

Molecular profiling and characterization of the tumor immune microenvironment (TME) in appendiceal carcinoma (AC).

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Background

The rarity of appendiceal adenocarcinoma (AC) presents challenges in understanding disease pathogenesis. We previously showed that AC has higher rates of mutations in *KRAS* and *GNAS* and lower rates of *TP53*, *APC*, and *PIK3CA* than CRC. The appendix also has many lymphoid clusters and regulates IgA production in the large bowel, suggesting that AC may be subject to more lymphocytic regulation than CRC. We sought to characterize the molecular profile and TME across AC histopathological types.

Methods

AC samples were analyzed by DNA sequencing (592 genes, NextSeq), or whole exome sequencing, NovaSeq), whole transcriptome sequencing (WTS, NovaSeq), and immunohistochemistry (IHC) for molecular profiling, including microsatellite instability (MSI), mismatch repair (MMR), PD-L1 (SP142), and tumor mutational burden (TMB). Microenvironment Cell Population-counter Quantiseq was used to quantify tumor immune contexture using WTS. AC histopathology was derived from pathology reports. Mann-Whitney U and ChiSquare tests were applied as appropriate, with P-values adjusted for multiple comparisons using Benjamini-Hochberg.

Table 1- Patient demographics and appendiceal histology distribution.

	Adenocarcinoma NOS	Goblet	High Grade Adeno	Low Grade Mucinous	Mucinous adenocarcinoma	PMP	Signet ring cell carcinoma
Count (N)	253	36	43	104	239	10	46
Median Age [range]	62 [28.0 - 90.0]	57 [22.0 - 85.0]	62 [25.0 - 87.0]	60.5 [33.0 - 84.0]	61 [29.0 - 90.0]	62 [34.0 - 75.0]	64 [43.0 - 86.0]
Female	50.2% (127/253)	55.6% (20/36)	60.5% (26/43)	66.4% (69/104)	51.1% (122/239)	50.0% (5/10)	50.0% (23/46)
Male	49.8% (126/253)	44.4% (16/36)	39.5% (17/43)	33.7% (35/104)	49.0% (117/239)	50.0% (5/10)	50.0% (23/46)

Results

Figure 1 – TMB distribution among appendiceal histology types.

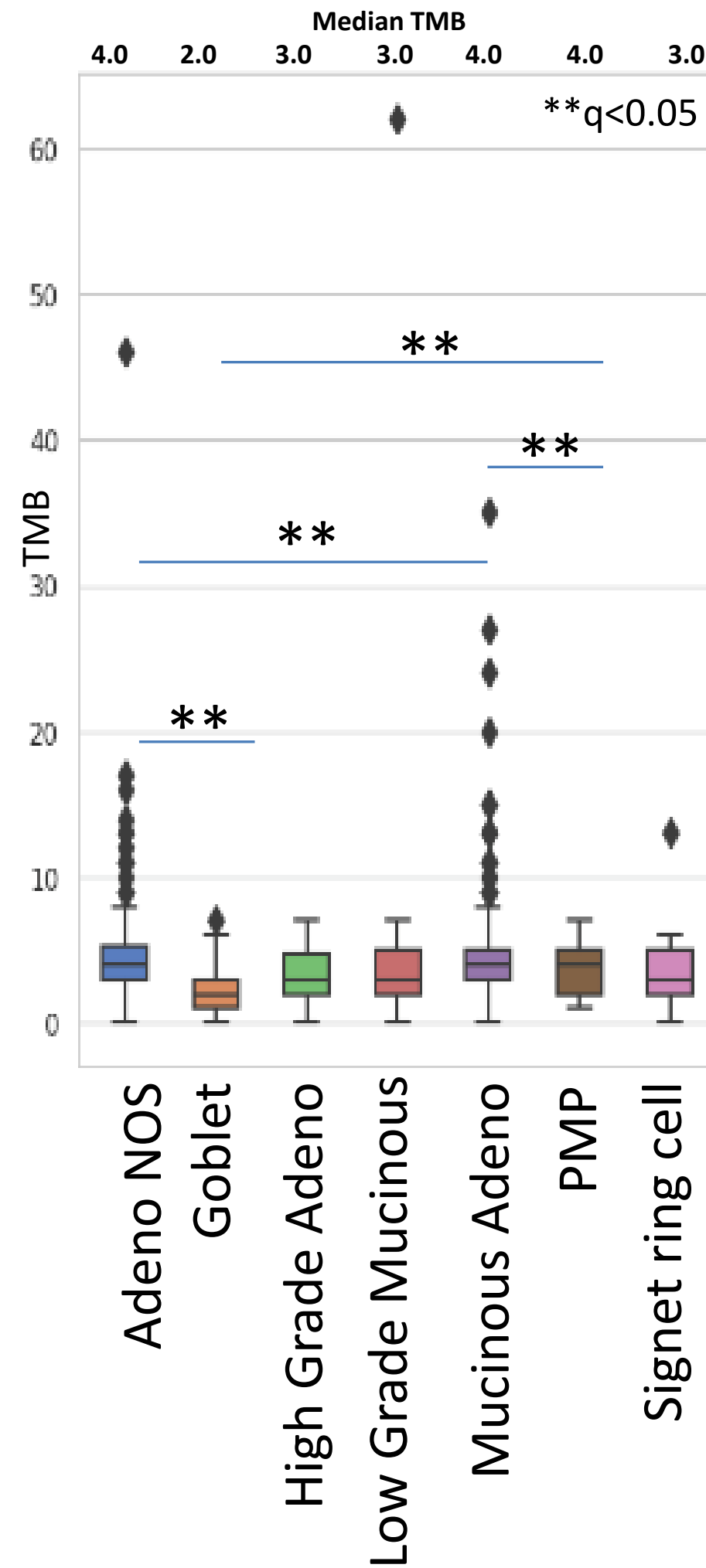


Figure 2- IFN signature comparison among appendiceal histotypes.

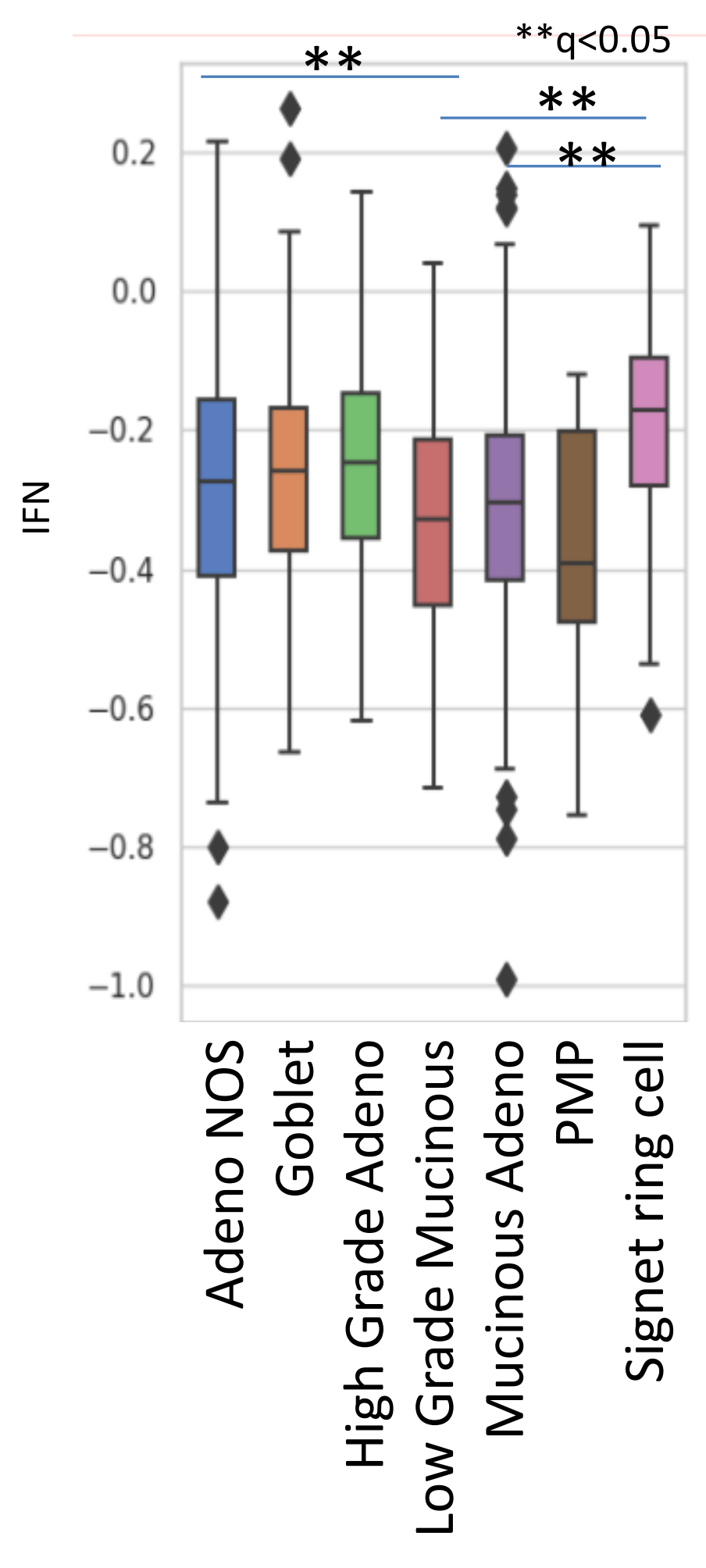


Figure 3 – % PDL-1 (SP142) high among appendiceal histology types (no statistical difference observed from pairwise comparisons).

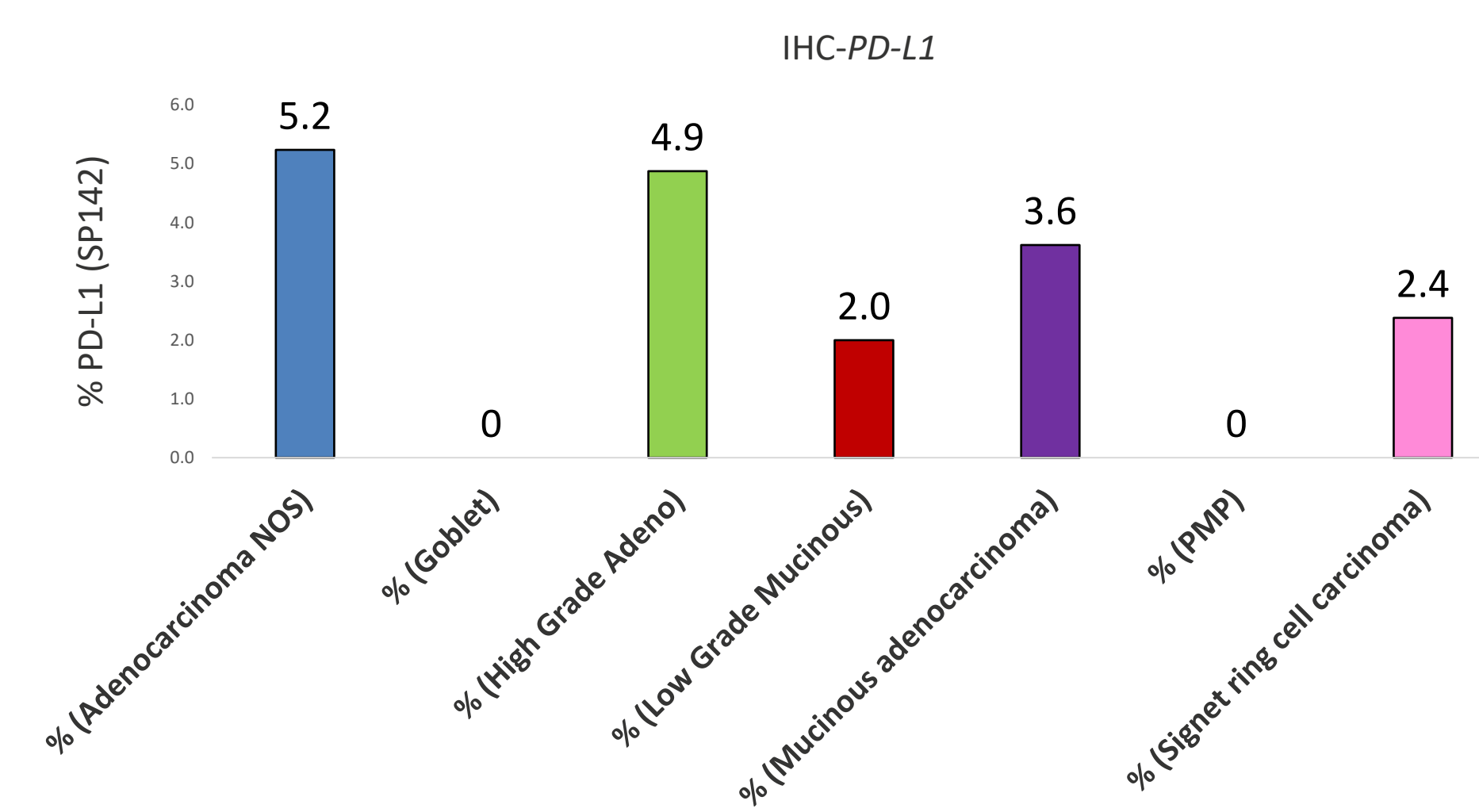


Table 2- Seven patients had dMMR (4 Adeno NOS and 3 Mucinous Adeno)

Histology	IHC-dMLH1	IHC-MSH2	IHC-MSH6	IHC-PMS2
Adeno NOS	Loss			Loss
Adeno NOS		Loss	Loss	
Adeno NOS			Loss	Loss
Mucinous adeno		Loss	Loss	
Mucinous adeno			Loss	
Mucinous adeno		Loss	Loss	

Table 3- Immune marker expression among appendiceal histotypes (highlighted q values indicate significance).

IO Gene	Median expression							pairwise q values						
	Adeno NOS	Goblet	High Grade Adeno	Low Grade Mucinous	Mucinous adeno	PMP	Signet ring cell	Adeno NOS vs High Grade Adeno	Adeno NOS vs Low Grade Mucinous	Adeno NOS vs Mucinous adeno	Goblet vs Low Grade Mucinous	Goblet vs High Grade Adeno	High Grade Adeno vs Low Grade Mucinous	High Grade Adeno vs Mucinous adeno
HAVCR2	16.1	16.2	25.1	14.4	15.5	14.4	25.9	0.045	0.55	0.717	0.605	0.043	0.028	
CD274	3.0	1.9	4.5	3.1	3.3	3.9	2.9	0.259	0.919	0.959	0.259	0.247	0.235	
PDCD1LG2	1.0	1.1	1.4	1.0	1.0	1.4	1.0	0.052	0.773	0.864	1	0.264	0.116	
CD80	3.5	2.7	4.9	3.1	3.7	6.3	3.6	0.497	0.911	0.754	0.522	0.773	0.692	
LAG3	0.8	1.1	1.1	0.6	0.7	0.8	0.8	0.289	0.052	0.148	0.022	0.031	0.055	
PDCD1	0.5	0.4	0.6	0.5	0.4	0.5	0.5	0.859	0.748	0.55	0.874	0.629	0.559	
IFNG	0.3	0.2	0.4	0.2	0.4	0.3	0.4	0.859	0.503	0.098	0.989	0.429	0.228	
CTLA4	1.8	1.8	2.2	1.2	1.2	1.4	2.3	0.717	0.025	0.063	0.047	0.051	0.179	
IDO1	1.7	1.2	1.2	1.0	1.3	0.7	1.8	0.981	0.041	0.047	0.596	0.2	0.325	
CD86	7.9	7.9	11.8	8.2	7.6	9.1	10.9	0.046	0.847	0.981	0.981	0.051	0.046	

Table 4- Pairwise comparisons for most prevalent alterations (p<0.05 bold)

Comparison	NGS-GNAS	NGS-KRAS	NGS-TP53	NGS-APC
(Adeno NOS vs Goblet)	1	0.017	0.001	0.246
(Adenocarcinoma NOS vs High Grade Adeno)	0.001	0.922	0.028	0.748
(Adeno NOS vs Low Grade Mucinous)	<0.001	<0.001	<0.001	0.0017
(Adeno NOS vs Mucinous adeno)	<0.001	<0.001	1	<0.001
(Adeno NOS vs PMP)	<0.001	0.035	0.109	1
(Adenoc NOS vs Signet ring cell carcinoma)	1	0.356	0.003	0.028
(Goblet vs High Grade Adeno)	0.083	0.0004	1	1
(Goblet vs Low Grade Mucinous)	<0.001	<0.001	1	1
(Goblet vs Mucinous adeno)	0.0004	<0.001	0.028	1
(Goblet vs PMP)	<0.001	<0.001	1	1
(High Grade Adeno vs Low Grade Mucinous)	<0.001	<0.001	0.634	1
(High Grade Adeno vs PMP)	0.028	0.770	1	1
(High Grade Adeno vs Signet ring cell carcinoma)	0.214	0.012	1	1
(Low Grade Mucinous vs Mucinous adeno)	<0.001	0.036	<0.001	1
(Low Grade Mucinous vs Signet ring)	<0.001	<0.001	1	1
(Mucinous adeno vs Signet ring)	0.0006	<0.001	0.123	1
(PMP vs Signet ring)	<0.001	0.001	1	1

Figure 4- Most prevalent alterations among appendiceal histotypes.

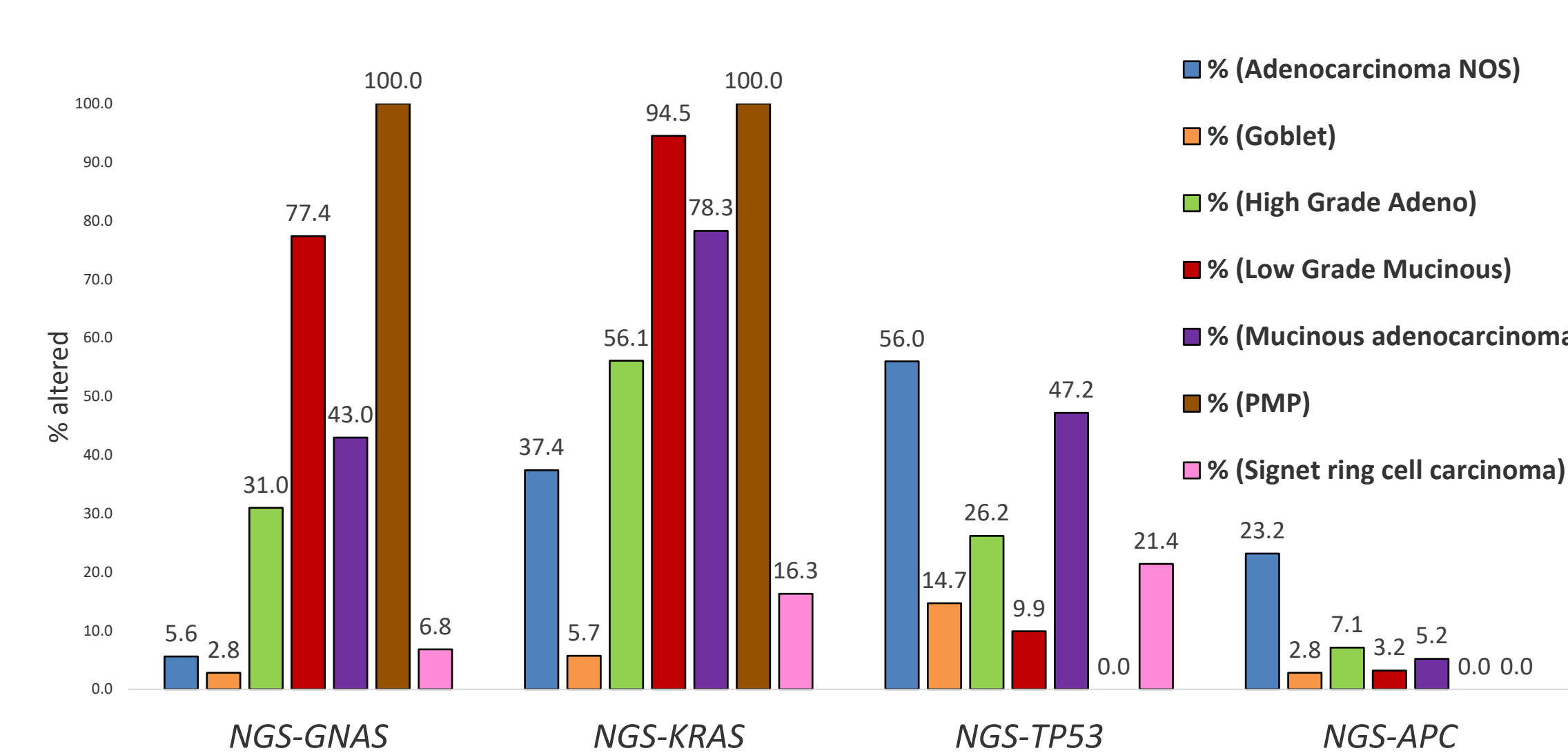


Table 5- TME (Quantiseq) comparison among appendiceal histotypes (highlighted q values indicate significance).

Immune Cell	Median cell infiltration						
	Adeno NOS	Goblet	High Grade Adeno	Low Grade Mucinous	Mucinous adeno	PMP	Signet ring cell
Macrophages M1	0.047	0.039	0.063	0.045	0.057	0.061	0.053
Dendritic cells	0.007	0.012	0.003	0.002	0	0	0.014
Uncharacterized cells	0.7004	0.6656	0.6554	0.7056	0.7077	0.6658	0.667
B cells	0.0467	0.0609	0.0536	0.0504	0.0495	0.0467	0.0624
Macrophages M2	0.0554	0.0739	0.0863	0.0491	0.0421	0.0461	0.0571
T cells CD8	0.0016	0.0038	0.0051	0.0001	0.0008	0.0058	0.0054
Monocytes	0 (0.40%)	0 (2.8%)	0 (0%)	0 (2.9%)	0 (1.3%)	0 (0%)	0 (4.3%)
Tregs	0.023	0.024	0.026	0.019	0.022	0.028	0.025
NK cells	0.034	0.041	0.032	0.037	0.031	0.039	0.035
Neutrophils	0.044	0.017	0.025	0.038	0.038	0.043	0.024
T cells CD4	0.006	0.002	0	0	0	0.014	0.007

Immune Cell	pairwise q values (Quantiseq)															
	Adeno NOS vs Goblet	Adeno NOS vs Low Grade Mucinous	Adeno NOS vs Mucinous adeno	Adeno NOS vs Signet ring	Goblet vs Low Grade Mucinous	Goblet vs High Grade Adeno	Goblet vs Mucinous adeno	Goblet vs PMP	High Grade Adeno vs Low Grade Mucinous	High Grade Adeno vs Mucinous adeno	High Grade Adeno vs Signet ring	Low Grade Mucinous vs Mucinous adeno	Low Grade Mucinous vs Signet ring	Mucinous adeno vs Signet ring	PMP vs Signet ring	
Macrophages M1	0.33	0.06	0.84	0.01	0.52	0.03	0.34	0.02	0.31	0.23	0.80	0.48	0.15	0.80	0.63	0.81
Dendritic cells	0.06	0.11	0.02	<0.001	0.16	0.01	<0.001	0.02	0.99	0.48	0.02	0.27	0.01	<0.001	0.03	0.91
Uncharacterized	0.07	0.05	0.78	0.55	0.02	1.00	0.13	0.06	0.99	0.10	0.04	0.95	0.99	0.04	0.01	0.91
B cells	0.01	0.17	0.21	0.64	<0.001	0.15	0.07	0.01	0.67	0.84	0.38	0.11	0.55	0.04	0.01	0.68
Macrophages M2	0.07	0.01	0.89	0.03	0.18	0.53	0.12	0.01	0.44	0.02	<0.001	0.46	0.17	0.15	0.01	0.38
T cells CD8	0.17	0.07	0.26	0.90	0.21	0.98	0.04	0.15	0.98	0.01	0.06	0.92	0.35	0.05	0.17	0.99
Monocytes	0.27	0.86	0.15	0.52	0.07	0.52	1.00	0.70	0.83	0.49	0.70	0.38	0.53	0.83	0.34	0.77
Tregs	0.80	0.15	0.09	0.84	0.33	0.55	0.18	0.63	0.92	0.01	0.08	0.81	0.15	0.03	0.21	0.99
NK cells	0.15	0.37	0.16	0.01	0.71	0.07	0.53	0.01	0.80	0.07	0.90	0.31	<0.001	0.82	0.06	0.87
Neutrophils	0.01	0.15	0.38	0.44	0.01	0.44	0.09	0.05	0.36	0.59	0.38	0.45	0.85	0.07	0.03	0.36
T cells CD4	0.90	0.44	0.64	0.04	0.70	0.54	0.68	0.34	0.96	0.80	0.92	0.33	0.44	0.48	0.12	0.99

Conclusions

- AC (N = 731) were grouped by histology: 5% goblet cell (GC), 6% high-grade adenocarcinoma (HGA), 14% low grade mucinous (LGM), 33% mucinous adenocarcinoma (MA), 1% pseudomyxoma peritonei (PMP), 6% signet ring cell carcinoma (SRC), and 35% adenocarcinoma not otherwise specified (NOS).
- Median TMB was significantly higher in NOS vs. GC (4 mutations/megabase vs 2, q < 0.001), NOS vs. LGM (4 vs 3, q = 0.048), MA vs. GC (4 vs 2, q < 0.001), and MA vs. LGM (4 vs 3, q = 0.037).
- Distinct TME patterns were observed in NOS vs. MA (median cell fraction: dendritic cells 0.07 vs 0, q < 0.01; M2 macrophages 0.055 vs 0.042, q = 0.030; natural killer cells 0.034 vs 0.031, q = 0.011; CD4 T cells 0.006 vs 0, q = 0.044) and GC vs. MA (M1 macrophages 0.039 vs 0.057, q = 0.021; dendritic cells 0.012 vs 0, q < 0.01; B cells 0.061 vs 0.050, q = 0.013; M2 macrophages 0.074 vs 0.042, q < 0.01; natural killer cells 0.041 vs 0.031, q = 0.01; and neutrophils 0.017 vs 0.038 q = 0.045).
- There is significant heterogeneity in TMB, TME, and mutational profiles across AC histologies. MA has a particularly immune-cold TME shown by lower infiltration of lymphocytes, TIS, and IO gene expression. These findings are critical to identify novel biomarkers and develop new therapeutic strategies for AC.