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ARTICLE

Prognostic model for patient survival in primary anorectal mucosal melanoma: stage at presentation determines relevance of histopathologic features

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Abstract

Pathological staging of primary anorectal mucosal melanoma is often performed according to the American Joint Commission on Cancer (AJCC) guidelines for cutaneous melanoma, as an anorectal melanoma-specific staging system does not exist. However, it remains unknown whether prognostic factors derived for cutaneous melanoma also stratify risk in anorectal melanoma. We retrospectively determined correlations between clinicopathological parameters and disease-specific survival in 160 patients. Patients were grouped by clinical stage at presentation (localized disease, regional or distant metastases). Cox proportional hazards regression models determined associations with disease-specific survival. We also summarized the somatic mutations identified in a subset of tumors analyzed for hotspot mutations in cancer-associated gene panels. Most of the patients were white (82%) and female (61%). The median age was 62 years. With a median follow-up of 1.63 years, median disease-specific survival was 1.75 years, and 121 patients (76%) died of anorectal melanoma. Patients presenting with regional (34%) or distant metastases (24%) had significantly shorter disease-specific survival compared to those with disease localized to the anorectum (42%). Of the 71 anorectal melanoma tumors analyzed for hotspot genetic alterations, somatic mutations involving the *KIT* gene (24%) were most common followed by *NRAS* (19%). Increasing primary tumor thickness, lymphovascular invasion, and absence of regression also correlated with shorter disease-specific survival. Primary tumor parameters correlated with shorter disease-specific survival in patients presenting with localized disease (tumor thickness) or regional metastases (tumor thickness, absence of regression, and lymphovascular invasion), but not in patients presenting with distant metastases. Grouping of patients according to a schema based on modifications of the 8th edition AJCC cutaneous melanoma staging system stratified survival in anorectal melanoma. Our findings support stage-specific associations between primary tumor parameters and disease-specific survival in anorectal melanoma. Moreover, the AJCC cutaneous melanoma staging system and minor modifications of it predicted survival among anorectal melanoma patients.

Introduction

Primary anorectal melanoma accounts for ~1.5% of all melanomas and 16.5% of all mucosal melanomas [1, 2], and represents the second most common subtype of mucosal melanoma after sinonasal. In a SEER database study of anorectal melanoma patients in the United States from 1973–2011, the annual anorectal melanoma incidence was 0.343 cases per 1 million (0.259 in men and 0.407 in women) and has steadily increased over time [2, 3]. Anorectal melanoma is an aggressive melanoma subtype with 5-year overall survival rates estimated between 14% [4] to 30% [5, 6] and an estimated median overall survival of

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9–19 months [3, 7–9]. Among mucosal melanoma subtypes, anorectal melanoma exhibits the shortest interval to disease progression and the highest rate of metastasis [10].

Mucosal melanomas, including melanomas arising in the sinonasal, anorectal, and genitourinary mucosa, present unique clinical and pathological challenges distinct from cutaneous melanoma. Mucosal melanomas arise in non-keratinizing epithelia (squamous, columnar, or junctional) in anatomic locations less amenable to examination, commonly lack pigmentation and frequently simulate common benign processes (e.g., sinonasal polyps, hemorrhoids)—all of which compromise early detection [8, 9]. The higher density of lymphovascular structures in mucosal sites also provides greater access to, and thus, may increase the likelihood of systemic dissemination of tumor cells. Taken together, it is not surprising that mucosal melanoma patients more commonly present with regional and/or distant metastases (~35–50%) compared to only 11% of patients with cutaneous melanoma at diagnosis [11]. Surgical extirpation of mucosal melanoma can also be challenging because of the less distinctive microanatomic boundaries in mucosal sites compared to skin; and anatomic and functional constraints such as sphincter preservation impact approaches to local control. Mucosal melanomas also harbor unique molecular-genetic alterations reflecting their origin in anatomic sites protected from exposure to ultraviolet light [12–14]. Finally, mucosal melanomas may have lower response rates to immune checkpoint blockade therapy compared to cutaneous melanoma [3, 13, 15–17].

Identifying risk stratification systems that accurately reflect the unique biology of anorectal melanoma remains an important challenge. Clinical stage at presentation, as defined by Ballantyne [18], is one predictor of survival in anorectal melanoma, and in previous studies, patients with localized disease (i.e., restricted to the anorectum) had longer survival compared to those that presented with regional and/or distant metastases [2, 8, 10, 18–21]. However, the Ballantyne system lacks granularity, particularly regarding risk stratification among patients with early-stage disease. In addition, owing to the rarity of anorectal melanoma, few studies have assessed the association between discrete pathological variables of the primary tumor and clinical outcomes [22–24]. Further, most prior studies have grouped the various subtypes of mucosal melanoma (sinonasal, genitourinary, and anorectal) together, precluding assessment of prognostic variables that may be unique to a given subtype [10]. As such, these studies were neither designed nor sufficiently powered to determine the relative contribution of conventional prognostic factors specifically in anorectal melanoma or to consider these in the context of stage at presentation [10].

Taken together, there is a critical unmet need to delineate the clinicopathological parameters that accurately predict the biological behavior of anorectal melanoma—particularly among patients presenting with localized and regional metastatic disease. Delineation of patients at highest risk for disease progression could identify populations that might benefit from early therapeutic intervention, such as neoadjuvant therapy or entry into clinical trials. Conversely, identification of individuals at low risk for relapse could spare such patients toxicities associated with additional treatments, and support surveillance-based approaches. Thus, we retrospectively determined associations between clinicopathological parameters of primary tumor and metastatic disease and disease-specific survival in 160 patients with anorectal melanoma.

Materials and methods

Selection of cases

The study was approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center. The pathology archives were searched to identify patients with anorectal melanoma referred to MD Anderson at some point during their care, during the period from March 1986 through October 2012 ($n = 254$). This time period was selected in order to ensure adequate follow-up for surviving patients and to avoid the potential impact on survival due to novel (targeted/immuno-) therapies. Data collected from all patients were examined for completeness and patients were selected for inclusion in the final analyses based on the availability of both: (i) hematoxylin-eosin (H&E)-stained slides of primary tumors (for reevaluation of histologic parameters, particularly in older cases) and/or detailed pathology reports (issued by one of the coauthors) documenting histopathological parameters that included at least primary tumor thickness, ulceration status, and mitotic rate (as required for staging of cutaneous melanoma according to criteria in the 8th [25] and/or 7th [26] editions of the *American Joint Commission On Cancer Staging Manuals*), and (ii) carefully annotated demographic, clinical management, and follow-up data (minimum of 4 years for surviving patients). Overall, 160 patients met these criteria and were included in the final analyses. Some patients in the current study may have been included in prior reports from MD Anderson [5, 7, 27], but the overall results here reported are not duplicated.

Clinical, histopathological, and mutational data

For each patient, the following demographic/clinical data were collected: date and age at diagnosis; sex; ethnicity; anatomic location of primary tumor (rectum, located above the dentate line; anorectal junction, referring to the area of

anal sinuses, valves and papillae, [i.e., the level of the dentate line]; anal canal, referring to the smooth anoderm or squamous zone located below the dentate line; and anal verge, referring to the anocutaneous junction); details of regional metastases (date of diagnosis, clinically occult or clinically evident), anatomic site, type of lymph node(s) removed (sentinel or non-sentinel), number of all lymph nodes removed and number of lymph nodes involved by metastatic melanoma, size of metastatic deposit, micro-anatomic location of the metastatic focus within the lymph node, and presence of extranodal extension; treatment approach, including type(s) of surgery for primary anorectal melanoma and regional lymph nodes as well as non-surgical therapies (radiation and systemic); details of distant metastases (date of diagnosis, anatomic location, and lactate dehydrogenase level at diagnosis, when available); and date and cause of death, when applicable.

H&E-stained slides of the primary tumor were reviewed/evaluated for each case by at least one dermatopathologist (PN, MTT, VGP, JLC, DI, CAT, AHD, WLW, and AJL). Discrepancies with outside diagnoses were adjudicated in a consensus conference setting with the majority opinion prevailing. The following primary tumor histopathological parameters were recorded: histologic type, tumor thickness [28] measured from top of the granular layer of squamous epithelium/columnar epithelium of colonic mucosa, or base of the ulcer to the deepest point of invasion and was categorized according to American Joint Commission on Cancer (AJCC) 8th edition pathological T-category criteria (T1: ≤1.0 mm; T2: 1.1–2.0 mm; T3: 2.1–4.0 mm; T4: >4.0 mm); level of rectal wall invasion (lamina propria, submucosa, muscularis propria, or serosa and beyond); vertical growth phase [29]; radial growth phase; mitotic rate of the invasive component [30], determined using the hot spot approach (highest number of mitotic figures in the invasive component/mm^2) [31]; tumor-associated epithelial ulceration [32]; regression, defined as spontaneous partial or complete disappearance of a previously documented melanoma with associated dermal fibrosis, dilated vessels and a variable density of pigmented macrophages without evidence to suggest these changes were due to therapeutic interventions or external trauma [33]; lymphovascular invasion [34]; perineural invasion [35]; microscopic satellitosis [36]; tumor-infiltrating lymphocytes [37] (absent, non-brisk, or brisk); precursor melanocytic nevus [38]; predominant cytology (epithelioid, nevoid, spindled, or mixed); and 'final resection margin' status after completion of all local surgical procedures (negative, involved by in situ melanoma, or involved by invasive melanoma). As we could not consistently demonstrate an obvious histopathologic origin from either glandular or squamous mucosa, we relied on the grossly defined anatomic landmarks as defined above to designate anatomic location.

We used a modified Ballantyne staging system [18] to assign clinical stage at presentation, defined as the period up to 120 days from the date of diagnosis to include imaging studies and definitive surgical management. The patients were grouped as follows: group L (patients with *localized* disease confined to the anorectum), which included patients with clinically negative lymph nodes that were not evaluated pathologically (LcN0) and patients with pathologically-negative lymph nodes (LpN0); group R (patients with *regional* lymph node metastases), which included patients with clinically occult regional metastases (RpN+ with pathologically confirmed microscopic metastasis) and clinically evident regional metastases (RcN+ determined by clinical evaluation/imaging); and group D (patients with *distant* metastases).

Group L patients were classified according to four systems: (i) the AJCC 8th edition [25] for cutaneous melanoma: stage I versus stage II; (ii) AJCC 8th edition T-categories for cutaneous melanoma [39]; (iii) modified T-categories based on three groups of different tumor thickness, thin: ≤1.0 mm (T1); intermediate: >1.0 to 4.0 mm (T2/3) and thick: >4.0 mm (T4); and (iv) the tumor thickness-mitotic rate system, previously derived for stratification of primary vulvar melanomas [40] (Supplemental Table 1).

For mutational analyses, areas containing predominantly viable tumor tissue were identified on H&E-stained slides and the corresponding formalin-fixed paraffin-embedded (FFPE) tissue was manually macrodissected from mirror-image unstained slides for genomic DNA extraction, which was then analyzed for mutations by PCR-based DNA sequencing or PCR-based primer extension or pyrosequencing of select exons of specific genes (*KIT*, *NRAS* and/or *BRAF*) ($n = 53$) or PCR-based primer extension assay of 12 genes ($n = 9$) or next generation sequencing-based assay 46 genes (AmpliSeq Cancer Panel, Life Technologies; San Francisco, CA, USA) ($n = 9$) [41]. Genomic alterations, including pathogenic mutations and potential germline variants were identified and recorded.

Statistical analysis

The clinical and histopathological characteristics were summarized using descriptive statistics. Fisher's exact test was used to determine the associations between histopathological and clinical characteristics [42]. Median follow-up period was quantified used the Kaplan–Meier estimated potential follow-up method [43]. Overall survival was defined as the time interval from the date of diagnosis to the date of death due to any cause. Disease-specific survival was defined as the time interval from the date of diagnosis to the date of death directly related to the progression of anorectal melanoma. Patients who were free of

anorectal melanoma at the time of death were censored in the analysis of disease-specific survival. The survival curves for overall survival and disease-specific survival were estimated by the Kaplan–Meier method [44]. Log-rank tests were used to assess the significance of differences in disease-specific survival between groups [45]. Univariate Cox proportional hazards regression models were used to determine the association of clinicopathological characteristics with disease-specific survival or overall survival [46]. A multivariable Cox proportional hazards model for disease-specific survival was obtained by first including all covariates with p-value < 0.10 from univariate Cox models and then performing a stepwise selection using a threshold of 0.05 for the significance level of the Wald chi-square to identify covariates to be retained in the final model. The Bayesian Information Criterion was used to compare the performance of staging systems and the model with a lower value was preferred considering both model fitting and model size. No adjustments were made for the multiple comparisons. We used R version 3.1.1 (R Core Team 2014) to conduct all analyses.

Results

Demographic characteristics of primary anorectal melanoma

The clinicopathological characteristics of patients included in the analysis ($n = 160$) are summarized in Table 1. Overall, 61% were women and the majority (82%) were white. The median age at diagnosis was 62 years (range: 26–89 years). Tumor thickness was >4.0 mm in 118 (73.7%) patients, with a median of 6.9 mm; 141 (88.1%) tumors were ulcerated. Sixty-two tumors (38.8%) extended into the submucosa, 28 (17.5%) extended into the muscularis propria, and 18 (11.2%) extended into the serosa and beyond.

The initial diagnostic biopsy procedure was polypectomy or hemorrhoidectomy in the majority of the patients (Supplemental Fig. 1). Sixty-six of 126 patients that underwent surgical management of anorectal melanoma had their surgeries performed at MD Anderson; wide excision and regional lymph node dissection were most frequent. Ninety patients that had achieved negative margins after planned excisional surgery (Table 1), of which seven developed local recurrence. Final margins of 15 and 50 patients were positive for melanoma in situ and invasive melanoma, respectively. Adjuvant radiation therapy was administered for disease control to the primary site only (45 patients) or to the primary and regional nodal basin (23 patients) (Supplemental Fig. 2). A variety of approaches to systemic therapy were employed in the neoadjuvant and adjuvant settings (Supplemental Fig. 2).

Table 1 Clinicopathological parameters and outcome of primary anorectal melanoma patients included in retrospective analysis

Clinicopathological parameter/outcome	Retrospective analysis cohort $n = 160$	
Median age at diagnosis (range)	62 (26–89) years	
	n	%
Sex		
Female	98	61
Male	62	39
Unknown	–	–
Ethnicity		
African American	6	4
Asian	7	4
White	131	82
Hispanic	13	8
Unknown	3	2
Anatomic site		
Anorectal junction	45	28
Rectum	51	32
Anal canal	59	37
Anal verge	4	2
Unknown	1	1
Clinical stage at presentation		
Localized disease	67	42
Regional metastases	55	34
Distant metastases	38	24
Histological type		
Unclassified	42	26
Acral lentiginous	65	41
Nodular	48	30
Superficial spreading	5	3
Tumor thickness, mm		
≤1.00	6	4
1.01–2.00	14	9
2.01–4.00	22	14
>4.00	118	73
Level of rectal wall invasion		
Lamina propria	6	4
Submucosa	62	39
Muscularis propria	28	17
Serosa and beyond	18	11
Unknown	46	29
Vertical growth phase		
Not identified	4	3
Present	156	97
Unknown	–	–
Radial growth phase		
Not identified	50	31
Present	70	44
Unknown	40	25
Mitotic rate of invasive component, mitotic figures/mm^2		
0	5	3
1	6	4
2–10	70	44
>10	79	49
Ulceration		
Not identified	19	12
Present	141	88

Table 1 (continued)

Clinicopathological parameter/outcome	Retrospective analysis cohort $n = 160$	
Median age at diagnosis (range)	62 (26–89) years	
	n	%
Regression		
Not identified	140	88
Present	18	11
Unknown	2	1
Lymphovascular invasion		
Not identified	86	54
Present	74	46
Perineural invasion		
Not identified	137	86
Present	23	14
Microscopic satellitosis		
Not identified	123	77
Present	34	21
Unknown	3	2
Tumor-infiltrating lymphocytes		
Absent or non-brisk	155	97
Brisk	3	2
Unknown	2	1
Precursor nevus		
Not identified	157	98
Present	2	1
Unknown	1	1
Predominant cytology		
Epithelioid	101	63
Spindled	13	8
Nevoid	1	1
Mixed	45	28
Unknown		
Final resection margin status		
Negative	90	56
Positive for in situ melanoma	15	10
Positive for invasive melanoma	50	31
Unknown	5	3
Outcome		
Dead	131	82
Alive	18	11
Lost to follow-up	11	7
Cause of death		
Anorectal melanoma	121	76
Other	4	2
Unknown or alive	35	22

Overall, 131 of 160 patients had died during follow-up, most ($n = 121$, 92.4%) due to progression of anorectal melanoma; 11 were lost to follow-up. The median follow-up of the 8 patients that were alive at their last encounter was 5.0 years (range: 0.4–20.8 years). The median overall survival was 1.8 years, and median disease-specific survival was 1.8 years (95% CI: 1.6–2.2 years) (Supplemental Fig. 4). The 5-year disease-specific survival was 22% (95% CI: 16–30%). Most patients in our cohort presented with

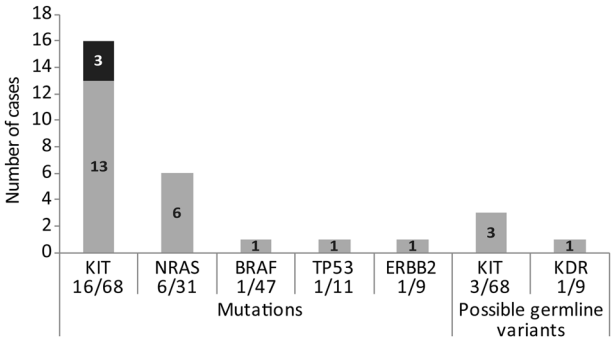


Fig. 1 Genomic mutations in anorectal melanoma. **a**, Prevalence of hotspot mutations in 71 cases of anorectal melanoma. Fractions indicate number of cases in which mutations were detected (numerator) compared to the number of cases evaluated (denominator) for each gene. Among tumors with *KIT* mutations, three cases had point mutations involving two different codons (black), while 13 had mutations involving only one (gray)

metastatic disease at diagnosis (Table 1), including 34% with regional nodal metastases and 24% with distant metastases (irrespective of regional disease); 42% had disease confined to the anorectum.

Seventy-one anorectal melanoma tumors (54 primaries, 14 regional metastases, 2 distant metastases, 1 unknown source) from patients in this cohort were evaluated for frequently reported (hotspot) mutations (Fig. 1, Table 2 and Supplemental Fig. 3). Among the genes evaluated, the frequency of mutations was 24% *KIT* (16 of 68 evaluated tumors, and the L576P point mutation was most common; $n = 5$), 19% *NRAS* (6 of 31; most commonly affecting codons Q61 and G12; $n = 2$ each), 2% *BRAF* (1 of 47; an activating K601E mutation).

Associations between clinicopathological parameters and disease-specific survival

Clinical stage at presentation (Fig. 2a) significantly correlated with disease-specific survival ($p < 0.001$) on univariate analysis (Table 3, Fig. 2b). Patients presenting with regional metastases (median 1.81 years, hazard ratio [HR] = 1.52; $p = 0.05$) or distant metastases (median 1.25 years, HR = 3.24; $p < 0.001$) had shorter disease-specific survival compared to patients presenting with localized disease (median 2.39 years). Five-year disease-specific survival rates were 32%, 18%, and 6% for patients presenting with localized, regional, and distant disease, respectively (Supplemental Fig. 4). In addition, the following primary tumor parameters correlated with shorter disease-specific survival on univariate analysis among all patients: tumor thickness, either categorized by cut-offs in the 8th edition *AJCC Staging Manual* for cutaneous melanoma ($p = 0.01$) or when considered as a continuous variable (HR = 1.03; $p < 0.001$) (Fig. 3a); lymphovascular invasion (HR = 2.19; $p < 0.001$;

Table 2 Summary of molecular genetic findings in anorectal melanoma

Tissue tested	Gene	Incidence	Type of mutation	Exon	Aminoacid change	Synchronous mutations in other genes
P	*KIT*	3	Missense	11	V560D	–
P	*KIT*	4	Missense	11	L576P	–
R	*KIT*	1	Missense	11	L576P	*KDR* C482R[a]
P	*KIT*	1	Ins30bp	11	–	–
P	*KIT*	1	Missense	13	K642E M541L[a]	
P	*KIT*	1	Missense	13	V654A	–
P[b]	*KIT*	1	Missense	13	K642Q N822Y	–
D[b]	*KIT*	1	Missense	11	K558N D579Y	–
P[b]	*KIT*	1	Missense	17	N822H V824M	–
P	*KIT*	1	Missense	17	D816H	–
P	*KIT*	1	Missense	17	N822I	–
P	*NRAS*	1	Missense	2	G12C	–
P	*NRAS*	2	Missense	2	G12S	–
P	*NRAS*	1	Missense	2	G13D	–
P	*NRAS*	1	Missense	3	Q61K	–
P	*NRAS*	1	Missense	3	Q61L	–
P	*BRAF*	1	Missense	15	K601E	–
R	*TP53*	1	Missense +del10bp	5	R196P	–
P	*ERBB2*	1	Missense	21	V842I	*KIT* M541L[a]
P	*KIT*	1	Missense	11	M541L[a]	

P primary, *R* regional metastasis, *D* distant metastasis

[a]Possible germline variant

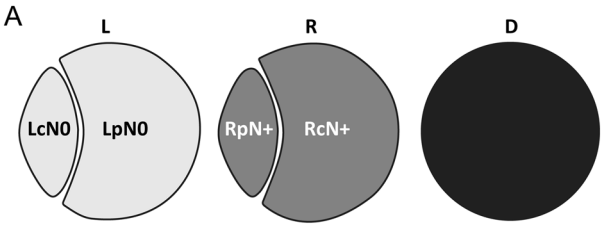
[b]Cases harboring point mutations involving two different codons of *KIT* gene

Fig. 3b); perineural invasioin (HR = 1.68; $p = 0.04$; Fig. 3c); and final surgical margins positive for invasive melanoma (HR = 2.4; $p < 0.001$; Fig. 3d) (Table 2). The presence of primary tumor regression correlated with longer disease-specific survival (HR = 0.37; $p = 0.01$; Fig. 3e). Ulceration, mitotic rate, and level of rectal wall invasion did not correlate with disease-specific survival (Fig. 3f–h). Multivariable models (Table 3) were constructed considering only factors that were significant on univariate analysis. These showed that the presence of distant metastases (HR = 2.71; $p < 0.001$), increased tumor thickness (as a continuous variable) (HR = 1.02; $p = 0.004$), and lymphovascular invasion (HR = 1.83; $p = 0.003$) independently correlated with shorter disease-specific survival, while primary tumor regression independently correlated with longer disease-specific survival (HR = 0.28; $p = 0.001$).

Stage-specific associations between clinicopathological parameters and disease-specific survival

Because clinical stage was among the most robust predictors of disease-specific survival in our cohort (Fig. 2, Table 3), we next considered factors associated with disease-specific survival among patients grouped separately according to their clinical stage at presentation (Fig. 2a), to determine stage-specific associations between histopathological parameters and disease-specific survival (Table 4). This analysis also incorporated measurements of disease burden among patients with metastases, including the number of regional lymph nodes involved by melanoma; clinical detectability (by imaging studies or pathological examination) for patients with regional metastasis; and serum lactate dehydrogenase levels and the location/number of anatomic sites involved for patients with distant metastasis. Of the primary tumor pathological parameters evaluated, tumor thickness, lymphovascular invasion and regression correlated with disease-specific survival among L and among R patients (Table 4). Parameters of regional (number of nodes involved and whether these were clinically evident or occult) and distant (lactate dehydrogenase levels, site and number of organs involved) metastatic disease burden did not significantly correlate with disease-specific survival among group R and D patients, respectively.

Multivariable models (Table 5) were constructed for each stage group considering only factors that were significant on univariate analysis. Among group L patients, only tumor



Retrospective analysis cohort n=160	
L: Disease localized to primary site n=67 (42%)	**LcN0:** Localized disease; regional lymph nodes not sampled n=27 (17%)
	LpN0: Localized disease; regional lymph nodes negative by pathologic evaluation n=40 (25%)
R: Disease involving regional lymph node(s) n=55 (34%)	**RpN+:** Clinical occult regional disease, i.e., imaging negative, but pathologically confirmed micrometastasis n=18 (11%)
	RcN+: Clinically evident regional lymph node metastasis, i.e., imaging positive macrometastasis n=37 (23%)
D: Distant metastasis n=38 (24%)	

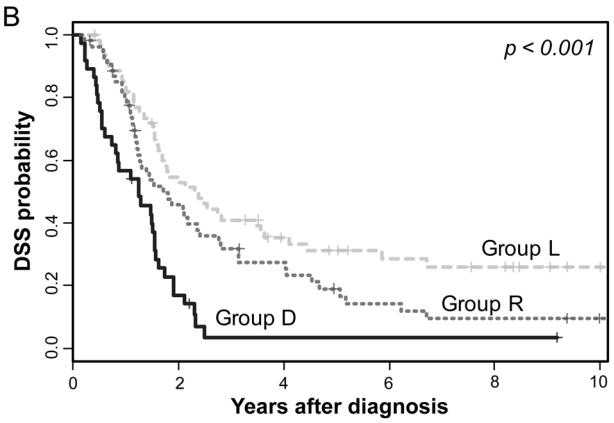


Fig. 2 Stratification of anorectal melanoma patients according to clinical stage at presentation. **a** Classification of patients using a modified Ballantyne clinical staging system. The patients were classified initially according to anatomic sites of involvement as having localized disease, regional metastases, or distant metastases. Patients with localized disease and regional disease were further subdivided on the basis of nodal status. **b** Kaplan–Meier estimates of disease-specific survival (DSS) based on clinical stage at presentation

thickness as a continuous variable (HR = 1.08; $p = 0.001$) independently correlated with shorter disease-specific survival in the multivariable model. In the LcN0 subgroup, tumor thickness as a continuous variable (HR = 1.14; $p = 0.03$) and age ≥62 years (HR = 4.35; $p = 0.02$) independently correlated with shorter disease-specific survival. In the LpN0 subgroup, only lymphovascular invasion (HR = 2.97; $p = 0.006$) independently correlated with shorter disease-specific survival. Among group R patients, tumor thickness as a continuous variable (HR = 1.04; $p = 0.001$) and lymphovascular invasion (HR = 2.23; $p = 0.02$) independently correlated with shorter disease-specific survival, while presence of regression (HR = 0.16; $p = 0.004$) independently correlated with longer disease-specific survival in the multivariable model. In the RpN+subgroup, tumor thickness as a continuous variable (HR = 1.16; $p = 0.01$) and lymphovascular invasion (HR = 6.4; $p = 0.008$) independently correlated with shorter disease-specific survival. In the RcN+subgroup, ulceration independently correlated with longer disease-specific survival (HR = 0.21; $p = 0.02$), while perineural invasion correlated with shorter disease-specific survival (HR = 2.98; $p = 0.05$). Among patients who did not present with distant metastases, (groups L + R; Fig. 2b), tumor thickness as a continuous variable (HR = 1.04; $p < 0.001$) and lymphovascular invasion (HR = 1.85; $p = 0.03$) independently correlated with shorter disease-specific survival, whereas primary tumor regression (HR = 0.12; $p = 0.005$) independently correlated with longer disease-specific survival.

Risk stratification of patients with localized anorectal melanoma

Whereas metastatic disease at presentation strongly correlated with shorter disease-specific survival, with few other features of the primary tumor impacting disease-specific survival in this setting, patients presenting with localized anorectal melanoma showed significant variability in their disease-specific survival. Therefore, we performed additional focused analyses on group L patients (Fig. 2) to compare candidate risk models. First, we applied the 8th edition cutaneous melanoma AJCC staging system (and modifications thereof) by grouping these patients according to (i) a two-group system: stage I (T1a through T2a) versus stage II (T2b through T4b); (ii) eight individual T-sub-categories; and (iii) a *modified* cutaneous melanoma T-category criteria to map to 3 tumor thickness groups: 'thin' (T1), 'intermediate' (T2/3) and 'thick' (T4) (Supplemental Table 1). Grouping of patients according to AJCC cutaneous melanoma stage I/II delineated two groups of patients with a trend to distinct disease-specific survival, but this did not achieve statistical significance ($p = 0.1$) (Fig. 4a, Table 6). Although there were too few events within some of the individual T-sub-categories to assess disease-specific survival, overall survival could be stratified according to the 8T-sub-categories (Fig. 4b, Table 6), albeit with significant overlap of the Kaplan–Meier-curves ($p = 0.14$). In contrast, the 3-category grouping based on *modified tumor thickness* ranges significantly differentiated disease-specific survival among anorectal melanoma patients with localized disease ($p = 0.03$) (Fig. 4c, Table 6).

We recently reported a 2-tier prognostic model (tumor thickness-mitotic rate system, Supplemental Table 1) [40] that accurately risk-stratified patients with primary vulvar melanoma according to primary tumor thickness and mitotic

Table 3 Univariate and multivariable Cox proportional hazards model for disease-specific survival

Clinical or histopathologic parameter ($n = 160$)	n	Univariate analysis		Multivariable analysis	
		HR (95% CI)	p	HR (95% CI)	p
Median age at diagnosis, years					
<62	80	–	–	–	–
≥62	80	1.20 (0.84, 1.72)	0.31	–	–
Sex					
Female	98	–	–	–	–
Male	62	0.76 (0.53, 1.10)	0.15	–	–
Anatomic site			0.15		
Anorectal junction	45	–	–	–	–
Rectum	51	0.74 (0.47, 1.15)	0.18	–	–
Anal canal	59	0.71 (0.46, 1.11)	0.13	–	–
Anal verge	4	0.18 (0.03, 1.34)	0.10	–	–
Clinical stage at presentation			**<0.001**		**<0.001**
Localized disease (Group L)	67	–	–	–	–
Regional metastases (Group R)	55	1.52 (0.99, 2.31)	**0.05**	1.38 (0.90, 2.13)	0.14
Distant metastases (Group D)	38	3.24 (2.03, 5.18)	**<0.001**	2.71 (1.66, 4.41)	**<0.001**
Histological type			**0.002**		
Unclassified	42	–	–	–	–
Acral lentiginous	65	0.47 (0.30, 0.73)	0.001	–	–
Nodular	48	0.58 (0.37, 0.92)	0.02	–	–
Superficial spreading	5	0.19 (0.05, 0.79)	0.02	–	–
Tumor thickness, mm					
As a categorical variable			**0.01**	–	**–**
≤1.0	6	–	–	–	–
1.0–2.0	14	4.36 (0.55, 34.40)	0.16	–	–
2.0–4.0	22	3.26 (0.43, 24.58)	0.25	–	–
>4.0	118	6.46 (0.90, 46.41)	0.06	–	–
As a continuous variable	160	1.03 (1.02, 1.05)	**<0.001**	1.02 (1.00, 1.05)	**0.004**
Level of rectal wall invasion			0.24		
Lamina propria	6	–	–	–	–
Submucosa	62	0.47 (0.20, 1.12)	0.09	–	–
Muscularis propria	28	0.68 (0.28, 1.67)	0.40	–	–
Serosa and beyond	18	0.59 (0.23, 1.53)	0.28	–	–
Vertical growth phase					
Not identified	4	–	–	–	–
Present	156	2.09 (0.29, 15.00)	0.46	–	–
Mitotic rate of invasive component, per mm^2 figures/mm^2			0.15		
0	5	–	–	–	–
1	6	4.84 (0.54, 43.37)	0.16	–	–
2–10	70	5.99 (0.83, 43.35)	0.08	–	–
>10	79	6.87 (0.95, 49.62)	0.06	–	–
Ulceration					
Not identified	19	–	–	–	–
Present	141	1.47 (0.79, 2.73)	0.23	–	–
Regression					
Not identified	140	–	–	–	–

Table 3 (continued)

Clinical or histopathologic parameter ($n = 160$)	n	Univariate analysis		Multivariable analysis	
		HR (95% CI)	p	HR (95% CI)	p
Present	18	0.37 (0.17, 0.79)	**0.01**	0.28 (0.13, 0.61)	**0.001**
Lymphovascular invasion					
Not identified	86	–	–	–	–
Present	74	2.19 (1.52, 3.17)	**<0.001**	1.83 (1.23, 2.72)	**0.003**
Perineural invasion					
Not identified	137	–	–	–	–
Present	23	1.68 (1.03, 2.72)	**0.04**	–	–
Microscopic satellitosis					
Not identified	123	–	–	–	–
Present	34	1.34 (0.87, 2.06)	0.18	–	–
Precursor nevus					
Not identified	157	–	–	–	–
Present	2	0.34 (0.05, 2.40)	0.28	–	–
Final resection margin status			**<0.001**		–
Negative	90	–	–	–	–
Positive for in situ melanoma	15	0.95 (0.51, 1.76)	0.86	–	–
Positive for invasive melanoma	50	2.40 (1.62, 3.55)	**<0.001**	–	–

Statistically significant p-values are bolded

rate. To determine if this system also stratified risk among other mucosal melanoma subtypes, we categorized the group L anorectal melanoma patients according to the tumor thickness-mitotic rate system (Supplemental Table 1), and observed a trend towards statistical significance ($p = 0.1$) for overall survival (Fig. 4d, Table 6).

Since risk stratification based predominantly on tumor thickness, either as a 3-group approach or a 2-group AJCC cutaneous melanoma-based staging system approach, appeared to stratify disease-specific survival among group L patients, we evaluated their performance on the whole cohort of 160 anorectal melanoma patients in the context of the overall AJCC 8th edition cutaneous melanoma staging system (i.e., assessed together with stage III and IV anorectal melanoma patients; Fig. 5a, b). Both systems significantly stratified disease-specific survival when considering all anorectal melanoma patients together, with comparable Bayesian Information Criterion values for each (Table 7).

Discussion

Our study represents the largest single-institution retrospective study of anorectal melanoma patients and encapsulates the disease course typical in the pre-targeted/immunotherapy era. Mutational analyses in a subset of our patient cohort confirmed a high incidence of *KIT* mutations in anorectal melanoma (16 of 68 patients)—a frequency similar to prior reports [13, 14], supporting that our patient cohort appropriately reflects the biological spectrum of anorectal melanoma in the population. Our study is the first to describe stage-specific clinicopathological prognostic parameters in this disease. The 5-year disease-specific survival was 22% (95% CI: 16–30%) in our cohort, which is similar to or slightly higher than previously reported 5-year disease-specific survival [47]. Clinical stage at presentation (defined as localized disease, regional metastases, or distant metastases) [18] robustly correlated with disease-specific survival. In addition, tumor thickness, lymphovascular invasion, and regression correlated with disease-specific survival in the complete cohort. Interestingly, patients whose final resection margins were positive for melanoma in situ had disease-specific survival similar to those with negative margins (Fig. 3d). This could be due to a number of different factors. First, 13/15 (87%) patients with margins positive for melanoma in situ eventually developed metastasis, which would ultimately determine patient outcome. In addition, since immunohistochemical studies were not routinely used in the assessment of margin status, margins with more subtle involvement may have been under-called. Additional significant associations between disease-specific survival and discrete histopathological parameters were identified in a stage-specific fashion.

Among patients with disease localized to the anorectum (group L), greater tumor thickness correlated with shorter

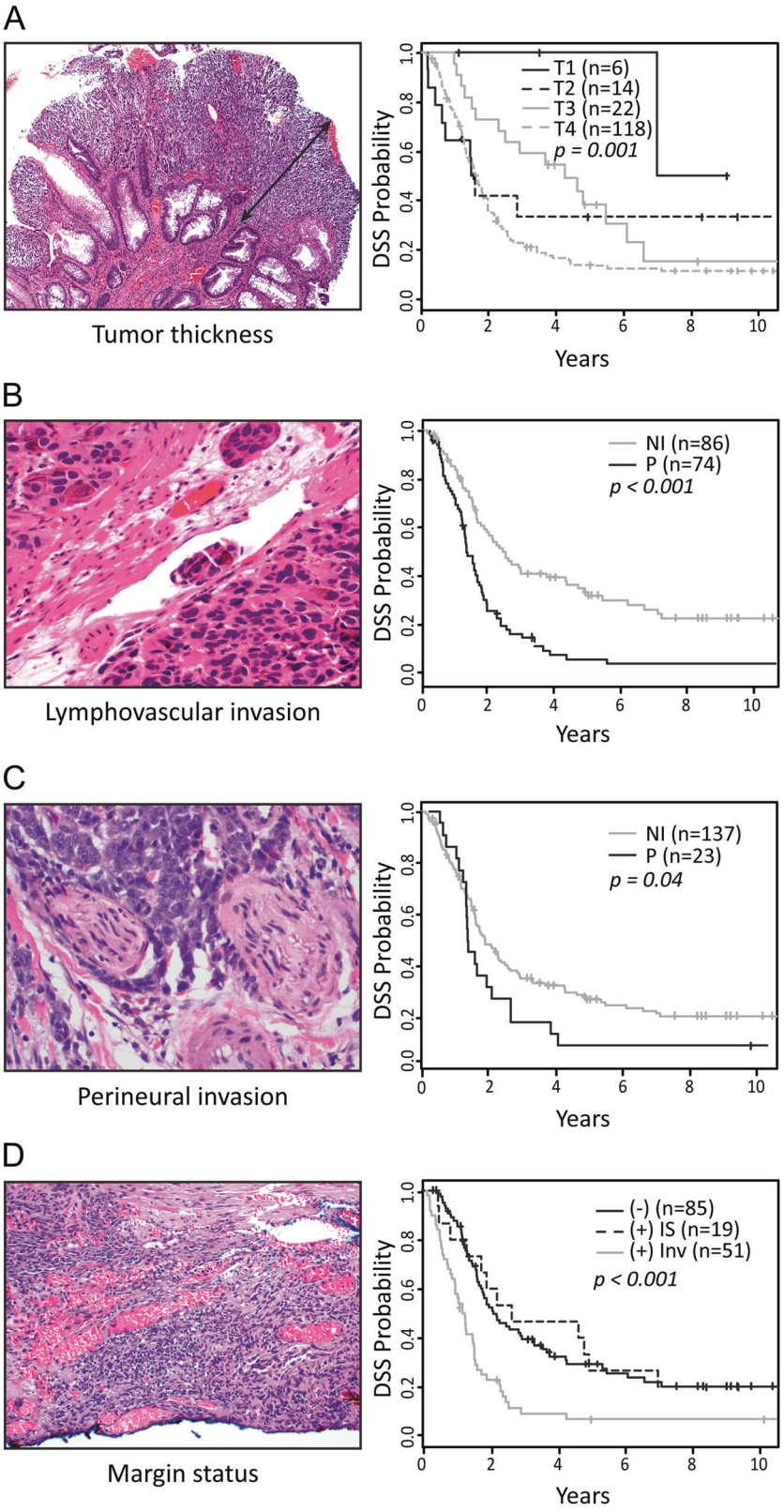
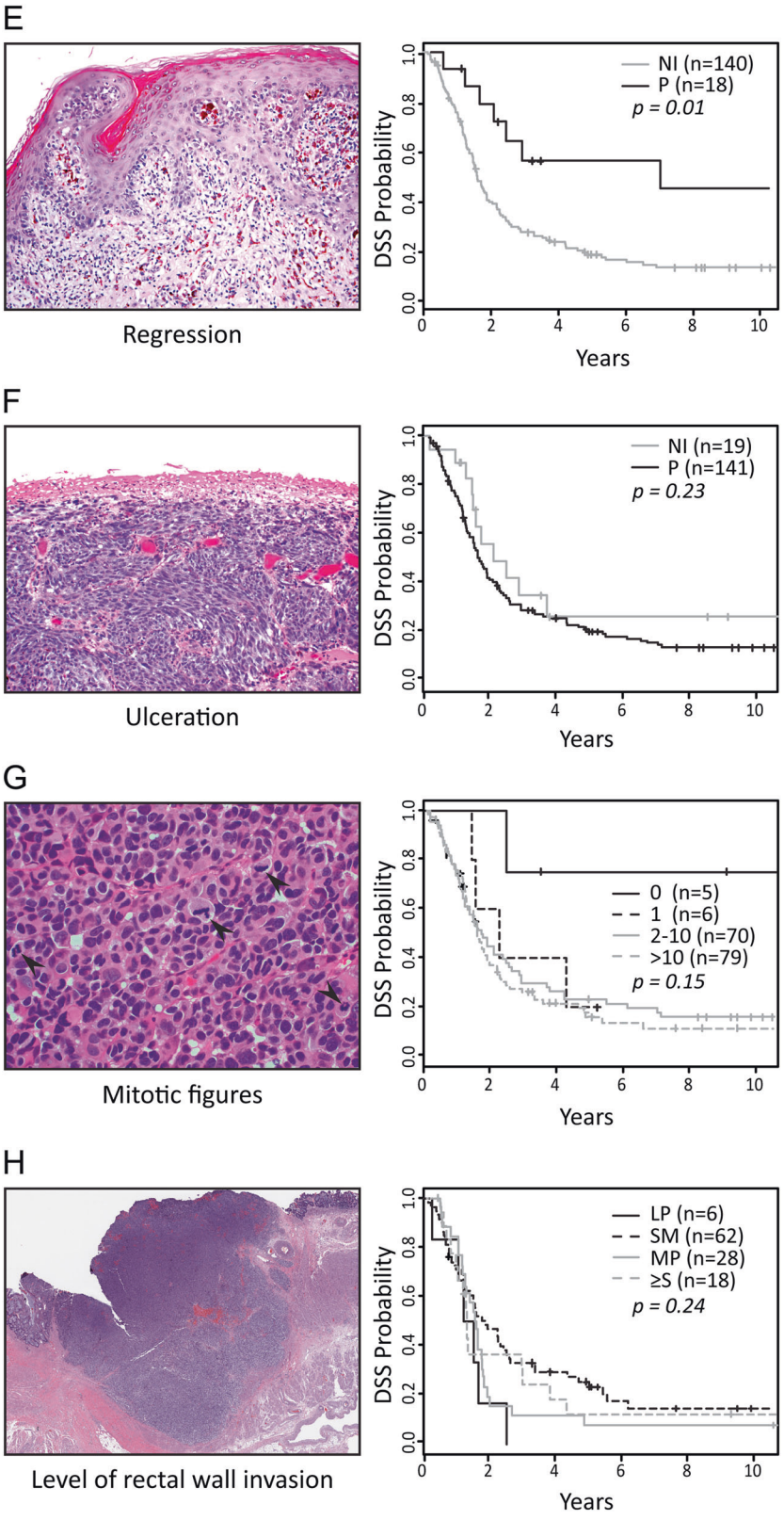


Fig. 3 Disease-specific survival (DSS) in patients with anorectal melanoma stratified according to primary tumor histopathologic parameters. Each pair of images consists of a representative micrograph of a histopathologic feature of anorectal melanoma (left) and the respective Kaplan–Meier disease-specific survival estimates (right). **a** Tumor thickness, measured according to American Joint Commission on Cancer (AJCC) 8th edition cut-offs for cutaneous melanoma: T1 ≤ 1.0 mm, T2 > 1.0–2.0 mm, T3 > 2.0–4.0 mm, T4 > 4.0 mm (arrow- tumor thickness measured perpendicular to the surface along the thickest aspect of anorectal melanoma involving colonic mucosa; H&E, 20×). **b** Lymphovascular invasion (H&E, 400×). **c** Perineural invasion of nerve fibers (H&E, 400×). **d** Margin status, (−): negative, (+) IS: positive for in situ (IS), (+) Inv: positive for invasive melanoma; pictured: involvement of resection margin by invasive melanoma (H&E, 200×). **e** Histologic regression (H&E, 200×). **f** Tumor-associated ulceration, characterized by absence of epithelium overlying melanoma and associated fibrinous exudate on the mucosal surface (H&E, 200×). **g** Mitotic figures/mm^2 (×4, arrows) within the invasive component (H&E, 400×). **h** Level of rectal wall invasion by melanoma, LP lamina propria, SM submucosa, MP muscularis propria and ≥S serosa and beyond; pictured: MP invasion (H&E, 20×). NI not identified, P present

disease-specific survival. However, correlations observed between tumor thickness within the discrete subgroups were inconsistent. For example, tumor thickness independently correlated with disease-specific survival among LcN0 patients, but not among LpN0 patients. This discrepancy might be at least partially attributed to the overall small

Fig. 3 (Continued)



number of patients in our cohort. Moreover, it is possible that presence of lymphovascular invasion in LpN0 patients may be a more prognostically relevant variable than tumor thickness, which is also supported by the higher hazard ratio in univariate analysis (2.97 higher risk for disease-specific survival when lymphovascular invasion was present

Table 4 Clinical stage specific univariate Cox proportional hazards model of clinicopathological parameters for disease-specific survival from the date of diagnosis

	Group							
Clinico-pathologic parameter	L ($n = 67$) p-value, HR 95% CI	LcN- ($n = 27$) p-value, HR 95% CI	LpN- ($n = 40$) p-value, HR 95% CI	R ($n = 55$) p-value, HR 95% CI	RpN+ ($n = 18$) p-value, HR 95% CI	RcN+ ($n = 37$) p-value, HR 95% CI	L+R ($n = 122$) p-value, HR 95% CI	D ($n = 38$) p-value, HR 95% CI
Age ≥ 62 y	0.56, 1.2 (0.65, 2.20)	**0.01**[a], 4.44 (1.38, 14.30)	0.1, 0.53 (0.25, 1.12)	0.41, 1.28 (0.71, 2.32)	0.88, 0.92 (0.34, 2.49)	0.32, 1.46 (0.69, 3.07)	0.34, 1.23 (0.80, 1.87)	0.20, 1.58 (0.79, 3.18)
TT	**0.001**[a], 1.08 (1.03, 1.13)	**0.02**[a], 1.12 (1.02, 1.23)	**0.03**[a], 1.06 (1.01, 1.12)	**0.004**[a], 1.04 (1.01, 1.06)	**0.03**[a], 1.11 (1.01, 1.23)	0.46, 1.02 (0.97, 1.06)	**<0.001**[a], 1.04 (1.03, 1.06)	0.89, 0.99 (0.92, 1.07)
LEV	0.8, 0.87 (0.23, 3.31)	NP	0.75, 0.79 (0.18, 3.47)	0.49, 0.48 (0.06, 3.91)	0.94, 0.95 (0.23, 3.90)	0.67, 0.62 (0.07, 5.35)	0.60, 0.76 (0.27, 2.14)	**0.02**[a], 0.02 (0.00, 0.49)
MIT	NP	NP	NP	0.98, 1.03 (0.14,7.67)	0.56, 1.90 (0.22,15.99)	0.48, 0.47 (0.06,3.69)	0.09, 5.53 (0.76,40.24)	0.38, 2.47 (0.32,18.85)
ULC	0.16, 2.09 (0.75, 5.86)	NP	0.21, 1.99 (0.69, 5.73)	0.15, 0.46 (0.16, 1.32)	0.86, 0.83 (0.10, 6.61)	**0.05**[a], 0.27 (0.08, 0.97)	0.32, 1.44 (0.70, 2.99)	0.55, 1.44 (0.44, 4.77)
−REG	0.19, 0.39 (0.09, 1.62)	0.55, 0.54 (0.07, 4.11)	0.27, 0.32 (0.04, 2.37)	**0.04**[a], 0.28 (0.09, 0.94)	0.31, 0.34 (0.04, 2.73)	0.09, 0.28 (0.07, 1.21)	**0.03**[a], 0.36 (0.15, 0.90)	**0.05**[a], 0.24 (0.05, 1.01)
LVI	**0.02**[a], 2.09 (1.12, 3.88)	0.65, 1.29 (0.43, 3.87)	**0.006**[a], 2.97 (1.36, 6.48)	**0.02**[a], 2.14 (1.16, 3.93)	**0.02**[a], 3.64 (1.25, 10.63)	0.27, 1.53 (0.73, 3.25)	**<0.001**[a], 2.23 (1.45, 3.43)	0.54, 1.25 (0.61, 2.55)
PNI	0.20, 1.69 (0.75, 3.81)	0.89, 0.86 (0.11, 6.60)	0.1, 2.19 (0.87, 5.49)	**0.01**[a], 3.01 (1.31, 6.95)	**0.03**[a], 6.01 (1.19, 30.31)	0.09[a], 2.42 (0.86, 6.77)	**0.01**[a], 2.05 (1.17, 3.59	0.059, 0.77 (0.29, 2.01)
SAT	0.64, 1.22 (0.54, 2.76)	0.98, 1.02 (0.22, 4.61)	0.60, 1.30 (0.49, 3.45)	0.48, 1.29 (0.64, 2.61)	0.20, 2.38 (0.62, 9.10)	0.82, 1.10 (0.48, 2.50)	0.31, 1.32 (0.78, 2.25)	0.74, 0.88 (0.42, 1.87)
+MAR	0.13, 1.79 (0.84, 3.83)	0.26, 1.93 (0.61, 6.13)	0.19, 2.06 (0.70, 6.12)	**0.01**[a], 2.35 (1.21, 4.58)	0.07, 3.28 (0.91, 11.85)	**0.05**[a], 2.26 (1.02, 5.01)	**0.004**[a], 2.12 (1.29, 3.48)	0.42, 1.45 (0.59, 3.60)
+RLN	NP	NP	NP	NP	NP	NP	NP	0.07[a], 3.90 (0.89, 17.11)
# of+RLN 1 Vs. >1	NP	NP	NP	0.69, 0.88 (0.46, 1.66)	0.81, 1.13 (0.41, 3.09)	0.55, 0.77 (0.32, 1.84)	NP	0.46, 0.63 (0.19, 2.10)
# of+RLN 1 Vs. 2-3	NP	NP	NP	0.39, 0.71 (0.33, 1.54)	0.93, 1.05 (0.34, 3.31)	0.25, 0.54 (0.18, 1.56)	NP	0.36, 0.52 (0.13, 2.10)
# of+RLN 1 Vs. >3	NP	NP	NP	0.68, 1.17 (0.56, 2.42)	0.72, 1.28 (0.33, 4.98)	0.98, 1.01 (0.39, 2.59)	NP	0.95, 0.95 (0.18, 4.93)
Macrometastasis	NP	NP	NP	1.00, 1.00 (0.54, 1.85)	NP	NP	NP	0.42, 1.82 (0.42, 8.29)
eLDH	NP	NP	NP	NP	NP	NP	NP	0.65, 1.24 (0.54, 2.71)
M1a vs. M1b	NP	NP	NP	NP	NP	NP	NP	–
≤M1b vs. M1c	NP	NP	NP	NP	NP	NP	NP	0.10, 3.37 (0.78,14.65)
≤M1c vs. M1d	NP	NP	NP	NP	NP	NP	NP	0.13, 3.28 (0.71,15.14)
# of M-sites 1 Vs. 2	NP	NP	NP	NP	NP	NP	NP	0.10, 2.69 (0.84, 8.61)
# of M-sites 1 Vs. 3	NP	NP	NP	NP	NP	NP	NP	0.08, 2.89 (0.90, 9.31)
# of M-sites 1 Vs. 4	NP	NP	NP	NP	NP	NP	NP	0.20, 2.25 (0.66, 7.73)

Age median patient age at diagnosis, *TT* tumor thickness as a continuous variable, *LEV* level of rectal wall invasion to serosa or beyond, *MIT* mitotic figures >10/mm^2, *ULC* ulceration, *−REG* absence of regression, *LVI* lymphovascular invasion, *PNI* perineural invasion, *SAT* microscopic satellitosis, *+MAR* final resection margin positive for invasive melanoma, *NP* univariate analysis could not be performed due to too few numbers in certain categories, *+RLN* metastasis to regional lymph nodes, *macrometastasis* clinically evident metastatic disease, *eLDH* elevated LDH levels at diagnosis of distant metastasis

Statistically significant p-values are bolded

[a]Parameters included in subsequent multivariable analysis

Table 5 Clinical stage specific multivariable Cox proportional hazards model of clinicopathological parameters for disease-specific survival from the date of diagnosis

	Groups							
Clinico-pathological parameter	L ($n = 67$) p-value, HR 95% CI	LcN0 ($n = 27$) p-value, HR 95% CI	LpN0 ($n = 40$) p-value, HR 95% CI	R ($n = 55$) p-value, HR 95% CI	RpN+ ($n = 18$) p-value, HR 95% CI	RcN+ ($n = 37$) p-value, HR 95% CI	L + R ($n = 122$) p-value, HR 95% CI	D ($n = 38$) p-value, HR 95% CI
Age ≥62 y	–	**0.02**, 4.35 (1.30,14.53)	–	–	–	–	–	–
TT	**0.001**, 1.08 (1.03, 1.13)	**0.03**, 1.14 (1.02, 1.28)	–	**0.001**, 1.04 (1.02, 1.07)	**0.01**, 1.16 (1.04, 1.30)	–	**<0.001**, 1.04 (1.02, 1.07)	–
LEV	–	–	–	–	–	–	–	**0.02**, 0.02 (0.00, 0.49)
ULC	–	–	–	–	–	**0.02**, 0.21 (0.06, 0.81)	–	–
−REG	–	–	–	**0.004**, 0.16 (0.05, 0.57)	–	–	**0.005**, 0.12 (0.03,0.53)	–
LVI	–	–	**0.006**, 2.97 (1.36, 6.48)	**0.02**, 2.23 (1.17, 4.26)	**0.008**, 6.40 (1.61, 25.45)	–	**0.03**, 1.85 (1.06, 3.22)	–
PNI	–	–	–	–	–	**0.05**, 2.98 (1.02, 8.69)	–	–
+MAR	–	–	–	–	–	–	–	–
+RLN	–	–	–	–	–	–	–	–

Statistically significant p-values are bolded

TT tumor thickness as a continuous variable, *LEV* level of rectal wall invasion to serosa or beyond, *MIT* mitotic figures >10/mm^2, *ULC* ulceration, *−REG* absence of regression, *LVI* lymphovascular invasion, *PNI* perineural invasion, *SAT* microscopic satellitosis, *+MAR* final resection margin positive for invasive melanoma, *+RLN* metastasis to regional lymph nodes

compared to 1.06 for increased tumor thickness as a continuous variable). Median tumor thickness has been reported to be greater for anorectal melanoma compared to other mucosal melanoma subtypes [10], and the relationship between tumor thickness and patient outcome in anorectal melanoma has generally confirmed worse outcome with increasing tumor thickness [5, 22, 23, 48–52]. However, the use of different tumor thickness cut-offs obscures direct comparison of results across the different reports. Some studies stratified anorectal melanoma according to the AJCC T-category criteria for cutaneous melanoma [23, 48], while others applied specific numerical cut-offs, including 4.00 mm [5], or 10.0 mm [52]. In addition, previous anorectal melanoma studies did not stratify patients according to clinical stage at presentation when determining relationships between primary tumor parameters. Taken together, studies on larger anorectal melanoma patient cohorts that incorporate stage at presentation are needed to define consensus criteria regarding the relationship between tumor thickness and patient survival.

Among patients with regional (lymph node) metastases (group R), greater tumor thickness and lymphovascular invasion correlated with shorter disease-specific survival (particularly among those with clinically occult regional lymph node metastases, RpN+). Our observed correlation between tumor thickness and disease-specific survival among anorectal melanoma patients with clinically occult regional lymph node metastases (RpN+) is consistent with the recent modifications to the 8th edition AJCC for cutaneous melanoma, in which T-category was integrated with the N-category to assign stage III subgroups [39]. However, the prognosis among RcN+patients is likely driven by their greater metastatic disease burden compared to RpN+ patients, and thus, the tumor thickness of the primary melanoma is possibly less critical in this disease setting. The significance of ulceration correlating with longer disease-specific survival in RcN+ patients is unclear, but may be attributable to limitations of statistical analysis on a small cohort of patients. In our anorectal melanoma cohort, regression was present in 11.3% of primary tumors and correlated with longer disease-specific survival among all patients and improved disease-specific survival among those presenting with clinically evident regional metastasis (RcN+). Although a recent meta-analysis revealed lower rates of sentinel lymph node metastasis in cutaneous melanoma with regression, the prognostic significance of primary tumor regression in cutaneous melanoma remains controversial and poorly understood [33, 53]. In our previous study of vulvar melanoma, regression was present in 25% of cases and also correlated with longer disease-specific survival [40], suggesting that an association between regression and improved patient survival may be common across anogenital mucosal melanoma subtypes. Of

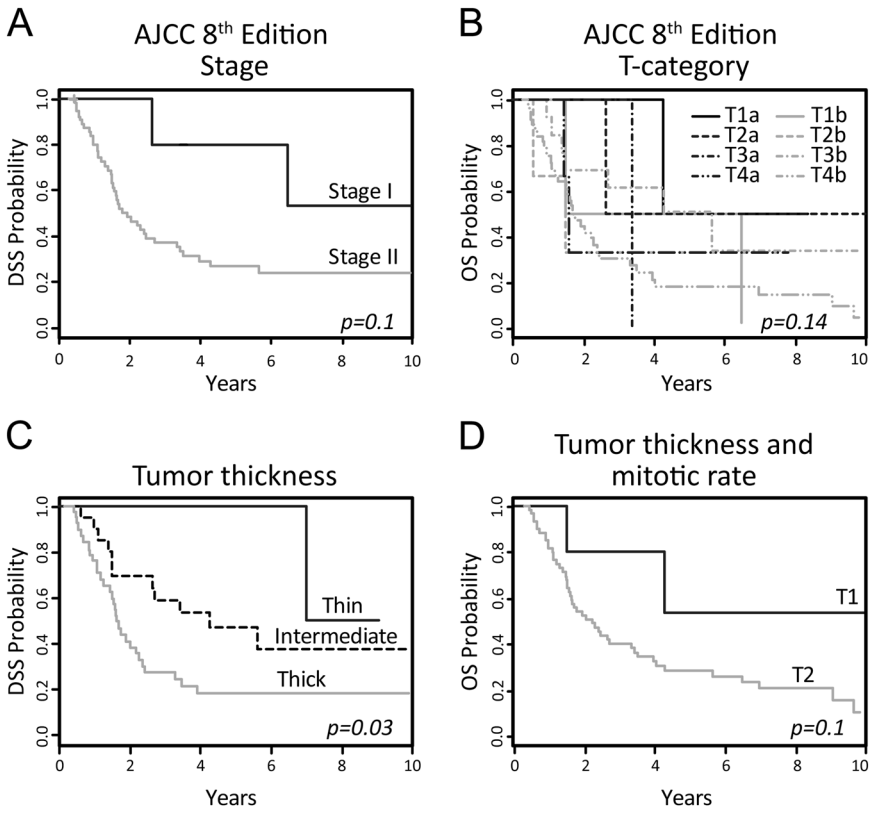


Fig. 4 Risk stratification of patients with localized anorectal melanoma using T-categories. Kaplan–Meier plots of group L anorectal melanoma patients only for **a** disease-specific survival using American Joint Commission on Cancer (AJCC) stage (stage I: T1a to T2a; stage II: T2b to T4b), **b** overall survival according to the AJCC 8th-edition T-category system (T1a to T4b), **c** disease-specific survival using modified tumor thickness system (thin: tumor thickness ≤ 1.0 mm [T1]; intermediate: tumor thickness > 1.0–4.0 mm [T2/3]; thick: tumor thickness > 4.0 mm [T4]) and, overall survival for tumor thickness-mitotic rate system (T1: tumor thickness ≤ 2.0 mm and mitotic rate < 2/mm^2; T2: tumor thickness > 2.0 mm and mitotic rate ≥ 2/mm^2) [40]

Table 6 Grouping of anorectal melanoma patients presenting with only localized disease using the various T-categories and modifications of the American Joint Commission on Cancer (AJCC) 8th-edition cutaneous melanoma and tumor thickness (TT)-mitotic rate staging systems and results of univariate analysis of overall survival (OS) and disease-specific survival (DSS)

System	Groups	Survival	Group L ARM patients, $n = 67$		
			n	HR (95% CI)	*p*-value
AJCC 8th edition stage	I	DSS	8	–	–
	II		59	3.37 (0.81, 13.97)	0.095
AJCC 8th edition T-category		OS			0.142
	T1a		3	Reference	–
	T1b		2	3.96 (0.36, 43.78)	0.262
	T2a		3	1.00 (0.06, 16.33)	1.000
	T2b		4	3.65 (0.33, 40.42)	0.291
	T3a		1	3.79 (0.24, 6.91)	0.348
	T3b		13	1.95 (0.24, 15.86)	0.534
	T4a		3	3.11 (0.28, 34.40)	0.355
	T4b		38	4.86 (0.66, 35.66)	0.120
Modified tumor thickness		DSS			**0.032**
	Thin (TT: ≤1.0 mm)		5	Reference	–
	Intermediate (TT: >1.0–4.0 mm)		21	2.36 (0.30, 18.31)	0.412
	Thick (TT: >4.0 mm)		41	4.98 (0.67, 36.69)	0.116
Tumor thickness-Mitotic rate	pT1	OS	5	Reference	–
	pT2		62	3.33 (0.80, 13.80)	0.097

Statistically significant *p*-values are bolded

note, since mucosal melanoma may respond less favorably to immune checkpoint blockade therapy compared to cutaneous melanoma [3, 13, 15–17], the relationship between regression, the composition and density of the tumor-associated immune infiltrate, and response to immune-modulatory therapy among mucosal melanoma

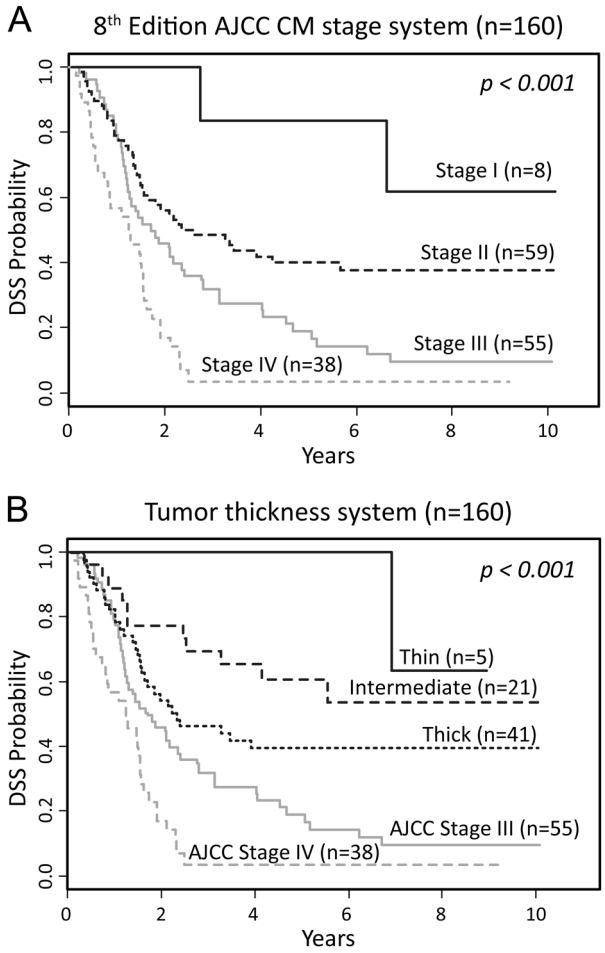


Fig. 5 Comparison of risk stratification of all anorectal melanoma patients using two staging systems derived from the 8th edition American Joint Commission on Cancer (AJCC) cutaneous melanoma criteria. A, 8th edition AJCC stage system for cutaneous melanoma, B, Combination of modified tumor thickness and AJCC stage systems

remains a provocative question. Analyses in contemporary patient datasets that include tumor burden, comprehensive immune profiling, and tumor mutational burden are necessary to address this systematically.

Among patients presenting with clinically evident regional or distant metastases, we found no meaningful correlations between most primary tumor parameters (including conventional high-risk features such as greater tumor thickness and lymphovascular invasion) and disease-specific survival. This is mostly similar to cutaneous melanoma in which the prognosis of these patients is largely driven by their advanced stage [54]. However, measures of regional disease burden (satellitosis, the number of nodes involved and whether these were clinically evident or occult) also did not independently correlate with disease-specific survival among patients with regional metastases. However, among the 55 patients in our cohort with regional metastases, 25% (14/55) underwent comparatively limited evaluation of the extent of their regional disease burden (without a formal pathological enumeration of the number and extent of regional lymph node burden) (Supplemental Table 2). Further, indices of distant metastatic disease burden, including lactate dehydrogenase levels and site(s) of distant metastasis (including central nervous system involvement), also did not correlate with disease-specific survival among patients with distant metastases. However, our cohort included only 38 patients presenting with distant metastases, significantly limiting the power of these analyses. Additional studies utilizing larger number of anorectal melanoma patients with regional and distant disease are likely needed to further evaluate the prognostic role of metastatic disease burden in anorectal melanoma.

Our primary goal was to optimize risk stratification of anorectal melanoma patients by integrating relevant clinicopathological variables, particularly among those

Table 7 Comparison of risk stratification of all anorectal melanoma patients using two staging systems derived from the 8th edition American Joint Commission on Cancer (AJCC) cutaneous melanoma criteria using Bayesian Information Criteria (BIC) model

Risk stratification systems	$n = 160$	HR (95% CI)	p-value	BIC value
8th Edition AJCC cutaneous melanoma staging system			**<0.001**	1037.1
Stage I (group L)	8	Reference	–	
Stage II (group L)	59	3.47 (0.84, 14.39)	0.09	
Stage III (group R)	55	4.57 (1.11, 18.86)	0.04	
Stage IV (group D)	38	9.85 (2.34, 41.42)	0.002	
Modified tumor thickness system			**<0.001**	1038.6
Thin (group L)	5	Reference	–	
Intermediate (group L)	21	2.42 (0.31, 18.76)	0.40	
Thick (group L)	41	5.07 (0.69, 37.28)	0.11	
Stage III (group R)	55	5.39 (0.74, 39.20)	0.10	
Stage IV (group D)	58	11.74 (1.59, 86.59)	0.02	

Statistically significant p-values are bolded

presenting with localized disease. Although a formal staging system for anorectal melanoma does not exist, most studies have applied the Ballantyne clinical system [6, 10, 18, 24, 49, 55–61]. For anorectal melanoma patients who presented with localized disease, we found that a 3-group system based on minor modifications of the AJCC 8th edition tumor thickness cut-offs for cutaneous melanoma (thin: T1, intermediate: T2-3 and thick: T4) most robustly stratified disease-specific survival (Fig. 3, Table 6), followed by the 2-group system also based on 8th edition AJCC for cutaneous melanoma cut-offs (Stage I: T1a to T2a versus Stage II: T2b to T4b). The 3-group modified 'tumor thickness' system was derived based on our initial observation that although tumor thickness correlated with disease-specific survival among all anorectal melanoma patients, there was considerable overlap between T2 and T3 patients (Fig. 2a) and also predicted disease-specific survival among patients with localized anorectal melanoma. When applied to the entire cohort in conjunction with AJCC stage III and IV patients, both the 'stage' and the modified 'tumor thickness' systems accurately risk-stratified patients according to disease-specific survival, supporting the application of 8th edition AJCC cut-offs for cutaneous melanoma to patients with anorectal melanoma.

Compared to the 2-group system based on 8th edition AJCC cutaneous melanoma stage I and II and the tumor thickness-mitotic rate system, the 3-group system based on modified tumor thickness alone delineated a third group of patients that were at intermediate risk for progression (Fig. 3d). Whether this group of patients and those designated with "thick" melanomas may benefit from more frequent follow-up and/or potential adjuvant therapy(ies) compared to the "thin" melanoma patients should be explored further. Taken together, our study supports that the 8th edition AJCC staging system for cutaneous melanoma (or slight modifications of it) represents a robust approach for risk stratification among patients with anorectal melanoma, including those with localized disease. Since there were too few events within some of the individual T-sub-categories to assess disease-specific survival, only overall survival could be derived for AJCC 8th edition T-category and tumor thickness-mitotic rate system. Additional studies utilizing larger patient cohorts are required to validate these observations.

There are several limitations to our study. First, this was a retrospective study and due to the long interval of time studied (since 1986), patients in our cohort were treated with a broad array of clinical and surgical management strategies; some patients were treated elsewhere, while majority had their disease management at MD Anderson (Supplemental Fig. 2). Our study was not powered to detect differences related to the different treatment strategies used or responses to such therapies. Additionally, only 6 patients in our cohort had tumor thickness ≤1.0 mm; therefore, the data on thin melanomas is quite limited in the current study. We note that the median tumor thickness in our series (6.9 mm) was less than that reported in prior large series on anorectal melanoma (range: 7.3–12.0 mm from 286 patients) [22, 48, 49, 52], supporting that our patient cohort reflects the anorectal melanoma disease spectrum typically encountered in clinical practice. A further limitation was the mutational analyses; the assays used to identify mutations in our cohort of anorectal melanoma was derived from our routine clinical practice. Thus, the assay platforms and the number of genes evaluated varied over time and were not comprehensive. Only a few cases tested carried mutations, so we were underpowered to determine whether discrete molecular-genetic alterations correlated with patient outcome; such investigations are warranted going forward.

In conclusion, our analysis of clinicopathological parameters in 160 anorectal melanoma patients supports the prognostic significance of tumor thickness in patients with clinically localized disease, while meaningful associations were not identified in anorectal melanoma patients with distant or clinically evident regional metastases. Classification according to minor modifications (modified tumor thickness) of the AJCC 8th edition cutaneous melanoma staging system robustly stratifies patients with localized anorectal melanoma. Our study supports the rationale to evaluate these systems in additional larger cohorts of anogenital melanoma patients. To support ongoing and future planned efforts, we strongly recommend that all prognostic indicators for primary cutaneous melanoma also be recorded in the diagnosis and stratification of risk for primary anorectal melanoma.

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Compliance with ethical standards

Conflict of interest MIR reports receiving speakers' bureau honoraria from Amgen Inc., Merck, and Provectus Biopharmaceuticals and is a consultant/advisory board member for Amgen Inc., GlaxoSmithKline, and Merck. JEG has served as a consultant or advisory board member for Merck, Bristol-Myers Squibb, Novartis, Syndax, and Castle Biosciences, unrelated to the content of this manuscript. MTT is a consultant/advisory board member for Novartis LLC, Myriad Genetics, and Seattle Genetics. VGP is a consultant for Myriad Genetics. MAD is an advisory board member for BMS, Novartis, Roche/Genentech, Sanofi-Aventis, Vaccinex, Array and Syndax; a consultant for Nanostring; and PI of research grants to The University of Texas MD Anderson Cancer Center from BMS, Roche/Genentech, Sanofi-Aventis, Merck, Oncothyreon. The remaining authors declare that they have no conflict of interest.

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