Heliyon 9 (2023) e22432

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Stromal score is a promising index in tumor patients' outcome determination

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ARTICLE INFO

Keywords:
Cancer-associated fibroblasts
Immune score
Immune-checkpoint blockade
Overall survival
Stromal score

ABSTRACT

Background: Immune status is widely acknowledged as a valuable marker for predicting cancer prognosis and therapy response. However, there has been a limited understanding of the stromal landscape in cancer.
Methods: By employing ESTIMATE, stromal- and immune-scores were inferred for 6193 tumor samples spanning 12 cancer types sourced from The Cancer Genome Atlas (TCGA). Subsequently, the samples were categorized into seven groups based on their stromal and immune scores. A comparison of prognosis, lymphocyte and stromal cell infiltration, and the response to programmed death ligand 1 (PD-L1) therapy was conducted among these subtypes.
Results: It was unveiled by the analysis that, in the majority of cancer types, stromal score exhibited a more potent predictive capability for outcomes compared to the immune score. Furthermore, it was observed that in four cancer types, intermediate immune infiltration coupled with low stromal infiltration correlated with the most favorable overall survival, whereas an unfavorable outcome was predicted in colorectal cancer (CRC) and stomach adenocarcinoma (STAD) when high immune infiltration coexisted with intermediate or high stromal infiltration.
Conclusion: In summary, while high immune scores frequently correlate with a positive prognosis, such correlation is not universal. A potential strategy to address the current limitations of the immune score in specific circumstances could involve a focus on stromal scores or a subtle integration of stromal and immune status.

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https://doi.org/10.1016/j.heliyon.2023.e22432
Received 16 May 2023; Received in revised form 9 November 2023; Accepted 13 November 2023
Available online 20 November 2023

1. Introduction

For more than 80 years, the American Joint Committee on Cancer (AJCC) TNM staging system has served as the most dependable reference for the routine prognosis and treatment of malignant tumors [1]. However, it is now acknowledged that clinical outcomes and therapy responses can greatly differ among patients within the same tumor stage [2]. Taking colorectal cancer (CRC) as an example, various approaches have been suggested to classify CRC into distinct subtypes, such as consensus molecular subtypes (CMSs), KRAS or BRAF mutation status, and other gene expression or methylation-based panel classifications [3,4]. The TNM classification often falls short in accurately predicting tumor patient outcomes, owing to the far greater complexity and significance of the tumor microenvironment (TME) than previously anticipated [5]. Recently, a methodology known as the 'Immunoscore,' proposed by Galon and based on the quantification of two lymphocyte populations (CD3/CD45RO, CD3/CD8, or CD8/CD45RO), has emerged as a superior prognostic factor compared to the TNM classification [6–10]. Nonetheless, in clear cell renal cell carcinoma (ccRCC), patients with elevated levels of infiltrating $CD8^+$ T cells in tumors typically exhibit poor outcomes [11], indicating that a higher immunoscore does not invariably correlate with a favorable prognosis. Moreover, the stromal score has been recognized as an independent prognostic factor associated with unfavorable overall survival in gastric cancer [12] and CRC [13]. However, in papillary thyroid carcinoma [14] and hepatocellular carcinoma [15], a high stromal score was significantly linked to improved overall survival (OS) and progression-free survival (PFS).

Consequently, there is an urgent need for a systematic exploration of stromal- and immune-scores at a pan-cancer level to better comprehend the prognostic relevance of the TME. In this context, our objective is to categorize 12 cancer types (bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), CRC, head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), ovarian serous adenocarcinoma (OV), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC)) into categories of high, moderate, and low stromal- and immune-scores. This categorization aims to assess the combined impact of stromal- and immune-scores on cancer prognosis stratification. The findings from this study may establish a groundwork for personalized cancer diagnosis and treatment from a fresh TME perspective, ultimately enhancing the precision of malignancy assessment when integrated with the clinical attributes of the tumor.

2. Materials and methods

2.1. Gene expression data processing

Level 3 tumor RNASeqV2 mRNA expression datasets were obtained from The Cancer Genome Atlas (TCGA) via https://portal.gdc.cancer.gov/. Raw CEL files of GSE39582 and GSE62254 (Affymetrix HG U133 Plus 2.0 arrays) were downloaded from the Gene Expression Omnibus (GEO) at https://www.ncbi.nlm.nih.gov/geo/. For the estimation of expression levels, the MAS5 algorithm was employed, following the methodology described in a prior publication [16]. Concerning PRJEB25780, the raw FASTQ sequencing files, encompassing 45 patients and associated clinical data, were acquired from the European Nucleotide Archive (ENA) at https://www.ebi.ac.uk/ena. The process involved the mapping of paired-end reads to human protein coding genes (Homo_sapiens.GRCh38.84) using HISAT2 (version 2.2.0) with the default parameters [17], and the quantification was performed using featureCounts [18]. The inference of stromal- and immune-score within tumor samples was carried out using the ESTIMATE method [19]. In brief, the stromal- and immune-score for each sample were calculated through single sample gene set enrichment analysis (ssGSEA), relying on 141 stromal gene signatures and 141 immune gene signatures, respectively. The determination of proportions of infiltrated lymphocytes and stromal cells in tumor tissue was executed using xCell [20]. Furthermore, the identification of synthetically correlated immune or stromal cells with patient survival within each cancer type was achieved via the LASSO method, employing the *glmnet* package's built-in *cv.glmnet* function [21].

2.2. Tumor mutation burden (TMB) calculation

Somatic mutation datasets including all 12 cancers, as called by MuTect, were sourced from the TCGA data portal (as of 11/13/2018). Mutations categorized as silent, RNA-related, or located within introns, 3'-untranslated regions (UTRs), 5'-UTRs, or flanking sequences were excluded from subsequent analyses.

2.3. Subdivide tumor patients according to integrated stromal- and immune-scores

To clarify the distinctive roles of immune and stromal infiltration in tumor progression, patients were categorized into immune-high, -mediate, -low, and stromal-high, -mediate, -low groups. Subsequently, the amalgamation of TME statuses, such as immune-high and stromal-high, -mediate, or -low, followed a similar methodology.

2.4. Survival analysis

Survival contrasts across various subtypes were assessed using the Kaplan-Meier method and subjected to the log-rank test through the application of the *survfit* and *survdiff* functions within the R *survival* package [22]. The execution of multivariate Cox regression

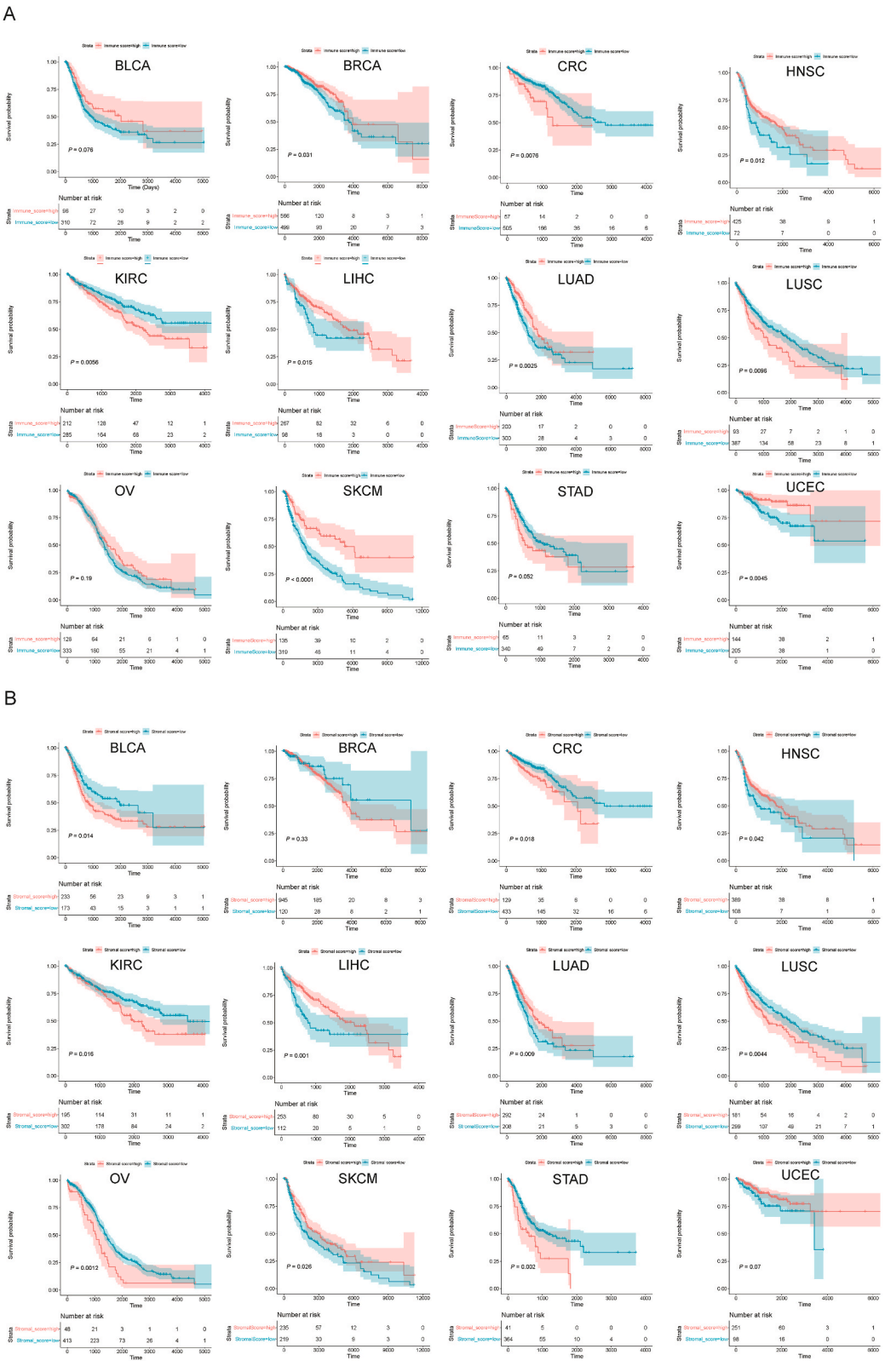

Fig. 1. Survival Disparities Based on Optimal Immune or Stromal Score Cutoff in 12 Cancer Types. A. Overall Survival (OS) differences stratified by immune score. B. OS differences stratified by stromal score.

analysis was carried out using the *coxph* function. The identification of optimal cutoff points for variables (be it immune- or stromal-score) was accomplished utilizing the surv_*cutpoint* function within the R *survminer* package.

2.5. Data and code availability

The codes employed throughout this study are publicly accessible at https://github.com/huwangxiong/Stromal-score-is-a-promising-index-in-tumor-patients-outcome-determination. The authors are prepared to provide all other data upon reasonable request.

3. Results

3.1. Generally stromal score has a better outcome predictive power than immune score

For the purpose of comparing the predictive capacities of immune- and stromal-score in determining tumor patients' outcomes, we initially determined the optimal cutoff point for the immune score, given its widely accepted significance in patient prognosis. Utilizing maximally selected rank statistics, we identified that the immune score failed to effectively distinguish cancer patients within BLCA, OV, and STAD (Log rank test, $P > 0.05$, Fig. 1A). Additionally, in the cases of CRC and KIRC, higher immune scores corresponded to unfavorable outcomes, and this observation was confirmed through validation with an independent cohort, GSE39582, encompassing 573 CRC cases (Fig. S1). Consequently, we proceeded to examine the correlation between stromal score and outcome across the 12 cancer types. The findings revealed that stromal score did not significantly differentiate cancer patients within BRCA and UCEC (Log rank test, $P > 0.05$, Fig. 1B). Notably, elevated stromal infiltration was linked to poorer prognosis in BLCA, CRC, KIRC, LUSC, OV, and

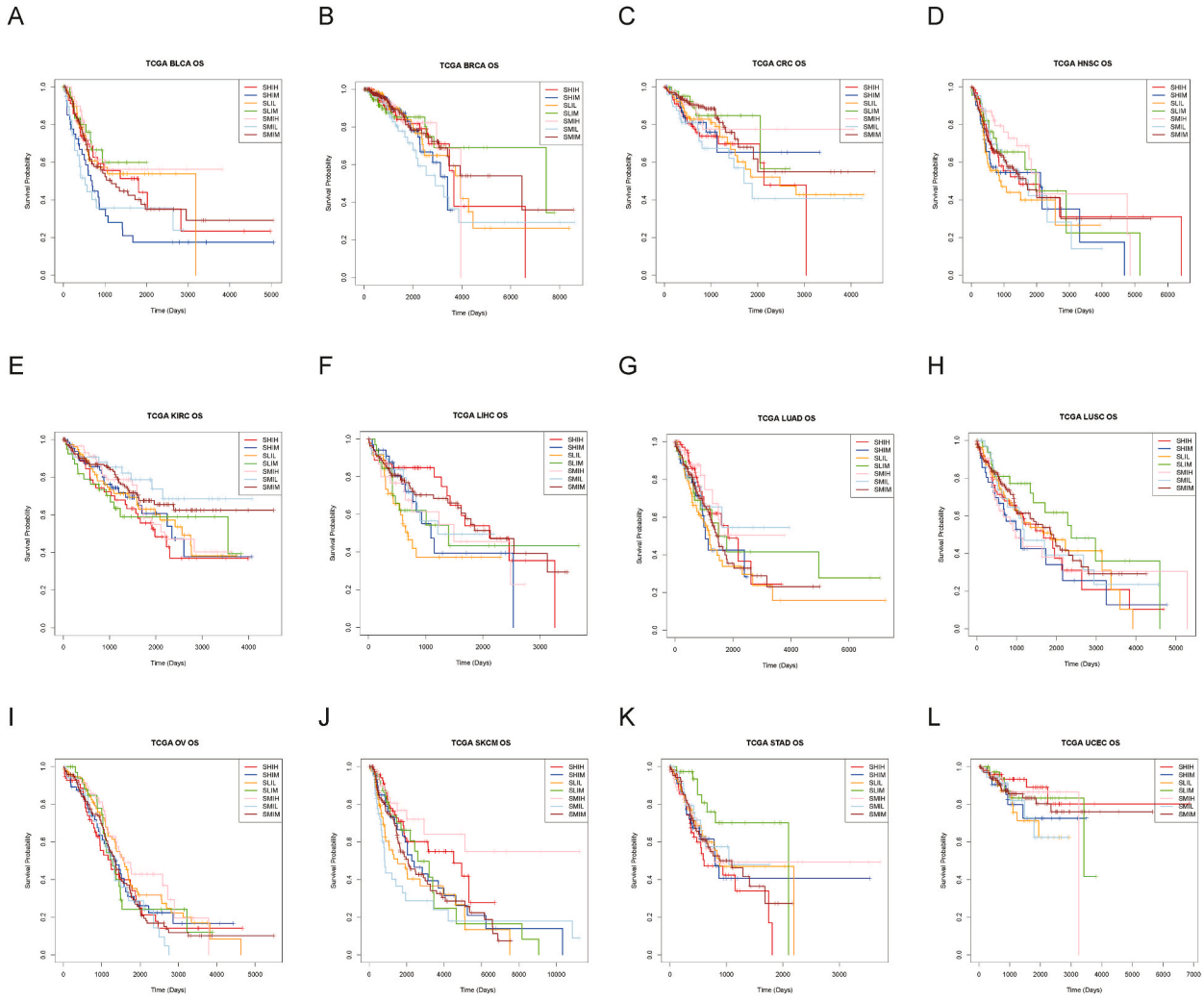

Fig. 2. Survival differences among seven subtypes defined by simultaneous integration of immune and stromal scores in 12 cancer types.

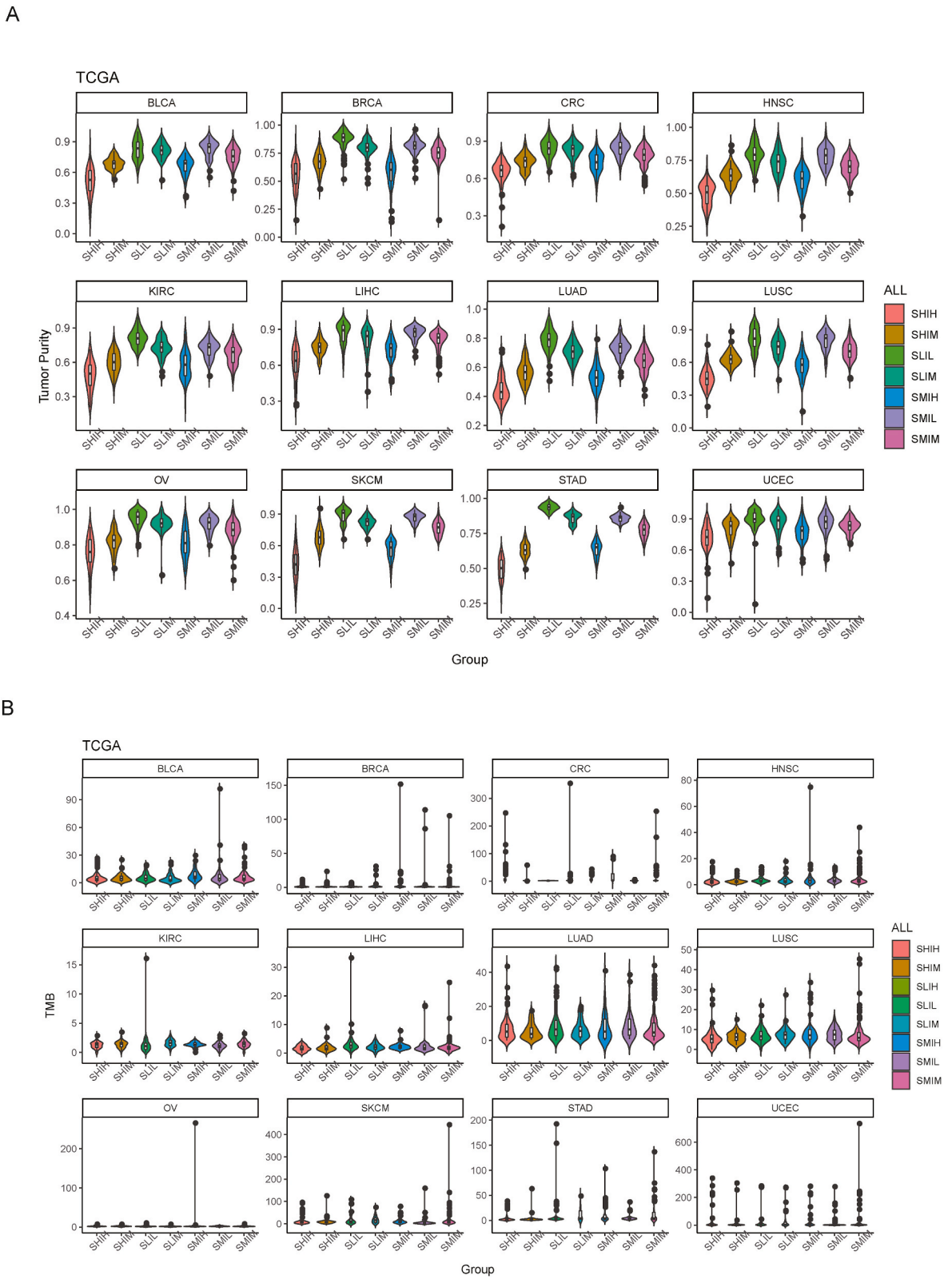

Fig. 3. Distinctive Characteristics Associated with Survival Disparities Across Different Subtypes. A. Tumor purity estimated using the CPE method. B. Comprehensive TMB in seven subtypes.

STAD, a trend strikingly counter to the inverse relationship observed in HNSC, LIHC, LUAD, and SKCM. Particularly for STAD, the distinguishing capability of the stromal score was confirmed through validation with the Asian Cancer Research Group study GSE62254 (Log rank test, $P < 0.0001$, Fig. S1). Overall, in BLCA, LIHC, LUSC, OV, and STAD, the stromal score exhibited more pronounced discriminatory power than the immune score (Log rank test, $P < 0.05$, Fig. 1). Consequently, there is a compelling indication that integrating stromal and immune statuses could potentially enhance the stratification of cancer patients in a prognosis-relevant manner.

3.2. Combing stromal and immune score in prognosis stratification

As singular stromal and immune scores proved insufficient to distinguish clinical outcomes within specific cancer types (Fig. 1), we proceeded by categorizing cancer patients into nine distinct subgroups: stromal-high and immune-high (SHIH), stromal-high and immune-mediate (SHIM), stromal-high and immune-low (SHIL), stromal-mediate and immune-high (SMIH), stromal-mediate and immune-mediate (SMIM), stromal-mediate and immune-low (SMIL), stromal-low and immune-high (SLIH), stromal-low and immune-mediate (SLIM), and stromal-low and immune-low (SLIL), employing the technique of quartile division, with "mediate" signifying the intermediate quartiles. It is pertinent to note that due to a lack of samples or an insufficient number of samples, the survival difference was examined solely among the remaining seven subgroups across all 12 cancer types. Intriguingly, our observations indicated that SLIM demonstrated the most favorable overall survival (OS) in BLCA, BRCA, LUSC, and STAD, whereas SMIH also exhibited a favorable outcome in BLCA, BRCA, CRC, HNSC, LUAD, OV, SKCM, and UCEC (Fig. 2A-L). These patterns were consistently reflected in progression-free survival (PFS) (Fig. S2). In contrast, SMIL was associated with the poorest prognosis in BLCA, BRCA, CRC, and SKCM. Remarkably, KIRC displayed the worst and best OS in SMIL and SLIM, respectively (Fig. 2E). An expected favorable outcome associated with high immune infiltration was exclusively observed in SKCM, where SMIH and SHIH showed the best OS and SLIL and SMIL were linked to the worst OS.

To examine the potential mechanisms contributing to the survival disparities among these subtypes, we investigated tumor purity among them. The results revealed that SLIL consistently exhibited the highest tumor purity, while SHIH consistently displayed the lowest tumor purity across all 12 cancer types (Fig. 3A). However, we noted no significant difference in tumor mutation burden (TMB) between the various subtypes (Wilcoxon test, $P > 0.05$, Fig. 3B). This observation suggests that the impact of TMB on immune score was attenuated when accounting for stromal infiltration.

3.3. Enrichment of specific stromal or immune cells in subdivided groups

As previously highlighted, considerable variability in tumor purity among diverse subtypes suggests that differences may be attributed to infiltrated immune and stromal cells aside from tumor cells. Consequently, we embarked on a comprehensive exploration of the presence of 64 immune and stromal cells infiltrating tumors. Interestingly, our findings revealed that chondrocytes and fibroblasts were enriched in the SHIH and SHIM subgroups of BLCA, HNSC, LIHC, LUAD, LUSC, and STAD. Notably, in the case of STAD,

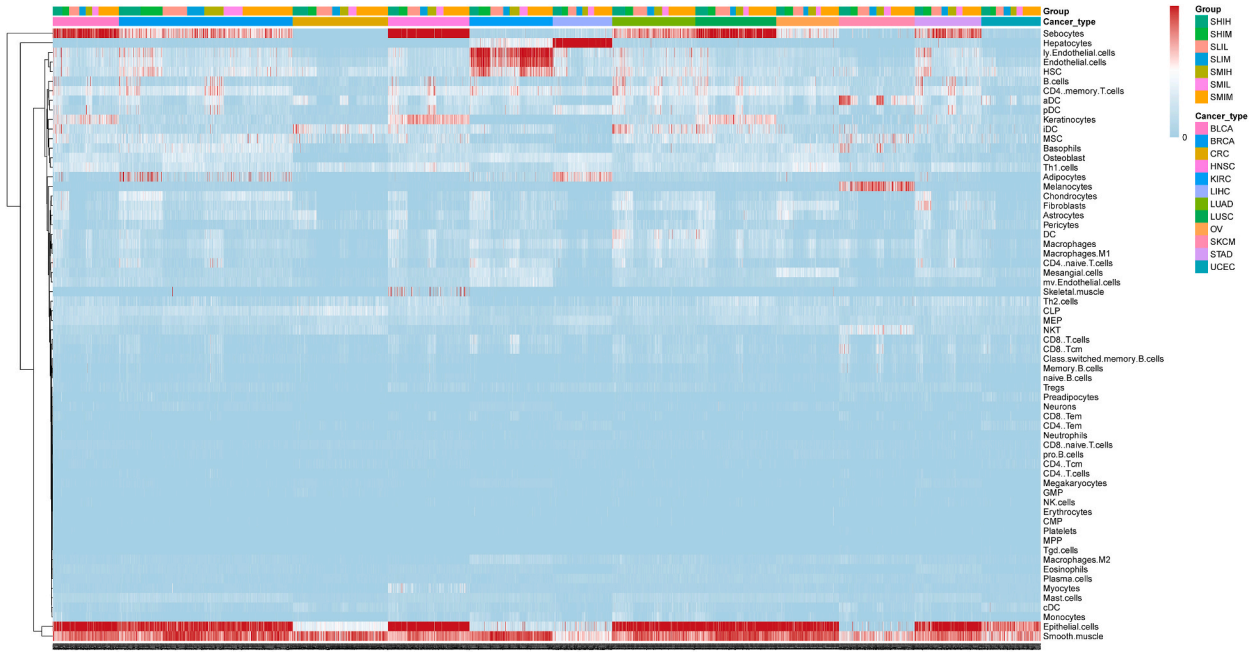

Fig. 4. Heatmap Illustrating Immune and Stromal Cells Identified via xCell Inference.

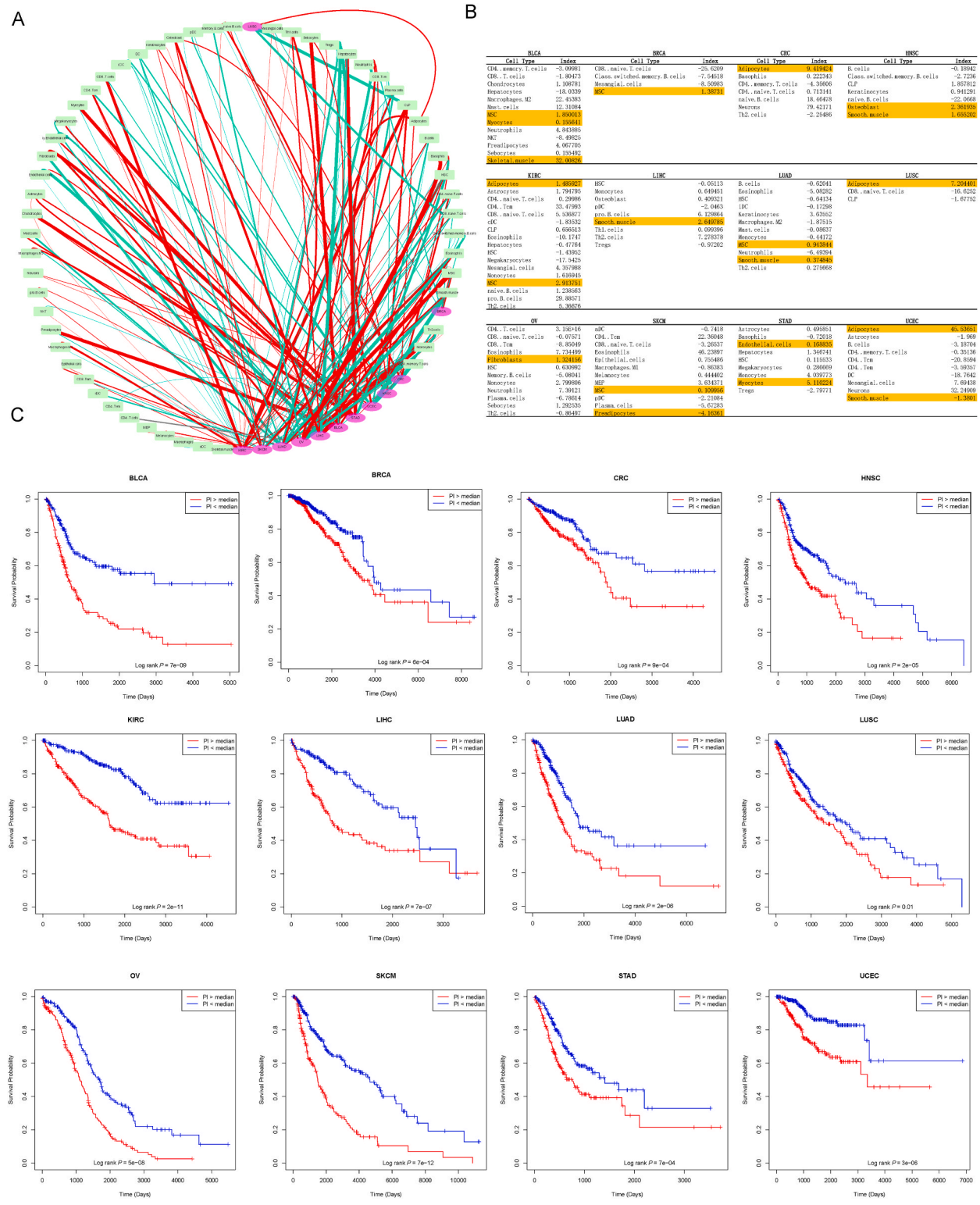

Fig. 5. A Compilation of Well-Defined Signatures Predicting Cancer Patient Prognosis. A. Survival-related immune or stromal cells shared across 12 cancer types, represented by red bands for adverse factors and green bands for protective factors. Band thickness indicates significance (P value) of a factor within the signature. B. List of parameters in the proportional hazards model, with stromal cells highlighted in orange background. C. KM plots depicting high- or low-risk subtypes in 12 cancer types derived from TCGA data following LASSO regression.

heightened lymphatic endothelial cell and HSC infiltration was further observed within the SHIH and SHIM subgroups (Fig. 4). Moreover, antigen-presenting dendritic cells (aDC) exhibited enrichment in the SHIH and SMIH subgroups of BRCA, CRC, HNSC, LUAD, LUSC, SKCM, STAD, and UCEC. The analysis also uncovered a relatively high infiltration of CD8 T cells and CD8 Tcm in the SHIH and SMIH subgroups of HNSC, KIRC, LUSC, SKCM, and UCEC (Fig. 4).

Beyond its potential to efficiently predict prognosis, another promising application of immune or stromal cell infiltration-based scoring lies in assessing the efficacy of immunotherapy. In this context, we employed the PRJEB25780 cohort comprising 45 metastatic gastric cancer patients treated with anti-PD-L1 pembrolizumab to gauge the therapeutic efficacy using immune and stromal scores. As anticipated, around 85 % of patients exhibited no response (PD and SD) within the high stromal infiltration group, while merely 38 % of patients displayed a response (CR and PR) within the high immune infiltration group (Fig. S3). This outcome underscores that a high immune score alone possesses unsatisfactory predictive value in tumors with abundant stromal infiltration, such as STAD.

3.4. Construction of prognostic model based on integrating immune and stromal cells

Considering the enrichment of distinct immune or stromal cell types in specific subtypes (Fig. 4), it is plausible to assume that these could be employed to stratify cancer patients into high- or low-risk subtypes based on various combinations of stromal and immune cells. Consequently, we employed LASSO to identify optimal cell types in all 12 cancer types. Remarkably, each of the 12 cancer types could be categorized into high- or low-risk subtypes based on a minimum of three (LUSC) to seventeen (KIRC) infiltrated cell types (with at least one stromal cell type), some of which (e.g., CLP, MSC, and Th1 cells) were common across different cancer types (Fig. 5A and B). Furthermore, the panels derived from LASSO exhibited enhanced discrimination between low-risk and high-risk subtypes (Log rank test, $P = 0.01 \sim 7e^{-12}$) compared to immune (Log rank test, $P = 0.19 \sim 2e^{-5}$) or stromal-based (Log rank test, $P = 0.33 \sim 0.0012$) scores alone in all 12 cancer types (Figs. 1 and 5C).

4. Discussion

In this study, we undertook the pioneering effort of systematically evaluating the prognostic predictive potential of combined immune and stromal infiltration across 12 distinct cancer types. Our approach introduced a novel perspective by categorizing cancer patients into nine subgroups through the integration of immune and stromal scores. This innovative methodology deviated from the conventional practice of solely investigating the connection between patient outcome and high immune- or stromal-score. Notably, we observed that immune score, which is generally positively correlated with outcome and typically holds greater influence than stromal score, exhibited effective predictive ability only within SKCM, aligning with previous studies [23,24]. Furthermore, we identified that the division of tumors into stromal high but immune low or vice versa, as previously practiced, was largely unsuitable, given the absence or minimal representation of such subtypes across all 12 cancer types, such as SHIL and SLIH. The current simplistic approach of combining immune and stromal scores into a singular TME score may not sufficiently capture the complexities, urging the adoption of a more dynamic simultaneous consideration of immune and stromal scores in the majority of cancer types. This caveat also carries implications for the broader context of tumor purity assessment [25].

Remarkably, our findings revealed that intermediate immune infiltration combined with low stromal infiltration conferred the best overall survival in BLCA, BRCA, LUSC, and STAD. In essence, the scenario of high immune infiltration accompanied by intermediate or high stromal infiltration might not universally translate to favorable outcomes, as demonstrated by CRC and STAD (Fig. 2). Moreover, in various cancer types, the potential anti-tumor benefits associated with high immune cell infiltration could be countered by stromal components, such as cancer-associated fibroblasts (CAF).

Elevated CAF infiltration is closely tied to poor outcomes and resistance to immune-checkpoint blockade (ICB) [26]. This could, in part, elucidate the superior predictive efficacy of stromal score in STAD. Furthermore, our study highlighted that the degree of survival distinction achieved by immune and stromal scores in OV was comparatively limited compared to other solid tumors. This suggests that the tumor itself may wield significant influence over invasiveness and immune evasion [27]. Consequently, immune or stromal scores might not universally suit all cancer types or molecular subtypes within the same tumor for prognostic evaluation, particularly as a primary choice in OV.

While acknowledging the achievements of this study, we recognize certain limitations. For instance, the newly introduced subtypes based on immune and stromal infiltration were not externally validated with an independent dataset. Furthermore, the relatively small sample size of tumor patients receiving ICB treatment stemmed from the challenges in collecting prognostic and sequencing data. We are committed to expanding our patient pool receiving ICB therapy to validate our findings. Additionally, the precise immune or stromal cell type most relevant to clinical outcomes remains to be elucidated. The degree of impact from these factors (immune or stromal cell types) and the determination of a weighted index for individual factors also warrant further investigation.

5. Conclusions

In conclusion, stromal score emerges as a pragmatic alternative to immune score alone in prognostic assessments of cancers characterized by infiltrated-excluded dynamics, due to the absence of cytotoxic lymphocytes in the tumor core. The dynamic integration of stromal and immune scores offers another valid approach to prognostic prediction, particularly in the context of poorly immunogenic or 'cold' cancers such as BLCA, OV, and STAD.

Data availability

The datasets generated and/or analysed during the current study are available in the TCGA repository (https://cancergenome.nih.gov/) or GEO database (https://www.ncbi.nlm.nih.gov/geo/) with accession number GSE39582 and GSE62254.

Funding

This research was supported in by the grant from the Zhejiang Provincial Medical and health Science and Technology Project, Grant/Award Number: 2021KY083, 2023KY063 and 2023KY591; Key project of Medical and health science and technology Plan of Zhejiang Province, Grant/Award Number: WKJ-ZJ-2020.

CRediT authorship contribution statement

Xiaoxian Xu: Conceptualization, Data curation, Writing – original draft. **Yu Xu:** Data curation, Visualization, Writing – original draft. **Wangxiong Hu:** Formal analysis, Writing – original draft. **Wenjie Hong:** Data curation, Software. **Yichen Wang:** Data curation, Writing – review & editing. **Xiaojing Zhang:** Data curation, Formal analysis. **Xiaoji Fan:** Data curation, Visualization. **Tingzhang Wang:** Formal analysis, Visualization. **Hanmei Lou:** Writing – review & editing. **Yanmei Yang:** Supervision, Writing – review & editing. **Jianhua Qian:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not Applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22432.

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