Prevalence of Secondary Immunotherapeutic Targets in the Absence of Established Immune Biomarkers in Solid Tumors

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1. Introduction

Immune checkpoint inhibitor-based therapies have achieved impressive success in the treatment of several cancer types. Predictive immune biomarkers, including PD-L1 by immunohistochemistry (IHC), microsatellite instability (MSI) and tumor mutational burden (TMB) are well established as surrogate markers for immune evasion and tumor-specific neoantigens across many tumors. Positive detection across cancer types varies but overall, approximately 50% of patients test negative for these primary immune biomarkers[1]. In this study, we investigated the prevalence of secondary immune biomarkers outside of PD-L1 IHC, TMB and MSI.

2. Methods

Comprehensive genomic and immune profiling, including PD-L1 IHC, TMB, MSI and gene expression of 395 immune related genes was performed on 6078 Formalin-Fixed Paraffin-Embedded (FFPE) tumors representing 34 cancer types (**Figure 1**), predominantly composed of lung cancer (37.6%), colorectal cancer (11.9%) and breast cancer (8.5%). Expression levels by RNA-seq of 34 genes targeted by immunotherapies in solid tumor clinical trials currently open in the United States, identified as secondary immune biomarkers, were ranked against a reference population (**Figure 2**). Genes with a rank value ≥75th percentile were considered high, and positive values for primary immune biomarkers were associated with PD-L1 IHC (≥1%), MSI (MSI-H) and TMB (High ≥10 Mut/Mb) status. Conversely, negative values for primary immune biomarkers were associated with PD-L1 IHC <1%, microsatellite stable (MSS), and TMB Not High (<10 Mut/Mb). Additionally, secondary immune biomarker status was segmented by tumor type and cancer immune cycle roles.

3. Results

In total, 41.0% of cases were PD-L1 positive, 6.4% TMB High, and 0.1% MSI-H. 12.6% of cases were positive for >2 of these biomarkers while 39.9% were negative (PD-L1 Negative, TMB Not High and MSS) for the three primary immune biomarkers (**Figure 3A**). Of these negative cases, 89.1% were high for at least one secondary immune biomarker and 10.1% were negative for secondary immune biomarkers (**Figure 3B**). Tumor types negative for primary immune biomarkers with ≥50% prevalence of high secondary immune biomarkers included brain, prostate, kidney, sarcoma, gallbladder, breast, colorectal, and liver cancer (**Figure 3C**). High expression of cancer testis antigen secondary immune biomarkers (e.g., NY-ESO-1, LAGE-1A, MAGE-A4) was most observed in bladder, prostate, sarcoma, ovarian, and liver cancer (**Figure 4A**). Tumors demonstrating T-cell priming (e.g., CD40, OX40, CD137), trafficking (e.g., TGFB1, TLR9, TNF) and/or recognition (e.g., CTLA4, LAG3, TIGIT) secondary immune biomarkers were most represented by kidney, gallbladder, sarcoma, and prostate cancers (**Figure 4C-E**), with melanoma, esophageal, head & neck, cervical, stomach, and lung cancer least represented (≥15%).

4. Conclusion

Our studies show comprehensive tumor profiling that includes gene expression can detect secondary immune biomarkers targeted by investigational immunotherapies in approximately 90% of cases that are negative for the three primary immune biomarkers. While genomic profiling could also provide therapeutic choices for a percentage of these patients, detection of secondary immune biomarkers by RNA-seq provides additional options for patients without a clear therapeutic path as determined by PD-L1 IHC, TMB, MSI, and genomic profiling alone.

Tables + Figures

Figure 1. Distribution of top 20 cancer types among 6078 analyzed tumor samples. An additional 14 cancer types comprise the remaining 2.5% of samples

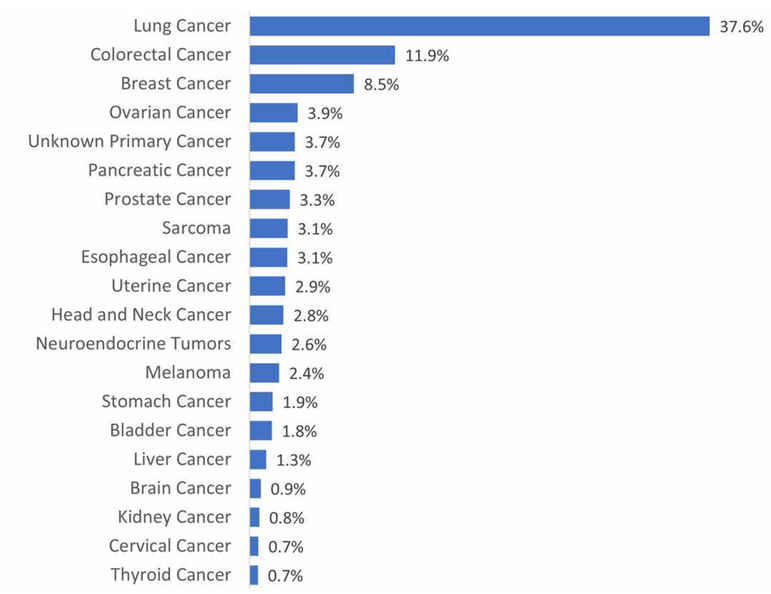
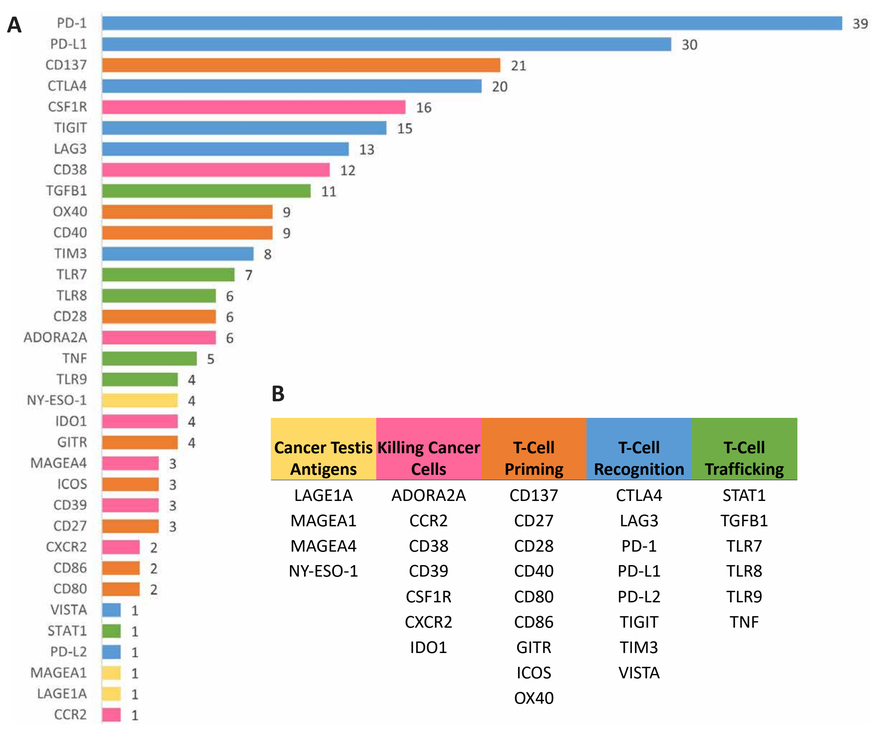


Figure 2. (A) Secondary immune biomarkers with the number of corresponding immunotherapies currently being investigated in open solid tumor US clinical trials. (B) Cancer immune cycle roles associated with secondary immune biomarkers.



B

Cancer Testis Antigens	Killing Cancer Cells	T-Cell Priming	T-Cell Recognition	T-Cell Trafficking
LAGE1A	ADORA2A	CD137	CTLA4	STAT1
MAGEA1	CCR2	CD27	LAG3	TGFB1
MAGEA4	CD38	CD28	PD-1	TLR7
NY-ESO-1	CD39	CD40	PD-L1	TLR8
	CSF1R	CD80	PD-L2	TLR9
	CXCR2	CD86	TIGIT	TNF
	IDO1	GITR	TIM3	
		ICOS	VISTA	
		OX40		

Figure 3. Distribution of primary and secondary immune biomarkers

(**A**) depicts the distribution of primary immune biomarker combinations across all 6078 tumor samples. Each slice is mutually exclusive and provides the distinction between the prevalence of combinations or lack of positive primary immune biomarkers. (**B**) provides a relative breakdown of secondary immune biomarker results within the tumor samples found to be negative for primary immune biomarkers. (**C**) cancer types where 50% or more samples negative for primary immune biomarkers had at least one high secondary immune biomarker.

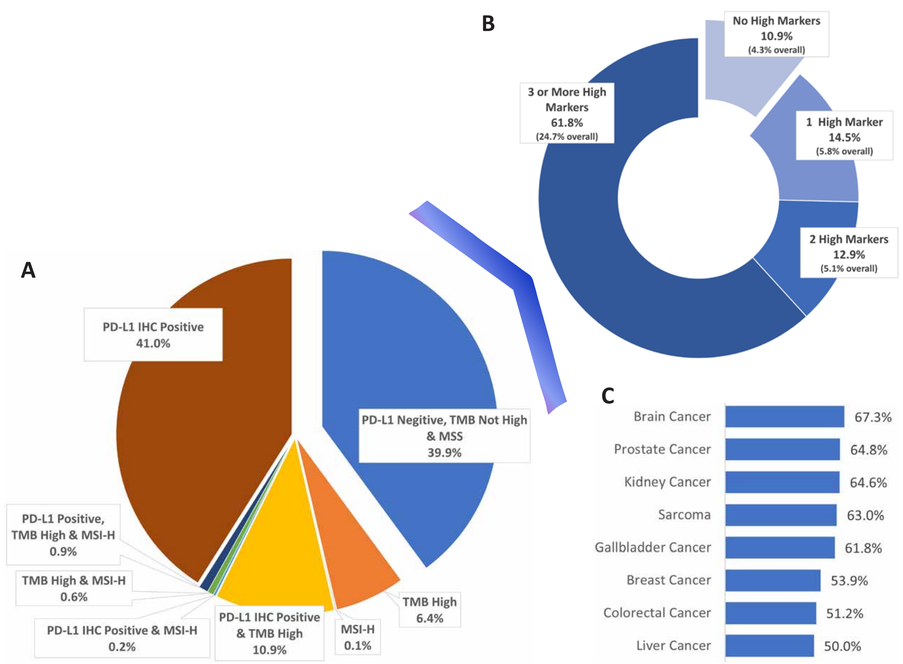
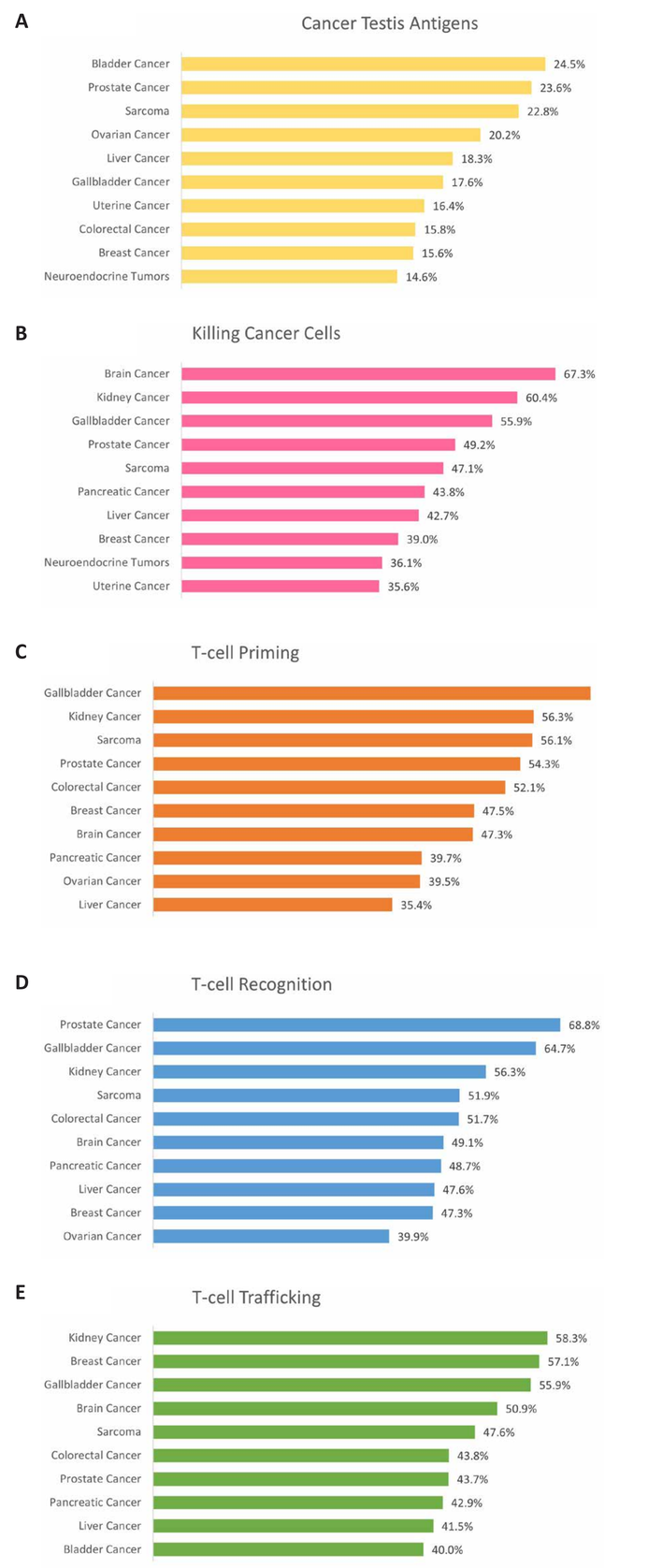


Figure 4. Prevalence of positive secondary immune biomarkers associated with select cancer immune cycle roles. Each figure (A-E) displays the top 10 cancer types for each role.



References

1. Huang, R.S.P., Haberberger, J., Severson, E. et al. A pan-cancer analysis of PD-L1 immunohistochemistry and gene amplification, tumor mutation burden and microsatellite instability in 48,782 cases. *Mod Pathol* 34, 252–263 (2021).

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