TERT promoter mutations are associated with poor prognosis in cutaneous squamous cell carcinoma

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Background: Telomerase reverse transcriptase gene (*TERT*) promoter (*TERT*p) mutations have been reported as potential predictors of poor prognosis in several cancers, but the prognostic value of *TERT*p mutations for cutaneous squamous cell carcinoma (cSCC) has not been determined.

Objective: To evaluate the frequency of *TERT*p mutations and correlate it with clinicopathologic features and patient outcome.

Methods: We performed genetic profiling of *TERT*p mutations in a retrospective series of cSCCs. The predictive value of *TERT*p mutations and clinicopathologic parameters were assessed by using logistic regression models.

Results: A total of 152 cSCCs from 122 patients were analyzed for *TERT*p mutations; the mutation rate was 31.6% (48 of 152), and it was higher in invasive cSCC (42 of 121 [34.7%]) than in in situ cSCC (6 of 31 [19.4%]). Age older than 75 years (odds ratio [OR], 14.84; $P = .013$] and *TERT*p mutation (OR, 8.11; $P = .002$) were independent predictors of local recurrence. *TERT*p mutation (OR, 15.89; $P = .022$) was independently associated with higher risk of lymph node metastasis.

Limitations: The restricted number of metastatic cases.

Conclusion: *TERT*p mutations may prove to be a molecular biomarker with prognostic significance in invasive cSCC, but larger studies are needed. (J Am Acad Dermatol 2019;80:660-9.)

Key words: biomarker; cutaneous squamous cell carcinoma; metastases; outcome; prognosis; prognostic biomarker; recurrence; squamous cell carcinoma; *TERT*; *TERT* promoter mutation.

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Dr Lopes and Dr Soares contributed equally to this article.

Funding sources: Supported by the Operational Program for Competitiveness and Internationalization (POCI), Portugal 2020; by Portuguese funds through the Fundação para a Ciência e a Tecnologia/ Ministério da Ciência, Tecnologia e Inovação within the framework of the project Institute for Research and Innovation in Health Sciences (POCI-01-0145-FEDER-007274); and by the project Advancing Cancer Research: From Basic Knowledge to Application; NORTE-01-0145-FEDER-000029; Projetos Estruturados de I&D&I, which is funded by Norte 2020-Programa Operacional Regional do Norte. Further support was provided by the European Regional Development Fund through the Operational Programme for Competitiveness and Internationalization COMPETE 2020 and Portuguese national funds via the Fundação para a Ciência e a Tecnologia, under the project POCI-01-0145-FEDER-016390: CANCEL STEM, and by the Portuguese Society of Dermatology and Venereology through a grant titled Bolsa Juvenal Esteves 2016/Bolsa de Investigação LEO to Dr Campos.

Conflicts of interest: None disclosed.

Accepted for publication August 16, 2018.

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Published online August 28, 2018.

0190-9622/$36.00

https://doi.org/10.1016/j.jaad.2018.08.032

Cutaneous squamous cell carcinoma (cSCC) is the second most common cancer in whites.[1] Ultraviolet radiation is the most common causal factor, and cSCC occurs most frequently in chronically sun-exposed areas, such as the face.[2] cSCC carcinogenesis includes premalignant lesions, actinic keratosis, in situ squamous carcinoma/Bowen disease, invasive carcinoma, and metastatic cSCC carcinoma, although a multistep model is not always present.[3] cSCC can recur and metastasize, and metastatic cases have a poor prognosis, with a 5-year survival rate of 25% to 35% and a 10-year survival rate less than a 10%.[4-6] Some clinicopathologic prognostic markers have been proposed in cSCC for local recurrence and metastasis (tumor thickness >6 mm; invasion beyond subcutaneous fat; perineural invasion; tumor size >2 cm; poor differentiation; and localization in the temple, lip, and ear).[7]

CAPSULE SUMMARY

- Patients with telomerase reverse transcriptase gene (*TERT*) promoter–mutated cutaneous squamous cell carcinoma (have higher risks for local recurrence and lymph node metastases.
- In the future, *TERT* promoter mutation may be included in the prognostic assessment of patients with cutaneous squamous cell carcinoma.

Telomerase reverse transcriptase gene (*TERT*), a ribonucleoprotein complex that synthesizes telomeric DNA (TTAGGG hexamers), is responsible for maintaining telomere length.[8] *TERT* promoter (*TERT*p) mutations create binding sites for E-twenty-six family transcription factors that result in telomerase expression and increasing telomere length and stability, allowing cancer cells to divide and preventing senescence or apoptosis. Recurrent somatic *TERT*p mutations have been found in a high percentage of melanomas, cancers of the central nervous system, bladder cancers, and thyroid cancers (follicular cell-derived).[9-13] In cutaneous carcinomas,[13,14] an ultraviolet light–induced damage signature has been attributed to *TERT*p mutations (cytidine-to-thymidine transitions at dipyrimidine motifs).[9,10] These *TERT*p mutations have been described as a potential biologic predictor of metastasis and/or mortality in melanoma, glioblastoma, medulloblastoma, and bladder and thyroid cancers.[12,15-18] However, in cSCC, only small series of cases were evaluated and no information about the putative prognostic value of these changes is available in the literature.

In this study, we assessed *TERT*p mutations in a large series of cSCCs and correlated these mutations with clinicopathologic features and patients outcome.

MATERIALS AND METHODS

Patient selection, sample selection, and clinicopathologic characterization

All the procedures described in this study were in accordance with national and institutional ethical standards and were previously approved by local ethical review committees. A more detailed description of methods is available in the Supplemental Methods (available at http://www.jaad.org).

A descriptive and statistical analysis of all consecutive cSCCs surgically removed at Centro Hospitalar Vila Nova de Gaia e Espinho between January 2004 and December 2013 was performed. The inclusion criteria included immunocompetence, histologic diagnosis of cSCC, and available follow-up data. The exclusion criteria included lesions in patients with genetic diseases associated with increased risk of cSCC (eg, xeroderma pigmentosum, epidermodysplasia verruciformis, and albinism). None of the cases in this retrospective series were treated with Mohs micrographic surgery. A total of 184 histologic specimens were revised by pathologists with experience in cutaneous neoplasms (J.M.L., J.P., and M.F.). Tumors were categorized according to the protocol for examination of specimens of the College of American Pathologists and the American Joint Committee on Cancer guidelines.[19] Representative tumor areas were marked by the pathologists on hematoxylin and eosin–stained slides to perform manual microdissection.

For statistical analysis, age at diagnosis was categorized as being in 1 of 2 groups according to the mean age (≤75 vs >75 years), and topographic locations were classified according to the *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision*,[20] and according to sun exposure. Specimens were classified as in situ cSCC or invasive cSCC according to histologic subtype. In addition to College of American Pathologists protocol variables, other recorded categories were pattern of invasion and presence of intratumoral and peritumoral infiltrate. The T stage of each tumor was classified according to the correlative tumor, node, and metastasis classification. As in other studies,[21,22] recurrence was defined as the development of a histologically confirmed cSCC in the same topographic area in

Abbreviations used:

cSCC: cutaneous squamous cell carcinoma
OR: odds ratio
*TERT*p: telomerase reverse transcriptase gene promoter

addition to being identified by the assisting dermatologist as recurrence. Progression-free survival was defined as the time until diagnosis of recurrence and/or metastasis. Progression-free survival and overall follow-up times were recorded in months.

DNA extraction and mutation analysis

DNA was retrieved from 10-μm cuts of formalin-fixed paraffin-embedded tissue samples after careful microdissection. A DNA extraction kit (Citogene, Citomed, Odivelas, Portugal) was used according to the manufacturer's instructions. Polymerase chain reaction was performed with Qiagen Multiplex kit (Qiagen, Hilden, Germany) using the recommended settings.[11] Direct sequencing reaction was performed with the BigDye Terminator Kit (Perkin-Elmer, Foster City, CA), and the fragments were run in an ABI prism 3100 Genetic Analyzer (Perkin-Elmer). Independent polymerase chain reaction amplification/sequencing was performed for both positive and inconclusive (not confirmed as positive or negative) samples. Mutations were detected by using Mutation Surveyor DNA variant analysis software (Softgenetics, State College, PA) and matched with reference sequences from GenBank. Sequences were obtained from base pairs −270 to −50 upstream of the start (ATG) codon, which include the recurrent *TERT*p mutations described in other cancers.[9,10] The described *TERT*p mutations evaluated included the following: −124C>T, −146C>T, tandem −124/−125CC>TT, and tandem −138/139CC>TT.

Statistical analysis

Statistical analysis was conducted with SPSS software (version 24.0, IBM Corp, Armonk, NY). Descriptive statistics, the chi-square test, Fisher exact test, and Student *t* test (unpaired, 2-tailed) were used when appropriate. The predictive value of *TERT*p and other variables for recurrence, metastasis, and progression-free survival was assessed by using univariate and multivariate logistic regression models. In the regression models, all the variables that were significantly associated with the specified outcome in the univariate model were included in the multivariate analysis. Confidence intervals were calculated with 95% coverage. Survival curves were plotted by the Kaplan-Meier method with the log-rank statistics. Results were considered statistically significant at P less than .05. The significance level was adjusted by Bonferroni correction (with 0.05 divided by the number of performed comparisons for each dependent variable) when multiple comparisons were performed (results displayed in Supplemental Table I (available at http://www.jaad.org).

RESULTS

Of the 184 histologically characterized cases, we were unable to determine *TERT*p status in 32 cases owing to the small size and/or low quality of the samples. In total, 152 lesions from 122 patients were analyzed for *TERT*p mutations. Of these, 31 corresponded to in situ cSCC and 121 to invasive cSCC. The overall frequency of *TERT*p mutations was 31.6% (48 of 152 cases). *TERT*p mutations were present in 6 of 31 in situ cSCCs (19.4%) and 42 of 121 invasive cSCCs (34.7%). The following mutations were detected: −124 (G>A mutation in 26 of 48 cases [54.2%]), −146 (G>A mutation in 18 of 48 cases [37.5%]), and tandem mutation at position −124/−125 in 4 cases (8.3%). The mutations were mutually exclusive.

Relationship between *TERT*p mutations and clinicopathologic features

Table I presents the clinicopathologic features and the frequency of *TERT*p mutations in the series. Clinicopathologic factors and their association with *TERT*p mutations are presented in Supplemental Table I.

We analyzed all the cSCCs and observed that *TERT*p mutations were present in the face, trunk, and upper and lower limb. *TERT*p mutations were more frequent in invasive cSCCs than in in situ cSCCs, although the difference was not statistically significant.

In in situ cSCCs, men showed a higher frequency of *TERT*p mutations (in 5 of 15 [33.3%]) than women did (in 1 of 16 [6.3%]). Of the 3 in situ cSCCs that recurred, none of had *TERT*p mutations.

In invasive cSCCs, *TERT*p mutation was associated with a larger maximum tumor thickness (4.8 ± 4.1 mm vs 3.4 ± 2.2 mm [P = .050]), and tumors with thicker than 6 mm displayed a higher frequency of *TERT*p mutations (in 10 of 19 cases [52.6%] vs 29 of 96 cases [30.2%] cases [P = .059]), although neither association reached statistical significance after adjustment of the P values with Bonferroni correction. Despite the difference not reaching statistical significance, tumors with *TERT*p mutations were larger than tumors with wild-type *TERT*p (2.3 ± 1.8 cm vs 1.6 ± 1.2 respectively

Table I. Clinicopathologic features and the frequency of *TERT*p mutations

Feature	All lesions	In situ cSCC	Invasive cSCC
No. of cases	152	31	121
Age at diagnosis, y, mean ± SD	76.8 ± 11.8	78.5 ± 7.0	76.5 ± 12.7
Male	73.8 ± 11.7	77.8 ± 6.8	73.0 ± 12.3
Female	80.8 ± 10.8	78.4 ± 7.8	81.6 ± 11.6
Sex, n (%)			
Male	87 (57.2)	15 (48.4)	72 (59.5)
Female	65 (42.8)	16 (51.6)	49 (40.5)
Sun exposure, n (%)			
Chronic	103 (67.8)	11 (35.5)	92 (76.0)
Intermittent	43 (28.3)	18 (58.1)	25 (20.7)
Undetermined	6 (3.9)	2 (6.5)	4 (3.3)
Localization, n (%)			
Face	97 (63.8)	9 (29.0)	88 (72.7)
Trunk	8 (5.3)	4 (12.9)	4 (3.3)
Upper limb	21 (13.8)	6 (19.4)	15 (12.4)
Lower limb	20 (13.2)	10 (32.3)	10 (8.3)
Undetermined	6 (3.9)	2 (6.5)	4 (3.3)
Follow-up, mo, mean ± SD	42.9 ± 28.8	39.7 ± 22.0	43.7 ± 30.3
Progression-free survival, mo, mean ± SD	39.8 ± 29.3	38.4 ± 22.2	40.1 ± 30.9
Adverse outcome, n (%)			
No	129 (84.9)	28 (90.3)	101 (83.5)
Yes	23 (15.1)	3 (9.7)	20 (16.5)
Recurrence, n (%)			
No	132 (86.8)	28 (90.3)	104 (86.0)
Yes	20 (13.2)	3 (9.7)	17 (14.0)
Metastases, n (%)			
No	144 (94.7)	31 (100)	113 (93.4)
Yes	8 (5.3)	0	8 (6.6)
*TERT*p mutations, n (%)			
Wild-type	104 (68.4)	25 (80.6)	79 (65.3)
Mutation	48 (31.6)	6 (19.4)	42 (34.7)
Procedure, n (%)			
Biopsy	34 (22.4)	6 (19.4)	28 (23.1)
Excision	118 (77.6)	25 (80.6)	93 (76.9)
Maximum tumor size, cm, mean ± SD	1.9 ± 1.5	1.9 ± 1.2	1.9 ± 1.5
Maximum tumor size, n (%)			
<2 cm	69 (45.4)	10 (32.3)	59 (48.8)
≥2 cm	39 (25.7)	9 (29.0)	30 (24.8)
Cannot be assessed	44 (28.9)	12 (38.7)	32 (26.4)
Peripheral margins, mm, mean ± SD	2.3 ± 2.9	1.6 ± 1.5	2.5 ± 3.1
Deep margins, mm, mean ± SD	2.6 ± 2.4	3.0 ± 1.7	2.5 ± 2.5
Ulceration, n (%)			
No	53 (34.9)	14 (45.2)	39 (32.2)
Yes	91 (59.9)	15 (48.4)	76 (62.8)
Undetermined	8 (5.3)	2 (6.5)	6 (5.0)
Actinic keratosis, n (%)			
No	50 (32.9)	6 (19.4)	44 (36.4)
Yes	92 (60.5)	25 (80.6)	67 (55.4)
Undetermined	10 (6.6)		10 (8.3)
Invasion, n (%)			
Noninvasive	31 (20.4)		
Invasive	121 (79.6)		
Histologic type, n (%)			
Acantholytic			9 (7.4)
Verrucous			2 (1.7)
NOS			110 (90.9)

Continued

Table I. Cont'd

Feature	All lesions	In situ cSCC	Invasive cSCC
Histologic grade, n (%)			
Well differentiated			43 (35.5)
Moderately/poorly differentiated			74 (61.2)
Cannot be assessed			4 (3.3)
Pattern of invasion, n (%)			
Expansive			66 (54.5)
Infiltrative			51 (42.1)
Cannot be assessed			4 (3.3)
Level of invasion, n (%)			
Papillary dermis			36 (29.8)
Reticular dermis			53 (43.8)
Subcutaneous tissue			25 (20.7)
Cannot be assessed			7 (5.8)
Maximum tumor thickness, mm, n (%)			3.8 ± 3.1
<6 mm			96 (79.3)
≥6 mm			19 (15.7)
Cannot be assessed			6 (5.0)
Intratumoral infiltrate, n (%)			
Moderate/intense			13 (10.7)
Few/absent			108 (89.3)
Peritumoral infiltrate, n (%)			
Moderate/intense			68 (56.2)
Few/absent			53 (43.8)
Lymphovascular invasion, n (%)			
Not present			116 (95.9)
Present			5 (4.1)
Perineural invasion, n (%)			
Not present			118 (97.5)
Present			3 (2.5)

cSCC, Cutaneous squamous cell carcinoma; *NOS*, not otherwise specified; *TERT*p, telomerase reverse transcriptase gene promoter.

[P = .068]). *TERT*p mutations were significantly more frequent in cases that recurred (in 13 out of 17 cases [76.5%] vs in 29 of 104 cases [27.9%] [P < .001]). *TERT*p mutations were also more common in metastatic cases (in 7 of 8 cases [87.5%]) than in nonmetastatic cases (35 of 113 cases [31.0%]), but statistical analysis was precluded owing to the small number of wild-type cases with metastasis (n = 1).

Relationship between *TERT*p mutation and outcome

In this analysis, we included only invasive cSCCs (n = 121). The mean follow-up time of the patients was 43.7 plus or minus 30.3 months (range, 6-156 months).

A total of 17 cases (14.0%) and 8 cases (6.6%) presented recurrence and metastasis (all of which were lymph node metastasis), respectively, during follow-up. Table II presents the main characteristics of the cases with adverse outcomes.

Regression modeling was performed for factors associated with an adverse outcome (recurrence or metastases) in invasive cSCC (Table III). When the factors associated with the risk of recurrence were analyzed, age older than 75 (odds ratio [OR], 13.19; P = .014], absence of ulceration (OR, 2.96; P = .048), and *TERT*p mutation (OR, 8.41; P = .001) were identified to be predictors in the univariate analysis. When the aforementioned factors were included in the multivariate analysis, *TERT*p mutation (OR, 8.11; P = .002) and age older than 75 years (OR, 14.84; P = .013] were identified as independent predictors of recurrence. As shown in Table II, 82.4% of the cases that recurred were located on the face. When facial and extrafacial lesions were compared, no statistical difference was observed between the mean values of their surgical margins (mean peripheral margins of 2.5 ± 3.5 vs 2.7 ± 1.8, respectively, and mean deep margins of 2.6 ± 2.7 vs 2.6 ± 2.1, respectively). Peripheral margins (OR, 1.12; P = .121) and deep margins (OR, 0.92; P = .924) were not associated with recurrence in the univariate analysis. Despite the fact that the differences between margins did not achieve significance in the univariate

Table II. Features of invasive cSCC in patients with recurrence and/or lymph node metastasis

Case	Patient age, y/sex	Location	AO	PFS, mo	TERTp mutation	Max, cm	PM/DM, mm	HT	HG	TT, mm	Peritumoral infiltrate	FU, mo	Status at FU
1	85/F	Ear	R	22	WT	2	3.5/3.0	NOS	Mod	4	Mod-int	56	NED
2	78/M	Forehead	R	16	WT	1.8	2.0/1.5	NOS	Mod	1.5	Few-abs	22	DNR
3	80/M	Ear	R	35	Mut	5	4.0/0.8	NOS	Mod	9	Mod-int	80	NED
4	81/M	Ear	R + Ms	11	Mut	1.5	4.0/8.0	Acan	Mod	7	Few-abs	36	DOD
5	83/M	Forehead	R + Ms	9	Mut	NA	0/0	NOS	Poor	NA	Few-abs	34	DNR
6	81/M	Ear	R	32	Mut	0.9	3.0/2.0	NOS	Well	2	Mod-int	41	NED
7	80/F	Arm	R + Ms	28	Mut	2.5	4.5/2.0	NOS	Well	2	Mod-int	96	NED
8	82/F	Hand	R + Ms	38	Mut	1.2	4.0/0.1	NOS	Mod	5	Few-abs	58	NED
9	75/M	Arm	R	39	Mut	NA	0.5/0.2	NOS	Mod	3	Few-abs	56	NED
10	80/M	Temple	R	25	Mut	1.1	1.5/2.0	NOS	Mod	4	Few-abs	38	NED
11	86/M	Ear	Ms	3	WT	NA	0/0	NOS	Mod	2	Few-abs	12	DOD
12	87/M	Cheek	R	7	WT	1.5	1.5/4.0	NOS	Mod	4	Few-abs	24	DNR
13	87/F*	Cheek	R	12	Mut	1	4.0/4.0	NOS	Mod	5	Mod-int	36	NED
14	87/F*	Forehead	R	12	Mut	0.8	2.0/3.0	NOS	Mod	1.5	Mod-int	36	NED
15	88/F	Nose	R	16	Mut	0.6	1.5/2.0	NOS	Mod	2	Few-abs	24	NED
16	82/F	Nose	R	20	WT	0.9	2.5/0.5	NOS	Poor	3	Mod-int	62	NED
17	80/M	Ear	R	8	Mut	3	0/0	NOS	Well	4	Few-abs	12	DNR
18	86/M	Ear	Ms	6	Mut	NA	0/0.2	NOS	Mod	5	Few-abs	12	DNR
19	86/F	Nose	Ms	10	Mut	2.5	3.0/5.0	NOS	Mod	11	Few-abs	24	NED
20	84/M	Scalp	R + Ms	3	Mut	NA	21.0/1.0	NOS	Mod	18	Few-abs	30	NED

Acan, Acantholytic; *AO*, adverse outcome; *cSCC*, cutaneous squamous cell carcinoma; *DNR*, death not related; *DOD*, dead of disease; *F*, female; *Few-abs*, few-absent; *FU*, follow-up; *HG*, histologic grade; *HT*, histologic type; *M*, male; *Max*, maximum tumor size; *Mod*, moderately differentiated; *Mod-int*, moderate-intense; *Ms*, lymph node metastasis; *Mut*, mutated; *NA*, not available/analyzed; *NED*, no evidence of disease; *NOS*, not otherwise specified; *PFS*, progression-free survival; *PM/DM*, peripheral margins and deep margins; *Poor*, poorly differentiated; *R*, recurrence; *SD*, standard deviation; *Sub*, subcutaneous tissue; *TERT*p, telomerase reverse transcriptase gene promoter; *TT*, tumor thickness; *Well*, well-differentiated; *WT*, wild-type.
*Tumor taken from the same patient on the same day.

analysis, we included margins in the multivariate analysis because lower margins may be a confounder in relation to *TERT*p mutation prognostic value in recurrence. After adjustment for margins, *TERT*p mutation (OR, 6.75; $P = .004$) and age older than 75 years (OR, 13.00; $P = .022$) continued to be independent predictors of recurrence in the multivariate analysis.

When we analyzed predictors of metastasis, univariate analysis demonstrated that few or absent peritumoral lymphocytes (OR, 10.20; $P = .033$), subcutaneous tissue invasion (OR, 5.46; $P = .034$), peripheral margins (OR, 1.16; $P = .048$), and *TERT*p mutation (OR, 15.60; $P = .012$) were associated with a higher likelihood of metastasis. In the multivariate analysis, only *TERT*p mutation (OR, 15.89; $P = .022$) was independently associated with a higher risk of metastasis.

The Kaplan-Meier survival analysis revealed that *TERT*p mutation (Fig 1, *A* and *B*) and age older than 75 years (Fig 1, *C* and *D*) were associated with a shorter time for recurrence (log rank $P < .001$ and $P < .001$) and a shorter time for metastasis to occur (log rank $P = .002$ and $P = .007$). Absent or few peritumoral lymphocytes (Fig 1, *E*) and subcutaneous tissue invasion (Fig 1, *F*) were associated with a shorter time for metastasis occurrence (log rank $P = .007$ and $P = .014$).

DISCUSSION

The most important added value provided by the present study is the finding that patients with mutated *TERT*p invasive cSCC have a substantially higher risk of adverse outcome (recurrence and metastasis). Our results are in accordance with those of previous studies in other cancers, in which *TERT*p mutation was a significant predictor of poor prognosis.[15,18,23] The usefulness of *TERT*p mutations as a prognostic marker is particularly relevant because invasive cSCC is highly frequent, a small percentage of such carcinomas behave aggressively, and there is a lack of good prognostic indicators in this setting.

Concerning invasive cSCC, we report a lower mutation rate (34.7%) than those reported in most previously published studies (50.0%-74.1%); however, those studies were conducted with a limited number of samples (5-37 cases).[13,14,24,25] Because our study included a larger consecutive series of cases in a hospital, we believe that it represents a

Table III. Predictive factors for recurrence and lymph node metastasis

	Recurrence				Metastasis			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
Factor	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Mean age, y								
≤75	1	**.014**	1	**.013**	NA	NA		
>75	13.19 (1.69-103.19)		14.84 (1.77-124.17)					
Ulceration								
Yes	1	**.048**	1	.116	1	.196		
No	2.96 (1.01-8.68)		2.69 (0.78-9.21)		2.78 (0.59-13.11)			
Level of invasion								
Dermis	1	.741			1	**.034**	1	.163
Subcutaneous tissue	0.80 (0.21-3.05)				5.46 (1.14-26.28)		3.95 (0.57-27.30)	
Peritumoral infiltrate								
Moderate-intense	1	.184			1	**.033**	1	.054
Few-absent	2.03 (0.72-5.74)				10.20 (1.21-85.68)		9.69 (0.96-97.75)	
Peripheral margins*								
	1.12 (0.97-1.28)	.121			1.16 (1.00-1.35)	**.048**	1.10 (0.90-1.33)	.352
*TERT*p								
Wild-type	1	**.001**	1	**.002**	1	**.012**	1	**.022**
Mutation	8.41 (2.53-27.90)		8.11 (2.22-29.59)		15.60 (1.85-131.65)		15.89 (1.48-170.70)	

Parameters with significant results in the univariate analysis of 1 of the adverse outcomes (recurrence or metastasis) are included. None of the other clinicopathologic features were associated with outcome in the univariate analysis. Not displayed are facial location and deep margins that were not associated with recurrence and metastasis in the univariate analysis. Boldface indicates statistical significance.

CI, Confidence interval; *NA*, not applicable (no metastasis occurred in patients age 75 years or younger); *OR*, odds ratio; *TERT*p, telomerase reverse transcriptase gene promoter.

*Peripheral margins were analyzed in the model as a continuous variable.

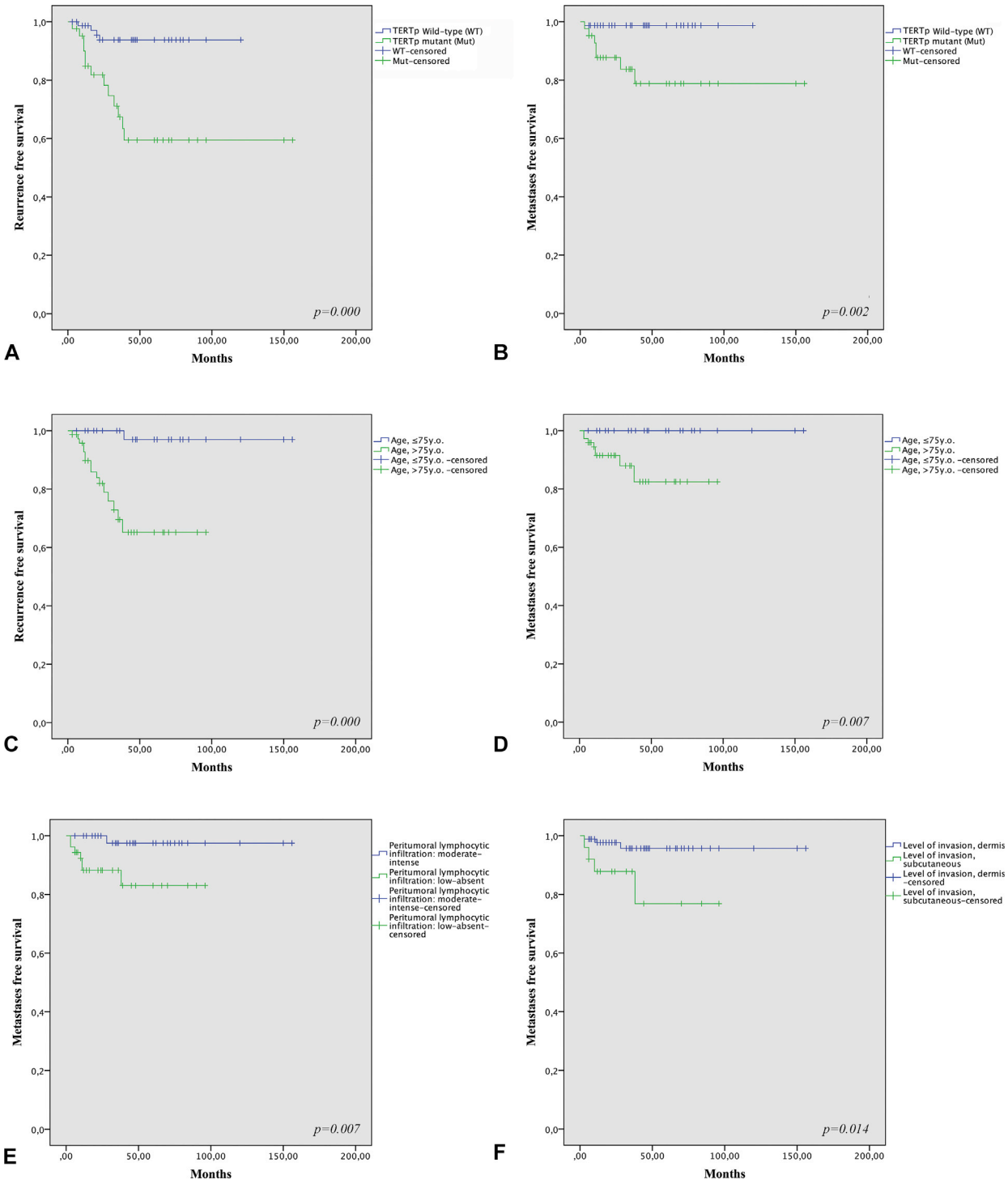

Fig 1. Kaplan-Meier curves for recurrence-free survival and metastasis-free survival of invasive cutaneous squamous cell carcinoma, respectively, according to telomerase reverse transcriptase gene (*TERT*) promoter (*TERT*p) status (**A** and **B**) and age (**C** and **D**). Kaplan-Meier curves for metastasis-free survival of invasive cutaneous squamous cell carcinoma according to peritumoral lymphocytic infiltration (**E**) and level of invasion (**F**). *MUT*, Mutation; *WT*, wild type.

more accurate estimation of *TERT*p mutation in cSCC. Our study indicates that *TERT*p mutation may be more frequent in invasive cSCC than in in situ cSCC, although studies with a larger number of in situ cases are necessary to confirm this premise.

As in melanoma[15,23] *TERT*p-mutated, invasive cSCCs presented a larger maximum tumor thickness than wild-type *TERT*p cases did, although the difference in our study was not statistically significant. Our results also showed a higher (but not statistically significantly so) frequency of *TERT*p mutations in tumors thicker than 6 mm, which is a parameter that has been described as being associated with a higher risk of metastasis.[21]

The role of *TERT*p mutation in early cutaneous squamous cell carcinogenesis remains to be clarified because our study revealed a higher rate of *TERT*p mutation in in situ cSCCs (19.4%) than the previously reported rate (9.1%),[13] even though recurrent in situ cSCCs did not have this mutation in our series. Thus, studies including cases of normal-appearing skin, actinic keratosis, in situ cSCCs, and invasive cSCCs are warranted to clarify the role of *TERT*p mutation in the putative pathogenic model(s) of cSCC.

Our rate of recurrence of invasive cSCC (14.0%) is within the range of those rates reported in previous studies (3.0%-16.0%).[21,22,26-28] We observed a lymph node metastasis rate (6.6%) slightly higher than that reported in the literature (3.7%-4.6%).[21,22] The fact that all cases were drawn from a hospital that often assists patients with more advanced disease may in part explain the differences in the aforementioned reported rates. Most of the recurrent invasive cSCC cases were located on the face, which is an anatomic region where larger clearance margins are difficult to attain. Despite this, facial location and mean margins were not associated with recurrence.

With regard to other prognostic factors in this series, age older than 75 years was an independent predictor of recurrence in invasive cSCC, as identified in previous studies.[22,28-30] Our results also indicated that little or no peritumoral infiltration was associated with metastasis in the univariate analysis and with a shorter time until this adverse outcome occurred. In other tumor models (including melanoma), the absence of or a reduced number of lymphocytes (without addressing the different subsets by immunohistochemistry) is an independent parameter associated with adverse prognosis.[31-33] Invasion of the subcutaneous tissue, which is a classic risk factor for recurrence and metastasis of cSCC,[7] was a predictor of metastasis in the univariate analysis and was associated with a shorter time until metastization. Despite these results, when adjusted for other variables, few or absent peritumoral infiltrates and invasion of the subcutaneous tissue failed to be independent prognostic predictors.

We are aware that our series lacks a substantial number of metastatic cSCC samples with longer follow-up. Nevertheless, we evaluated a consecutive series and found that the prevalence of advanced cSCC in our cohort is actually slightly higher when compared with the frequency reported by others. Another limitation is that there could be a potential selection bias because not all initially selected cases had histologic specimens available.

We conclude that *TERT*p mutations may be potential markers for aggressive behavior in cSCC because they may be more frequently observed in invasive cSCC than in situ cSCC and were associated with recurrence and metastasis in invasive cSCC. Moreover, recurrence and metastasis in invasive cSCC were likely to occur sooner in cases with *TERT*p mutation. As in other cancers,[34] the inclusion of *TERT*p mutation in management guidelines should be considered in cSCC.

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SUPPLEMENTAL METHODS

Patient selection, sample selection, and clinicopathologic characterization

All the procedures described in this study were in accordance with national and institutional ethical standards and were previously approved by the local ethical review committees. According to Portuguese law, informed consent is not required for retrospective studies.

A descriptive and statistical analysis of all consecutive cutaneous squamous cell carcinomas (cSCCs) surgically removed at Centro Hospitalar Vila Nova de Gaia e Espinho (CHVNGE) between January 2004 and December 2013 was performed. The inclusion criteria included immunocompetence, a histologic diagnosis of cSCC, and available follow-up data. The exclusion criteria included lesions in patients with genetic diseases associated with increased risk of cSCC (eg, xeroderma pigmentosum, epidermodysplasia verruciformis, and albinism). None of the cases in this retrospective series were treated with Mohs micrographic surgery. We identified and gathered available formalin-fixed paraffin-embedded tissue samples from the pathology department of Centro Hospitalar Vila Nova de Gaia e Espinho. A total of 184 histologic specimens were examined by pathologists with experience in cutaneous neoplasms (J.M.L., J.P., and M.F.). Tumors were characterized on the basis of the protocol for examination of specimens of the College of American Pathologists (CAP) and the American Joint Committee on Cancer guidelines.[S1,S2] The included College of American Pathologists criteria were as follows: procedure, tumor site, tumor size, histologic type, histologic grade, thickness of the tumor, status of surgical (peripheral and deep) margins of the excised tumors, lymphovascular invasion, perineural invasion, lymph node status, and pathologic staging (pathologic tumor, node, and metastasis stating). We also included additional criteria such as type of infiltration (expansive or infiltrative), presence of ulceration, peritumoral and intratumoral lymphocytic infiltrate, presence of actinic keratosis in adjacent epidermis, and presence of ulceration in the histologic specimens. We used transected tumor biopsy specimens of 26 cases (5 in situ cSCCs and 21 invasive cSCCs) to evaluate pristine primary tumors, without alterations derived from subsequent complete re-excision of each tumor. There was no evidence of invasive components in any of the re-excised specimens of the in situ cSCC cases (data not shown). Similarly, the values of the parameters evaluated in the invasive cSCC cases did not differ. Importantly, none of these 26 cases were used to evaluate the impact of surgical margins, except for the other parameters (as stated in the Results and Tables I-III) whenever they were adequately assessed. Representative tumor areas were marked by the pathologists on hematoxylin and eosin–stained slides to carry out manual microdissection.

Age at diagnosis was recorded and categorized into 2 groups according to the mean age (≤75 years vs >75 years) for statistical analyses. Topographic locations of the specimens were classified according to *International Statistical Classification of Diseases and Related Health Problems*, *Tenth Revision*[S3] (including lips, eyelid, ear, face, scalp/neck, trunk, upper limb, lower limb, and undetermined). The topographic locations were then grouped into 5 locations for statistical analyses, including the face (including the neck), trunk, upper limb, lower limb, and not specified. Moreover, topographic locations were subdivided into chronically sun-exposed (scalp/neck, face, ears, eyelids, and hands) and intermittently sun-exposed (trunk, upper limb, lower limbs, and feet) locations. Because 82.4% of the recurrent cases and 75.0% of the metastatic cases were from invasive cSCCs located on the face, topographic location was further divided into facial lesions and extrafacial lesions for statistical purposes. Specimens were classified as in situ cSCC or invasive cSCCs, with the latter category subdivided by histologic type into acantholytic, spindle cell, verrucous, pseudovascular, adenosquamous, and not otherwise specified. Histologic grade was classified as well differentiated, moderately differentiated, poorly differentiated, and undifferentiated. Pattern of invasion was classified as expansive or infiltrative. The tissue level of the tumors was classified as invading the papillary dermis, invading the reticular dermis, or invading the subcutaneous tissue and beyond. Maximum tumor thickness was measured in millimeters and categorized as being in 1 of 2 groups (<6 mm vs >6 mm), in which only invasive cSCCs were included. Peripheral and deep surgical margins were measured in millimeters. The presence of ulceration and actinic keratosis was identified. Actinic keratosis was identified in the adjacent normal epidermis. Intratumoral and peritumoral infiltrates were classified as moderate/intense and few/absent. The presence of lymphovascular and perineural invasion was annotated. The T stage of each tumor was classified according to the correlative tumor, node, and metastasis classification. As in previous studies,[S4,S5] recurrence was defined as the development of a histologically confirmed cSCC in the same topographic area in addition to being identified by the assisting dermatologist as

recurrence. Progression-free survival was defined as the time until diagnosis of recurrence and/or metastasis. Progression-free survival and overall follow-up times were recorded in months.

DNA extraction and mutation analysis

DNA from the formalin-fixed paraffin-embedded tissue samples was retrieved from 10-μm cuts after careful microdissection. A DNA extraction kit (Citogene, Citomed, Odivelas, Portugal) was used according to the manufacturer's instructions. The extracted DNA was quantified with a Nanodrop N-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and stored at −20°C. Polymerase chain reaction (PCR) was performed with a Qiagen Multiplex Kit (Qiagen, Hilden, Germany) using the recommended settings for Q solution DNA amplification. Genomic DNA (25-100 ng) was amplified by PCR under the following cycling conditions: 30 seconds at 95°C; 90 seconds at 62°C, and 20 seconds at 72°C for 40 cycles. The primers used can be found in a previous publication by our group.[S6] Direct sequencing reaction was performed with the BigDye Terminator Kit (Perkin-Elmer, Foster City, CA), and the fragments were run in an ABI prism 3100 Genetic Analyzer (Perkin-Elmer). The sequencing reaction was performed in a forward direction, and an independent PCR amplification/sequencing was performed in both a forward and reverse direction for positive and inconclusive (not confirmed as positive or negative) samples. Mutations were detected by using Mutation Surveyor DNA variant analysis software (Softgenetics, State College, PA) and matched with reference sequences from GenBank. Sequences evaluated corresponded to the loci in the TERTp ranging from −270 to −50 base pairs upstream of the TERT gene start codon that include the recurrent TERTp mutations described in other cancers.[S7,S8] The described *TERT*p mutations evaluated included the following: −124C>T, −146C>T, tandem −124/−125CC>TT and tandem −138/139CC>TT.

Statistical analysis

Statistical analysis was conducted with SPSS software (version 24.0, IBM Corp, Armonk, NY). The results were expressed as a percentage or mean plus or minus SD. Statistical analysis was performed on both the whole series of cSCCs and 2 different groups of cSCCs (in situ and invasive cSCCs). The chi-square test, Fisher exact test, and Student *t* test (unpaired, 2-tailed) were used when appropriate. The predictive value of *TERT*p mutations and other variables (age, sex, sun exposure, localization, presence of ulceration, presence of actinic keratosis, tumor size, histologic type, histologic grade, pattern of invasion, level of invasion, maximum tumor thickness, intratumoral infiltrate, peritumoral infiltrate, lymphovascular invasion, and perineural invasion) for recurrence, metastasis, and progression-free survival were assessed by using univariate and multivariate logistic regression models. Survival curves were plotted by the Kaplan-Meier method with the log-rank statistics. In the regression models, all the variables that were significantly associated with the specified outcome in the univariate model were included in the multivariate analysis. Confidence intervals were calculated with 95% coverage. Results were considered statistically significant at P less than .05. The significance level was adjusted by Bonferroni correction (with 0.05 divided by the number of performed comparisons for each dependent variable) when assessing *TERTp* mutations according to different clinicopathologic and molecular characteristics (results displayed in Supplemental Table I; available at http://www.jaad.org), as multiple comparisons were performed.

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STATISTICAL ANALYSIS PLAN

Telomerase reverse transcriptase gene (*TERT*) promoter (*TERTp*) mutations are associated with poor prognosis in cutaneous squamous cell carcinoma (cSCC).

Study objectives

Assess *TERTp* mutations, clinicopathologic features, and outcome in a series of cSCCs.

Primary objective

Assess *TERTp* mutations in a large series of cSCCs and correlate these mutations with clinicopathologic features and patients' outcome (recurrence or metastasis).

Study design

Retrospective study (genetic profiling of *TERTp* mutations in a retrospective series of cSCCs).

Study population

Consecutive cSCCs surgically removed at Centro Hospitalar Vila Nova de Gaia and Espinho between January 2004 and December 2013.

Definition of population for analysis

The study population included cSCCs that were surgically removed at Centro Hospitalar Vila Nova de Gaia and Espinho.

Statistical methodology

Statistical procedures. Continuous variables were summarized by mean and standard deviation. Categoric data were summarized as the number and percentage of subjects in each category.

The comparison of groups was performed by using parametric tests, such as *t* tests for continuous variables and the chi-square test (or Fisher exact test) for categoric variables or the equivalent nonparametric test, as appropriate.

A logistic regression model was used to find associations between clinicopathologic features, *TERTp* status, and adverse outcome, controlling for possible confounding variables. In the regression models, all the variables significantly associated with the specified outcome in the univariate model were included in the multivariate analysis.

Kaplan-Meier survival curves were presented to investigate the effect of *TERTp* mutation and other variables in progression-free survival.

All statistical tests were performed under a 2-sided significance level of 5%. The significance level was adjusted by Bonferroni correction (with 0.05 divided by the number of comparisons performed for each dependent variable) when assessing *TERTp* mutations according to different clinicopathologic and molecular characteristics (results displayed in Supplemental Table I; available at http://www.jaad.org), as multiple comparisons were performed.

Supplemental Table I. Clinicopathologic and molecular associations with *TERT*p mutations in cSCC

	All lesions				In situ cSCC				Invasive cSCC			
Clinicopathologic feature	**Total**	***TERT*p WT**	***TERT*p Mut**	***P* value**	**Total**	***TERT*p WT**	***TERT*p Mut**	***P* value**	**Total**	***TERT*p WT**	***TERT*p Mut**	***P* value**
No. of cases, n (%)	152	104 (68.4%)	48 (31.6%)		31	25 (80.6%)	6 (19.4%)		121	79 (65.3%)	42 (34.7%)	
Age at diagnosis, mean ± SD	76.9 ± 11.7	77.2 ± 11.4	75.9 ± 12.6	.544	78.5 ± 7.0	78.6 ± 7.4	76.2 ± 6.9	.476	76.5 ±12.7	76.8 ± 12.5	75.9 ± 13.3	.726
Male	73.9 ± 11.6	73.1 ± 11.7	75.1 ± 11.8	.451	77.8 ± 6.8	78.7 ± 6.6	76.0 ± 7.7	.490	73.0 ± 12.3	71.8 ± 12.3	74.9 ± 12.5	.314
Female	80.8 ± 10.7	81.8 ± 9.3	77.7 ± 14.5	.189	78.4 ± 7.8	78.5 ± 8.1	77.0	.863	81.6 ± 11.6	83.3 ± 9.5	77.7 ± 15.0	.124
Sex, n (%)				.119				NA				.435
Male	87 (57.2)	55 (52.9)	32 (66.7)		15 (48.4)	10 (40.0)	5 (83.3)		72 (59.5)	45 (57.0)	27 (64.3)	
Female	65 (42.8)	49 (47.1)	16 (33.3)		16 (51.6)	15 (60.0)	1 (16.7)		49 (40.5)	34 (43.0)	15 (35.7)	
Sun exposure, n (%)				NA				NA				NA
Chronic	103 (67.8)	67 (64.4)	36 (75.0)		11 (35.5)	9 (36.0)	2 (33.3)		92 (76.0)	58 (73.4)	34 (81.0)	
Intermittent	43 (28.3)	32 (30.8)	11 (22.9)		18 (58.1)	14 (56.0)	4 (66.7)		25 (20.7)	18 (22.8)	7 (16.7)	
Undetermined	6 (3.9)	5 (4.8)	1 (2.1)		2 (6.5)	2 (8.0)	0		4 (3.3)	3 (3.8)	1 (2.4)	
Localization, n (%)				NA				NA				NA
Face	97 (63.8)	64 (61.5)	33 (68.8)		9 (29.0)	7 (28.0)	2 (33.3)		88 (72.7)	57 (72.2)	31 (73.8)	
Trunk	8 (5.3)	3 (2.9)	5 (10.4)		4 (12.9)	1 (4.0)	3 (50.0)		4 (3.3)	2 (2.5)	2 (4.8)	
Upper limb	21 (13.8)	14 (13.5)	7 (14.6)		6 (19.4)	6 (24.0)	0		15 (12.4)	8 (10.1)	7 (16.7)	
Lower limb	20 (13.2)	18 (17.3)	2 (4.2)		10 (32.3)	9 (36.0)	1 (16.7)		10 (8.3)	9 (11.4)	1 (2.4)	
Undetermined	6 (3.9)	5 (4.8)	1 (2.1)		2 (6.5)	2 (8.0)	0		4 (3.3)	3 (3.8)	1 (2.4)	
Localization, n (%)*				.506				NA				.942
Extrafacial	49 (33.6)	35 (35.4)	14 (29.8)		20 (69.0)	16 (69.6)	4 (66.7)		29 (24.8)	19 (25.0)	10 (24.4)	
Face	97 (66.4)	64 (64.6)	33 (70.2)		9 (31.0)	7 (30.4)	2 (33.3)		88 (75.2)	57 (75.0)	31 (75.6)	
Procedure, n (%)				.252				NA				.092
Biopsy	34 (22.4)	26 (25.0)	8 (16.7)		6 (19.4)	4 (16.0)	2 (33.3)		28 (23.1)	22 (27.8)	6 (14.3)	
Excision	118 (77.6)	78 (75.0)	40 (83.3)		25 (80.6)	21 (84.0)	4 (66.7)		93 (76.9)	57 (72.2)	36 (85.7)	
Maximum tumor size, cm, mean ± SD	1.9 ± 1.5	1.7 ± 1.2	2.2 ± 1.8	.149	1.9 ± 1.2	2.0 ± 1.2	1.8 ± 1.5	.765	1.9 ± 1.5	1.6 ± 1.2	2.3 ± 1.8	.068
Maximum tumor size, n (%)				.489				NA				.384
<2 cm	69 (45.4)	47 (66.2)	22 (59.5)		10 (32.3)	8 (53.3)	2 (50.0)		59 (48.8)	39 (69.6)	20 (60.6)	
≥2 cm	39 (25.7)	24 (33.8)	15 (40.5)		9 (29.0)	7 (46.7)	2 (50.0)		30 (24.8)	17 (30.4)	13 (39.4)	
Cannot be assessed	44 (28.9)				12 (38.7)				32 (26.4)			
Peripheral margins, mm, mean ± SD	2.3 ± 2.9	2.0 ± 2.5	2.9 ± 3.4	.125	1.6 ± 1.5	1.6 ± 1.5	1.5 ± 1.3	.900	2.5 ± 3.1	2.2 ± 2.8	3.0 ± 3.6	.191
Deep margins, mm, mean ± SD	2.6 ± 2.4	2.5 ± 2.3	2.7 ± 2.5	.627	3.0 ± 1.7	2.6 ± 1.5	4.8 ± 1.0	.017	2.5 ± 2.5	2.5 ± 2.5	2.5 ± 2.5	.963
Ulceration, n (%)				.173				NA				.389
No	53 (34.9)	32 (33.0)	21 (44.7)		14 (45.2)	9 (39.1)	5 (83.3)		39 (32.2)	23 (31.1)	16 (39.0)	
Yes	91 (59.9)	65 (67.0)	26 (55.3)		15 (48.4)	14 (60.9)	1 (16.7)		76 (62.8)	51 (68.9)	25 (61.0)	
Undetermined	8 (5.3)				2 (6.5)				6 (5.0)			

Continued

Supplemental Table I. Cont'd

Clinicopathologic feature	All lesions				In situ cSCC				Invasive cSCC			
	Total	*TERT*p WT	*TERT*p Mut	*P* value	Total	*TERT*p WT	*TERT*p Mut	*P* value	Total	*TERT*p WT	*TERT*p Mut	*P* value
Actinic keratosis, n (%)				.750				NA				.553
No	50 (32.9)	35 (36.1)	15 (33.3)		6 (19.4)	5 (20.0)	1 (16.7)		44 (36.4)	30 (41.7)	14 (35.9)	
Yes	92 (60.5)	62 (63.9)	30 (66.7)		25 (80.6)	20 (80.0)	5 (83.3)		67 (55.4)	42 (58.3)	25 (64.1)	
Undetermined	10 (6.6)								10 (8.3)			
Invasion, n (%)				.101								
Noninvasive	31 (20.4)	25 (24.0)	6 (12.5)									
Invasive	121 (79.6)	79 (76.0)	42 (87.5)									
Histologic type, n (%)												NA
Acantholytic									9 (7.4)	6 (7.6)	3 (7.1)	
Verrucous									2 (1.7)	2 (2.5)	0	
NOS									110 (90.9)	71 (89.9)	39 (92.9)	
Histologic grade, n (%)												.275
Well differentiated									43 (35.5)	31 (40.3)	12 (30.0)	
Moderately/poorly differentiated									74 (61.2)	46 (59.7)	28 (70.0)	
Cannot be assessed									4 (3.3)			
Histologic grade, n (%)												NA
Well/moderately differentiated									106 (87.6)	70 (90.9)	36 (90.0)	
Poorly differentiated									11 (9.1)	7 (9.1)	4 (10.0)	
Cannot be assessed									4 (3.3)			
Pattern of invasion, n (%)												.960
Expansive									66 (54.5)	43 (56.6)	23 (56.1)	
Infiltrative									51 (42.1)	33 (43.4)	18 (43.9)	
Cannot be assessed									4 (3.3)			
Level of invasion, n (%)												.667
Papillary dermis									36 (29.8)	23 (30.7)	13 (33.3)	
Reticular dermis									53 (43.8)	37 (49.3)	16 (41.0)	
Subcutaneous tissue									25 (20.7)	15 (20.0)	10 (25.6)	
Cannot be assessed									7 (5.8)			
Level of invasion, n (%)												.490
Dermis									89 (73.6)	60 (80.0)	29 (74.4)	
Subcutaneous tissue									25 (20.7)	15 (20.0)	10 (25.6)	
Cannot be assessed									7 (5.8)			
Maximum tumor thickness, n (%)									3.8 ± 3.1	3.4 ± 2.2	4.8 ± 4.1	.050
<6 mm									96 (79.3)	67 (88.2)	29 (74.4)	.059
≥6 mm									19 (15.7)	9 (11.8)	10 (25.6)	
Cannot be assessed									6 (5.0)			

Continued

Supplemental Table I. Cont'd

	All lesions				In situ cSCC				Invasive cSCC			
Clinicopathologic feature	**Total**	***TERT*p WT**	***TERT*p Mut**	***P* value**	**Total**	***TERT*p WT**	***TERT*p Mut**	***P* value**	**Total**	***TERT*p WT**	***TERT*p Mut**	***P* value**
Intratumoral infiltrate, n (%)												NA
Moderate-intense									13 (10.7)	8 (10.1)	5 (11.9)	
Few-absent									108 (89.3)	71 (89.9)	37 (88.1)	
Peritumoral infiltrate, n (%)												.816
Moderate-intense									68 (56.2)	45 (57.0)	23 (54.8)	
Few-absent									53 (43.8)	34 (43.0)	19 (45.2)	
Lymphovascular invasion, n (%)												NA
Not present									116 (95.9)	75 (94.9)	41 (97.6)	
Present									5 (4.1)	4 (5.1)	1 (2.4)	
Perineural invasion, n (%)												NA
Not present									118 (97.5)	78 (98.7)	40 (95.2)	
Present									3 (2.5)	1 (1.3)	2 (4.8)	
Recurrence, n (%)								NA				**<.001**
No					28 (90.3)	22 (88.0)	6 (100.0)		104 (86.0)	75 (94.9)	29 (69.0)	
Yes					3 (9.7)	3 (12.0)	0		17 (14.0)	4 (5.1)	13 (31.0)	
Metastasis, n (%)												NA
No					31 (100)				113 (93.4)	78 (98.7)	35 (83.3)	
Yes					0				8 (6.6)	1 (1.3)	7 (16.7)	
Progression-free survival, mo, mean ± SD	39.8 ± 29.3	38.3 ± 25.8	43.0 ± 35.8	.418	38.4 ± 22.2	34.7 ± 19.8	53.8 ± 26.7	.057	40.1 ± 30.9	39.4 ± 27.4	41.4 ± 36.9	.736
Follow-up, mo, mean ± SD	42.9 ± 28.8	39.7 ± 25.5	49.8 ± 34.1	.071	39.7 ± 22.0	36.3 ± 19.9	53.8 ± 26.7	.080	43.7 ± 30.3	40.8 ± 27.0	49.3 ± 35.3	.144

Values in bold are statistically significant with the following *P* value cutoffs after Bonferroni correction: *P* less than .003 for all lesions and in situ cSCC and *P* less thasn .002 for invasive cSCC. An unpaired Student *t* test was used to compare means in continuous variables; chi-square and Fisher exact tests were applied to evaluate a possible association between categoric variables. *cSCC*, Cutaneous squamous cell carcinoma; *Mut*, mutated; *NA*, not applicable (the chi-square or Fisher exact test is not applicable because more than 20% of the expected counts are less than 5); *SD*, standard deviation; *TERTp*, telomerase reverse transcriptase gene promoter; *WT*, wild type.

*Cases of undetermined location were excluded (n = 6).