Cancer Cell

CellPress

Letter

Mutation burden and anti-PD-1 outcomes are not universally associated with immune cell infiltration or lymphoid activation

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https://doi.org/10.1016/j.ccell.2024.10.017

Cancers are conventionally classified as "hot" tumors that are associated with high tumor mutation burdens (TMBs) and tumor-infiltrating immune cells or "cold" tumors associated with a dearth of neoantigens and immune cell exclusion.[1] This dichotomy is frequently used to define the degree of pre-existing immune cell reactivity within the tumor microenvironment and has been linked to clinical outcomes including the efficacy of immune checkpoint inhibitor (ICI) treatment.[1] Recently, cancer-agnostic immune gene expression signatures (IGESs) denoting "hot" tumors, including the interferon-gamma (IFN-γ) and T cell-inflamed scores, have been derived from clinical cohorts as putative cancer-agnostic predictors of ICI treatment response in diverse cancer types.[2–4] Whether TMB or ICI benefit is robustly associated with "hot" tumors defined by pre-existing immune characteristics across diverse cancer histologies remains ambiguous. Herein, we sought to address the following outstanding questions: (1) are IGESs used to identify "hot" and ICI-sensitive tumors associated with TMBs or long-term ICI responders across diverse cancer types; (2) similarly, is the composition of infiltrating immune cells agnostically associated with TMBs or long-term survivors after ICI; and lastly (3) do immune characteristics account for the variable response rates to ICIs across cancer types?

To assess how immune characteristics are related to TMB and ICI outcomes, we analyzed a large real-world clinico-genomic dataset (Caris) comprised of 27,810 patients treated with anti-PD-1/L1 therapies for advanced stage microsatellite-stable cancers distributed across 26 different histological entities. Cancer specimens were subject to PD-L1 immunostaining, DNA sequencing, and whole-transcriptome sequencing followed by immune cell deconvolution. Details of our methodology are included in the supplemental information.

To evaluate whether cancers harboring high TMBs are associated with particular immune cell phenotypes, we compared the immune characteristics of TMB-high (cancers with TMBs in the top 80th percentile for that histology) versus TMB-low cancers (TMBs less than 80th percentile in that histology). Among TMB-high cancers across 26 histologies, PD-L1 expression was significantly enriched ($q < 0.05$) in 6 cancers but depleted in breast cancer (Figure S1A). In TMB-high cancers, the IFN-γ and T cell-inflamed score was enriched in 9 and 4 cancer types, respectively (Figure S1A). CD8$^+$ T cells were enriched in TMB-high cancers for 10 cancer histologies, while 11 histologies showed no evidence of increased infiltration of any immune cell population in TMB-high cancers (Figure S1A). Similar results were observed when TMB-high cancers were defined using a flat cut-off of 10 mut/Mb. These results indicate that IGESs and infiltrating immune cells implicated in anti-tumor responses are only associated with TMB in a minority of cancer types. Sensitivity analyses were performed after stratifying patients by age and sex (for relevant cancer types), which demonstrated conserved associations between gene signatures and immune features with TMB.

We next assessed whether immune activity differed between long-term responders to ICIs, defined as patients with a duration of ICI treatment in the top 80th percentile in each histology. Across 26 histologies, PD-L1 expression, T cell inflamed, and IFN-γ scores were increased in long-term responders in 6, 6, and 9 cancer types, respectively (Figure S1A). CD8$^+$

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IPR2024-00648

T cells were enriched in long-term responders across 8 histologies with considerable overlap with cancer types that also demonstrated increased gene signature scores (Figure S1A). Other immune cell types including B, M1 macrophage, dendritic, $CD4^+$ T, and Treg cells were co-enriched with $CD8^+$ T cells among long-term responders in several histologies (Figure S1A). Similar results were observed when we examined immune features among long-term ICI survivors, defined as patients with an overall survival length calculated from the time of first ICI administration to last contact or death among the top 80th percentile in each histology. PD-L1 expression, T cell inflamed, and IFN-γ scores were increased in long-term responders in 3, 8, and 8 cancer types, respectively (Figure S1A). $CD8^+$ T cells were enriched in long-term survivors across 8 histologies, of which 7 cancer types also demonstrated $CD8^+$ T cell enrichment in long-term responders. Among long-term survivors, unique immune features included increased natural killer cell infiltration in renal cell carcinoma, non-small cell lung adenocarcinoma, and mesothelioma and the depletion of M1 macrophages in head and neck and neuroendocrine cancers (Figure S1A). A notable finding among long-term responders and survivors of vulvar cancers was the enrichment and depletion of dendritic cells and neutrophils, respectively (Figure S1A). Collectively, these results indicate that IGESs and infiltrating immune cells are not associated with ICI long-term responders or survivals in many histologies and that many immune features associated with outcomes across histologies appear idiosyncratic, without consistent patterns of immune cell enrichment or depletion between cancer types. Importantly, subgroup analyses stratifying patients by sex (male versus female), line of ICI therapy use (first versus subsequent line), and tissue location (primary versus metastatic site) for relevant cancer types showed nearly identical associations between immune features and ICI outcomes across subgroups.

Given that IGESs and immune infiltration were not universally increased among TMB-high cancers or long-term ICI responders within individual histologies, we examined whether immune features may explain variations in ICI outcomes across cancer types. To explore this, we plotted the previously reported objective response rates (ORRs) to monotherapy ICIs observed in prospective trials against PD-L1 expression, IGES scores, and $CD8^+$ T cell infiltration across the 26 cancer histologies examined in the Caris cohort (Figure S1B).[5,6] Because survival lengths after ICIs vary between different cancer types due to differences in tumor biology and post-treatment clinical progression patterns, we analyzed ORRs to ICIs, which provides a comparable metric of drug activity across cancer types. This analysis revealed no significant correlations between PD-L1 expression, T cell inflamed, and IFN-γ scores with the ORRs to ICIs (Figure S1B). In contrast, there was a low but significant correlation (Pearson $r = 0.4$, p value $= 0.04$) between $CD8^+$ T cell infiltration and ORRs, with an explained variance of 16.3% (Figure S1B). By comparison, TMB and ORRs were found to be strongly correlated (Pearson $r = 0.75$, p value < 0.001) with an explained variance of 55.5%, consistent with prior studies. Examining correlations between TMB versus PD-L1 expression, IGES scores, and $CD8^+$ T cell infiltration showed no significant associations. Taken together, these results indicate that the immune characteristics examined are not instructive of ICI sensitivity across many cancer types or TMBs. A caveat of this analysis is that the underlying patient population used to derive ICI ORRs was not from the Caris cohort (which lacks ORR data). Nonetheless, the ICI ORRs used in this analysis were derived from pivotal or large late-phase trials, suggesting that they may be generalizable to other patients.[5,6]

Our results challenge prior conclusions regarding the universal predictive value of pre-existing immune tumor activity and highlight the limited utility of immune classes to describe the clinical behaviors of cancers. Rather, the utility of immune and genetic biomarkers for predicting anti-PD-1/L1 therapy efficacy is histology dependent. For instance, IGESs were infrequently associated with high-TMB or long-term ICI responders in most histologies studied and were not instructive of the variance in ORR to ICIs across multiple cancer types. In addition, there were no specific immune cell subsets commonly associated with TMBs or long-term ICI outcomes across multiple cancer entities. Nonetheless, we identify new histologies where IGESs may be clinically applicable but have not been well studied in past studies, including carcinoma of unknown primary and bladder, biliary tract, and colorectal cancer.

A limitation of this study is that some tumor specimens analyzed may have been procured from earlier stages of the disease as new diagnostic or surgical specimens from advanced diseases may not have been obtained due to clinical reasons, and the impact of this on our results is unclear. Additionally, whether other clinical factors may influence the relationship between tumor immune activity and TMBs and ICI outcomes remains ambiguous. Nonetheless, results from a nationally represented and large real-world cohort suggest that immunological stratification of tumors has limited bearing on the immunogenicity of tumors or ICI outcomes. Moreover, the development of pan-cancer biomarkers for ICIs requires more rigorous scrutiny of cancer types being studied, as most investigations have been largely focused on only a few histologies. Lastly, our results serve as a resource for examining how specific immune cell subsets may be associated with TMBs or ICI outcomes in a broad range of human malignancies.

ACKNOWLEDGMENTS

D.H. is supported by a Cancer Prevention and Research Institute of Texas Early Clinical Investigator Award (RP200549) and the Josephine Hughes Sterling Foundation. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors received no specific funding for this work.

AUTHOR CONTRIBUTIONS

D.H. and H.Z. conceived the study. D.H., A.E., and J.X. performed data analyses. D.H., A.S., W.E.-D., E.S.A., S.L.G., M.J.H., H.B., D.S.B.H., S.V.L., P.C.M., R.R.M., T.W.-D., J.M., G.W.S., D.S., and H.Z. contributed to the assembly of the CARIS cohort. D.H. drafted the paper, and all authors participated in the review and editing of the manuscript.

DECLARATION OF INTERESTS

A.E., J.X., G.W.S., and D.S. are employees of Caris Life Sciences.

S.L.G. serves as a paid consultant/advisor to Pfizer, Daiichi Sankyo, Eli Lilly, AstraZeneca, Genentech, SeaGen, Novartis, and Menarini and has stock ownership in HCA Healthcare.

E.S.A. serves as a paid consultant/advisor to Janssen, Astellas, Sanofi, Dendreon, Bayer, BMS, Amgen, Constellation, Blue Earth, Exact Sciences, Invitae, Curium, Pfizer, Merck, AstraZeneca, Clovis, and Eli Lilly; has received research support (to his institution) from Janssen, J&J, Sanofi, BMS, Pfizer, AstraZeneca, Novartis, Curium, Constellation, Celgene, Merck, Bayer, and Clovis; and is the co-inventor of a patented AR-V7 biomarker technology that has been licensed to Qiagen.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2024.10.017.

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