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Prognostic significance of human telomerase reverse transcriptase promoter region mutations C228T and C250T for overall survival in spinal chordomas

Chetan Bettegowda,* Stephen Yip,* Bowen Jiang, Wei-Lien Wang, Michelle J. Clarke, Aron Lazary, Marco Gambarotti, Ming Zhang, Daniel M. Sciubba, Jean-Paul Wolinsky,# C. Rory Goodwin, Edward McCarthy, Niccole M. Germscheid, Arjun Sahgal, Ziya L. Gokaslan, Stefano Boriani, Peter Pal Varga, Charles G. Fisher, and Laurence D. Rhines

Department of Neurosurgery, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA (C.B., B.J., D.M.S., J-P.W.); Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada (S.Y.); Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA (W-L.W.); Department of Neurosurgery, Mayo Clinic, Rochester, Minnesota, USA (M.J.C.); National Center for Spinal Disorders, Buda Health Center, Budapest, Hungary (A.L., P.P.V.); Department of Pathology, IRCCS Rizzoli Orthopedic Institute, Bologna, Italy (M.G.); Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA (M.Z.); Department of Neurosurgery, Duke University Medical Center, Durham, North Carolina, USA (C.R.G.); Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA (E.M.); Research Department, AOSpine International, Davos, Switzerland (N.M.G.); Department of Radiation Oncology, Odette Cancer Centre, Sunnybrook Health Sciences Centre and University of Toronto, Toronto, Ontario, Canada (A.S.); Department of Neurosurgery, Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA (Z.L.G.); IRCCS Istituto Ortopedico Galeazzi, Milan, Italy (S.B.); Division of Spine, Department of Orthopaedics, University of British Columbia and Vancouver Coastal Health, Vancouver, British Columbia, Canada (C.G.F.); Department of Neurosurgery, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA (L.D.R.)

Corresponding Author: Chetan Bettegowda, MD, PhD, The Johns Hopkins Hospital, 600 North Wolfe Street, Phipps 118, Baltimore, MD 21287 (cbetteg1@jhmi.edu).

*Co-first authors

#Current address: Department of Neurosurgery, Feinberg School of Medicine, Northwestern University, Chicago, Illinois

Abstract

Background. Spinal chordomas, a subtype of primary spinal column malignancies (PSCM), are rare tumors with poor prognosis, and we have limited understanding of the molecular drivers of neoplasia.

Methods. Study design was a retrospective review of prospectively collected data with cross-sectional survival. Archived paraffin embedded pathologic specimens were collected for 133 patients from 6 centers within Europe and North America between 1987 and 2012. Tumor DNA was extracted and the human telomerase reverse transcriptase (*hTERT*) promoter was sequenced. The *hTERT* mutational status was correlated with overall survival (OS) and time to first local recurrence.

Results. Ninety-two chordomas, 26 chondrosarcomas, 7 osteosarcomas, 3 Ewing's sarcomas, and 5 other malignant spinal tumors were analyzed. Median OS following surgery was 5.8 years (95% CI: 4.6 to 6.9) and median time to first local recurrence was 3.9 years (95% CI: 2.5 to 6.7). Eight chordomas, 2 chondrosarcomas, 1 Ewing's sarcoma, and 1 other malignant spinal tumor harbored either a C228T or C250T mutation in the *hTERT* promoter. In the overall cohort, all patients with *hTERT* mutation were alive at 10 years postoperative with a median OS of 5.1 years (95% CI: 4.5 to 6.6) ($P = 0.03$). *hTERT* promoter mutation was observed in 8.7% of spinal chordomas, and 100% of chordoma patients harboring the mutation were alive at 10 years postoperative compared with 67% patients without the mutation ($P = 0.05$).

Conclusions. We report for the first time that *hTERT* promoter mutations C228T and C250T are present in approximately 8.7% of spinal chordomas. The presence of hTERT mutations conferred a survival benefit and could potentially be a valuable positive prognostic molecular marker in spinal chordomas.

Key Points

1. *hTERT* promoter mutations C228T and C250T are present in 8.7% of spinal chordomas.
2. *hTERT* promoter mutations are associated with a positive overall survival benefit in spinal chordomas.

Importance of the Study

Primary spinal column malignancies are rare tumors with poor prognosis and few systemic treatment options, and we have limited understanding of the molecular drivers of neoplasia. The current study consists of 133 patients from an international, multicenter database and represents the largest surgical cohort study on molecular genetics of these rare tumors. We report for the first time that *hTERT* promoter mutations C228T and C250T are present in 8.7% of spinal chordomas. All patients with *hTERT* mutation were alive at 10 years postoperative compared with those patients who lacked the mutations. *hTERT* could potentially be a valuable molecular biomarker in prognostication of spinal chordomas.

Primary spinal column malignancies (PSCMs) are a rare and heterogeneous group of oncologic lesions.[1] Chordoma, chondrosarcoma, osteosarcoma, Ewing's sarcoma, and other malignant soft tissue tumors of the spine are typically classified as PSCM. Clinical presentations are usually insidious and up to 33% of patients are found to have metastatic disease at initial evaluation.[2] While chordoma and chondrosarcoma can be indolent but locally invasive, Ewing's sarcoma and osteosarcoma are more aggressive and associated with higher rates of tumor progression and recurrence.[3]

Surgical, radiation, and systemic treatment options may be limited in the management of PSCM. En bloc resection is the evidence-based treatment, particularly for chordoma and chondrosarcoma.[4,5] Unfortunately the spine's anatomical restraints make achieving wide margins exceedingly difficult, frequently resulting in neurological sacrifice, high morbidity, and sometimes limited ability to achieve tumor-free resection margins. Even with adjuvant chemo and radiation therapy,[6–11] PSCMs are known to have a poorer prognosis, even worse than similar tumors in the appendicular skeleton. Because of these high stakes, surgeons and oncologists need more precise prognostic variables to help guide treatment. Despite the evolving use of molecular markers in many cancers, there is currently limited understanding of the molecular drivers of carcinogenesis in PSCM.

Human telomerase reverse transcriptase (*hTERT*) is an important catalytic subunit of the telomerase complex and contributes to telomere maintenance during tumorigenesis and cellular immortalization.[12] *hTERT* overexpression is observed in many human cancer types, including renal cell carcinoma,[13] melanoma,[14] urothelial carcinoma,[15,16] oral squamous cell carcinoma,[17] thyroid carcinomas,[18] and gliomas.[19,20] Two hotspot somatic mutations in the *hTERT* promoter region, 1,295,228 C > T (C228T) and 1,295,250 C > T (C250T), have been identified in a recurrent fashion, particularly in human melanoma and gliomas.[21,22] The C228T and C250T promoter mutations generate a de novo binding motif for E-twenty-six transcription factors and can upregulate the transcriptional activity of *hTERT* by 2- to 6-fold in human melanomas.[23,24] Although *hTERT* promoter mutations are frequently associated with worse survival outcomes in thyroid carcinoma, non–small cell lung cancer, and melanoma,[25–27] the prognostic value of these mutations in central nervous system neoplasms is unclear. Recent research in glioma patients demonstrated a survival benefit in patients with *hTERT* promoter mutations in isocitrate dehydrogenase (IDH) wildtype/O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylated glioblastoma (GBM) and is strongly associated with IDH mutant–1p/19q codeleted oligodendroglial neoplasm.[28,29]

Few studies have investigated the role of *hTERT* promoter region mutations in PSCM.[30–32] The present study investigates the prognostic value of *hTERT* promoter mutations on overall survival (OS) and time to local recurrence in a large international cohort of PSCM patients, with a subgroup analysis focused on spinal chordoma.

Materials and Methods

Design

Study design was a retrospective review of prospectively collected data with cross-sectional survival in the AOSpine Knowledge Forum Tumor (AOSKFT) primary

database. The AOSKFT model has been described in previous publications.[33–35] An initial cohort of 1495 patients with primary spinal column tumors were treated at 13 centers within Europe, North America, and Australia between December 1985 and January 2013. Ethics approval was received at each center from appropriate institutional review boards; likewise, written informed consent was obtained from each subject. Excluded from the current study were 1322 patients because the pathologic specimens were not evaluated for *hTERT* promoter mutation or the specimens were not definitively identified as PSCM. Archived paraffin embedded pathologic specimens were available for 133 patients from 6 of the 13 centers; 92 spinal chordomas were then selected for subgroup analysis.

A secure web-based application (REDCap, Vanderbilt University) was used to gather demographic, clinical, diagnostic, therapeutic, local recurrence, perioperative morbidity, and survival data. Information regarding patient mortality and disease-free survival was acquired cross-sectionally.

Definitions and Staging

Histologic classification and tumor grading were performed by a musculoskeletal tumor pathologist at each individual center. Chordoma histologic subtypes included classic (notochordal cells with regions of chondroid), chondroid (containing chordoma and chondrosarcoma components), and de-differentiated (loss of the integrase interactor 1 gene, increased mitotic potential). Chondrosarcoma histologic subtypes included de-differentiated and myxoid. The validated Enneking classification was used to characterize surgical margins of resected tumors (intralesional, marginal, or wide). If wide or marginal margins were achieved, the specimen was classified as Enneking appropriate (EA) resection. If intralesional resection was performed, the specimen was classified as Enneking inappropriate (EI) resection. Tumor grade and stage were classified based on the Enneking staging system for malignant musculoskeletal tumors.[36]

Patient Follow-Up

Follow-up examinations were performed based on individual institutional protocols. Local recurrence was defined as time interval from tumor resection to radiographic tumor reappearance at or near the surgical resection cavity. OS was defined as the interval between time of surgery and death.

hTERT Promoter Mutation Genotyping

Tumor DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) specimens using the DNA FFPE Tissue Kit (Qiagen, #56404). Genotyping of the *hTERT* promoter was performed using Sanger sequencing. The small amplicon, 163 base pair fragment was amplified using the following primers: hTERT-seq-for 5′- CAGCGCTGCCTGAAACTC-3′ and hTERT-seq-rev 5′-GTCCTGCCCCTTCACCTT-3′. The large amplicon, 193 base pair fragment of the *hTERT* promoter region spanning the hotspot mutations on chromosome 5 (C228T and C250T) was amplified using the following primers: hTERT-seq-for 5′-CACCCGTCCTGCCCCTTCACCTT-3′ and hTERT-seq-rev 5′-GGCTTCCCACGTGCGCAGCAGGA-3′. Primer sequences were tagged with T7 forward (caggaaacagctatgac) and M13 reverse tags (taatacgactcactataggg). DNA was first quantitated using NanoDrop and diluted to 10 ng/μL. PCR was performed on Tetrad (Bio-Rad) with the following conditions: 94°C for 2 minutes; 35 cycles with 94°C (30 seconds), 62°C (30 seconds), and 68°C (30 seconds); 68°C for 5 minutes; and 4°C for hold. Post-PCR products were treated with ExoSAP-IT (Thermo Fisher Scientific, #78201.1.ML). Finally, BigDye Terminator v3.1 (Thermo Fisher Scientific, #4337455) was utilized for cycle sequencing on the ABI capillary electrophoresis platform.

Statistical Analysis

Data were described using descriptive statistics (mean ± standard deviation or median/interquartile range for continuous variables; absolute number/percentage for categorical variables). Comparisons between wildtype and mutational subgroups were done in a supervised manner. Further statistical subgroup analysis was performed in the spinal chordoma group. The chi-square test (Pearson's or Fisher's exact) and Student's *t*-tests or Wilcoxon Mann–Whitney tests were used for comparison between cohorts. Kaplan–Meier survival analyses were performed over a 10-year period and the Mantel–Cox log-rank test was used to evaluate factors associated with OS and overall time to first local recurrence. Statistical significance was set at $P \leq 0.05$. All statistical analyses were performed using Stata v12.0.

Results

Patient Population and Baseline Characteristics

Of the 133 patients included in the study, approximately 64% were male and 84% Caucasian. Patients had a mean age of 56 ± 16 years at the time of surgery. Although over 90% of the patients had pain as a presenting symptom at diagnosis, only 7% of the patients had a diagnosis of pathological fracture. Of the 133 patients, 15 (11%) had undergone previous resection, with intralesional resection achieved in 10/15 (67%) of the cases. Tissue diagnosis of PSCM was obtained by CT-trocar guided biopsy in 59% of the cases and intraoperative biopsy in 17%. Lesions of the mobile spine were seen in 36% of the patients, while 64% had lesions of the fixed spine (Table 1). The 133-patient cohort comprised 92 chordomas, 26 chondrosarcomas, 7 osteosarcomas, 3 Ewing's sarcomas, and 5 other malignant spinal tumors. Patient follow-up ranged from 2 days to 22.5 years postoperatively.

Treatment

All patients underwent surgical resection of their lesion. In the overall cohort, 125/133 had Enneking appropriateness

Table 1 Patient and tumor characteristics

Variables	
Sex (n = 133)	
Male	85 (63.9)
Female	48 (36.1)
Ethnicity (n = 115)	
African	3 (2.6)
Asian/Pacific Islander	4 (3.5)
Caucasian	96 (83.5)
East Indian	2 (1.7)
Hispanic	8 (7.0)
Other	2 (1.7)
Age at time of surgery (years) (n = 133)	55.8 ± 16.4
Pain at Diagnosis (n = 126)	
No	11 (8.7)
Yes	115 (91.3)
Pathologic Fracture at Diagnosis (n = 126)	
No	117 (92.9)
Yes	9 (7.1)
Previous Spine Tumor Operation (n = 133)	
No	118 (88.7)
Yes	15 (11.3)
Intralesional	*10 (66.7)*
Marginal	*0 (0.0)*
Wide	*1 (6.7)*
Unknown	*4 (26.7)*
How the Diagnosis Was Performed (n = 123)	
Open biopsy	*17 (13.8)*
CT-trocar biopsy	*72 (58.5)*
Intraoperative biopsy	*21 (17.1)*
Other	*13 (10.6)*
Spinal Level (n = 132)	
Mobile	*47 (35.6)*
Fixed	*85 (64.4)*
Diagnosis (n = 133)	
Chordoma	92 (69.2)
Chondrosarcoma	26 (19.5)
Ewing's sarcoma	3 (2.3)
Osteosarcoma	7 (5.3)
Other malignant soft tissue tumors	5 (3.8)
Tumor Grade (n = 133)	
Low (Ia/Ib)	83 (62.4)
High (IIa/IIb)	50 (37.6)
TERT Promoter Mutation (n = 133)	
No	121 (91.0)
Yes	12 (9.0)
TERT Status (n = 133)	
Wildtype	121 (91.0)
C228T	11 (8.3)
C250T	1 (0.7)

Data are presented as N (%), mean ± standard deviation or median (p25, p75).

information available. Surgery was EA in 83/125 patients (66%) and EI in 42/125 patients (34%). The 15 patients with prior tumor resection were regraded on the Enneking resection scale after the last surgical resection. Of 130 patients, 111 (85%) of patients did not receive any chemotherapy and 96/131 (73%) did not receive any radiation therapy.

Comparison of hTERT Mutant to Wildtype in the Overall Cohort

Of 133 patients, 121 (91%) were identified with *hTERT* wildtype, and 12/133 (9%) harbored a promoter mutation (Table 2). Eleven of 12 of the mutations were C228T with only 1 C250T. The *hTERT* mutations were identified in 8 chordomas, 2 chondrosarcomas, 1 Ewing's sarcoma, and 1 other malignant spinal tumor. The wildtype and C228T/C250T cohorts were evenly matched in terms of sex distribution (P = 0.76), age at time of surgery (P = 0.52), ethnicity (P = 0.39), and location of lesion in mobile versus fixed spine (P = 0.10). There was no statistical difference between the 2 cohorts in use of adjuvant therapy (P = 0.34), timing of chemotherapy (P = 1.00), and timing of radiation therapy (P = 0.28). Tumor grade was low (Ia/Ib) in 62% of the wildtype cohort and 67% of the *hTERT* mutation cohort (P = 1.00). The adequacy of surgical resection was not statistically different between the 2 cohorts (EA 65% in wildtype vs 80% in mutation, P = 0.49).

Subgroup Analysis in Spinal Chordoma

The histologic subtype was known in 46 chordoma patients, with 35 (76%) with of subtype, 4 (9%) chondroid, 2 (4%) de-differentiated, and 5 (11%) classified as other. Eighty-four (91.3%) chordoma patients were wildtype compared with 8 (8.7%) patients with the *hTERT* mutation. The wildtype and C228T/C250T chordoma cohorts were evenly matched in terms of sex distribution (P = 0.43), age at time of surgery (P = 0.27), ethnicity (P = 0.34), and Enneking appropriateness (P = 1.00). All 8 patients with *hTERT* mutations received adjuvant therapy compared with 56 (68%) of the wildtype patients. No patients in the mutation group received chemotherapy compared with 6 (7.3%) of the wildtype group (P = 1.00). Of the patients in the mutation group, 88% had low-grade tumor compared with 80% in the wildtype group (P = 1.00). Local recurrence at 10 years postoperative was 62.5% in the mutation group compared with 63.1% in the wildtype group (Table 3).

Patient Outcomes

Median OS for the entire cohort following surgery was 5.8 years (95% CI: 4.6 to 6.9) (Fig. 1) and median time to first local recurrence was 3.9 years following surgery (95% CI: 2.5 to 6.7) (Fig. 2). OS was worse in the high tumor grade group of Enneking II, with the estimated median OS of 4.8 years following surgery compared with 6.4 years in Enneking I patients (P = 0.05) (Supplementary

Table 2 Comparison of cohorts

Variables	TERT promoter mutation		*P*-value
	No (*n* = 121)	Yes (*n* = 12)	
Sex (*n* = 133)			0.76*
Male	78 (64.5)	7 (58.3)	
Female	43 (35.5)	5 (41.7)	
Age at time of surgery (y) (*n* = 133)	56.1 ± 16.5	52.9 ± 15.8	0.52†
Spinal Level (*n* = 132)			0.10*
Mobile	46 (38.0)	1 (9.1)	
Fixed	75 (62.0)	10 (90.9)	
Ethnicity (*n* = 115)			0.39*
Non-caucasian	16 (15.4)	3 (27.3)	
Caucasian	88 (84.6)	8 (72.7)	
Adjuvant Therapy Given (*n* = 131)			0.34*
No	77 (64.7)	10 (83.3)	
Yes	42 (35.3)	2 (16.7)	
Timing of Chemotherapy (*n* = 130)			1.00*
Preop	8 (6.8)	1 (8.3)	
Postop	4 (3.4)	0 (0.0)	
Both	6 (5.1)	0 (0.0)	
Neither (no chemo)	100 (84.8)	11 (91.7)	
Timing of Radiation Therapy (*n* = 131)			0.28*
Preop	9 (7.6)	2 (16.7)	
Postop	20 (16.8)	0 (0.0)	
Both	4 (3.4)	0 (0.0)	
Neither (no radiation)	86 (72.3)	10 (83.3)	
Tumor Grade (*n* = 133)			1.00*
Low (I)	75 (62.0)	8 (66.7)	
High (II)	46 (38.0)	4 (33.3)	
Enneking Appropriateness (*n* = 125)			0.49*
EA	75 (65.2)	8 (80.0)	
EI	40 (34.8)	2 (20.0)	
Local Recurrence at 10 Years Postoperative (*n* = 130)			0.75*
No	77 (65.3)	9 (75.0)	
Yes	41 (34.7)	3 (25.0)	
Survival at 10 Years Postoperative (*n* = 133)			0.01*
Alive	74 (61.2)	12 (100.0)	
Dead	47 (38.8)	0 (0.0)	

Data are presented as *N* (%) or mean ± standard deviation; EA: Enneking appropriate, EI: Enneking inappropriate.
**Fisher's exact test,* †*Student's t-test*

Fig. 1). There was no statistical difference in OS between EA and EI patients (P = 0.76) (Supplementary Fig. 2). Likewise, whether adjuvant therapy was used had no significant impact on OS following surgery (P = 0.42) (Supplementary Fig. 3).

Patients who lacked the *hTERT* mutations in the overall cohort had a significantly worse OS, with an estimated median OS of 5.1 years (95% CI: 4.5 to 6.6) compared with the 12 patients with *hTERT* mutations, where death was not observed and median OS was not reached by 10 years post-operative (P = 0.03) (Fig. 3). In the chordoma-specific cohort, all 8 patients harboring the mutation were alive at 10 years postoperative compared with 56 (67%) of chordoma patients without the mutation (P = 0.05) (Fig. 4) (Supplementary Fig. 4).

Table 3 Subgroup analysis specific to spinal chordomas

Variables	TERT Promoter Mutation		*P*-value
	No (*n* = 84)	Yes (*n* = 8)	
Sex (*n* = 92)			0.43*
Male	58 (69.1)	4 (50.0)	
Female	26 (30.9)	4 (50.0)	
Age at time of surgery (y) (*n* = 92)	60.4 ± 13.9	54.6 ± 14.0	0.27†
Spinal Level (*n* = 92)			0.05*
Mobile	32 (38.1)	0 (0.0)	
Fixed	52 (61.9)	8 (100.0)	
Ethnicity (*n* = 80)			0.34*
Non-caucasian	13 (18.1)	0 (0.0)	
Caucasian	59 (81.9)	8 (100.0)	
Adjuvant Therapy Given (*n* = 90)			0.10*
No	56 (68.3)	8 (100.0)	
Yes	26 (31.7)	0 (0.0)	
Timing of Chemotherapy (*n* = 90)			1.00*
Preop	2 (2.4)	0 (0.0)	
Postop	3 (3.7)	0 (0.0)	
Both	1 (1.2)	0 (0.0)	
Neither (no chemo)	76 (92.7)	8 (100.0)	
Timing of Radiation Therapy (*n* = 90)			0.61*
Preop	5 (6.1)	0 (0.0)	
Postop	16 (19.5)	0 (0.0)	
Both	3 (3.7)	0 (0.0)	
Neither (no radiation)	58 (70.7)	8 (100.0)	
Tumor Grade (*n* = 92)			1.00*
Low (I)	67 (79.8)	7 (87.5)	
High (II)	17 (20.2)	1 (12.5)	
Enneking Appropriateness (*n* = 89)			1.00*
EA	53 (64.6)	5 (71.4)	
EI	29 (35.4)	2 (28.6)	
Local Recurrence at 10 Years Postoperative (*n* = 92)			1.00*
No	53 (63.1)	5 (62.5)	
Yes	31 (36.9)	3 (37.5)	
Survival at 10 Years Postoperative (*n* = 92)			0.10*
Alive	56 (66.7)	8 (100.0)	
Dead	28 (33.3)	0 (0.0)	

Data are presented as *N* (%) or mean ± standard deviation; EA: Enneking appropriate, EI: Enneking inappropriate.
**Fisher's exact test,* †*Student's t-test*

Discussion

PSCMs are rare lesions, with most studies limited to single-center experiences.[37] The current study consists of 133 patients from an international, multicenter database and represents the largest surgical cohort study on the molecular genetics of these rare tumors. Overall, the median OS was 5.8 years and is reflective of the heterogeneous makeup of the cohort, which consists of both indolent tumors like chordomas and more aggressive tumors like Ewing's sarcoma. In the subgroup analysis, we report the presence of *hTERT* promoter mutations in 8.7% of spinal chordomas, which is associated with a statistically significant positive OS benefit (100% vs 67% alive at 10 years) without affecting the time to first tumor recurrence.

The interplay between *hTERT* and OS in chordoma or other primary spinal neoplasms is not well understood;

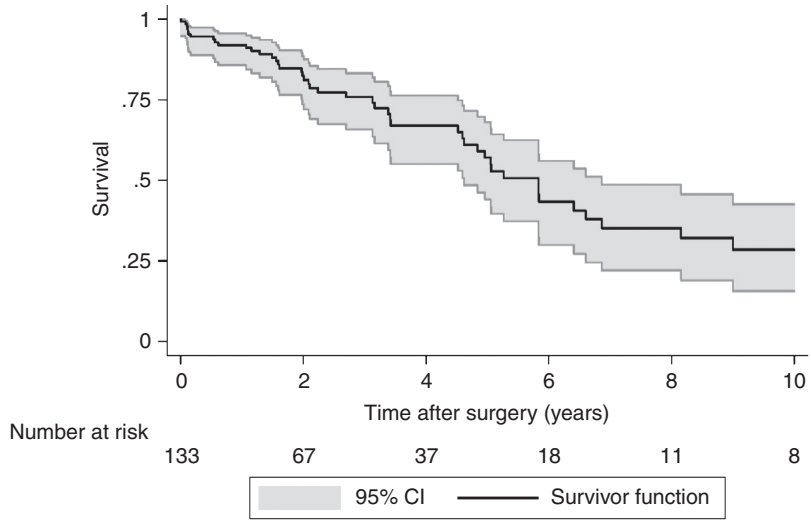

Fig. 1 Overall Kaplan–Meier survival curve.

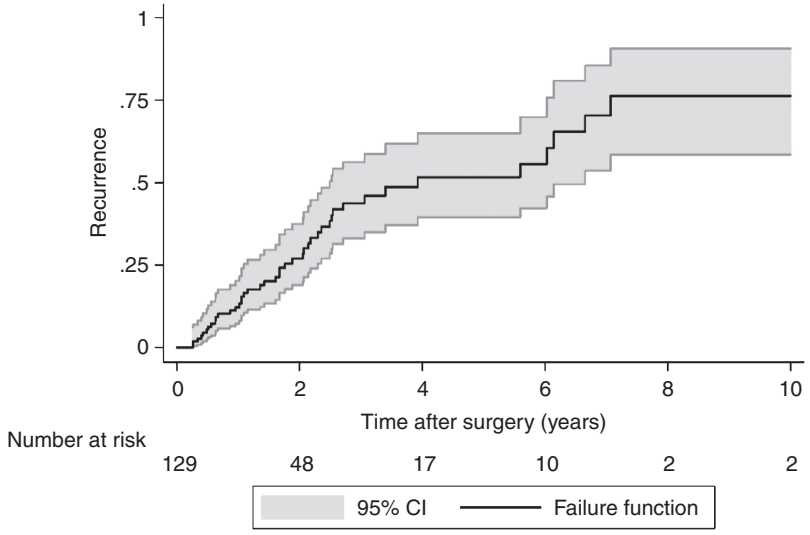

Fig. 2 Overall time to first local recurrence curve.

several studies on the topic have focused on *hTERT* expression and its effects on tumor growth and invasion. In one study, Zou et al reported *hTERT* expression in 54 spinal chordoma tissue samples but not in 20 nucleus pulposus control samples; *hTERT* expression was significantly associated with local tumor invasion and cellular proliferation based on Ki-67 staining index.[30] Additionally, high *hTERT* expression was an independent predictor of poor local recurrence-free survival, but no differences were seen in OS. In a series of 26 patients with clival chordomas, Pallini et al found that *hTERT* mRNA expression was frequently associated with increased doubling time for residual tumor and probability of local tumor recurrence.[38] Similarly, Hu et al reported higher expression of telomerase in 20 patients with sacral chordoma recurrence.[31] In another study, on chondrosarcomas, Chi et al reported a positive correlation between *hTERT* overexpression and telomere attrition and concluded that *hTERT* overexpression is associated with malignant sarcoma potential.[39] For both the Hu et al and Chi et al studies, overall survival was not evaluated. These reports suggest an association between *hTERT* overexpression and tumor aggressiveness, akin to previous studies in melanoma[14,40] and papillary thyroid carcinoma.[24] Despite these findings, the relationship between *hTERT* overexpression and OS in chordoma patients remains unclear. To our knowledge, there are no reports

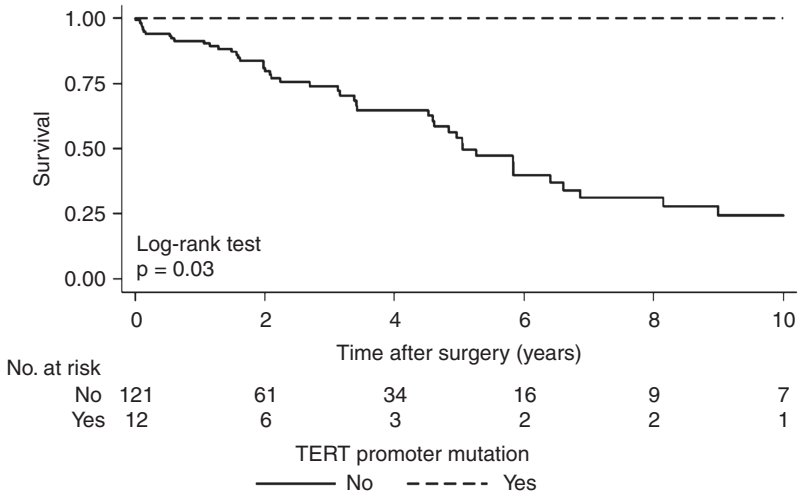

Fig. 3 Survival curve following surgery by presence of TERT promoter mutation in the overall cohort.

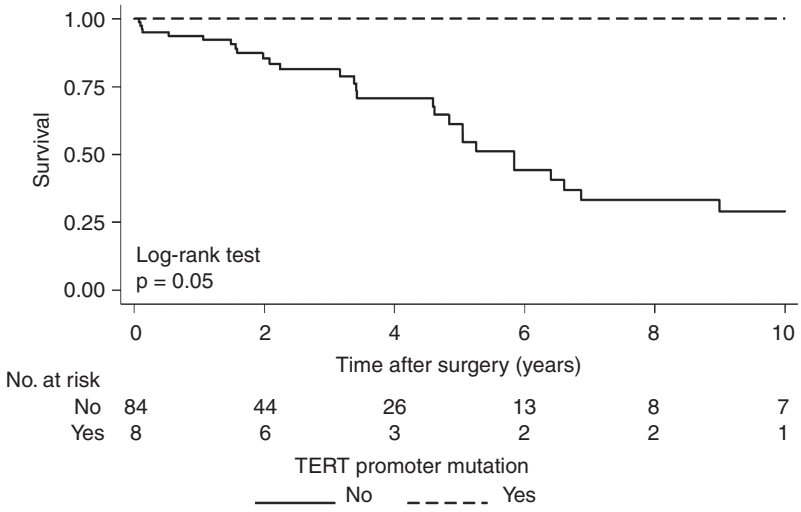

Fig. 4 Survival curve following surgery by presence of TERT promoter mutation in the chordoma specific cohort.

that specifically evaluate the role of *hTERT* promoter mutations in prognosticating survival of PSCM.

In many human cancers, *hTERT* promoter mutations are associated with higher *hTERT* expression. The C228T and C250T promoter mutations generate a binding motif for E-twenty-six transcription factors and are found to upregulate the transcriptional activity of *hTERT* by 2- to 6-fold in human melanomas.[23,24] Another study, in 48 GBMs, also reported significantly higher *hTERT* expression in C228T or C250T mutated tumors.[41] Although *hTERT* promoter mutations are associated with poor prognosis in many human cancers, there are reports in CNS tumors suggesting a survival benefit in patients harboring these mutations. *hTERT* mutations are seen in as many as 75% of glioma and GBM patients and do not appear to be a prognostic biomarker when evaluated in isolation;[27] despite this, patients with both MGMT methylation and *hTERT* promoter mutation are found to have improved survival (OS 28.3 vs 15.9 mo).[27] Conversely, *hTERT* mutations were found to be negatively prognostic in MGMT unmethylated GBM patients. In a study by You et al, patients with *hTERT* mutations exhibited improved prognosis when paired with IDH 1 or 2 (*IDH1/2*) mutations and 1p/19q loss of heterozygosity.[28] Poor prognosis was seen when *hTERT* mutations were paired with mesenchymal subtype or tumor protein p53 and epidermal growth factor receptor alteration.[28] In gliomas, the prognostic influence of *hTERT* promoter mutation appears to be dependent on the coexistence of other molecular biomarkers and drivers.[42]

This dual prognostic nature of the *hTERT* promoter mutation can help explain incongruent results in the literature, which mainly suggest that *hTERT* mutation is a negative prognostic biomarker. The pro-survival benefit of *hTERT* mutations observed in our study is also likely related to yet to be determined molecular pathways. A recent study by Tarpey et al defined the somatic molecular drivers of 104 cases of sporadic chordoma and found duplication of the notochordal transcription factor brachyury in 27% of cases. In addition, phosphatidylinositol-3 kinase signaling mutations and *LYST* inactivation mutations were implicated as potential novel oncologic markers.[43] The complex interactions between *hTERT* and other molecular drivers in PSCMs are still undefined and warrant further investigation.

Despite the use of large-scale, population-based, multicenter data, the current study remains limited by the rarity of PSCM and chordomas, variability between institutions with respect to treatment and follow-up time, and number of viable tissue samples. Intrinsic to the rarity of these tumors, the small sample sizes between the wildtype and hTERT mutation cohorts could potentially mask important differences between the groups that may only be detectable with larger sampling. As such, the 2 small groups being evenly matched is not definitively a negative lack of association. The compound analysis of all PSCM in the overall cohort can be confounded by biological differences and tumor heterogeneity, which prompted a subgroup analysis specific to spinal chordoma. We also did not specifically evaluate *hTERT* expression in this study. Further studies are needed to fully elucidate the interplay between *hTERT* promoter mutations, telomerase expression, and other molecular markers. Nevertheless, this study remains the largest contemporary series of primary spinal column tumors and is the first time the *hTERT* promoter mutations C228T and C250T have been implicated as a positive prognostic factor for survival in chordoma patients. We report for the first time that *hTERT* promoter mutations C228T and C250T are present in approximately 8.7% of spinal chordomas. The presence of hTERT mutations conferred a statistically significant survival benefit and could potentially be a valuable positive prognostic molecular marker in spinal chordomas.

Supplementary Material

Supplementary data are available at *Neuro-Oncology* online.

Keywords

chordoma | hTERT promoter mutation | primary spinal column malignancy | survival

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Conflict of interest statement

- Chetan Bettegowda reports consulting for Depuy-Synthes, which is outside of the submitted work.
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Authorship statement

- All authors contributed to writing the manuscript
- Analysis and interpretation: CB, SY, BJ, MZ, NMG, LR
- Data acquisition: CB, SY, WW, MJC, AL MG, MZ, CRG, DMS, JW, EM, ZLG, SB, PPV, CF, LR
- Experimental design: CB, SY, WW, AL, MG, NMG, AS, ZLG, SB, PPV, CF, LR

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