2021 ASCO® ANNUAL MEETING

LARGE SCALE MULTI-OMIC ANALYSIS SUGGESTS MECHANISMS OF RESISTANCE TO IMMUNOTHERAPY IN LEIOMYOSARCOMA

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CARIS POA™ PRECISION ONCOLOGY ALLIANCE

Background

Leiomyosarcoma (LMS)

- Rare mesenchymal tumors of smooth muscle differentiation
- 10-20% of newly diagnosed soft tissue sarcomas
- Most commonly arise in the uterus, retroperitoneum, skin, other soft tissue sites
- Genetically complex tumors without clear oncogenic drivers
- Limited effective systemic treatment options

Immune Microenvironment (IME) in LMS

- Immunophenotyping of 19 LMS tumors suggests an inflamed IME relative to other sarcoma subtypes (Pollack et al. Cancer 2017)
    - Higher expression of genes related to antigen presentation and T-cell-mediated immunity, higher T cell receptor clonality compared to LPS and SS, 35% of LMS with high PD-L1 expression by IHC

- A subset of LMS have high densities of tumor associated macrophages (TAMs); in non-gynecologic LMS increased TAM density is a negative predictor of disease specific survival (Lee et al. Clinical Cancer Research 2008)

Clinical Experiences with Immunotherapy in Leiomyosarcoma

SARC 028- phase II trial of pembrolizumab in sarcomas (Tawbi et al Lancet Oncology 2017)

- 0/10 LMS patients with treatment response

Phase II trial of nivolumab in uterine leiomyosarcoma (Ben-Ami et al. Cancer 2017)

- 0/12 patients with treatment response

Alliance A091401- two phase II trials of nivolumab+/- ipilimumab in STS (D'Angelo et al Lancet Oncology 2018)

- 1/15 LMS patients with response to nivolumab monotherapy
- 2/14 LMS patients with response to nivolumab/ipilimumab

Study Design

Study Objectives

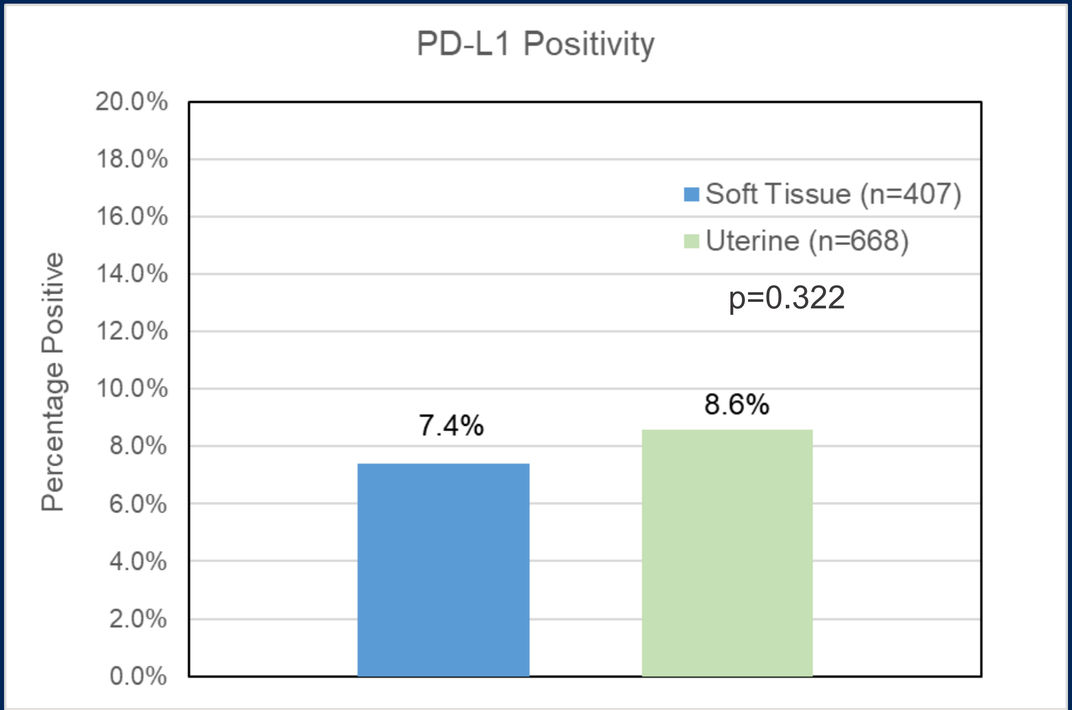
- Explore potential mechanisms of immunotherapy resistance in LMS with comprehensive genomic profiling
- Characterize the immune microenvironment and immune related gene expression within LMS subtypes and compare with melanoma

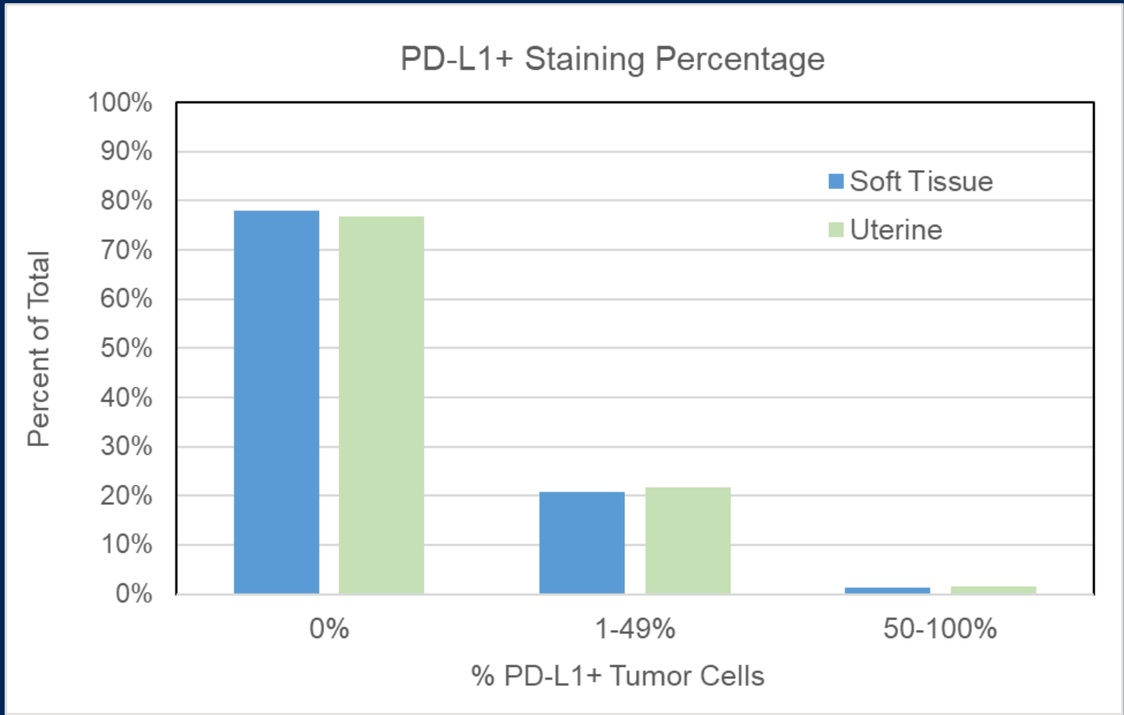
Retrospective genomic and transcriptomic analysis of 1115 LMS specimens sequenced through Caris Life Sciences

Characteristic	All cases	Soft tissue LMS	Uterine LMS
Total, N specimens (%) - N patients (%)	1115 (100%) 1077 (100%)	414 (37.1%) 407 (36.5%)	701 (62.9%) 671 (62.3%)
Median Age, years (range)	59 (9-90+)	64 (9-90+)	57 (23-90+)
Female, N (%)	961 (86.2%)	260 (62.8%)	701 (100%)
Metastatic+Local recurrence, N(%)	561 (50.3%)	205 (49.5%)	356 (50.8%)
Analyzed by WTS, N (%)	535 (48.0%)	315 (76.1%)	220 (31.4%)

PD-L1 Expression

- Immunohistochemistry for PD-L1 (SP142)
  - PD-L1 positive = 2+ stain intensity, ≥5% positive tumor cells

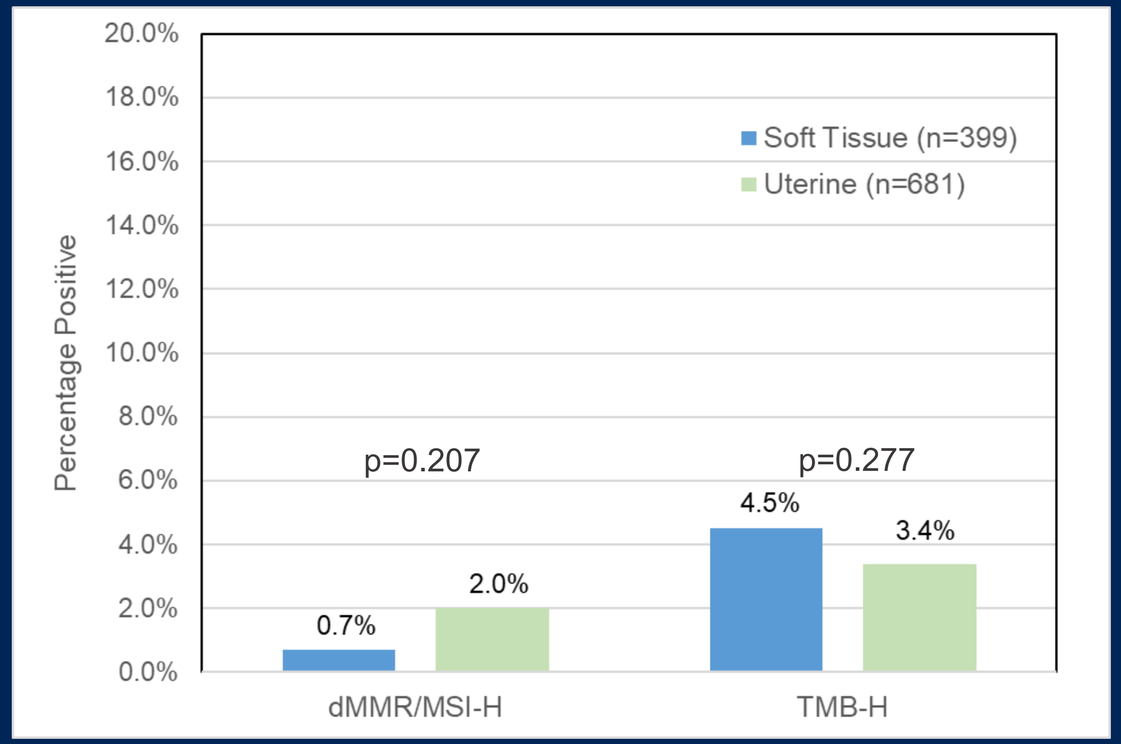


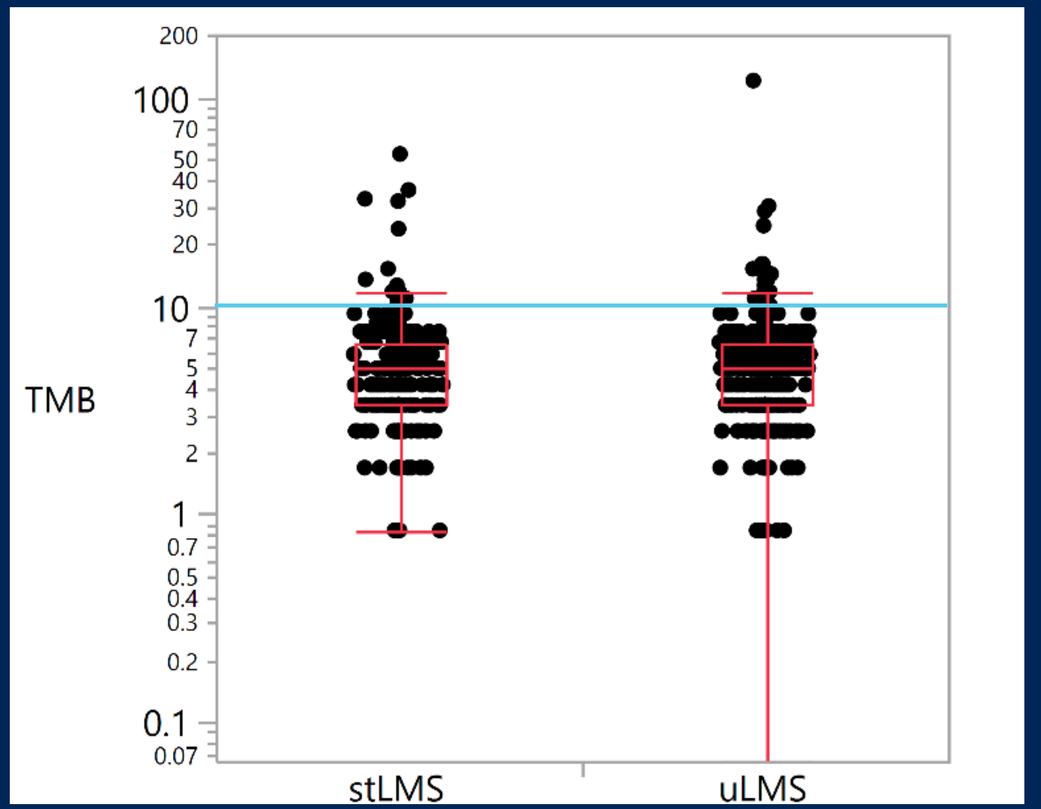


Tumor Mutation Burden and dMMR/MSI-H Status

- dMMR evaluated with IHC, MSI-H with NGS
- TMB-H defined as ≥10 mutations/Mb

	Median TMB (mut/mb)	IQR	P-value
Soft Tissue	5	3.3-6.7	0.498
Uterine	5	3.3-6.7	





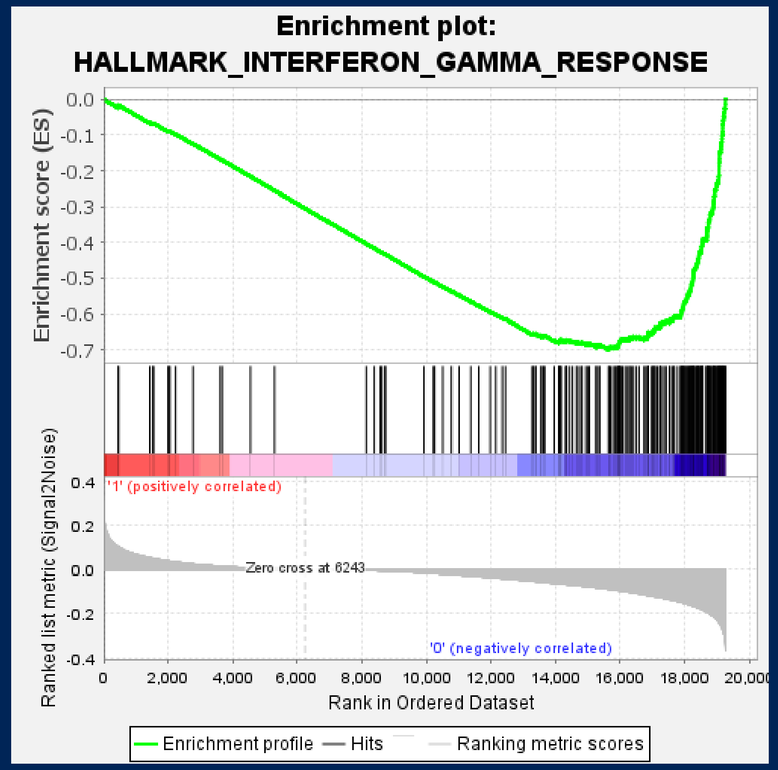
Gene Set Enrichment Analysis – stLMS vs. uLMS

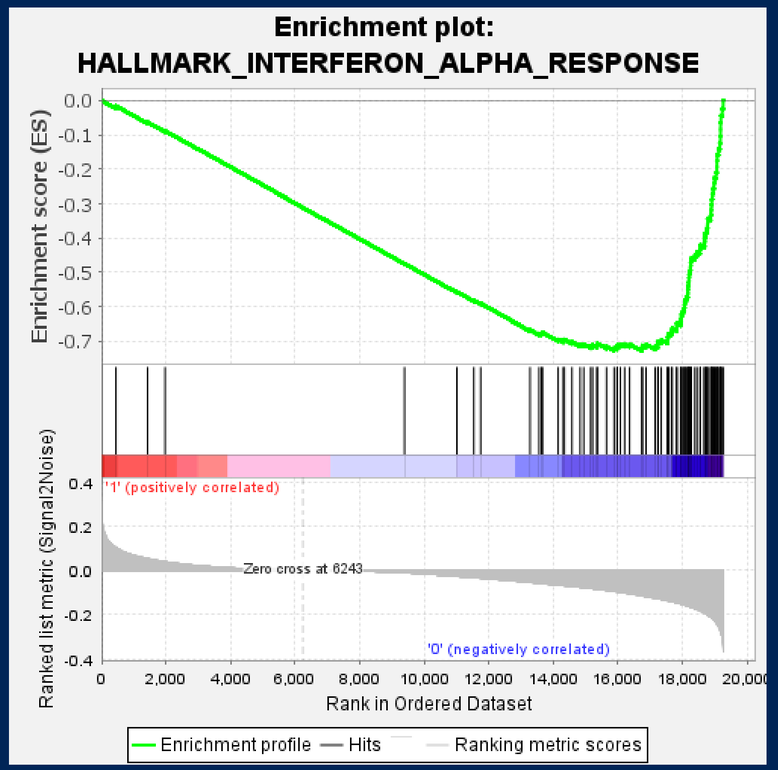
- Immune related gene sets are enriched in stLMS compared to uLMS

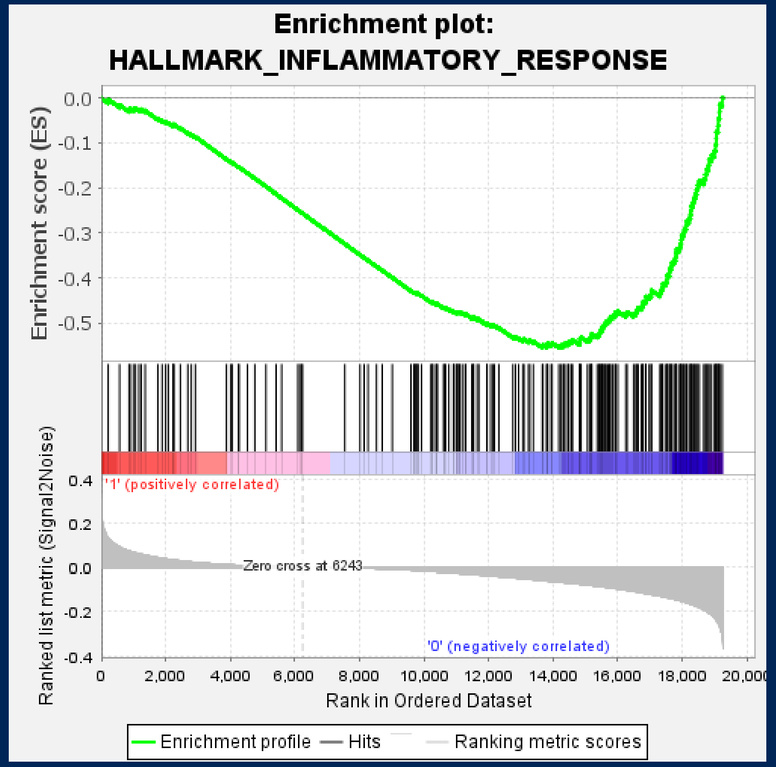
Hallmark Gene Set	NES	FDR q-value
Interferon Gamma Response	-1.744	0.021
Interferon Alpha Response	-1.675	0.028
Inflammatory Response	-1.658	0.024
IL-6/JAK/STAT3 Signaling	-1.611	0.031
TNFα Signaling via NF-KB	-1.610	0.025

NES= normalized enrichment score; FDR=false discovery rate, (-)NES = enriched in stLMS

Red= uLMS; Blue=stLMS



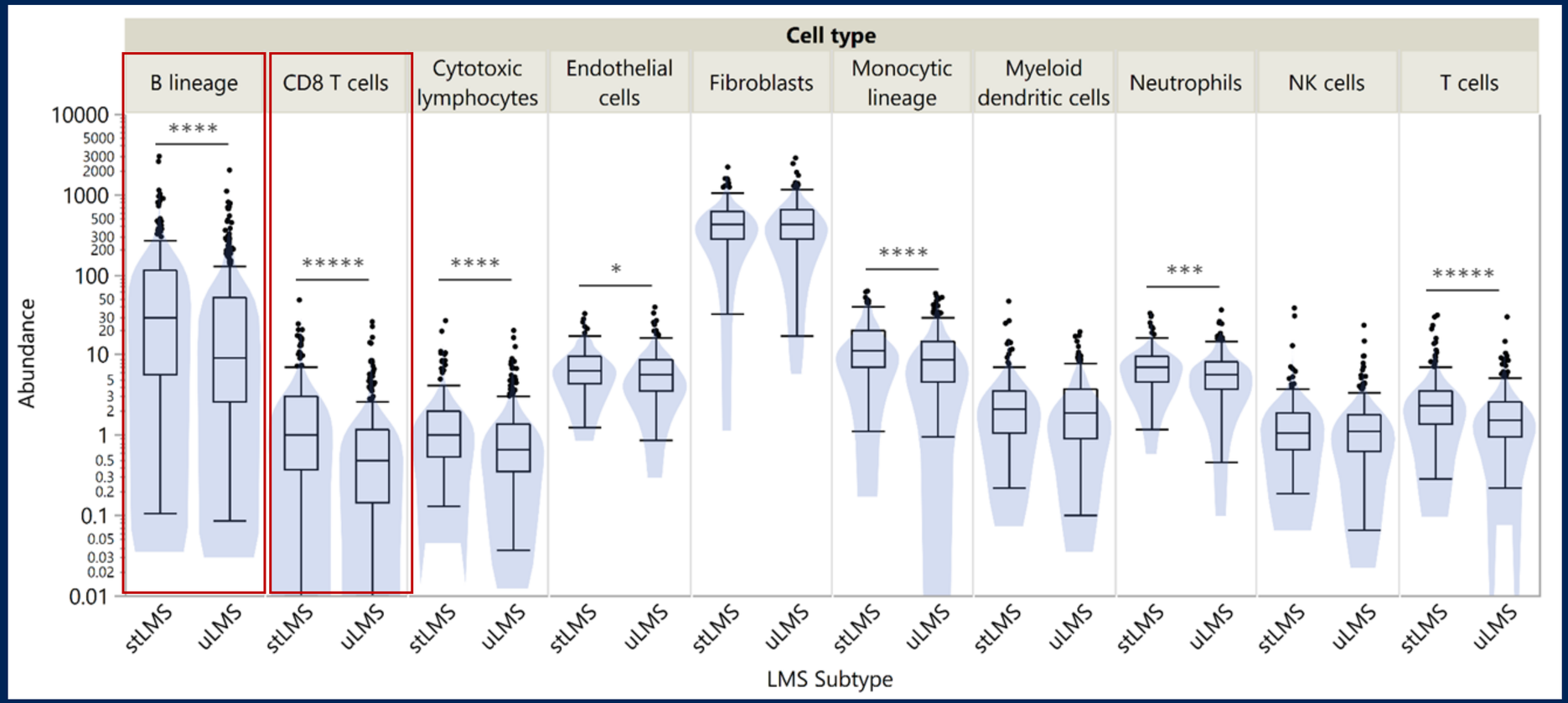




Immune cell infiltration derived from transcriptomic analysis – stLMS vs. uLMS

Immune cell infiltration is increased in stLMS over uLMS

- >2-fold increase in CD8 T cell and B cell abundance ($p<0.0001$)



*$p<0.05$, adjusted for multiple hypothesis testing (Benjamini-Hochberg)

Association between markers of IO response and immune cell infiltration in the overall LMS cohort

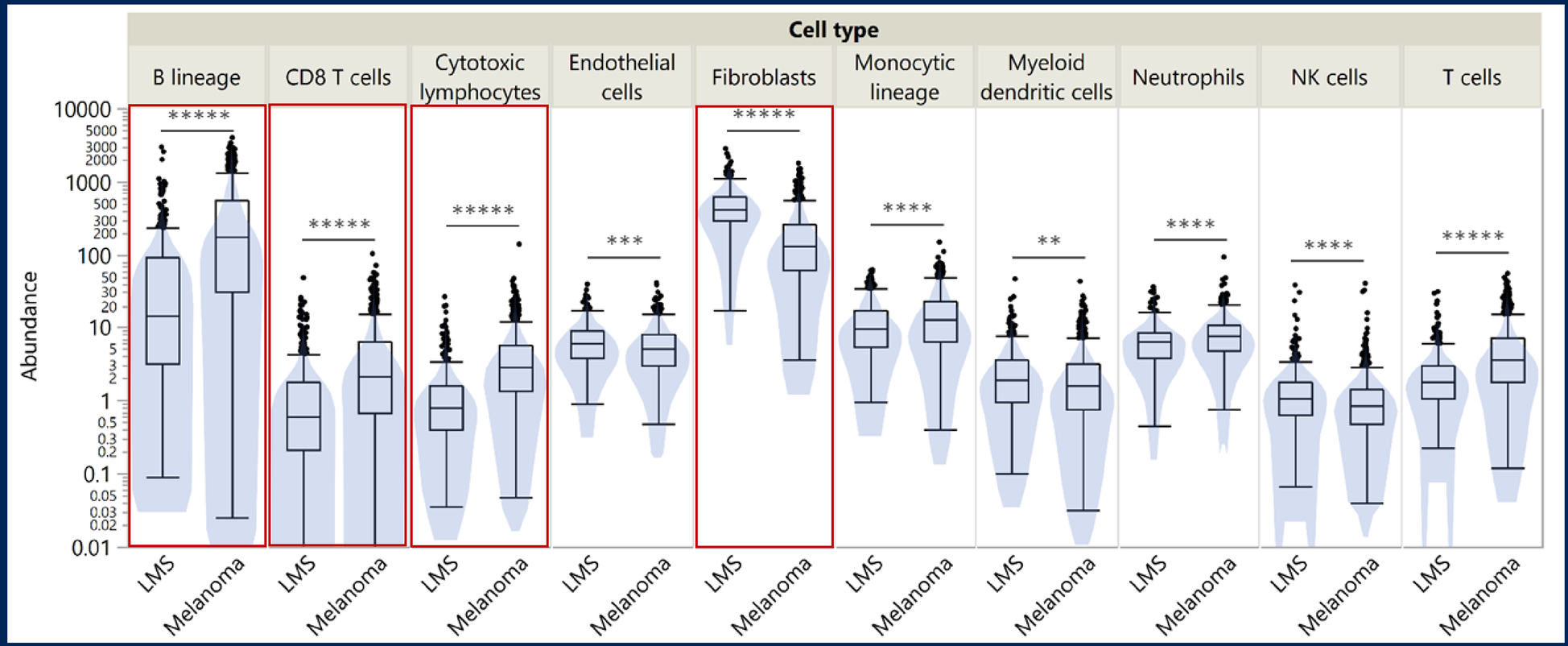
Cell Type	Biomarker	Median abundance	P-value
CD8+ T cell	MSS	0.6	0.0259
	MSI-High	2.5	
	TMB-Low	0.6	0.0116
	TMB-High	1.6	
	PD-L1-neg	0.6	0.0015
	PD-L1-pos	2.0	
B cell	MSS	14.3	0.0687
	MSI-High	92.2	
	TMB-Low	13.9	0.0082
	TMB-High	113.8	
	PD-L1-neg	13.9	0.0091
	PD-L1-pos	25.5	

Immune cell infiltration derived from transcriptomic analysis– LMS vs. melanoma

Immune cell infiltration is increased in melanoma over LMS

- >11-fold increase in B cell abundance
- >3 fold increase in CD8+ and cytotoxic T cell abundance

Fibroblasts more prevalent in LMS over melanoma (>3 fold increase)



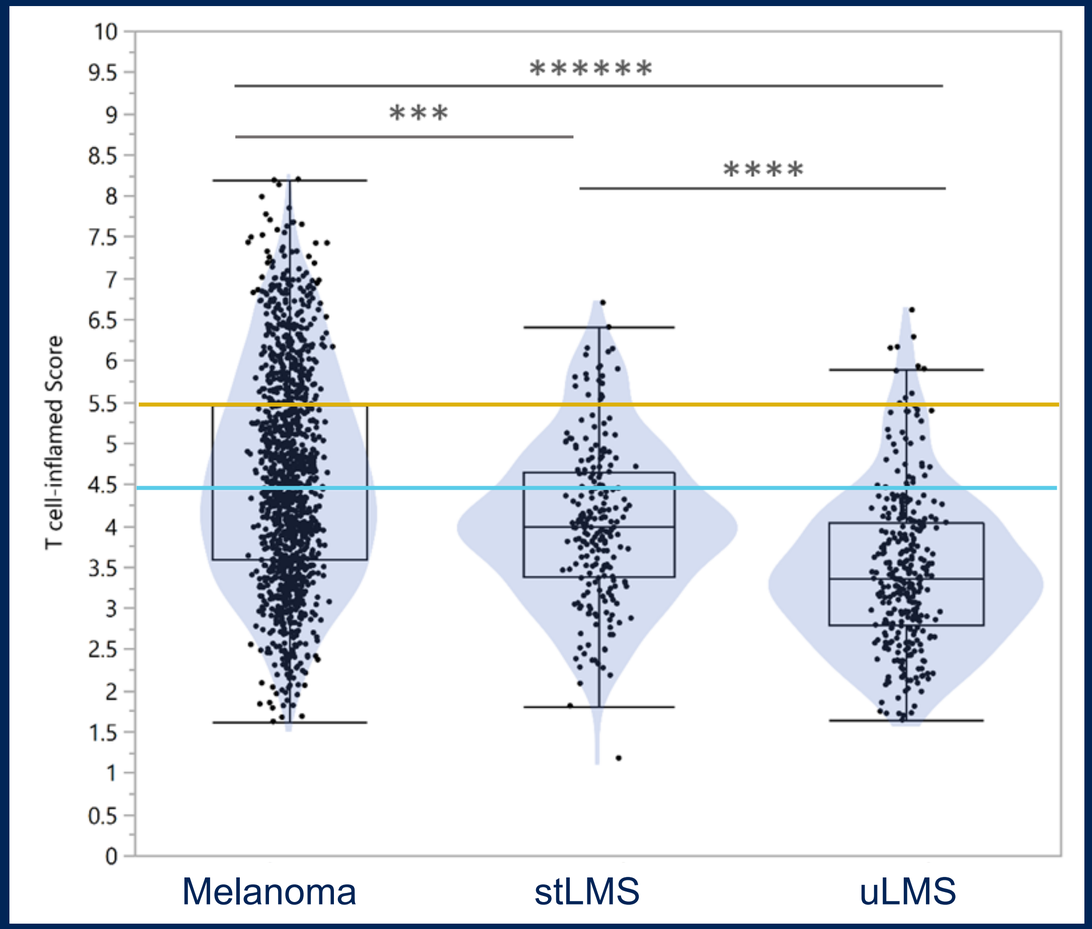
LMS n=537, Melanoma n=1255

*p<0.05, adjusted for multiple hypothesis testing (Benjamini-Hochberg)

T-cell Inflamed Signature– LMS vs. melanoma

- T-cell Inflamed Signature (TIS)= 18 gene panel indicative of a T cell activated TME that is associated with response to pembrolizumab across multiple solid tumors (Ayers et al JCI 2017, Danaher et al JITC 2018)
- TIS scores are significantly higher in melanoma relative to both stLMS and uLMS

Melanoma Quartile	stLMS	uLMS
Q4	9.1%	3.2%
Q3	20.5%	9.2%
Q2	39.5%	26.7%
Q1	30.9%	61.0%



Conclusions

- Small proportion of LMS with classic markers of IO response
- stLMS has a more immune active microenvironment relative to uLMS
- TME in LMS has low T cell and high fibroblast abundance relative to melanoma
- A smaller proportion of LMS have a T cell inflamed microenvironment relative to melanoma

Future Directions

- Incorporate clinical data to determine the predictive role of these biomarkers in LMS for immunotherapy response
- Validate and refine our findings with IHC studies and single cell sequencing
- Design IO trials using combination therapy in LMS to overcome the observed T-cell exclusion/desmoplastic phenotype

