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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, Amgen, 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 Serono, Pfizer, Sequenom, Foundation Medicine, and Guardant Health as well as consultant 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 al[2] 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.[7] 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.[8,9] 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).[8,10,11] 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 Foundatio-nOne (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 (covariables). Unless otherwise specified, only variables with *P* values ≤ .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 (*NF1*), 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

TERT expression.[19,30,31] The prevalence of *TERT* promoter mutations is lower in pediatric patients with glioblastomas (approximately 11%).[32] In agreement with our findings, in brain tumors, *TERT* promoter mutations have been associated with *EGFR* amplification and inversely correlated with altered *TP53*.[33] However, these associations were not maintained in the multivariate analysis. Labussiere et al[19] 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 =.002] and 12 vs 20 months [P =.04] for C228T and C250T, respectively).[34] Recently, a new molecular classification of gliomas using the *TERT* promoter mutation status has been reported to be highly predictive for survival.[24]

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.[8,10] 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.[22] 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–*BRAF* V600 mutation (a *BRAF* G466E mutation). Macerola et al[35] showed the association between *TERT* promoter and *BRAF* mutations to be an independent poor prognostic factor. Vinagre et al[8] 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.[36]

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.[23] 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.[31,37]

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).[15] 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.[38,39] This may explain the observed lack of *TERT* promoter mutations in gastrointestinal cancers (that continually self-renew) other than hepatocellular cancer.[15,17]

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.[40] 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.[41,42] In addition, the transcription factor GABPA/B can bind to and activate the *TERT* promoter.[43] 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.

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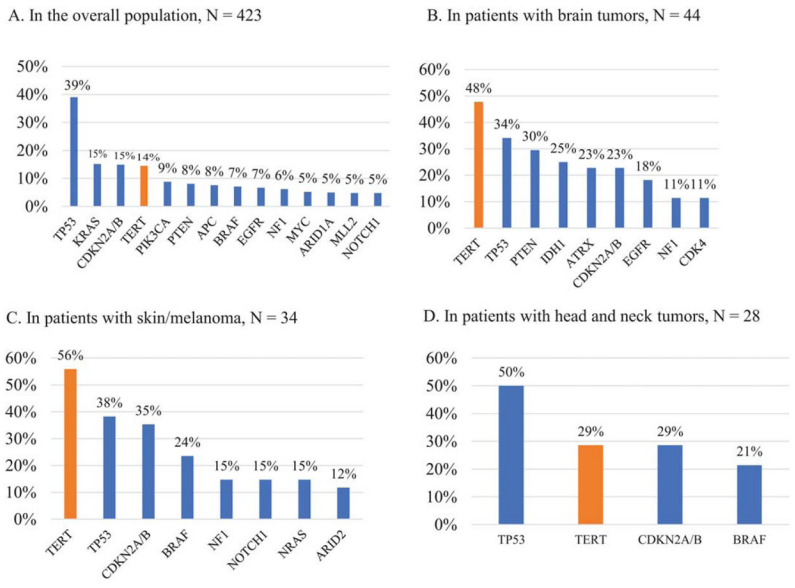

Figure 1.

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.

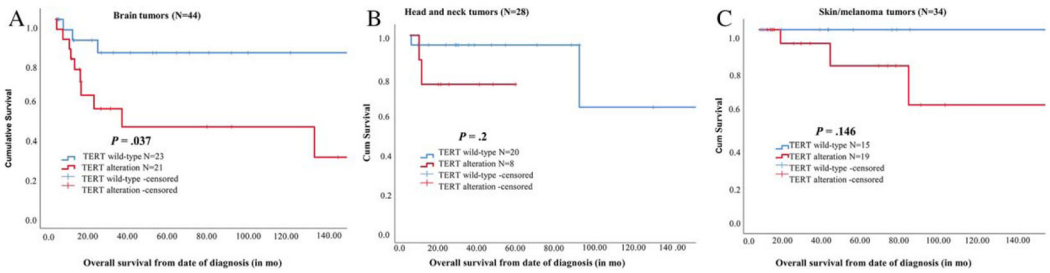

Figure 2.

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

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

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.

[a] 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.

[b] *Other* includes the following: sarcomas (n =6), fibromatosis (n =2), neurofibromas (n =2), neuroendocrine tumors (n =2), and unknown primaries (n =9).

[c] 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

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

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.

[a] 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.

[b] Mainly glioblastomas.

TABLE 3

Co-Alterations in the Presence of *TERT* Promoter Anomalies (Multivariate Analysis)

Alteration	*TERT* Promoter Alterations (n =61), %	*TERT* Promoter Wild Type (n =362), %	P^a	Wald Statistic	P^b
CDKN2A/B	39.3	11.0	<.0001	12.1	**.001**
PTEN	19.7	6.4	.001	1.6	.205
NF1	14.8	4.7	.004	1.4	.235
BRAF	19.7	5.0	.0001	13.0	**.0003**
Tumor Site	Overall *TERT* Promoter Alterations, %	Overall *TERT* Promoter Wild Type, %	P^a	Wald Statistic	P^b
Brain	34.4	6.4	<.0001	39.8	**<.0001**
Skin/melanoma	31.1	4.1	<.0001	34.7	**<.0001**

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

	Univariate		Multivariate	
Variable	**HR (95% CI)**	***P*[a]**	**HR (95% CI)**	***P*[b]**
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.