A). *TERT* promoter mutations have been reported in 55% to 84% of glioblastomas and have been associated with increased
The prevalence of TERT promoter mutations is lower in pediatric patients with glioblastomas (approximately 11%). In agreement with our findings, in brain tumors, TERT promoter mutations have been associated with EGFR amplification and inversely correlated with altered TP53. However, these associations were not maintained in the multivariate analysis. Labussiere et al showed that TERT promoter mutations were an independent factor associated with a poor prognosis in glioblastomas (overall survival, 13.8 vs 18.4 months), as were older age and EGFR amplification. In addition, TERT promoter mutations were associated with shorter overall survival for patients with primary glioblastomas in another study (11 vs 20 months [P=0.002] and 12 vs 20 months [P=0.04] for C228T and C250T, respectively). Recently, a new molecular classification of gliomas using the TERT promoter mutation status has been reported to be highly predictive for survival.

Overall, 56% of the patients with a skin/melanoma malignancy carried a TERT promoter mutation in our analysis. The −146 C>T mutation is the previously reported most frequently detected somatic base change in the TERT promoter. In our skin cancer/melanoma population (as in our overall cancer population), −124 C>T was the most frequent somatic base change, with 8 of 19 TERT promoter–altered skin/melanoma tumors (42%) harboring this specific base change, whereas only 6 patients (31.6%) had a −146 C>T base change. TERT promoter alterations are associated with poorer survival for patients with cutaneous melanomas. In patients with skin/melanoma tumors (n=34), there was an association between TERT promoter alterations and BRAF alterations (37% vs 7%). There was only 1 melanoma patient with a concurrent non-BRAFV600 mutation (a BRAFG466E mutation). Macerola et al showed the association between TERT promoter and BRAF mutations to be an independent poor prognostic factor. Vinagre et al also demonstrated that TERT messenger RNA levels are higher when TERT promoter and BRAF mutations coexist in melanomas. There is some evidence that BRAF mutations coexisting with TERT promoter mutations are associated with aggressive behavior in papillary thyroid cancers.

Interestingly, 29% of the patients with head and neck cancers in our cohort had the TERT promoter mutation. TERT promoter mutations are predictive of worse survival for patients with laryngeal cancer. In our study population, urothelial cancers accounted for only 3% of the cancers, with 3 of 13 having a TERT promoter mutation. TERT mutations are frequent in both noninvasive and invasive bladder tumors.

Tumor types with high levels of TERT promoter alterations almost always originate in tissues with relatively low rates of self-renewal (eg, melanomas and gliomas). It is speculated that TERT promoter mutations in these cancers maintain telomerase at levels that may lead to immortalization or at least prolong shortening of telomere length and senescence. This may explain the observed lack of TERT promoter mutations in gastrointestinal cancers (that continually self-renew) other than hepatocellular cancer.

Our study has some limitations. Most of our analysis evaluated patients with diverse cancers, although it is possible that this suggests generalizability of the observations across tumor types. Several subanalyses were performed in specific tumor types; the smaller number of patients in these subanalyses may have diminished the statistical power. For some other
cancer types of interest such as those in the bladder, there were only a small number of patient specimens available, and statistical analysis in this subgroup was not feasible.

Therapeutic options for targeting tumors with TERT promoter mutations are currently limited, although a variety of treatment approaches to affecting TERT are under development, including immunotherapies that use TERT as a tumor-associated antigen. Common aberrations that coexist with TERT promoter mutations include BRAF and CDKN2A/B anomalies. Further work is needed to ascertain the responses to BRAF inhibitors in the presence of coexisting TERT promoter mutations. TERT promoter mutations lead to increased telomerase activity, which can be targeted with inhibitors. In addition, the transcription factor GABPA/B can bind to and activate the TERT promoter. Therefore, combinations of experimental drugs that target this pathway and coexisting molecular aberrations can also be explored.

In conclusion, abnormalities in the TERT promoter are frequent across diverse cancers, with 14.4% of our patients harboring these aberrations; this makes aberrations in the TERT promoter among the most prevalent aberrations after TP53 (39% of patients) and KRAS and CDKN2A/B alterations (15% each) in our population. TERT promoter alterations were more frequent in men and were associated with brain, skin/melanoma, and head and neck tumors. Conversely, TERT promoter alterations were significantly less commonly observed in gastrointestinal, hematologic, breast, and lung cancers. TERT promoter mutations were associated with higher numbers of alterations, and this feature correlated with poorer survival. Targeting TERT and telomerase should be a goal of future studies.

Acknowledgments

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References


Figure 1.
Gene alteration frequencies. The bar graphs show the frequencies of the most common genes in the most represented tumor types. Only TERT promoter alterations have been tested and included. (A) Genes with 20 or more patients carrying the alteration are shown. (B–D) Genes with 5 or more patients carrying the alteration are shown. APC indicates adenomatous polyposis coli; ARID, AT-rich interaction domain; CDK4, cyclin-dependent kinase 4; CDKN2A/B, cyclin-dependent kinase inhibitor 2A/B; EGFR, epidermal growth factor receptor; MLL2, mixed-lineage leukemia 2; NF1, neurofibromin 1; NOTCH1, notch homolog 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; PTEN, phosphatase and tensin homolog; TERT, telomerase reverse transcriptase; TP53, tumor protein 53.
Figure 2.
Kaplan-Meier curves for (A) brain tumors, (B) head and neck cancers, and (C) skin/melanoma tumors from the date of diagnosis. The log-rank test was used to compare variables. *TERT* indicates telomerase reverse transcriptase.
TABLE 1

Patient Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total Patients (n =423 [100%])</th>
<th>TERT Promoter Alterations (n =61 [14.4%])</th>
<th>TERT Promoter Wild Type (n =362 [85.6%])</th>
<th>P (Univariate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, median (95% CI), y</td>
<td>57.2 (55.1–58.5)</td>
<td>59.1 (55.9–62.5)</td>
<td>56.7 (54.7–58.4)</td>
<td>.060</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td>.0001</td>
</tr>
<tr>
<td>Women</td>
<td>228 (53.9)</td>
<td>19 (8.3)</td>
<td>209 (91.7)</td>
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</tr>
<tr>
<td>Men</td>
<td>195 (46.1)</td>
<td>42 (21.5)</td>
<td>153 (78.5)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>293 (69.3)</td>
<td>49 (16.7)</td>
<td>244 (83.3)</td>
<td>.051</td>
</tr>
<tr>
<td>Asian</td>
<td>44 (10.4)</td>
<td>3 (6.8)</td>
<td>41 (93.2)</td>
<td>.173</td>
</tr>
<tr>
<td>Other</td>
<td>39 (9.2)</td>
<td>2 (5.2)</td>
<td>37 (94.8)</td>
<td></td>
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<tr>
<td>African American</td>
<td>20 (4.7)</td>
<td>3 (15.0)</td>
<td>17 (85.0)</td>
<td></td>
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<tr>
<td>Hispanic</td>
<td>20 (4.7)</td>
<td>4 (20.0)</td>
<td>16 (80.0)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>7 (1.7)</td>
<td>0 (0)</td>
<td>7 (100)</td>
<td></td>
</tr>
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<td>Type of cancer, No. (%)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>128 (30.3)</td>
<td>8 (6.3)</td>
<td>120 (93.7)</td>
<td>.001</td>
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<tr>
<td>Hematologic</td>
<td>49 (11.6)</td>
<td>0 (0)</td>
<td>49 (100)</td>
<td>.0004</td>
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<td>Breast</td>
<td>46 (10.9)</td>
<td>0 (0)</td>
<td>46 (100)</td>
<td>.001</td>
</tr>
<tr>
<td>Brain</td>
<td>44 (10.4)</td>
<td>21 (47.7)</td>
<td>23 (52.3)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Lung</td>
<td>43 (10.2)</td>
<td>0 (0)</td>
<td>43 (100)</td>
<td>.002</td>
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<tr>
<td>Skin/melanoma</td>
<td>34 (8.0)</td>
<td>19 (55.9)</td>
<td>15 (44.1)</td>
<td>&lt;.0001</td>
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<tr>
<td>Head and neck</td>
<td>28 (6.6)</td>
<td>8 (28.6)</td>
<td>20 (71.4)</td>
<td>.045</td>
</tr>
<tr>
<td>Other&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21 (5.0)</td>
<td>1 (4.8)</td>
<td>20 (95.2)</td>
<td>.336</td>
</tr>
<tr>
<td>Gynecologic</td>
<td>17 (4.0)</td>
<td>1 (5.9)</td>
<td>16 (94.1)</td>
<td>.487</td>
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<td>Genitourinary</td>
<td>13 (3.1)</td>
<td>3 (23.1)</td>
<td>10 (76.9)</td>
<td>.413</td>
</tr>
<tr>
<td>No. of alterations, median (95% CI)</td>
<td>4 (3–4)</td>
<td>5 (5–6)</td>
<td>3 (3–4)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Biopsy site used for testing, No. (%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>251 (59.6)</td>
<td>41 (16.3)</td>
<td>210 (83.7)</td>
<td>.358</td>
</tr>
<tr>
<td>Primary</td>
<td>170 (40.4)</td>
<td>20 (11.8)</td>
<td>150 (88.2)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval; TERT, telomerase reverse transcriptase.

Percentages in the Total Patients column are based on the total number of patients (n =423); percentages in the next 2 columns are based on the numbers in the Total Patients columns. Bolded values are significant.

<sup>a</sup> Fisher’s exact test was used for categorical variables, and Mann-Whitney tests were used for linear variables (age at diagnostic and number of alterations). For ethnicity, P values were calculated for the 2 most common ethnicities.

<sup>b</sup> Other includes the following: sarcomas (n =6), fibromatosis (n =2), neurofibromas (n =2), neuroendocrine tumors (n =2), and unknown primaries (n =9).

<sup>c</sup> All were tested with the FoundationOne assay; the biopsy site was unknown for 2 patients.
**TABLE 2**

Multivariate Analysis of Characteristics Associated With *TERT* Promoter Alterations

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>TERT</em> Promoter Alterations (n =61 [14.4%])</th>
<th><em>TERT</em> Promoter Wild Type (n =362 [85.6%])</th>
<th>Wald Statistic</th>
<th>Pd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women (n =228)</td>
<td>19 (8.3)</td>
<td>209 (57.7)</td>
<td>4.67</td>
<td>.031</td>
</tr>
<tr>
<td>Men (n =195)</td>
<td>42 (21.5)</td>
<td>153 (42.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of cancer, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal (n =128)</td>
<td>8 (6.3)</td>
<td>120 (93.7)</td>
<td>1.51</td>
<td>.219</td>
</tr>
<tr>
<td>Hematologic (n =49)</td>
<td>0 (0)</td>
<td>49 (100)</td>
<td>0</td>
<td>.997</td>
</tr>
<tr>
<td>Breast (n =46)</td>
<td>0 (0)</td>
<td>46 (100)</td>
<td>0</td>
<td>.997</td>
</tr>
<tr>
<td>Brain (n =44)</td>
<td>21 (47.7)</td>
<td>23 (52.3)</td>
<td>11.8</td>
<td>.001</td>
</tr>
<tr>
<td>Lung (n =43)</td>
<td>0 (0)</td>
<td>43 (100)</td>
<td>0</td>
<td>.997</td>
</tr>
<tr>
<td>Skin/melanoma (n =34)</td>
<td>19 (55.9)</td>
<td>15 (44.1)</td>
<td>10.5</td>
<td>.001</td>
</tr>
<tr>
<td>Head and neck (n =28)</td>
<td>8 (28.5)</td>
<td>20 (71.5)</td>
<td>1.8</td>
<td>.184</td>
</tr>
<tr>
<td>No. of alterations, median (95% CI)</td>
<td>5 (5–6)</td>
<td>3 (3–4)</td>
<td>14.5</td>
<td>.0001</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval; *TERT*, telomerase reverse transcriptase.

All percentages are based on the total number of patients with the variable. Bolded values are significant.

Ad A logistic regression model was used. Variables with *P* <.05 in the univariate model (Table 1) were included in the multivariate model. The Wald test is a way of testing the significance of variables in a statistical model; the higher the Wald statistic is, the higher the association is in the model.

bMainly glioblastomas.
<table>
<thead>
<tr>
<th>Alteration</th>
<th>TERT Promoter Alterations (n = 61), %</th>
<th>TERT Promoter Wild Type (n = 362), %</th>
<th>( p^{a} )</th>
<th>Wald Statistic</th>
<th>( p^{b} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDKN2A/B</td>
<td>39.3</td>
<td>11.0</td>
<td>&lt;.0001</td>
<td>12.1</td>
<td>.001</td>
</tr>
<tr>
<td>PTEN</td>
<td>19.7</td>
<td>6.4</td>
<td>.001</td>
<td>1.6</td>
<td>205</td>
</tr>
<tr>
<td>NF1</td>
<td>14.8</td>
<td>4.7</td>
<td>.004</td>
<td>1.4</td>
<td>235</td>
</tr>
<tr>
<td>BRAF</td>
<td>19.7</td>
<td>5.0</td>
<td>.0001</td>
<td>13.0</td>
<td>.0003</td>
</tr>
<tr>
<td>Tumor Site</td>
<td>Overall TERT Promoter Alterations, %</td>
<td>Overall TERT Promoter Wild Type, %</td>
<td>( p^{a} )</td>
<td>Wald Statistic</td>
<td>( p^{b} )</td>
</tr>
<tr>
<td>Brain</td>
<td>34.4</td>
<td>6.4</td>
<td>&lt;.0001</td>
<td>39.8</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Skin/melanoma</td>
<td>31.1</td>
<td>4.1</td>
<td>&lt;.0001</td>
<td>34.7</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Abbreviation: CDKN2A/B, cyclin-dependent kinase inhibitor 2A/B; NF1, neurofibromin 1; PTEN, phosphatase and tensin homolog; TERT, telomerase reverse transcriptase.

Alterations and tumor sites are expressed as percentages of TERT promoter alteration-positive patients and TERT promoter wild-type patients (eg, 34.4% of the overall TERT promoter alterations were in brain tumors). Bolded values are significant.

\( ^{a} \) Fisher's exact test was used. Only genes altered in 25 or more patients (n = 13) were tested in the univariate analysis. Bonferroni's correction\(^{12} \) was used to adjust for multiple testing to select variables to be included in the subsequent multivariate analysis. Because 13 genes were tested, the adjusted significance level chosen was <.004 (0.05/13). Therefore, only genes with a \( p \) value < .004 in the univariate analysis were selected for inclusion in the multiple logistic regression model and were included in this table.

\( ^{b} \) A multiple logistic regression model was used. The Wald test is a way of testing the significance of variables in a statistical model; the higher the Wald statistic is, the higher the association is in the model.
**TABLE 4**

Overall Survival Analysis: Univariate and Multivariate Correlates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>$p^a$</td>
</tr>
</tbody>
</table>
| Alterations
c | | | | |
| $TERT$ promoter | 0.441 (0.22–0.88) | .017 | 0.635 (0.31–1.31) | .220 |
| $TP53$ | 0.506 (0.27–0.94) | .027 | 0.637 (0.33–1.23) | .179 |
| $CDKN2A/B$ | 0.404 (0.20–0.80) | .008 | 0.613 (0.29–1.31) | .207 |
| No. of alterations ≥ 4 | 0.242 (0.11–0.53) | .0001 | 0.337 (0.15–0.78) | .012 |

Abbreviations: $CDKN2A/B$, cyclin-dependent kinase inhibitor 2A/B; CI, confidence interval; HR, hazard ratio; $TERT$, telomerase reverse transcriptase; $TP53$, tumor protein 53.

The bolded value is significant.

$^a$ The log-rank test was used. Only significant variables are represented in the univariate analysis.

$^b$ A Cox regression model was used. The median overall survival was not reached at the time of this analysis. The median follow-up time from diagnosis was 27.3 months (95% CI, 23.2–31.4 months).

$^c$ Patients with alterations in the $TERT$ promoter, $TP53$, or $CDKN2A/B$ did worse than those without alterations; patients with 4 or more alterations did worse than those with fewer alterations.