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ESMO

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Atlas of Tertiary Lymphoid Structures in Solid Tumors: Genomic Features and Prediction of Response to Immunotherapy

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Declaration of Interests

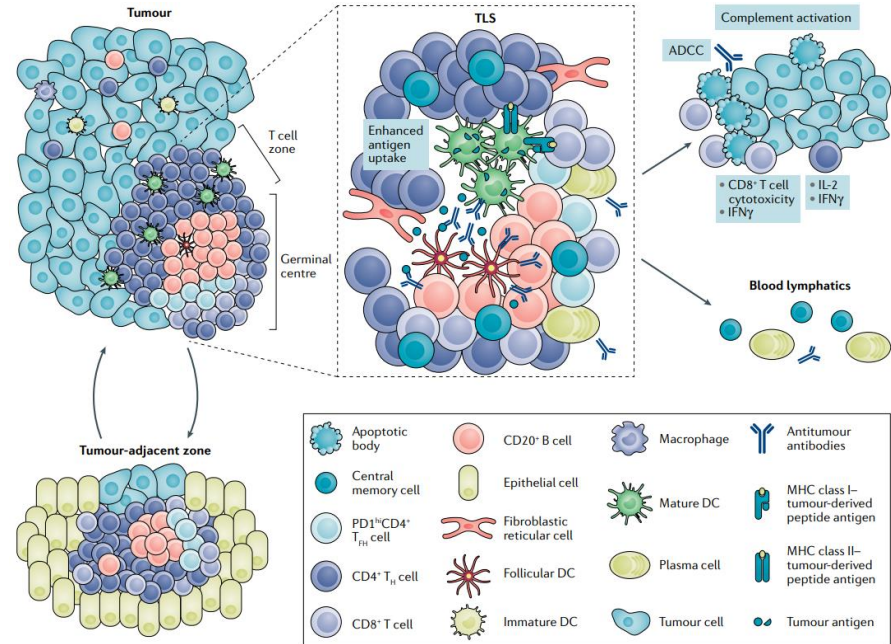
Antoine ITALIANO

- Consulting or Advisory Role .
- Research Funding (Institution) .

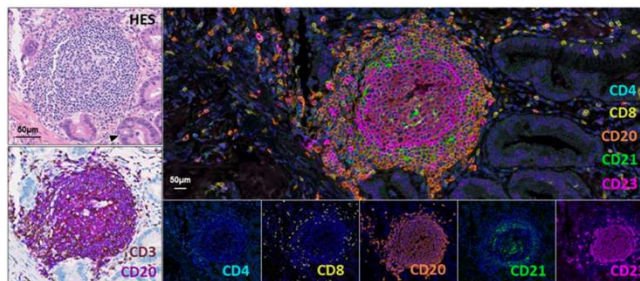
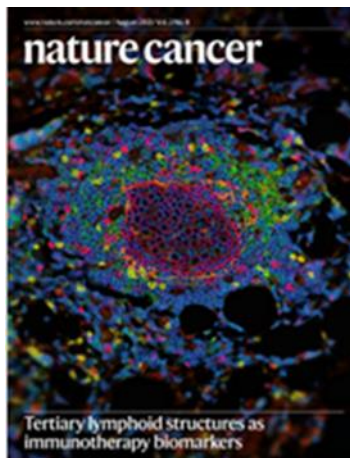
Tertiary Lymphoid Structures in Cancer

TLS in within the tumour microenvironment are critical regulators of the antitumor immune responses.

Associated with improved prognosis in several tumor types

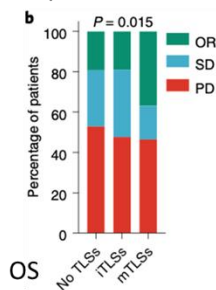


Predictive Role of Tertiary Lymphoid Structures

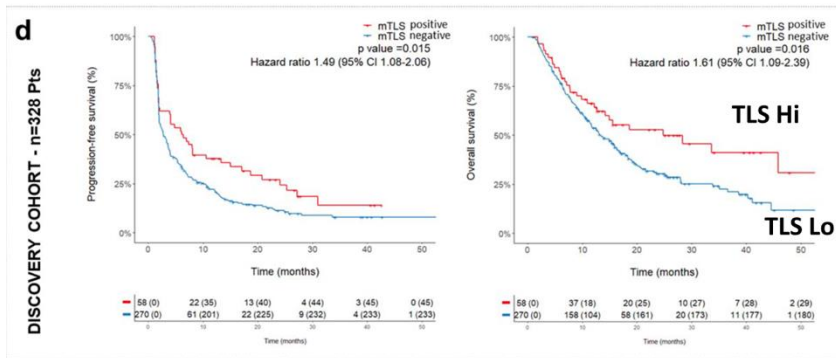
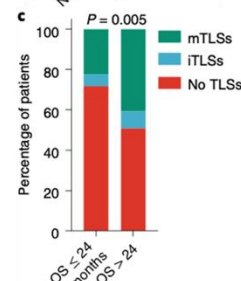


Mature TLS are predictive of response in cancer patients treated with immune-checkpoint inhibitors (anti-PD1 or anti-PD-L1)

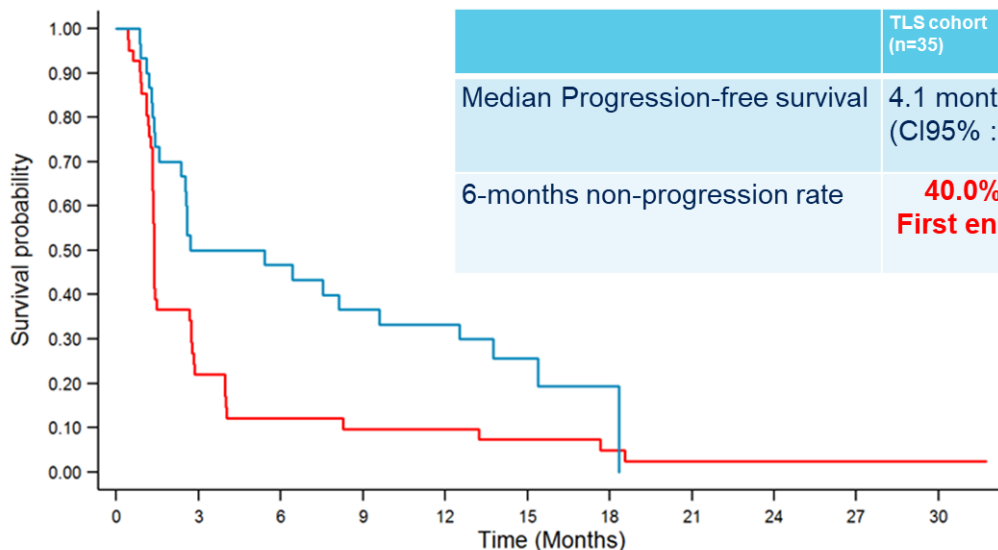
Response



OS



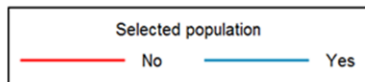
Predictive Role of Tertiary Lymphoid Structures



	TLS cohort (n=35)	Previous cohorts (all comers) n=41
Median Progression-free survival	4.1 months (CI95% : 2.4-12.5)	1.4 months* (CI95% : 1.3-2.7)
6-months non-progression rate	40.0% (22.7 – 59.4) First endpoint reached	4.9% (0.6 – 16.5)

At risk (Events)

No	41	(32)	9	(4)	5	(1)	4	(0)	4	(1)	3	(1)	2	(1)	1	(0)	1	(0)	1	(0)	1
Yes	30	(15)	15	(1)	14	(3)	11	(1)	10	(2)	4	(2)	1	(0)	0	(0)	0	(0)	0	(0)	0



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<https://doi.org/10.1038/s41591-022-01821-1>

Check for updates

Pembrolizumab in soft-tissue sarcomas with tertiary lymphoid structures: a phase 2 PEMBROSARC trial cohort

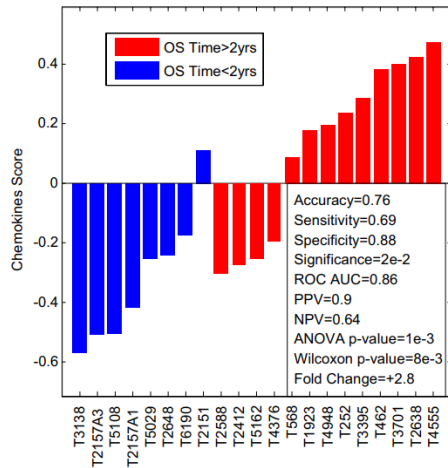
A. Italiano^{1,2,3,10,12}, A. Bessedé^{4,10}, M. Pulido^{5,6,10}, E. Bompas⁷, S. Piperno-Neumann⁸, C. Chevreau⁹, N. Penel¹⁰, F. Bertucci¹¹, M. Toulmonde¹, C. Bellera^{3,6}, J. P. Guegan⁴, C. Rey⁴, C. Sautès-Fridman^{12,13}, A. Bougouin^{12,13}, C. Cantarel¹⁴, M. Kind¹⁴, M. Spalato¹, B. Dadone-Montaudie¹⁵, F. Le Loarer^{3,16}, J. Y. Blay¹⁷ and W. H. Fridman^{12,13}

Immune checkpoint inhibitors (ICIs) show limited clinical activity in patients with advanced soft-tissue sarcomas (STSs). Retrospective analysis suggests that intratumoral tertiary lymphoid structures (TLSs) are associated with improved outcome in these patients. PEMBROSARC is a multicohort phase 2 study of pembrolizumab combined with low-dose cyclophosphamide in patients with advanced STS (NCT02406783). The primary endpoint was the 6-month non-progression rate (NPR). Secondary endpoints included objective response rate (ORR), progression-free survival (PFS), overall survival (OS) and safety. The 6-month NPR and ORRs for cohorts in this trial enrolling all comers were previously reported; here, we report the results of a cohort enrolling patients selected based on the presence of TLSs (n = 30). The 6-month NPR was 40% (95% confidence interval [CI], 22.7–59.4), so the primary endpoint was met. The ORR was 30% (95% CI, 14.7–49.4). In comparison, the 6-month NPR and ORR were 4.9% (95% CI, 0.6–16.5) and 2.4% (95% CI, 0.1–12.9), respectively, in the all-comer cohorts. The most frequent toxicities were grade 1 or 2 fatigue, nausea, dysthyroidism, diarrhea and anemia. Exploratory analyses revealed that the abundance of intratumoral plasma cells (PCs) was significantly associated with improved outcome. These results suggest that TLS presence in advanced STS is a potential predictive biomarker to improve patients' selection for pembrolizumab treatment.

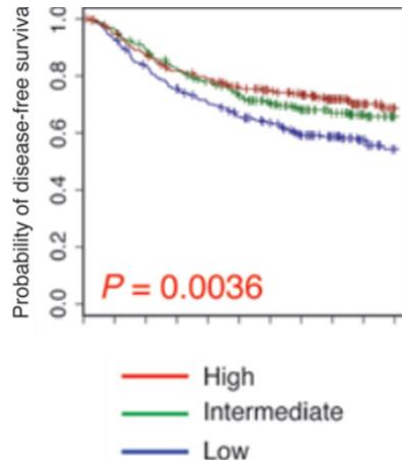
TLS gene expression signatures

- Various TLS and B cells related gene signatures have been proposed in the literature
 - Generally focus on chemokines related to the formation of TLS – E.g. CXCL13

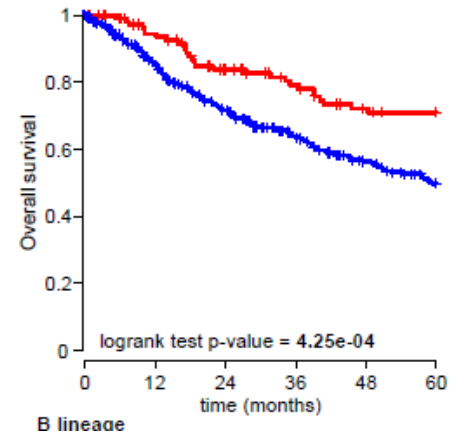
2 gene TLS signature associates with OS in CRC



Tfh signature associates with Breast Cancer DFS



B Lineage signature associates with Sarcoma outcomes



CARIS Real World Evidence database

- Caris molecular profiling utilizes a comprehensive approach including exome and RNA sequencing to guide more precise and individualized treatment decisions.
- **204,231 patients** with RNA-seq data for TLS signature analysis and linked insurance claims for clinical outcome association. Represents **over 50 cancer types**.
- **Treatment data** includes:
 - 26,868 patients treated with pembrolizumab
 - 9,654 patients treated with nivolumab
 - 4,483 patients treated with ipilimumab
 - 3,773 patients treated with durvalumab
 - 3,430 patients treated with atezolizumab
 - 546 patients treated with avelumab

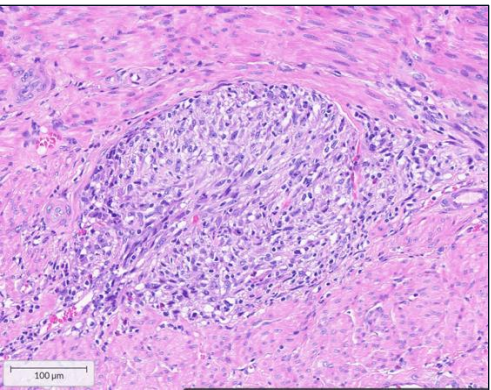
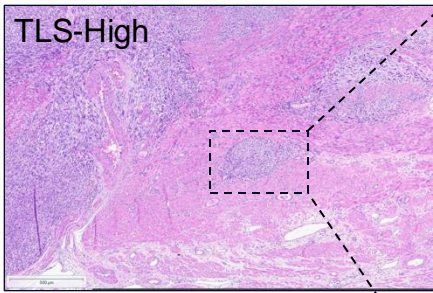
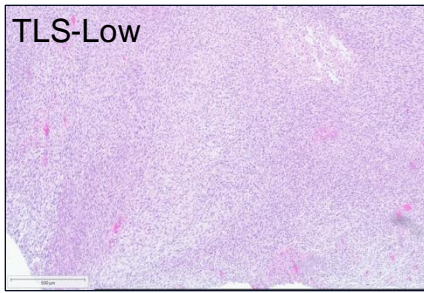
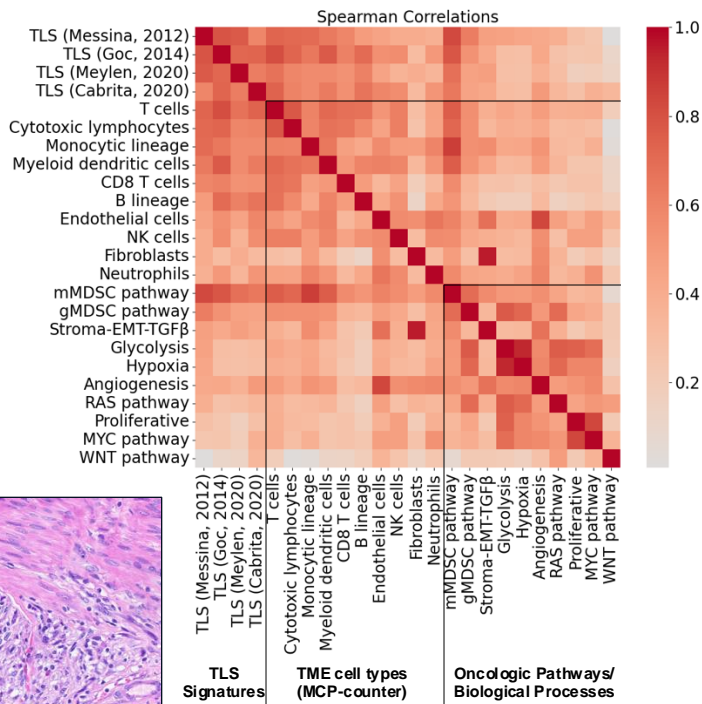
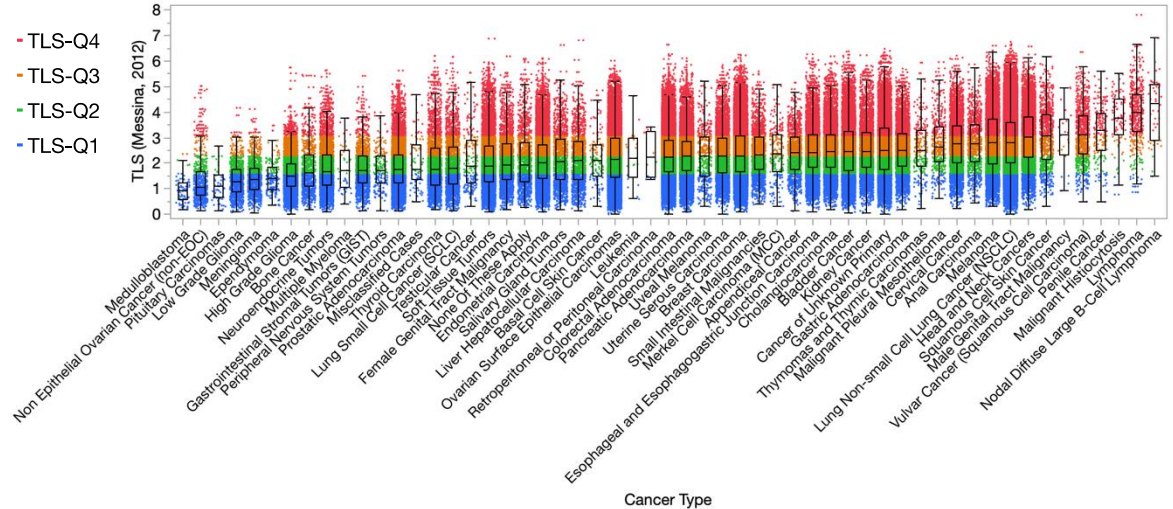
Study objectives

- **Evaluate predictive value of B cell/TLS signatures** identified through RNA-seq for patient survival following immune checkpoint inhibitor (ICI) versus other therapies in solid tumors.
- **Characterize the genomic landscape** (mutational status, copy number variations) of tumors enriched with B cell/TLS signatures compared to those without.
- **Compare genomic profiles** (mutational status, copy number variations) between responders and non-responders to ICI therapy in B cell/TLS signature-enriched tumors.

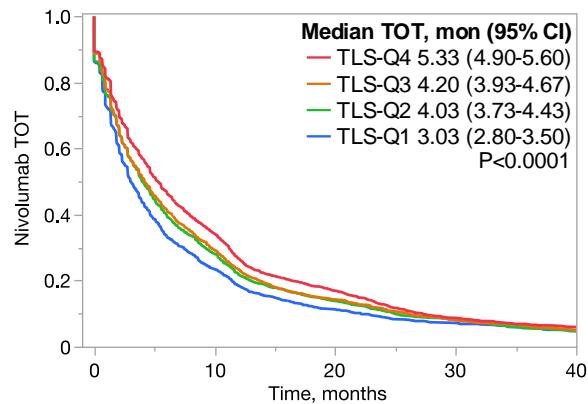
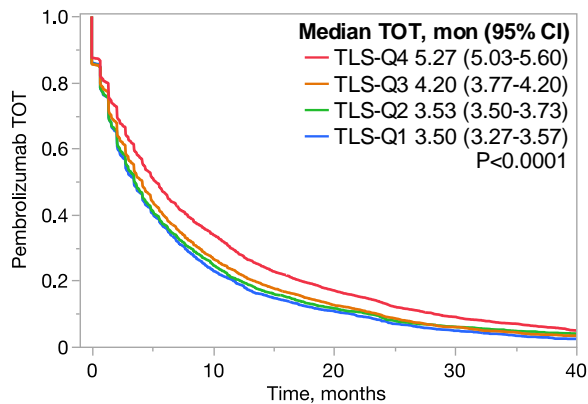
Study population

Age	Median	Range	Median TLS score
Years, at time of biopsy collection	66	0-90+	2.30
Sex	N	%	Median TLS score
Male	88340	43.24%	2.31
Female	115981	56.76%	2.30
Cancer Type	N	%	Median TLS score
Lung Non-small Cell Lung Cancer (NSCLC)	35324	17.29%	2.81
Colorectal Adenocarcinoma	24630	12.05%	2.24
Ovarian Surface Epithelial Carcinomas	16033	7.85%	2.16
Breast Carcinoma	15936	7.80%	2.30
Pancreatic Adenocarcinoma	9607	4.70%	2.26
Endometrial Carcinoma	8692	4.25%	2.02
Prostatic Adenocarcinoma	7619	3.73%	1.75
Cancer of Unknown Primary	7002	3.43%	2.50
Bladder Cancer	6854	3.35%	2.45
High Grade Glioma	6401	3.13%	1.51
Esophageal and Esophago-gastric Junction Carcinoma	6293	3.08%	2.42
Soft Tissue Tumors	6055	2.96%	1.90
Cholangiocarcinoma	5536	2.71%	2.44
Melanoma	5282	2.59%	2.79
Head and Neck Cancers	4556	2.23%	3.01
Uterine Serous Carcinoma	3823	1.87%	2.28
Gastric Adenocarcinoma	3650	1.79%	2.51
Cervical Cancer	3499	1.71%	2.76
Neuroendocrine Tumors	3211	1.57%	1.66
Kidney Cancer	3033	1.48%	2.46
Other	21285	10.42%	2.03
Tumor Mutational Burden (≥ 10 mut/Mb)	N	%	Median TLS score
TMB-High	35240	18.69%	2.74
TMB-Low	153268	81.31%	2.21
Not tested/indeterminate	15813	NA	NA
Microsatellite Instability	N	%	Median TLS score
MSI-High	6355	3.76%	2.67
MSS	162880	96.24%	2.28
Not tested/indeterminate	35086	NA	NA
PD-L1 (22c3) IHC	N	%	Median TLS score
0%	17037	17.05%	2.07
1-49%	38629	38.65%	2.72
$\geq 50\%$	44284	44.31%	3.42
Not tested/indeterminate	104371	NA	NA
PD-L1 (28-8) IHC	N	%	Median TLS score
0%	15037	45.20%	2.43
1-49%	11530	34.66%	2.83
$\geq 50\%$	6703	20.15%	3.36
Not tested/indeterminate	171051	NA	NA
PD-L1 (SP142) IHC	N	%	Median TLS score
0%	123948	76.57%	1.99
1-49%	32064	19.81%	2.50
$\geq 50\%$	5873	3.63%	3.35
Not tested/indeterminate	42436	NA	NA
Treatment	N	%	Median TLS score
Pembrolizumab	26868	13.15%	2.64
Nivolumab (any)	9654	4.72%	2.58
Ipilimumab (any)	4483	2.19%	2.59
Nivolumab+Ipilimumab	4308	2.11%	2.58
Durvalumab	3773	1.85%	2.76
Atezolizumab	3430	1.68%	2.48
avelumab	546	0.27%	2.42

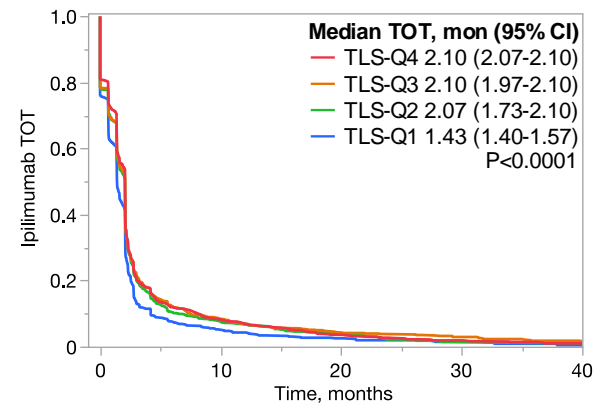
TLS Signatures correlates with Immune Cell Composition and Histological Presence in Various Tumor Types



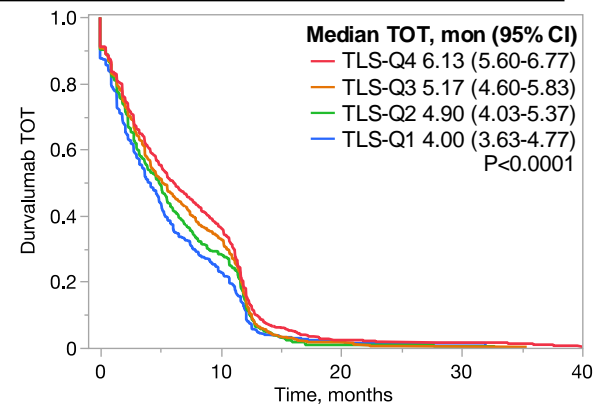
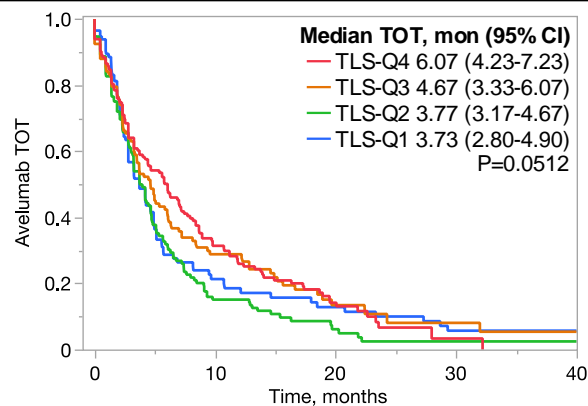
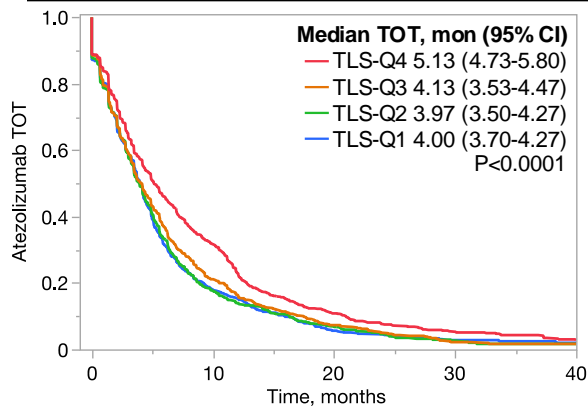
Anti-PD1 treatments



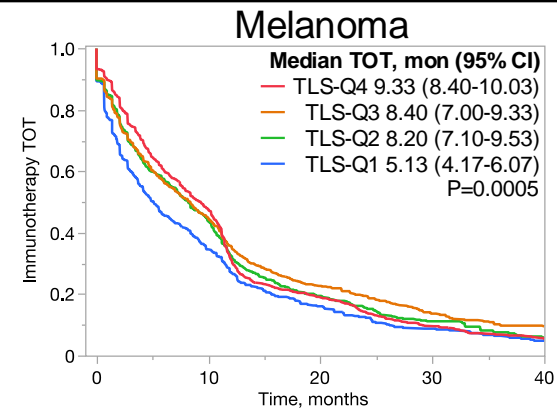
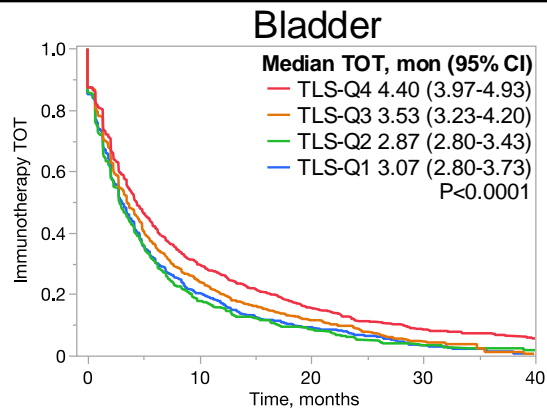
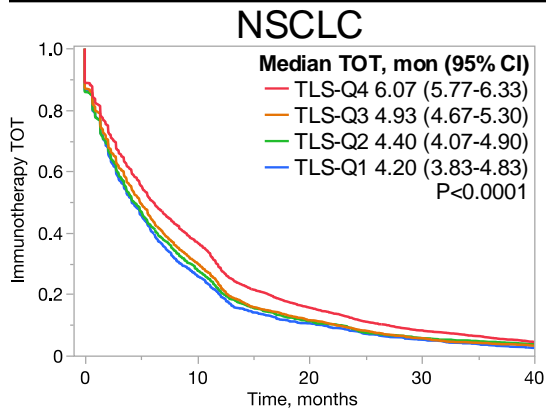
Anti-CTLA4 treatment



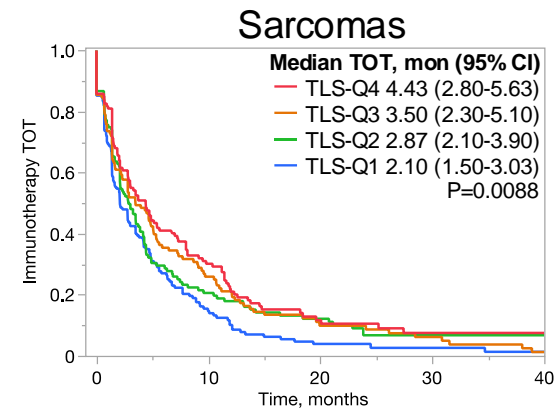
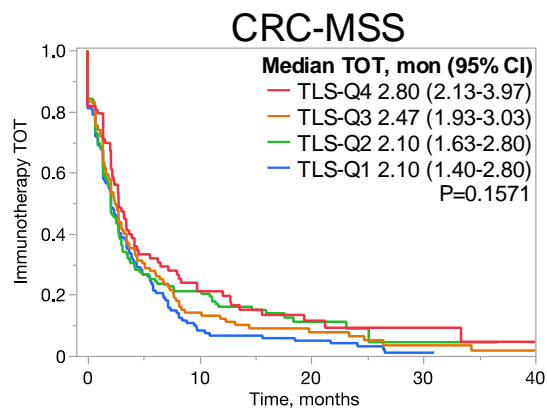
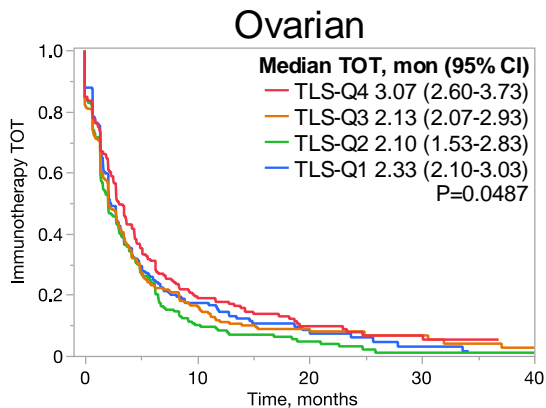
Anti-PDL1 treatments



Cancers commonly treated with Immunotherapy



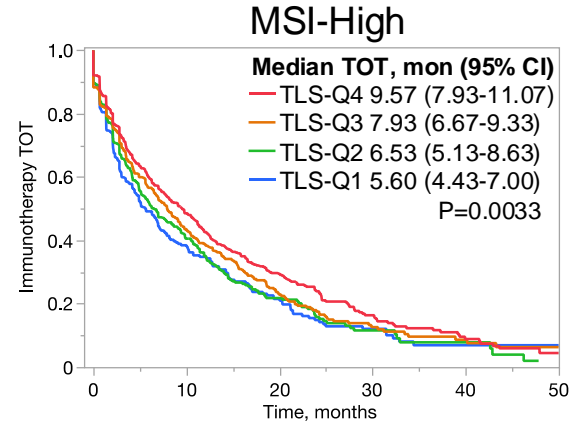
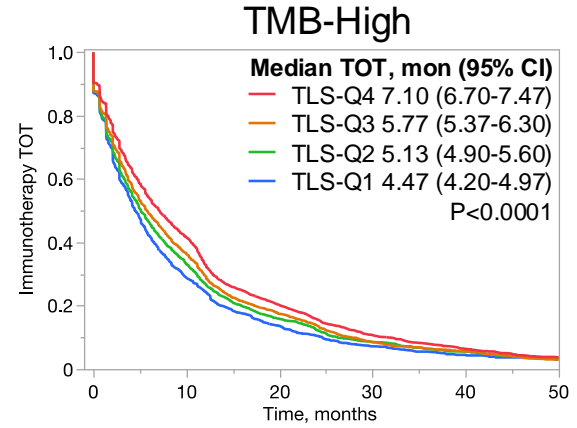
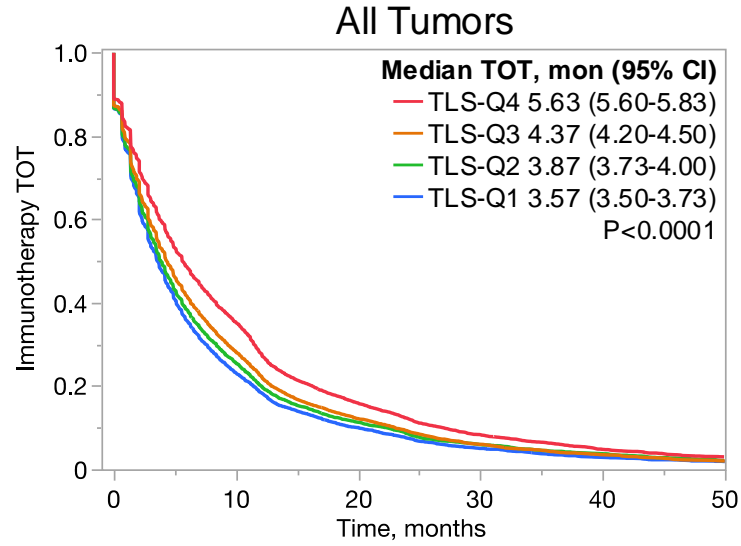
Cancers hard to treat with Immunotherapy



High TLS gene expression score is an independent predictor of improved TOT on immune-checkpoint inhibitor

Variable	Subgroup	N Total	Median TOT	Lower 95%	Upper 95%	Univariate Model				Multivariate Model			
						Uni HR	Lower 95%	Upper 95%	P-value	Multi HR	Lower 95%	Upper 95%	P-value
Cancer Type	NSCLC	19655	4.93	4.90	5.13	0.94	0.92	0.95	<0.0001	0.99	0.96	1.02	0.4822
	Other	38791	4.17	4.00	4.20	Ref	---	---	---	Ref	---	---	---
Sex	Male	29024	4.40	4.27	4.47	0.99	0.97	1.00	0.1188	0.97	0.94	0.99	0.0055
	Female	29422	4.23	4.20	4.33	Ref	---	---	---	Ref	---	---	---
Age at Biopsy	Above	29785	4.20	4.20	4.33	1.01	0.99	1.03	2.21E-01	1.03	1.01	1.06	0.0066
	Below	28661	4.43	4.27	4.47	Ref	---	---	---	Ref	---	---	---
MSI Status	MSI-High	2418	7.37	6.93	8.17	0.69	0.66	0.73	<0.0001	0.75	0.71	0.80	<0.0001
	MSS	40588	4.23	4.20	4.37	Ref	---	---	---	Ref	---	---	---
TMB Status	High	17328	5.83	5.63	6.03	0.75	0.74	0.77	<0.0001	0.83	0.81	0.86	<0.0001
	Low	34608	3.73	3.67	3.73	Ref	---	---	---	Ref	---	---	---
PD-L1 expression	≥50%	8788	5.83	5.60	6.23	0.80	0.78	0.82	<0.0001	0.89	0.85	0.92	<0.0001
	1-49%	20105	4.40	4.23	4.47	0.95	0.93	0.96	<0.0001	0.97	0.95	1.00	0.0414
	0%	24532	3.93	3.73	4.00	Ref	---	---	---	Ref	---	---	---
TLS Score Quartiles	TLS-Q4	14547	5.63	5.60	5.83	0.76	0.74	0.78	<0.0001	0.80	0.77	0.83	<0.0001
	TLS-Q3	10756	4.37	4.20	4.50	0.89	0.86	0.92	<0.0001	0.91	0.87	0.94	<0.0001
	TLS-Q2	8907	3.87	3.73	4.00	0.94	0.91	0.97	0.0005	0.95	0.91	0.99	0.0086
	TLS-Q1	7501	3.57	3.50	3.73	Ref	---	---	---	Ref	---	---	---

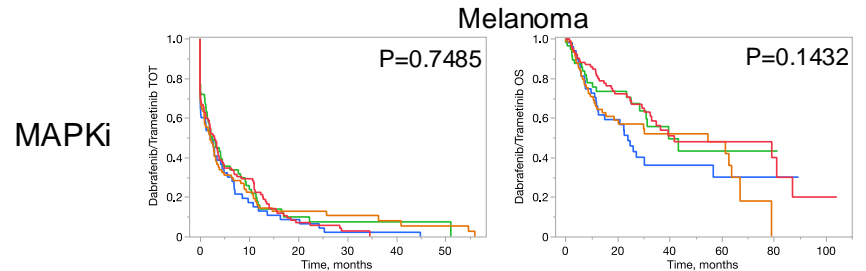
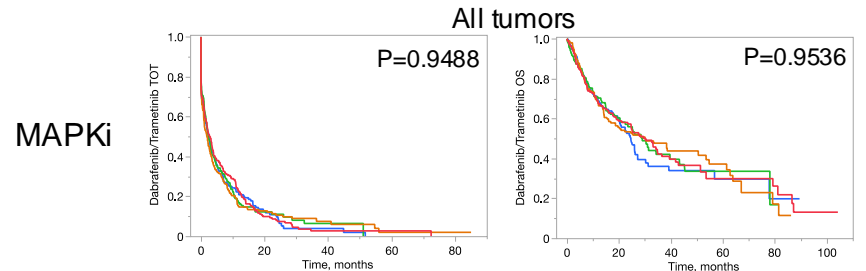
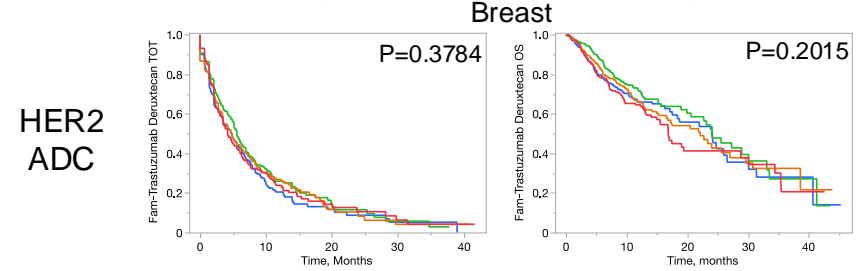
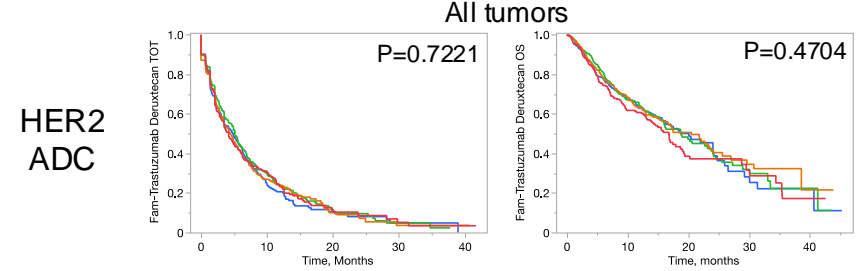
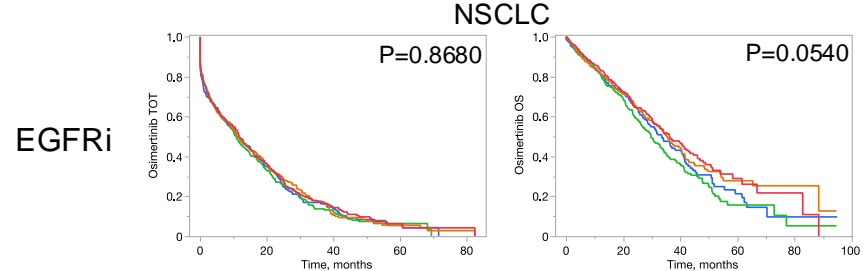
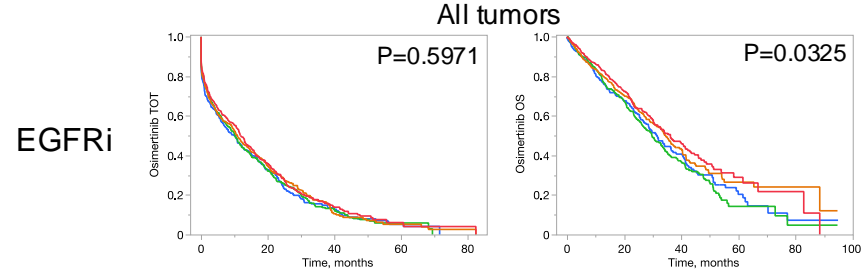
High TLS gene expression score is an independent predictor of improved TOT on immune-checkpoint inhibitor



High TLS gene expression score is an independent predictor of improved OS on immune-checkpoint inhibitor

Variable	Subgroup	N Total	Median OS	Lower 95%	Upper 95%	Univariate Model				Multivariate Model			
						Uni HR	Lower 95%	Upper 95%	P-value	Multi HR	Lower 95%	Upper 95%	P-value
Cancer Type	NSCLC	19655	19.33	18.90	19.83	1.00	0.98	1.02	<0.0001	1.04	1.00	1.08	0.0353
	Other	38791	18.57	18.20	18.97	Ref	---	---	---	Ref	---	---	---
Sex	Male	29024	18.17	17.70	18.63	1.07	1.05	1.09	<0.0001	1.07	1.03	1.10	0.0001
	Female	29422	19.70	19.17	20.17	Ref	---	---	---	Ref	---	---	---
Age at Biopsy	Above	29785	17.70	17.33	18.20	1.11	1.09	1.13	<0.0001	1.13	1.10	1.17	<0.0001
	Below	28661	20.00	19.60	20.50	Ref	---	---	---	Ref	---	---	---
MSI Status	MSI-High	2418	37.50	33.57	41.70	0.63	0.59	0.67	<0.0001	0.75	0.69	0.82	<0.0001
	MSS	40588	18.97	18.67	19.37	Ref	---	---	---	Ref	---	---	---
TMB Status	High	17328	25.63	24.73	26.43	0.71	0.70	0.73	<0.0001	0.78	0.76	0.81	<0.0001
	Low	34608	16.13	15.77	16.50	Ref	---	---	---	Ref	---	---	---
PD-L1 expression	≥50%	8788	23.63	22.47	24.87	0.81	0.78	0.83	<0.0001	0.94	0.89	0.99	0.0146
	1-49%	20105	18.60	18.03	19.00	0.95	0.93	0.97	<0.0001	1.01	0.97	1.04	0.6483
	0%	24532	17.47	17.03	17.97	Ref	---	---	---	Ref	---	---	---
TLS Score Quartiles	TLS-Q4	14547	24.63	23.83	25.77	0.70	0.68	0.73	<0.0001	0.72	0.69	0.76	<0.0001
	TLS-Q3	10756	19.20	18.63	19.93	0.87	0.83	0.90	<0.0001	0.87	0.83	0.91	<0.0001
	TLS-Q2	8907	16.67	16.00	17.43	0.96	0.92	1.00	0.0294	0.96	0.91	1.01	0.0796
	TLS-Q1	7501	15.43	14.73	16.17	Ref	---	---	---	Ref	---	---	---

High TLS gene expression score did not predict outcome of non-immunotherapy regimen



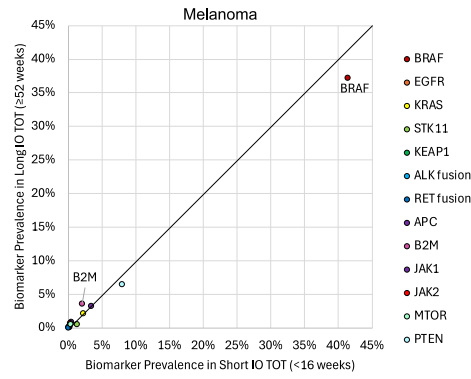
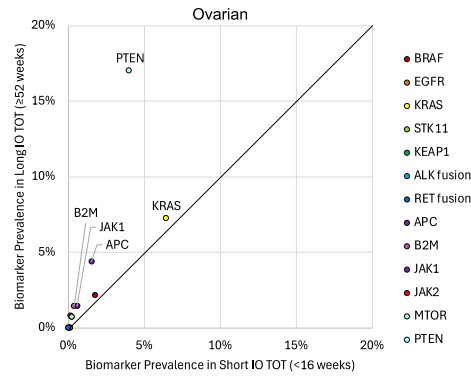
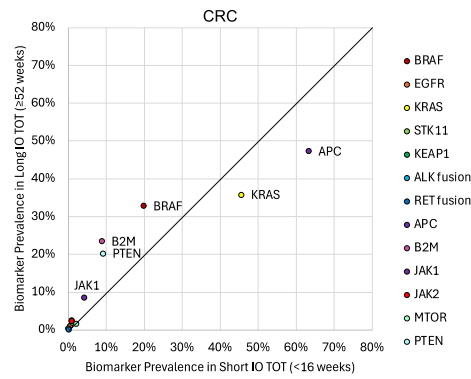
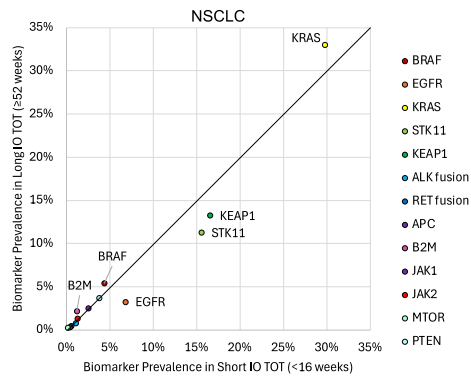
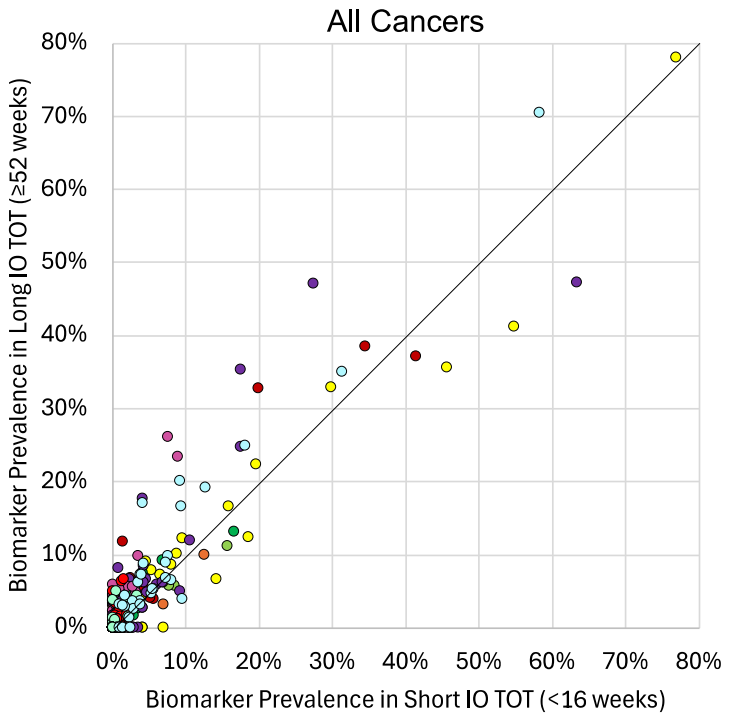
TLS gene expression scores did not correlate with TMB but with some genomic features according depending on tumor types

Signature	Spearman ρ Correlation with TMB	Prob> ρ
TLS (Messina, 2012)	0.1975	<.0001
TLS (Goc, 2014)	0.0684	<.0001
TLS (Cabrita, 2020)	-0.0097	<.0001
TLS (Meylan, 2022)	0.1409	<.0001
IFNg (Cristescu, 2018)	0.1295	<.0001

For instance, in NSCLC, EGFR mutation frequency decreased across TLS quartiles, with a significant difference noted between TLS-Q1 (11.3%) and TLS-Q4 (8.7%), $P < 0.0001$ (Supplementary Table 5).

The same trend was observed for the KRAS gene in digestive malignancies (colorectal cancer, small intestine cancer, cholangiocarcinoma)

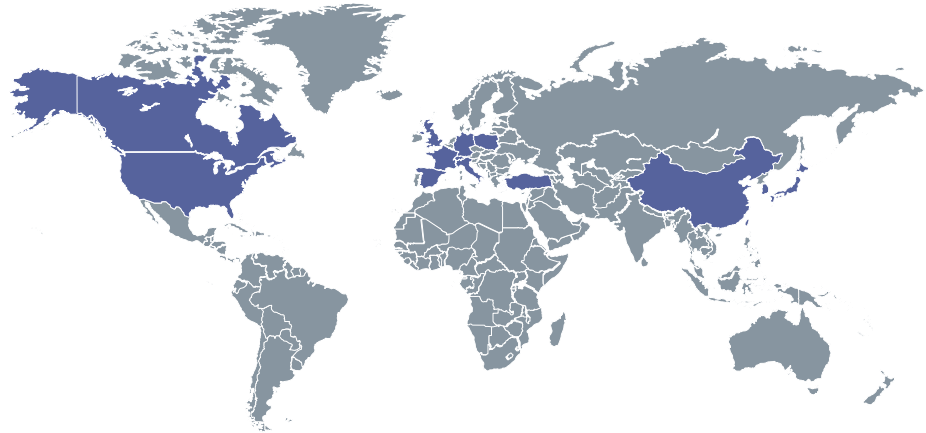
.... Mutation/amplification/deletion is associated with resistance to ICI in patients with high TLS gene expression scores



CONCLUSION

- Pioneering histology-agnostic RWE study leveraging TLS as a biomarker for patient selection in immune checkpoint inhibitor treatment.
- Notable inclusion of enduring responses, even in traditionally immunotherapy-resistant cases (MSS CRC, sarcoma...)
- Strong validation of TLS status-based selection as an effective approach for customizing immunotherapy in advanced solid tumor patients including patients with MSH high/TMB high status.
- ??? Based on additional analyses ongoing

Acknowledgements



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