The Impact of Tumor Microbiome on Anti-tumor Immunity in Anorectal Mucosal Melanoma

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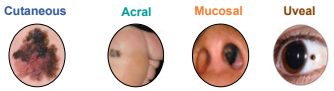
Introduction

Mucosal melanoma is a rare but lethal subtype of malignant melanoma



	Cutaneous	Acral	Mucosal	Uveal
% Malignant melanoma cases[1]:	87%	3%	1%	5%
5-year survival from diagnosis[1]:	81%	75%	25%	80%
Average anti-PD-1 ORR:	43%	32%	22%	8%

Figure 1. Cutaneous *vs* the different rare, non-sun related melanoma subtypes. Mucosal melanomas are typically not detected until late stage, are challenging to surgically resect, and respond poorly to standard melanoma therapy, including immune checkpoint blockade (ICB).

Approximately 25% of mucosal melanomas originate in the anal canal/rectum (Anorectal)

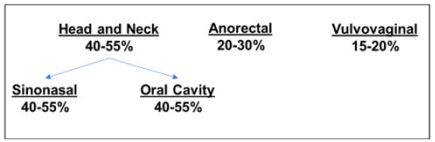


Figure 2. Mucosal melanoma anatomic sites of origin. Anorectal MM accounts for a significant portion of MM cases, with worse survival rates and poorer ICB response rates.

Anorectal mucosal melanomas have poor response to Immune Checkpoint Blockade therapies, including PD-1

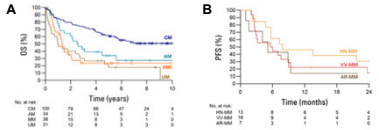


Figure 3. Immune checkpoint blockade (ICB) response rates across melanoma subtypes. (A) Mucosal melanoma (MM) patients have significantly loweroverall survival rates after ICB therapy. **(B)** Anorectal mucosal melanomas (AR-MM) perform particularly poor compared to head and neck (HN-MM) and vulvovaginal (VV-MM) mucosal melanomas.

Objective and Methods

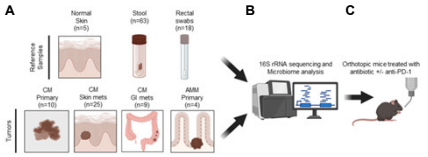


Figure 4. Investigating the potential role of the tumor microbiome on AR-MM immunotherapy responses. To determine how the unique bacterial "microbiome" present in the anorectal tumors may contribute to immunity, we analyzed **(A)** Patient specimens of tumors, stool, and swabs using **(B)** 16S rRNA bacteria sequencing. We then used **(C)** a novel orthotopic mouse model of rectal melanoma to evaluate the impact of antibiotic treatment on immunotherapy response.

Results

16S rRNA sequencing reveals anorectal mucosal melanoma tumor microbiomes reflect the gut, whereas cutaneous melanoma GI metastases do not fully adopt a gut-like tumor microbiome

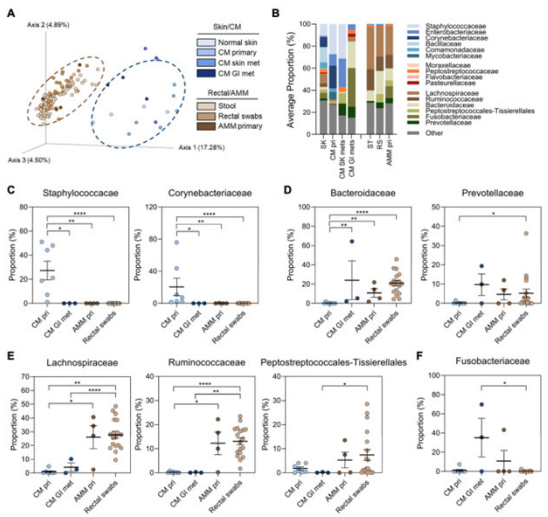


Figure 5. Microbiome composition across melanoma subtypes and normal tissues. (A) Principal coordinates analysis (PCoA) of 16S rRNA sequencing data reveals separation between primary cutaneous melanoma tumors (CM pri, blue hues) and anorectal mucosal melanoma tumors (AMM pri, brown hues) along axes explaining ~26% of total variance. **(B)** Stacked bar plots of average bacterial family-level relative abundance across major sample groups, including references (NS, normal skin; ST, stool; RS, rectal swabs) and tumors (CM pri, CM GI met, AMM pri). **(C–F)** Selected bacterial families with significant differences across CM pri, CM GI met, AMM pri, and rectal swabs from CM patients. Plots display median ± interquartile range; significance calculated by Kruskal-Wallis with Dunn's multiple comparisons. $P < 0.05$ (*), < 0.01 (**), < 0.001 (***), < 0.0001 (****).

Anorectal primary melanoma tumors are enriched for Gram-positive *Firmicutes Clostridia* bacteria

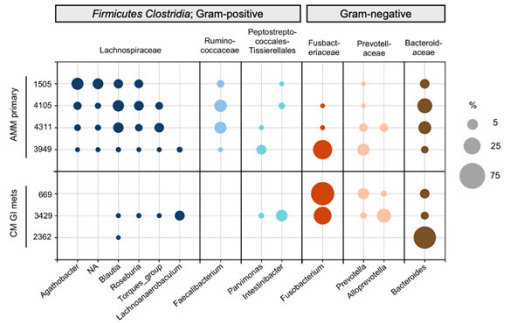


Figure 6. Microbiome species distribution in anorectal primary melanomas compared to cutaneous GI metastases. Bubble plot depicting the relative abundance of select bacterial families associated with gastrointestinal and mucosal environments across individual tumor samples from CM GI metastases and AMM primaries. Bubble size indicates relative abundance (%); samples are grouped by tumor type and patient ID. Families highlighted include Gram-positive Firmicutes Clostridia taxa (*Lachnospiraceae*, *Ruminococcaceae*, *Peptostreptococcales-Tissierellales*) and Gram-negative taxa (*Fusobacteriaceae*, *Prevotellaceae*, *Bacteroidaceae*).

Patients with cutaneous melanoma GI metastases respond better to immunotherapy than anorectal mucosal melanoma patients, raising the potential role of distinct tumor microbiomes in immunotherapy response

A

Characteristic	CM GI Mets (n = 6)	AMM Primary (n = 18)
Sex, No. (%)		
Male	4 (67)	9 (50)
Ethnicity, No. (%)		
Caucasian	6 (100)	15 (83)
Age at first ICI, years		
Median (Range)	52 (27-80)	60 (37-73)
ICI treatments, No. (%)		
Anti-CTLA-4	5 (83)	9 (50)
Anti-PD-1	3 (50)	7 (39)
Combination	1 (17)	10 (56)
Metastatic Sites, No. (%)		
Lymph Node	5 (83)	11 (61)
Subcutaneous	3 (50)	3 (17)
Lung	5 (83)	7 (39)
Liver	2 (33)	7 (39)
Brain	6 (100)	1 (6)
GI	6 (100)	N/A

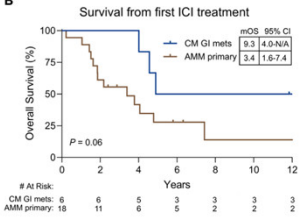


Figure 7. Comparison of ICI response in patients with CM GI mets and AMM primary tumors. (A) Clinical characteristics of patients with CM GI metastases (n=6) and AMM primary tumors (n=18), including age, sex, ethnicity, ICI treatment type, and metastatic sites. **(B)** Kaplan-Meier survival analysis showing overall survival from first immune checkpoint inhibitor (ICI) treatment in patients with CM GI metastases versus AMM primary tumors. Median overall survival (mOS) was longer in CM GI met patients (9.3 years) compared to AMM (3.4 years), though the difference did not reach statistical significance (P = 0.06).

Results (Cont.)

Antibiotic treatment improves anti-PD-1 immunotherapy response in rectal melanomas *in vivo*

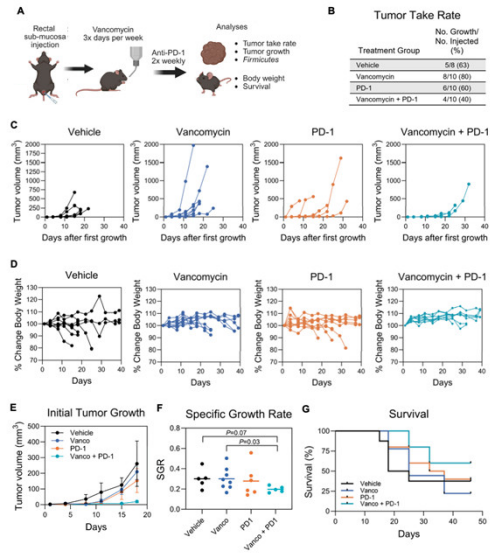


Figure 8. Vancomycin and anti–PD-1 combination treatment in a novel rectal mucosal melanoma mouse model. (A) Schematic of the treatment and analysis workflow. Mice were pre-treated with vancomycin (3×/week) and injected with YUMM3.3 melanoma cells into the rectal submucosa. Anti-PD-1 antibody was administered twice weekly (200 ug/mouse) beginning one week after tumor injection. Mice were monitored for tumor take rate, growth, body weight, survival, and microbiome composition. **(B)** Tumor take rate (% of mice with measurable tumor growth) for each treatment group. **(C)** Tumor growth curves over time, normalized to initial growth detection, in individual mice treated with vehicle, vancomycin, anti-PD-1, or combination vancomycin + anti-PD-1. **(D)** Percent change in body weight over time across treatment groups. **(E)** Average tumor volume during the initial growth phase (Days 0–18). Error bars represent SEM. **(F)** Specific Growth Rate (SGR) calculated per mouse, showing significantly reduced tumor growth with combination treatment ($P = 0.03$, Kruskal-Wallis with Dunn's multiple comparisons). **(G)** Kaplan–Meier survival curves for each treatment group.

Conclusions

- AMM tumors have a distinct microbial signature compared to CM GI metastases, reflecting the immunosuppressive gut microbiome
- MM tumors are enriched for *Firmicutes, Clostridia* taxa associated with immunomodulatory metabolite production
- Modulation of the tumor microbiome may represent a novel therapeutic strategy to enhance immunotherapy responses in MM

Acknowledgements

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