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ANATOMICAL PATHOLOGY

Primary anorectal melanoma: clinical, immunohistology and DNA analysis of 43 cases

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Summary

Primary melanoma involving the anorectal region is rare, accounting for <1% of all melanomas in most Western countries. It characteristically presents at an advanced clinical stage and is associated with poor clinical outcomes. Preliminary reports suggest that response rates to immunotherapies in patients with advanced stage mucosal melanoma are much lower than in cutaneous (or acral) melanoma patients but reasons for this are unclear. Comprehensive characterisation of the immune microenvironment in anorectal melanoma has not previously been performed. A single-institution cohort of 43 primary anorectal melanoma patients was examined to describe clinicopathological features and characterise the immune microenvironment to provide insights into the behaviour of this rare melanoma subtype. The tumours displayed multiple adverse prognostic attributes including deep thickness (median 11.5 mm), ulceration (81%) and high mitotic rate (median 12/mm^2). The median overall survival was 24 months and the median recurrence-free survival was 9 months. Tumour-infiltrating lymphocytes (TILs) were absent or mild in most tumours (75%); PD-L1 positive staining (>1% of tumour cells) was present in 44% of cases, however in 86% of positive tumours the percentage of positive cells was $\leq$10%. Four tumours underwent whole genome sequencing; no ultraviolet signature was identified, and there was a lower mutational load but higher structural chromosomal variant load compared with cutaneous melanomas. Poor responses of anorectal melanomas to immunotherapy may be caused by lower immunogenicity of these tumours as characterised by low mutation burden (and therefore low neoantigenicity), low TILs infiltrates and low PD-L1 expression. Further investigation is required to determine whether TILs and PD-L1 expression predict response to immunotherapy in patients with mucosal melanoma.

Key words: Melanoma; pathology; TILs; prognosis; PD-L1; immunotherapy; treatment.

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INTRODUCTION

Primary anorectal melanoma is rare, accounting for approximately 1% of all anal malignancies, 0.05% of all colorectal malignancies and 0.4–1.6% of all melanomas.[1–4] It is the third most common site for primary mucosal melanoma after the head and neck and vulvo-vaginal regions.[5] Because of its location, the diagnosis is often delayed and is commonly misdiagnosed as haemorrhoids initially, as it often presents with rectal bleeding, a mass, or a change in bowel habit.[6]

Whereas the prognosis of most patients with cutaneous melanoma is good, overall survival remains poor in patients with anorectal melanoma despite the use of multiple treatment modalities including surgery, radiotherapy and systemic therapies. Reported 5-year survival rates are of the order of 20%;[7,8] a review of 183 patients from the Surveillance, Epidemiology, and End Results (SEER) database found 5-year survival rates of 26.7% (Stage I), 9.8% (Stage II) and 0% (Stage III).[6]

Abdominoperineal resection, a procedure with significant morbidity, may be used in patients with bulky localised disease. However, more recently wide local excision has been used as an alternative with no apparent difference in overall survival, although possibly higher rates of local recurrence.[9,10] Adjuvant radiotherapy may also improve locoregional control, but again no survival benefit has been demonstrated.[11] A series of 54 patients treated over a 20 year period by local excision followed by hypofractionated radiotherapy achieved local control in 82% of patients; however, the 5-year overall survival rate was only 30%.[12]

Data on outcomes following systemic immunotherapy for metastatic mucosal melanoma are limited; a multicentre retrospective analysis of 33 patients treated with anti-CTLA-4 antibody (ipilimumab) showed one complete response and 22 cases of progression.[13] A retrospective analysis of six studies, including 86 patients with metastatic mucosal melanoma treated with anti-Programmed cell death-1 (PD-1) therapy alone (nivolumab) versus 35 patients treated with combination therapy (ipilimumab plus nivolumab)[14] found that the objective response of patients treated with nivolumab alone was 23%, versus 41% for patients with cutaneous melanoma, with median progression-free survivals of 3 months versus 6.2 months; for those patients treated with

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combination therapy, the objective response was 37% for mucosal melanoma versus 60% for those with cutaneous melanoma, progression-free survival 5.9 versus 11.7 months. A study of 706 patients with mucosal melanoma found that the presenting stages, incidence of nodal and distant metastases, the site predilection of distant metastases and overall survival are similar across primary sites of mucosal melanoma.[15]

Responses to anti-PD-1/PD-L1 (Programmed death-ligand 1) monotherapy in patients with advanced melanoma have typically been associated with PD-L1 expression in the tumour microenvironment.[16] As far as the authors are aware, there has been no large study assessing the tumour immune microenvironment in anorectal melanoma. Given the apparently lower rates of response to immunotherapies for metastatic mucosal melanoma when compared to metastatic cutaneous melanoma, understanding the tumour immune microenvironment is a crucial step towards elucidating reasons for this difference and how to overcome them.

MATERIAL AND METHODS

Patients and pathology

The databases of Melanoma Institute Australia (MIA) and the Tissue Pathology and Diagnostic Oncology Department at the Royal Prince Alfred Hospital, Sydney, were searched for all patients coded as having anorectal melanoma. Cases that had colorectal melanomas recorded as metastases from another site were excluded. The demographics, tumour characteristics, surgery, medical treatments and outcome data for the cases were recorded. Pathological assessment was based on review of the histopathology slides where possible, otherwise the pathology report was reviewed.

As anorectal melanoma is excluded from the American Joint Committee on Cancer (AJCC) cutaneous melanoma staging system, many previous reports have used a simplified three-level staging system for mucosal melanoma; in this classification, the disease is classified as local (stage I), regional (stage II) and disseminated (stage III).[4,9,17,18] We expanded this system to be more directly comparable with the AJCC cutaneous melanoma staging system, using four categories (Stage 1 = local disease with tumour thickness $\leq$2 mm; Stage 2 = local disease with tumour thickness >2 mm; Stage 3 = regional node involvement; and Stage 4 = disseminated disease).

The tumour site was determined by collating information recorded in the MIA database and by reviewing the pathology report and, if available, slides. If melanoma *in situ* was identified in squamous epithelium, these tumours were deemed to be in perianal skin, anal or anorectal but not rectal.

Immunohistology

The tumour microenvironment was assessed by grading the tumour-infiltrating lymphocytes (TILs) on haematoxylin and eosin stains using a previously described four-tier system,[19] and also with immunohistochemistry for CD4, CD8 and PD-L1 (Fig. 1). PD-L1 was deemed positive if there was $\geq$1% staining of tumour cells on the examined slide. The CD4 antibody was sourced from Cell Marque, USA, (clone SP35) with a 1/50 dilution. The CD8 antibody was obtained from Dako, Denmark, (clone C8144B) with a 1/100 dilution. The PD-L1 antibody was obtained from Cell Signaling, USA, (clone E1L3N) with a 1/75 dilution. The Bond-III Autostainer and Epitope Retrieval Solution 2 (pH 9; Leica, Germany) were used for all three antibodies. Assessment of PD-L1 was performed in a similar fashion as per previously described.[20]

DNA analysis

DNA were analysed using a MassArray System, OncoCarta platform (230 somatic mutations in 19 oncogenes) or OncoFocus platform (230 DNA changes in five genes *BRAF*, *EGFR*, *KIT*, *KRAS*, *NRAS*) (Agena Bioscience, USA) to screen for known DNA changes in genes involved in solid tumours. Select patients also underwent whole genome sequencing as part of a previously published wider cohort.[21]

RESULTS

Clinicopathological features

This study included 43 patients diagnosed with primary anorectal melanoma between 1958 and 2016 at Melanoma Institute Australia. The median age of the cohort was 61 years (range 28–89 years), and 22 (51%) were females (Table 1). Staging could be determined in 41/43 patients; one patient was Stage I (2%), 21 were Stage II (49%), 13 were Stage III (30%) and six were Stage IV (14%). The site of origin was deemed anal

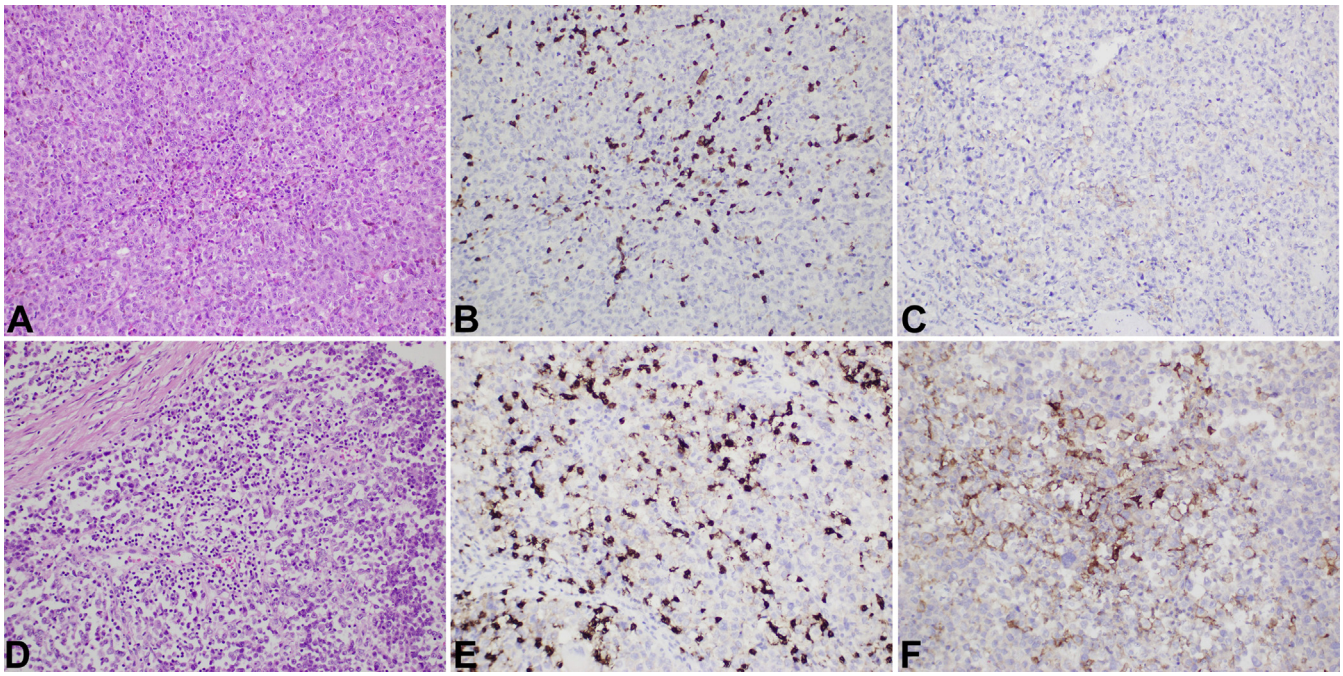

Fig. 1 (A,D) H&E, (B,E) CD8, and (C,F) PD-L1 staining of two cases of anorectal melanoma. (A–C) A 50-year-old male showing 3+ tumour infiltrating lymphocytes (TILs) on H&E and CD8 and focal PD-L1 staining. (D–F) A 36-year-old female showing 3+ TILs on H&E and CD8 and focal PD-L1 staining.

Table 1 Clinicopathological characteristics of patients with anorectal melanoma

Features	Patients (n = 43)	%
Age, years		
Median (range)	61 (28-89)	N/A
≤50	8	19
>50	35	81
Sex		
Male	21	49
Female	22	51
Site		
Anal	20	47
Anorectal	9	21
Rectal	9	21
Perianal	5	12
Decade of diagnosis		
1950s	1	2
1970s	3	7
1980s	6	14
1990s	6	14
2000s	15	35
2010s	12	28
Thickness, median mm	11.5	
Ulceration		
Present	35	81
Absent	3	7
Unknown	5	12
Mitotic count, median / mm^2	12	
In situ component		
Present	14	33
Absent	11	26
Unknown	18	42
Predominant cell type		
Epithelioid	14	33
Spindled	3	7
Mixed	11	26
Unknown	15	35
Lymphovascular invasion		
Absent	17	40
Present/suspicious	9	21
Unknown	17	40
Pigmentation		
Absent	8	19
Present	13	30
Unknown	22	51
Staging		
Stage I	1	2
Stage II	21	49
Stage III	13	30
Stage IV	6	14
Unknown	2	5
Surgery		
Abdominoperineal resection	20	47
Local excision	15	35
Biopsy only	4	9
Pelvic exenteration	1	2
Unknown	3	7

in 20 patients (47%), anorectal in nine patients (21%), rectal in nine patients (21%) and in peri-anal skin in five patients (12%).

The histopathological slides were available for review in 21 patients and for the remaining cases, the pathology reports could be examined. The tumours were generally thick (mean 12.9 mm, median 11.5 mm) and the level of invasion for the non-perianal tumours was either submucosal (10/38, 26%), to muscularis propria (5/38, 13%) and beyond the muscularis propria into peri-rectal fat (5/38, 13%). For the perianal tumours, 4/5 (80%) involved the reticular dermis (Clark level IV) and 1/5 (20%) involved the subcutis (Clark level V). The level of invasion was unknown for 18 patients.

Most of the tumours were ulcerated (81%). A melanoma *in situ* component within squamous mucosa could be identified in 33% of cases. Melanoma *in situ* involving rectal mucosa was not identified. The mitotic rate was high (median 12/mm^2). The predominant cell type was epithelioid (33%), spindled (7%) or mixed (26%). Lymphovascular invasion was present in 21% of cases. Satellite lesions were seen in six of 14 cases that could be assessed (43%). One of the perianal skin melanomas was associated with a dysplastic compound naevus; however, in none of the remaining cases was an associated pre-existing naevus identified.

Four of the cases also had a history of a separate invasive cutaneous primary melanoma; however, in all these an anorectal metastasis was deemed unlikely based on careful clinicopathological assessment. One patient had an upper back primary melanoma four years previously (Breslow thickness 0.55 mm); one had a 0.5 mm thick melanoma on the forearm 20 years previously; one had a 1.1 mm upper back primary melanoma 12 years previously; and one had a 1.2 mm vulval melanoma diagnosed 39 years previously. Two of these four cases had an *in situ* component associated with the anal tumour in keeping with primary tumours. None of the patients developed locoregional or distant metastasis from their non-anorectal primary prior to their diagnosis of anorectal melanoma.

Tumour microenvironment: immunohistology

TILs were absent in 45%, mild in 30%, moderate in 15% and marked in 10% of cases. The peritumoural lymphocyte densities were either absent (29%) or mild (71%) (Table 2). The average proportion of TILs that were CD8-positive was 78% (when combined with CD4-positive cells). The majority of peritumoural lymphocytes were also CD8-positive (mean 63%). PD-L1 staining of tumour cells was negative in 9/16 (56%) of cases and in the remaining seven cases the

Table 2 Characterisation of tumour-infiltrating lymphocytes, peritumoural lymphocytes and PD-L1 staining of tumour cells

		No. patients	%
Tumour infiltrating lymphocytes (TILs) (n = 20)	Absent (Grade 0)	9	45
	Mild (Grade 1)	6	30
	Moderate (Grade 2)	3	15
	Marked (Grade 3)	2	10
Peritumoural lymphocytes (n = 14)	Absent	4	29
	Mild	10	71
	Moderate	0	0
	Marked	0	0
PD-L1 staining of tumour cells (n = 16)	Positive	7	44
	1–5%	4	24
	6–10%	2	13
	>10%	1	6
	Negative	9	56

proportion of positive tumour cells ranged from 1% to 20%. Of the eight cases that had TILs (and had PD-L1 immunohistochemistry performed), 5/8 (63%) had at least some PD-L1 staining of tumour cells whereas of the eight cases with no TILs, 2/8 (25%) had positive staining with PD-L1 ($p = 0.31$; Fisher's exact test). Staining occurred most commonly at the periphery of the tumour.

Molecular data

Twelve tumours had OncoFocus or OncoCarta mutation testing, and four patients also had whole genome sequencing (Table 3). One tumour had both an *NRAS* (G12D) mutation and an *SF3B1* (R625H) mutation (rectal melanoma) and one tumour had a *C-KIT* (K642E) mutation (anorectal melanoma). A *GNAQ* mutation (R183Q) was present in one rectal tumour, and an *NF1* mutation (structural variant resulting in a deletion causing loss of function) was present in an anal tumour. No hot spot mutations were identified in the remaining eight tumours. In the four tumours that had whole genome sequencing (as previously described), no ultraviolet (UV) signature was identified in any case; the average total mutational load was 8682 (median 7713, range 6764–12,538) and this included single nucleotide polymorphisms and in-frame insertions/deletions. This compares with an average total mutational load of 147,497 in cutaneous melanoma assessed using the same platform and methodology ($p < 0.0001$). The mean structural variant counts for the four cases was 247.8 (median 250.5, range 215–275); this compares with mean structural variant rate of 101 in cutaneous melanoma using the same methodology ($p = 0.0011$).[21]

Surgery

Twenty patients received an abdominoperineal resection (47%) and in two of these an *en bloc* vaginectomy was also performed. One patient had a pelvic exenteration (2%) and 15 patients had local excision (35%). Four patients had a biopsy only (9%), while the surgical treatment in the remaining three patients was unknown. The margin could be determined for 14/20 of the abdominoperineal resections; the closest margin was 0.8 mm to a radial margin, and the next closest was 1.8 mm to the distal anal margin, while in the remaining specimens the tumours were well clear of all margins. Four of the 15 local excisions required re-excision (27%) for incomplete or close margins; uninvolved margins ranged from 0.5 mm to >10 mm.

Immunotherapy

Eight patients were treated with immunotherapy (Table 4) including anti-PD-1 therapy (nivolumab, pembrolizumab), anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) therapy (ipilimumab), and one patient received an anti-glucocorticoid-induced tumour necrosis factor receptor (GITR) drug. One patient had stable disease after 18 weeks of combination therapy (pembrolizumab plus ipilimumab) and this was followed by a complete response after 6 weeks of monotherapy (pembrolizumab), however progressive disease developed at week 29. The remaining seven patients all had progressive disease on all combinations of immunotherapy with time to progression ranging from 3.4 to 23.7 weeks (mean 7.7 weeks, median 7.5 weeks). The median progression free survival after the first line of treatment was 7.6 weeks 95% confidence interval (CI; 6.3, 18.7).

TILs/PD-L1 data were available for six of the eight patients treated with immunotherapy. Three of the six (50%) had TILs scores of 0. The remaining three patients had TILs scores of 1, 2 and 3. Three of the six (50%), had PD-L1 scores of 0, one patient had positive staining in 5% of tumour cells and two patients had positive staining in 10% of cells. The one patient who had a complete response at 6 weeks had a TILs score of 0 and negative PD-L1 staining.

Recurrence and survival

Outcome data were available for 42/43 patients with a median follow-up of 15.5 months (mean 30 months, range 1–91 months). The median recurrence-free survival was 9 months; there were six local recurrences, 10 regional lymph node recurrences and 15 distant recurrences. The 1-year and 5-year recurrence-free survivals were 48% (95% CI 34–66%) and 16% (95% CI 7–36%), Fig. 2A. The median overall survival was 24 months (95% CI 15–45%), Fig. 2B. The 5-year survival rate was 15% (5/34 patients alive).

For the patients with tumours containing TILs, overall survival was 86% at 12 months (95% CI 67–100%), whilst the overall survival for those patients without TILs was 52% (95% CI 27–100%). The overall survival at 5 years in those patients with tumours containing TILs was 16% (95% CI 7–35%), whilst no patients with tumours containing no TILs were alive at 5 years. Of the patients with PD-L1 expression in their tumours, five of six were alive at 12 months (83%) but none were alive at 5 years. For the patients with no PD-L1 expression in their tumours, three of six were alive at 12 months (50%) while none were alive at 5 years.

DISCUSSION

This case series provides a detailed description of clinicopathological, immunohistopathological, DNA and treatment features of anorectal melanoma patients. With a median recurrence-free survival of 9 months and median overall survival of 24 months, the study demonstrates that, in line with other studies,[6–8] anorectal melanoma is an aggressive malignancy with a very poor clinical outcome in most cases.

Table 3 Whole genome sequencing on anorectal melanoma cases

	Anorectal melanomas ($n = 4$)	Cutaneous melanoma[21]	p value[a]
Average total mutational load (SNPs and IFID) [b]	8682	147,497	<0.0001
Mean structural variant counts	247.8	101	0.0011
UV signature	Absent	Present	–

[a] p value calculated using the Mann–Whitney U test.
[b] Single nucleotide polymorphisms and in-frame insertions and deletions.

Table 4 Summary of anorectal melanoma patients treated with immunotherapy in the current series

Immunotherapy received (n = 8)[a]	Response			Median PFS after first treatment (95% CI)
	Complete response	Stable disease	Progressive disease	
PD-1 monotherapy (n = 4)	1	0	3	7.6 weeks (6.3, 18.7)
PD-1 based combination therapy (n = 4)	0	1	3	
Ipilimumab monotherapy (n = 4)	0	0	4	

CI, confidence interval; PFS, progression free survival.
[a] Some patients had multiple lines of therapy and are listed more than once. PD-1 therapy includes nivolumab and pembrolizumab.

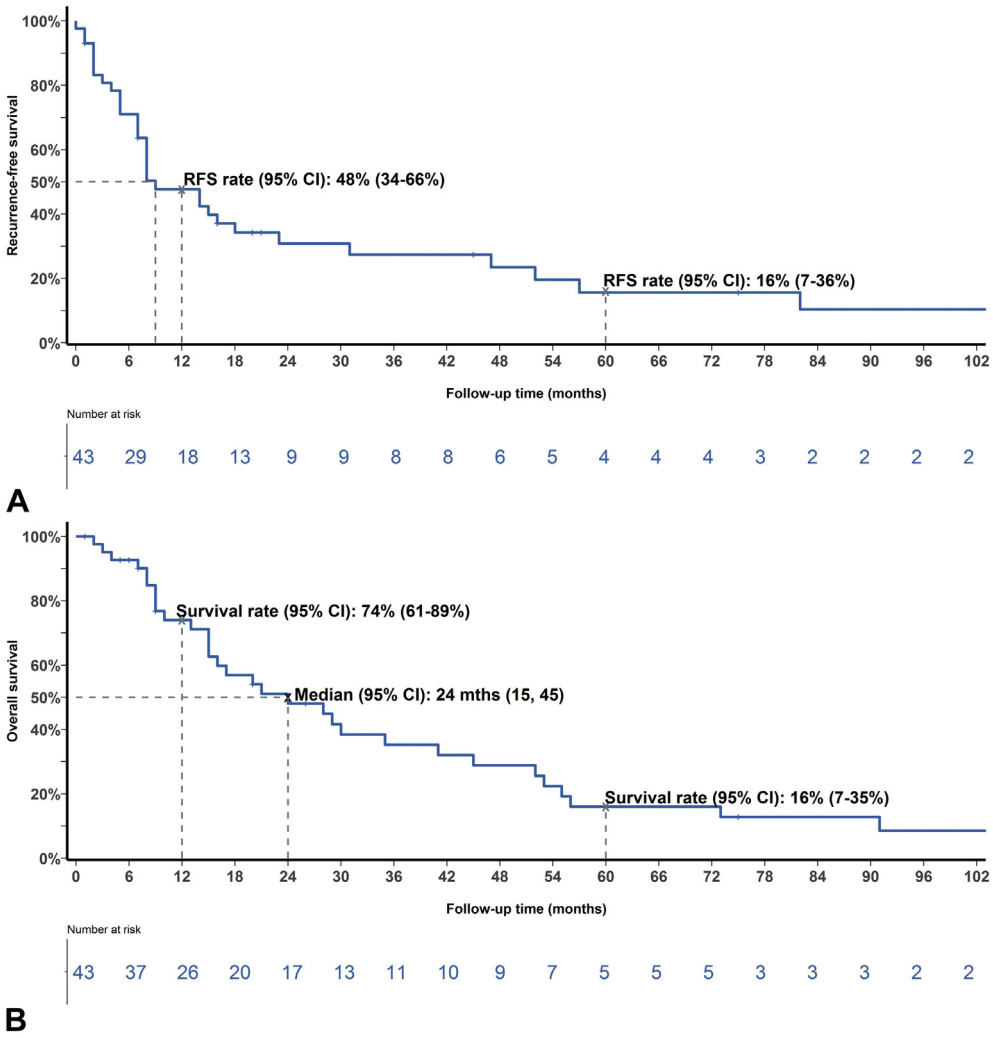

Fig. 2 Kaplan–Meier plots. (A) Recurrence-free survival and (B) overall survival in anorectal melanoma patients.

A review of the histopathology revealed multiple adverse prognostic pathological factors including deep tumour thickness, ulceration, high tumour mitotic rate, satellite lesions and lymphovascular invasion. In contrast, for cutaneous melanomas, approximately 85% of tumours were stage T1 or T2 (≤2 mm thick), and the mean number of mitoses/mm^2 was usually much lower, especially for thin tumours (1.0/mm^2 for melanomas <1 mm and 3.5/mm^2 for melanomas 1.01–2.0 mm thick).[22,23] Ulceration was also uncommon in thin cutaneous melanomas, ranging from 2.5% in T1 tumours to 55.2% for T4 tumours.[24]

Patients with pronounced TILs have an excellent prognosis in clinically localised cutaneous melanoma.[19] For those patients with metastatic melanoma, increased PD-L1 expression is associated with improved survival and predicts response to anti-PD-1 therapy.[16,20,22] There have been few studies assessing the immune microenvironment in mucosal melanoma; a study of 23 mucosal melanomas of the head and neck region found PD-L1 expression was associated with longer recurrence-free survival.[25] A study of PD-L1 and CD8 expression in 200 melanomas, including 36 mucosal tumours (two primary anorectal), showed that PD-L1

expression correlated with a moderate-severe grade of CD8+ TILs.[26] Immunohistochemical PD-L1 expression was also associated with better clinical outcomes following anti-PD-L1 treatment in one study of 18 melanomas (10 acral and 8 mucosal).[27] In our study, at least some TILs were present in 55% of cases; however, only two of 20 cases (10%) had marked TILs, and only five of 20 cases (25%) had TIL scores of moderate or more. Trends towards improved survival were identified in our study for tumours containing TILs and expressing PD-L1, however the number of patients included for analysis was relatively small. TILs/PD-L1 did not appear to correlate with a response to immunotherapy in our anorectal melanoma patient cohort, as the only patient of eight who responded to treatment had a TILs score of 0 and negative PD-L1 staining.

In our cohort, one rectal melanoma had an *SF3B1* mutation and this was accompanied by another *NRAS* driver mutation. *SF3B1* mutations have been identified in previous studies of anorectal melanoma; one study identified three of 15 tumours with *SF3B1* mutations. This included one R625H and two R625C mutations.[28] All three cases were also accompanied by other driver mutations (two *KIT* mutations and one *NF1* mutation). A *GNAQ* mutation was also identified in a rectal tumour in our cohort. Interestingly, the morphology of this tumour did not have a blue naevus-like appearance but did show osteo-cartilagenous differentiation, a rare phenomenon that has been reported in small numbers of melanomas, mostly acral and mucosal melanomas.[29–33] *GNAQ* and *GNA11* mutations are common in blue naevi and are present in up to 80% of uveal melanomas.[34–36] The vast majority of mutations are at Q209 (95%) with around 5% at R183.[37] *GNAQ* and *GNA11* mutations were reported in up to 9.5% of mucosal melanomas in one study of 284 cases (including 26 anorectal melanomas). The most common mutation within *GNAQ* and *GNA11* in the study by Sheng *et al.* was in codon 209 in exon 5. Some mutations of the 183 codon were found in the *GNA11* gene (R183C), however no R183Q mutations were reported.[38]

The average mutational load calculated in the four anorectal melanomas in our study (8682) was similar to that found in other mucosal melanomas and much lower than occurs in cutaneous melanoma.[21] The number of structural variations in anorectal melanomas was consistent with mucosal melanomas in general.[21] The lack of a significant ultraviolet (UV) signature was expected and supports the assumption that these tumours are primary to the anorectum and not cutaneous melanoma metastases (from occult primaries). UV is a well-recognised mutagen, while potential exposures for mucosal membranes, such as food/alcohol/drugs/viral and bacterial load, may have lower mutagenic capability. It remains to be determined whether the advanced stage of diagnosis or the differing aetiology of this form of the disease, or a combination of these, explains the poor response to immunotherapies.

This study demonstrates that anorectal melanoma has low rates of TILs and a poor response to immunotherapy. Whilst PD-L1 expression is common, it is present in a low proportion of tumour cells. Although significant improvements have been achieved for advanced cutaneous melanoma with the advent of immune checkpoint inhibitors and targeted therapies, very little improvement has been achieved to date for mucosal melanoma patients. Further studies are needed to clarify the mechanisms of resistance to immunotherapy for anorectal melanoma and for mucosal melanoma in general to improve outcomes.

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References

1. Bullard KM, Tuttle TM, Rothenberger DA, *et al*. Surgical therapy for anorectal melanoma. *J Am Coll Surg* 2003; 196: 206–11.
2. Meguerditchian A-N, Meterissian SH, Dunn KB. Anorectal melanoma: diagnosis and treatment. *Dis Colon Rectum* 2011; 54: 638–44.
3. Yap LB, Neary P. A comparison of wide local excision with abdominoperineal resection in anorectal melanoma. *Melanoma Res* 2004; 14: 147–50.
4. Cagir B, Whiteford MH, Topham A, *et al*. Changing epidemiology of anorectal melanoma. *Dis Colon Rectum* 1999; 42: 1203–8.
5. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American College of Surgeons Commission on Cancer and the Americal Cancer Society. *Cancer* 1998; 83: 1664–78.
6. Iddings DM, Fleisig AJ, Chen SL, *et al*. Practice patterns and outcomes for anorectal melanoma in the USA, reviewing three decades of treatment: is more extensive surgical resection beneficial in all patients? *Ann Surg Oncol* 2010; 17: 40–4.
7. Thibault C, Sagar P, Nivatvongs S, *et al*. Anorectal melanoma–an incurable disease? *Dis Colon Rectum* 1997; 40: 661–8.
8. Brady MS, Kavolius JP, Quan SH. Anorectal melanoma. A 64-year experience at Memorial Sloan-Kettering Cancer Center. *Dis Colon Rectum* 1995; 38: 146–51.
9. Pessaux P, Pocard M, Elias D, *et al*. Surgical management of primary anorectal melanoma. *Br J Surg* 2004; 91: 1183–7.
10. Droesch JT, Flum DR, Mann GN. Wide local excision or abdominoperineal resection as the initial treatment for anorectal melanoma? *Am J Surg* 2005; 189: 446–9.
11. Tchelebi L, Guirguis A, Ashamalla H. Rectal melanoma: epidemiology, prognosis, and role of adjuvant radiation therapy. *J Cancer Res Clin Oncol* 2016; 142: 2569–75.
12. Kelly P, Zagars GK, Cormier JN, *et al*. Sphincter-sparing local excision and hypofractionated radiation therapy for anorectal melanoma. *Cancer* 2011; 117: 4747–55.
13. Postow MA, Luke JJ, Bluth MJ, *et al*. Ipilimumab for patients with advanced mucosal melanoma. *Oncologist* 2013; 18: 726–32.
14. D'Angelo SP, Larkin J, Sosman JA, *et al*. Efficacy and safety of nivolumab alone or in combination with ipilimumab in patients with mucosal melanoma: a pooled analysis. *J Clin Oncol* 2017; 35: 226–35.
15. Lian B, Cui C, Zhou L, *et al*. The natural history and patterns of metastases from mucosal melanoma: an analysis of 706 prospectively-followed patients. *Ann Oncol* 2016; 28: 868–73.
16. Daud AI, Wolchok JD, Robert C, *et al*. Programmed death-ligand 1 expression and response to the anti-programmed death 1 antibody pembrolizumab in melanoma. *J Clin Oncol* 2016; 34: 4102–9.
17. Ross M, Pezzi C, Pezzi T, *et al*. Patterns of failure in anorectal melanoma. *Arch Surg* 1990; 125: 313.
18. Roumen RM. Anorectal melanoma in The Netherlands: a report of 63 patients. *Eur J Surg Oncol* 1996; 22: 598–601.

19. Azimi F, Scolyer RA, Rumcheva P, *et al*. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. *J Clin Oncol* 2012; 30: 2678–83.
20. Madore J, Vilain RE, Menzies AM, *et al*. PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. *Pigment Cell Melanoma Res* 2015; 28: 245–53.
21. Hayward NK, Wilmott JS, Waddell N, *et al*. Whole-genome landscapes of major melanoma subtypes. *Nature* 2017; 545: 175–80.
22. Thompson JF, Soong S-J, Balch CM, *et al*. Prognostic significance of mitotic rate in localized primary cutaneous melanoma: an analysis of patients in the multi-institutional American Joint Committee on Cancer Melanoma Staging Database. *J Clin Oncol* 2011; 29: 2199–205.
23. Criscione VD, Weinstock MA. Melanoma thickness trends in the United States, 1988–2006. *J Invest Dermatol* 2010; 130: 793–7.
24. Eigentler TK, Buettner PG, Leiter U, *et al*. Impact of ulceration in stages I to III cutaneous melanoma as staged by the American Joint Committee on cancer staging system: an analysis of the German central malignant melanoma registry. *J Clin Oncol* 2004; 22: 4376–83.
25. Thierauf J, Veit JA, Affolter A, *et al*. Identification and clinical relevance of PD-L1 expression in primary mucosal malignant melanoma of the head and neck. *Melanoma Res* 2015; 25: 503–9.
26. Kaunitz GJ, Cottrell TR, Lilo M, *et al*. Melanoma subtypes demonstrate distinct PD-L1 expression profiles. *Lab Invest* 2017; 97: 1063–71.
27. Cho J, Ahn S, Yoo KH, *et al*. Correlation between PD-L1 expression and PD-1 immune checkpoint blockade: a retrospective analysis for advanced melanoma. *J Clin Oncol* 2016; 34: e21007.
28. Yang HM, Hsiao SJ, Schaeffer DF, *et al*. Identification of recurrent mutational events in anorectal melanoma. *Mod Pathol* 2017; 30: 286–96.
29. Sundersingh S, Majhi U, Murhekar K, *et al*. Malignant melanoma with osteocartilaginous differentiation. *Indian J Pathol Microbiol* 2010; 53: 130.
30. Banerjee SS, Coyne JD, Menasce LP, *et al*. Diagnostic lessons of mucosal melanoma with osteocartilaginous differentiation. *Histopathology* 1998; 33: 255–60.
31. Grunwald MH, Rothem A, Feuerman EJ. Metastatic malignant melanoma with cartilaginous metaplasia. *Dermatologica* 1985; 170: 249–52.
32. Moreno A, Lamarca J, Martinez R, *et al*. Osteoid and bone formation in desmoplastic malignant melanoma. *J Cutan Pathol* 1986; 13: 128–34.
33. Pellegrini AE, Scalamogna PA. Malignant melanoma with osteoid formation. *Am J Dermatopathol* 1990; 12: 607–11.
34. Van Raamsdonk CD, Griewank KG, Crosby MB, *et al*. Mutations in *GNA11* in uveal melanoma. *N Engl J Med* 2010; 363: 2191–9.
35. Van Raamsdonk CD, Bezrookove V, Green G, *et al*. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature* 2009; 457: 599–602.
36. Ambrosini G, Pratilas CA, Qin L-X, *et al*. Identification of unique MEK-dependent genes in GNAQ mutant uveal melanoma involved in cell growth, tumor cell invasion, and MEK resistance. *Clin Cancer Res* 2012; 18: 3552–61.
37. Shoushtari AN, Carvajal RD. GNAQ and GNA11 mutations in uveal melanoma. *Melanoma Res* 2014; 24: 525–34.
38. Sheng X, Kong Y, Li Y, *et al*. GNAQ and GNA11 mutations occur in 9.5% of mucosal melanoma and are associated with poor prognosis. *Eur J Cancer* 2016; 65: 156–63.