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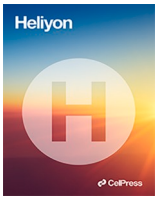

Research article

Cancer specific up-regulated lactate genes associated with immunotherapy resistance in a pan-cancer analysis

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ABSTRACT

Background: Although the lactate pathway has been reported to lead to immune escape through the inhibition of effector T cells, the cancer-intrinsic lactate signature has not been identified, and the immunotherapeutic efficacy and potential mechanism of the lactate signature are still unclear.
Methods: We defined a pan-cancer up-lactate score by comparing malignant tissues and normal tissues in the TCGA cohort. The immunotherapeutic efficacy was evaluated in non-small cell lung cancer (NSCLC), metastatic renal cancer (mRCC), bladder cancer (BLCA) and melanoma cohorts. The cancer cell-intrinsic mechanism to immune checkpoint inhibitors (ICIs) resistance was measured using single cell sequencing (scRNA-seq) data. Pathway activation was evaluated in the TCGA cohort and CPTAC cohort with transcriptomics and proteomics. The co-occurrence of up-lactate signature and mTOR signaling was determined by spatial transcriptomics of the tissue samples. Immunotherapy resistance and pathway regulation were validated in the in-house NSCLC cohort.
Results: Patients with the high up-lactate scores had significantly short overall survival (OS) than those with the low up-lactate scores ($p < 0.001$) across multiple types of cancers. The up-

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regulated lactate signature exhibited higher expression in the malignant cells compared with stromal cells and immune cells in multiple scRNA-seq datasets. A high up-lactate score was associated with poor OS in NSCLC, mRCC, BLCA and melanoma patients who received anti-PD(L)1 antibody. The up-lactate score was higher in the responders of cancer cells, but not in immune cells and stromal cells compared with the non-responders ($p < 0.05$). Moreover, up-lactate score was positively correlated with mTOR signaling across multiple cancers. In patients with NSCLC who received anti-PD-1 antibody, higher up-lactate scores were associated with significantly shorter PFS compared to lower up-lactate scores ($p < 0.001$). Additionally, the up-lactate score was associated with cold tumor, and was positively correlated with mTOR signaling.

Conclusion: Collectively, we defined a pan-cancer up-lactate signature, which is a feature of malignant cells and is associated with ICIs resistance. This reveals a coherent program with prognostic and predictive value that may be therapeutically targeted.

1. Introduction

Lactate is a product of glycolysis, produced by tumor cells [1], stromal fibroblasts [2] and infiltrating immune cells [3] in the tumor microenvironment (TME). It has been reported to contribute to immune escape by inhibiting effector T cells [4,5]. Lactate derived from tumor cell glycolysis reduced the antitumor activity of $CD8^+$ T cells and natural killer (NK) cells, exerts immunosuppressive effects and promotes tumor growth [4,5]. Previous research showed that increased lactate dehydrogenase (LDH) activity leads to tumor immune escape by inhibiting immune cells function [4]. Moreover, the expression levels of LDHA can be regulated by HIF1α, MYC and p53, which facilitated the epithelial-to-mesenchymal transition (EMT), angiogenesis and increased invasion, while high LDHA expression was associated with unfavorable patient survival outcomes in multiple types of cancer [6]. These studies highlighted the crucial targetable potential of the lactate pathway for cancer inhibition and facilitating the anti-tumor immune response.

Although these findings suggest that targeting lactate production and accumulation in tumors is an attractive approach for cancer treatment or mitigating immunosuppression, several questions remain unexplored. (i). The cancer intrinsic genes in the lactate pathway, which play a predominant role in fueling tumor growth and anti-immunity have not been identified; (ii). Association between the cancer intrinsic lactate genes and the immunotherapeutic efficacy has not been explored before, particularly in the context of large-scale cancer cohorts and immunotherapeutic cohorts; (iii). The mechanisms mediated by the lactate pathway in intracellular programs of malignant cells involved in immunosuppression are not explicit.

Therefore, in this study, we defined a pan-cancer up-lactate signature derived from cancer cells, representing the cancer-intrinsic malignant feature. The up-lactate signature was associated with a deserted immune infiltration phenotype, and a high up-lactate score was associated with poor overall survival (OS). Moreover, we investigated the potential mechanisms through which the up-lactate signature induces immune resistance. These findings substantially extend our understanding of the factors associated with ICI resistance and provide new perspectives on anticancer therapies or overcoming primary resistance to ICIs treatment.

2. Methods

2.1. The in-house cohort

Totally, we respectively analyzed 32 advanced-stage NSCLC patients who were progressed after first-line tyrosine kinase inhibitors (TKIs) and treated with anti-PD-1 antibody at National Cancer Center/Cancer Hospital and Chinese Academy of Medical Sciences from December 1st, 2022 to August 1st, 2023. Tissue samples were sequenced before anti-PD-1 antibody. Eligibility criteria included being aged 18–75 years; having an Eastern Cooperative Oncology Group score of 0 or 1; having histologically confirmed and standard treatment–recurrent or standard treatment–intolerant stage IV NSCLC; This study was approved by the ethics committees of the Cancer Hospital, Chinese Academy of Medical Sciences, and conducted in accordance with the Declaration of Helsinki and the international standards of good clinical practice. Finally, 16 patients who met the inclusion and exclusion criteria with available QC-passed mRNA data were included for analysis. The median follow-up time was 4.3 months. All patients have informed consent, and this study was approved by the ethics committees of the National Cancer Center (No.23/340–4082). RNA extraction, sequencing library construction, sequencing and FASTQ data quality control were performed in accordance with the protocol by Nick D.L. Owens et al. [7].

2.2. The public cohort

TCGA and CPTAC pan-cancer cohort and immunotherapeutic cohorts included in this study were summarized in Table S1 and the **Supplementary Materia and Methods**.

2.3. Single-cell RNA sequencing (scRNA-seq) datasets

Multiple types of cancers including NSCLC (GSE117570) [8], CHOL (GSE125449) [9], LIHC (GSE125449) [9], Merkel Cell Carcinoma (MCC) (GSE117988) [10], OV (GSE118828) [11], SKCM (GSE115978) [12], and STAD (GSE134520) [13] with available single cell transcriptomic sequencing data were leveraged and download from the Tumor Immune Single-cell Hub (TISCH) (http://

tisch.comp-genomics.org/search-gene/). The standardized analysis workflow for processing all the collected datasets, including quality control, batch effect removal, cell clustering, differential expression analysis, cell-type annotation, malignant cell classification and gene set enrichment analysis were described as previous study [14]. The differences of up lactate or down signatures across

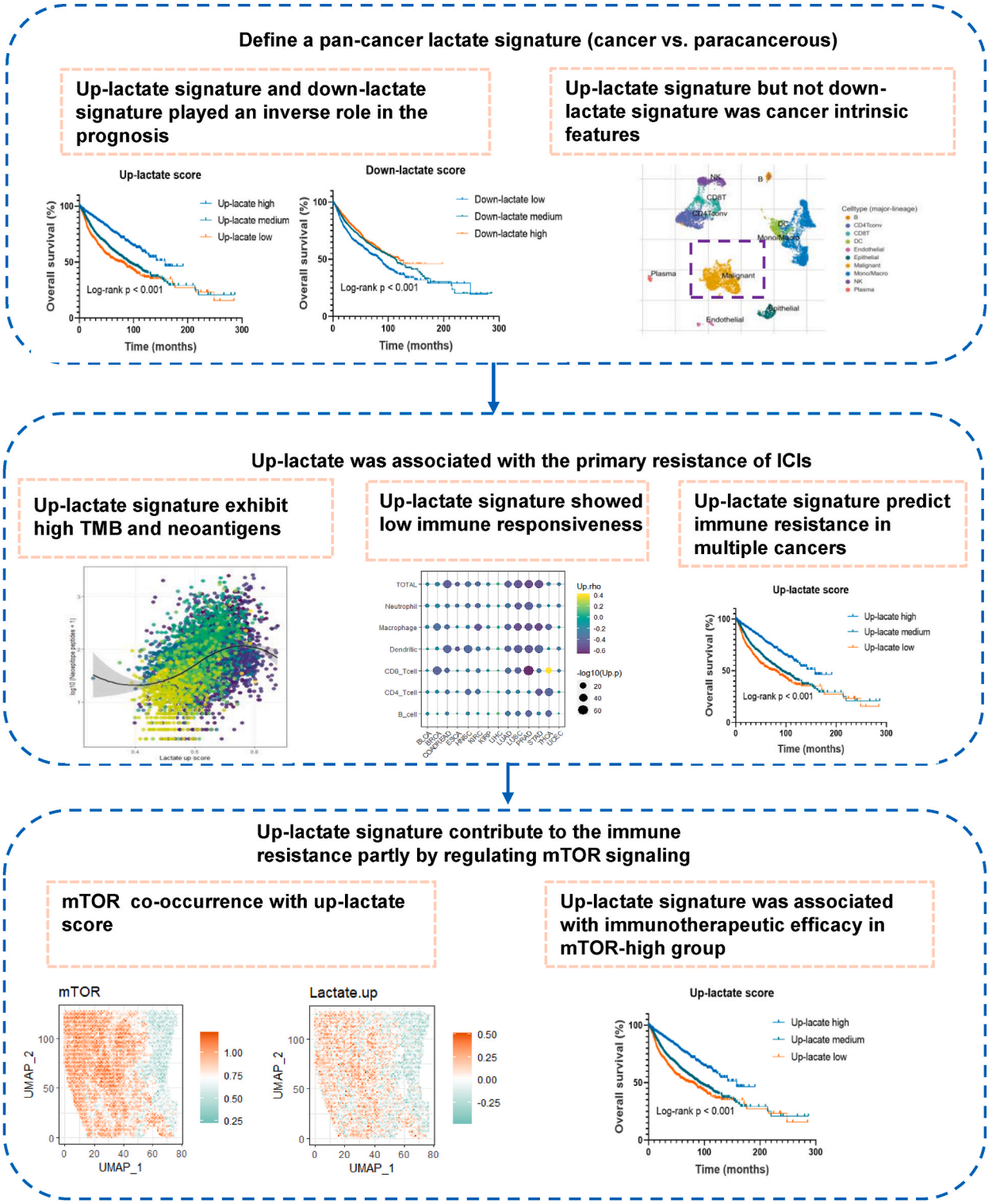

Fig. 1. Flow chart of this study.

different cell types were measured by Kruskal-Wallis's test.

The processed scRNA-seq data from HNSCC dataset (GSE103322) and SKCM (GSE115978, anti-PD-L1 combined with anti-CTLA4 antibody, n = 25) was derived from previous study [12,15]. HNSCC dataset was also leveraged to analyze the Spearman's correlations of up- or down lactate scores and immune inhibitory checkpoint expressions in the malignant cells. SKCM dataset was used to calculate the Spearman's correlations of up-lactate scores and immune resistant index (T cell resistant index was calculated by the ssGSEA score of the immune resistant program defined by Jerby et al. [12]) in the malignant cells.

2.4. Spatial transcriptome data of prostate cancer

The hematoxylin-eosin (HE)-stained formalin-fixed and paraffin-embedded (FFPE) samples, spatial positions and sequencing data of prostate cancer were download from (https://www.10xgenomics.com/resources/datasets/human-prostate-cancer-adjacent-normal-section-with-if-staining-ffpe-1-standard.). The case was male, original diagnosed as stage II adenocarcinoma, and the total Gleason score was 7.

Specifically, we totally obtained 3460 spots under tissue, with mean reads per spot of 29,191, and the median genes per spot was 4614. Median UMI Counts per spot were 11,444. Gene-barcode counts matrices were analyzed with the *Seurat* R package (version 4.2.0)[24,25]. Cells with <200 genes detected and >10 % mitochondrial gene mapped reads were filtered from downstream analyses. The up-lactate and down-lactate and mTOR signaling scores of individual cells were computed with normalized data using the ssGSEA method. The ssGSEA scores were added into the Seurat object with the *AddMetaData* function, and then visualization by the *FeaturePlot* function (Seurat version 4.2.0).

2.5. Statistical analysis

Statistical analyses were performed with GraphPad Prism version 9 (GraphPad Software, La Jolla, CA) or R versions 4.1.2. Spearman's correlation analysis was performed using the R package *stats* version 4.1.2. For continuous data, the Wilcoxon test was used to compare two groups, while the Kruskal-Wallis's test was used to compare multiple groups. Fisher's exact test was used to compare two groups for categorical data. Benjamini-Hochberg FDR adjustment was utilized for multiple tests correlation. For survival analyses, Up-lactate scores and down-lactate scores were divided into quartiles for categorization and the log-rank test to calculate P-

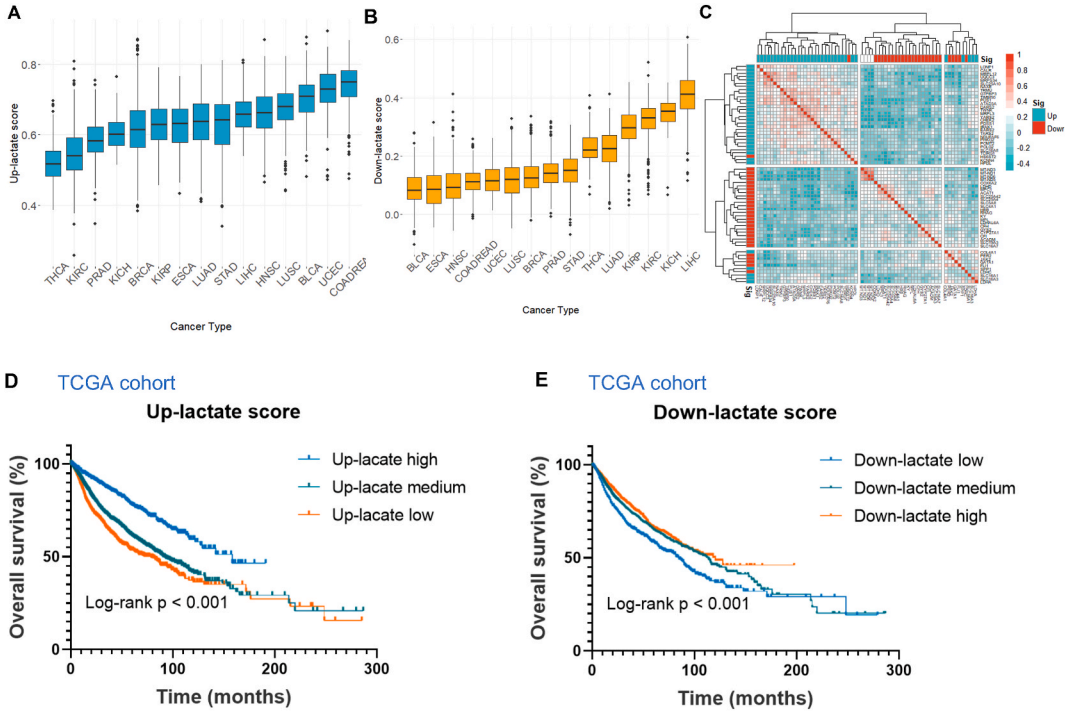

Fig. 2. Landscape of lactate pathway in the pan-cancer and the association with the prognosis in TCGA cohort and CPTAC cohort.
(A) Boxplots of up-lactate scores and down-lactate scores in multiple cancer types of the TCGA cohort.
(B) The intergene Spearman's correlations among up-regulated genes and down-regulated genes of lactate pathway between cancer and normal samples in the TCGA cohort.
(D–E) Unadjusted Kaplan-Meier curves showing survival by the quartile of the up-lactate or down-lactate score. P value was calculated by the log-rank test.
P value was calculated by the log-rank test.

values. Cox proportional hazards (PH) regression model was used to calculate the Hazard Ratio (HR), the 95 % confidence interval (95 % CI), and P values.

3. Results

3.1. Pan-cancer analysis of dysregulation in lactate pathways

The flow chart of study is shown in Fig. 1. Initially, we defined a pan-cancer lactate transcriptional signature to investigate the dysregulation in the lactate across different cancer types (Fig. S1A). We obtained 259 lactate-related genes through an integrative analysis of lactate-related pathways in MSigDB [16] (Table S2). A total of 34 genes were up-regulated and 30 genes were down-regulated in the lactate pathway by comparing malignant tissues to normal tissues (Supplementary Figs. S1B–1C, Table S3). Given the potential differences in biological roles of these dysregulated genes across different tumors, we integrated up-regulated and down-regulated lactate genes using ssGSEA to generate the up-lactate and down-lactate scores, respectively. The up-lactate scores and down-lactate scores showed broad variation across tumor types (Fig. 2A-B). Of note, cancer types with higher up-lactate scores, such as COADREAD, UCEC, BLCA, LUSC, had relatively low down-lactate scores. We observed a similar tendency in the CPTAC cohort (Figs. S1D–1E). Moreover, clustering the Pearson correlation matrix of intergene correlations across TCGA cancers identified generally distinct blocks of up-regulated and down-regulated genes (Fig. 2C–Table S4), suggesting co-regulation within the up- or down-regulated genes.

The up-lactate and down-lactate scores were further segregated into 3-quartiles (Q1–Q3) to investigate their prognostic significance. Patients with low up-lactate scores had significantly longer overall survival (OS) than those with the high up-lactate scores (Fig. 2D, log-rank $p < 0.001$). In contrast, patients with the low down-lactate scores showed an increased risk of death than those with the high down-lactate scores (Fig. 2E, log-rank $p < 0.001$). We then performed a multivariable cox regression to examine the prognostic role in the TCGA cohort based on 3-quartile thresholded up- or down-lactate scores by adjusting clinical stage and tumor type. Both up- and down-lactate scores remained significantly associated with superior or inferior prognosis, respectively (Supplementary Figs. S1F–1G), suggesting that the dysregulated lactate genes were clinically relevant and up-lactate and down-lactate score had opposite prognostic roles in cancers.

3.2. Up-lactate signature was an intrinsic property of tumor cells

Although lactate could extensively express in various cell types, different cells may exert a distinct role in determining the biological function of lactate (1). To identify the cellular source of the pan-cancer lactate signature, we conducted a multi-omics analysis. Initially, we calculated the correlations between the up- or down-lactate scores and estimated purity by different approaches [17–20], given that if the lactate signature came from the tumor and it would be a positive association with tumor purity [21]. As a result, the purity was positively correlated with the up-lactate scores (Rho = 0.31, $p < 2.2e\text{-}16$, Spearman's correlation. Fig. 3A), but was inversely correlated with the down-lactate scores (Rho = −0.24, $p < 2.2e\text{-}16$, Spearman's correlation, Fig. 3A). The consistent positive or inverse correlations between the up-lactate or down-score and purity were observed when stratified by cancer type (Table S5). Besides, this finding was further supported by the positive correlation between the up-lactate scores and the median VAF (Fig. 3B-C). Collectively, these results suggested the up-regulated lactate genes were cancer cell origin instead of the stromal cell origin.

To further ensure the cancer origin instead of the stromal or immune cells origin of the up-regulated lactate signature. Multiple types of cancers (n = 8) including HNSCC (GSE103322) [15], NSCLC (GSE117570) [8], CHOL (GSE125449) [9], LIHC (GSE125449) [9], MCC (GSE117988) [10], OV (GSE118828) [11], SKCM (GSE115978) [12], and STAD (GSE134520) [13] with available scRNA-seq data were leveraged, in which the cells were separated into cancer, stromal or immune cells origin by cell sorting technology at high resolutions. Consistently, we observed that the up-lactate score exhibited significantly higher expression in the malignant cancer cells compared with the stromal cells or immune cells in multiple datasets (Fig. 3D-G, Figs. S3A–3E, Kruskal-Wallis's test, $p < 0.001$). In contrast, the down-lactate scores were associated with lower, higher, or no significant differential expression in the cancer cells compared with stromal or immune origin cells (Kruskal-Wallis's test, $p > 0.5$; Fig. 3D-G, Figs. S3A–3E).

Altogether, these results highlighted that the up-regulated but not the down-regulated lactate genes were cell-intrinsic features of malignant tumor cells, which were not perturbed by tumor purity, stroma, or immune infiltration, providing insights into exploring intracellular tumor signaling and immunoregulatory properties.

3.3. Pan-cancer up-lactate signature was associated with immune cold tumor

To explore the intracellular immune regulation of the lactate pathway in tumors, we first investigated the correlations between lactate scores and TMB, neoantigens and immune infiltration, which represented immunogenicity and immune responsiveness in tumors, respectively. Although the up-lactate scores but not down-lactate scores were associated with high TMB, and neoantigens in pan-cancer analysis (TMB and neoantigens: up-lactate score Rho >0.50, $p < 2.2e\text{-}16$ for; Fig. 4A-B; down-lactate score: Rho < −0.20, $p < 2.2e\text{-}16$; Figs. S4A–4B, Spearman's correlation), the total predicted immune cells infiltration was inversely correlated with the up-lactate scores (Rho = −0.29, $p < 2.2e\text{-}16$, Fig. 4C), but showed a positive correlation with down-lactate scores (Rho = 0.27, $p < 2.2e\text{-}16$, Fig. S4C) across tumor types. The negative correlation remained after adjusting purity (linear regression, adjusted by purity, mean Rho = −0.33; Table S6) for the up-lactate scores, suggesting that the immune exclusion of up-lactate signatures was not influenced by tumor purity.

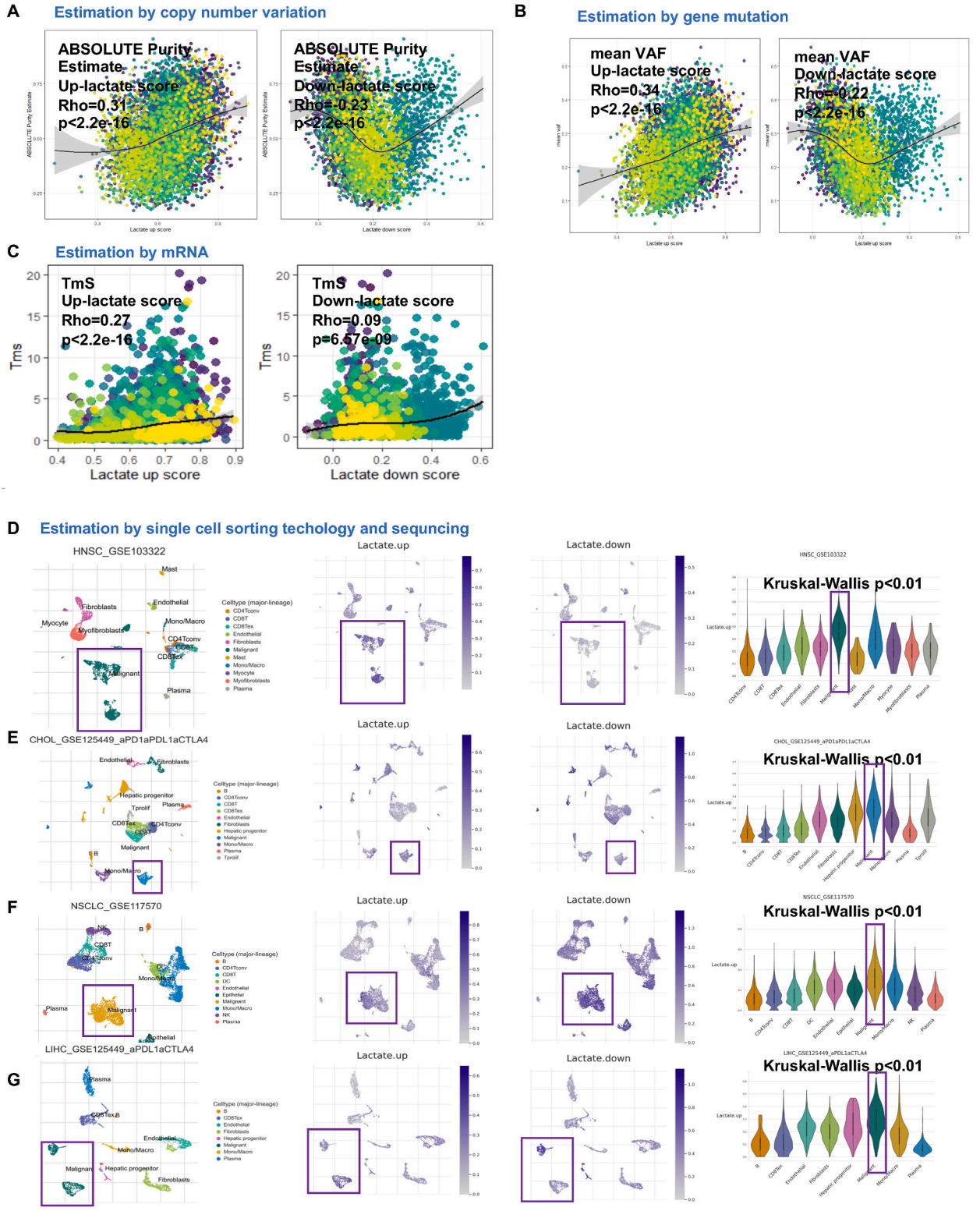

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Fig. 3. Up-regulated lactate signature instead of down-regulated lactate signature was cancer cell origin.
(A) Positive or inverse correlations between up-lactate scores or down-lactate scores and purity estimated by ABSOLUTE.
(B–C) Positive or inverse correlations between up-lactate scores or down-lactate scores and mean variation allele frequency and total cancer specific mRNA.
(D–G) The up-lactate and down-lactate scores in different cell types in the HNSCC dataset, CHOL dataset, NSCLC dataset, LIHC dataset.
Note: Different cancer types were marked by different colors for **(A–C).**

Since the functional gene expression signatures (Fges) [22] represented the major functional components and immune, stromal and other cellular populations in tumor defined previously [22], we further explored the association between the Fges and up-lactate scores stratified by quantiles. Signatures representing immune-depleted features and various immune cell components (e.g., macrophages, MDSC, Treg) were dramatically decreased in the high up-lactate score group compared with the low up-lactate score group (Mann-Whitney, $p < 0.001$, Fig. 4D). In contrast, signatures depicting cancer proliferation properties (e.g., EMT signature, proliferation) were increased in the high up-lactate score group compared with the low up-lactate score group (Mann-Whitney, $p < 0.001$, Fig. 4D). Furthermore, the up-lactate scores were positively correlated with a high proliferation rate, and higher homologous recombination defects (Figs. S4D–4F and Fig. 4E). Furthermore, we observed lower frequency immune cells count in the low up-lactate score group (bottom 25 % score) compared with the high up-lactate score group (top 25 % score) (Chi-square-test, $p < 0.05$, Fig. 4F-H) in multiple scRNA-seq datasets.

Collectively, these results suggest the up-lactate score is associated with high TMB, high PD-L1 expression and T cell inhibitor ligands expression, and decreased immune cell infiltration, suggesting that it is associated with immune "cold" tumor according to the previous definition [24].

3.4. Pan-cancer up-lactate signature was associated with anti-PD-1/PD-L1 antibody failure in multiple immunotherapeutic cohorts

We were motivated to investigate the immunotherapeutic efficacy of the up-lactate score given its association with higher PD-L1 expression, neoantigens but desert immune cell infiltration. We focused on patients who received anti-PD-1/anti-PD-L1 regimens, which reinvigorated dysfunctional tumor-infiltrating $CD8^+$ T cells [25].

We observed a gradual survival benefit from the low to high of the up-lactate scores consistently in patients with mRCC, NSCLC, bladder (BLCA), and melanoma who received anti-PD-1 regimens (Fig. 5A-D; log-rank $p < 0.05$). Even though the statistical difference was not significant in BLCA (log-rank $p = 0.164$), a tendency towards a survival benefit for patients with a low up-lactate score was observed. In the melanoma cohort, low up-lactate group had the best survival benefit from the anti-PD-1 antibody, both in IPI-treatment naïve or IPI-treatment progressed patients (Figs. S5A–5B). Moreover, we also observed an increased up-lactate score in the ICI non-responders (defined as PD and SD) compared to ICI responders (defined as PR and CR) in BLCA and mRCC cohort (Figs. S5C–5D).

The results suggest that a high up-lactate score is associated with a poor survival in patients who received anti-PD-1 regimens.

3.5. Pan-cancer up-lactate signature associated with the cancer-intrinsic ICI resistance

The above results suggest that the up-lactate signature is an intrinsic property of tumor cells. It was associated with immune "cold" tumor, and partially contributes to the failure of anti-PD(L)-1 regimens. In immunotherapy cohort, we observed that high up-lactate scores were associated with higher TMB (Kruska-Walis's test, $p = 0.028$) and neoantigens (Kruska-Walis's test, $p = 0.026$, Fig. 6A-B). The highest quantile of up-lactate score was associated with the higher PD-L1 expression (IHC IC > 2, $p < 0.05$, Chi-square test) compared with the lowest quantile of the score (Fig. 6C). Moreover, immune cells infiltration revealed an overall negative correlation with up-lactate scores (Figs. S6A–6B). Besides, the $CD8^+$ T cell counts at the tumor margin or at the tumor center, measured by immunofluorescence detection, were inversely collected with the up-lactate score (Figs. S6C–6D), suggesting an immune desert phenotype. Considering the higher immunogenicity and decreased immune infiltration in the tumors with high up-lactate scores, the up-lactate signature may be primarily associated with primary ICI resistance.

Primary ICI resistance could be driven by two mechanisms, one is cancer intrinsic, induced by the activation of various oncogenic pathways, and the other is extrinsic, dependent on the immunosuppressive factors produced by the TME [26,27]. To explore whether cancer-intrinsic up-lactate signature contributed to the primary ICI resistance, we explored a melanoma cohort (GSE115978, anti-PD-L1 combined with anti-CTLA4 antibody, $n = 25$), which separated the malignant cells and immune cells by flow cytometry and sequenced by scRNA-seq. As a result, the up-lactate scores and T cell resistant index (defined by Jerby et al. [12]) of malignant cancer cells were strongly positively correlated (Rho $= 0.65$, $p < 2.2e\text{-}16$, Spearman's correlation; Fig. 6D). Moreover, the up-lactate scores were higher in the malignant cells of ICI-resistant patients compared to ICI-response patients (Mann-Whitney test $p < 0.001$; Fig. 6E). In contrast, the up-lactate scores displayed no significant differences between ICI-resistant patients and ICI-response patients in the immune cells (e.g., B cells, CD4 T cells, CD8 T cells, NK cells, and monocyte cells) and stromal cells (e.g., endothelial cells and fibroblast cells) (Fig. 6F-G), suggesting that the up-lactate signature, which is cancer intracellular signaling, contributes to the immune "cold" tumor phenotype.

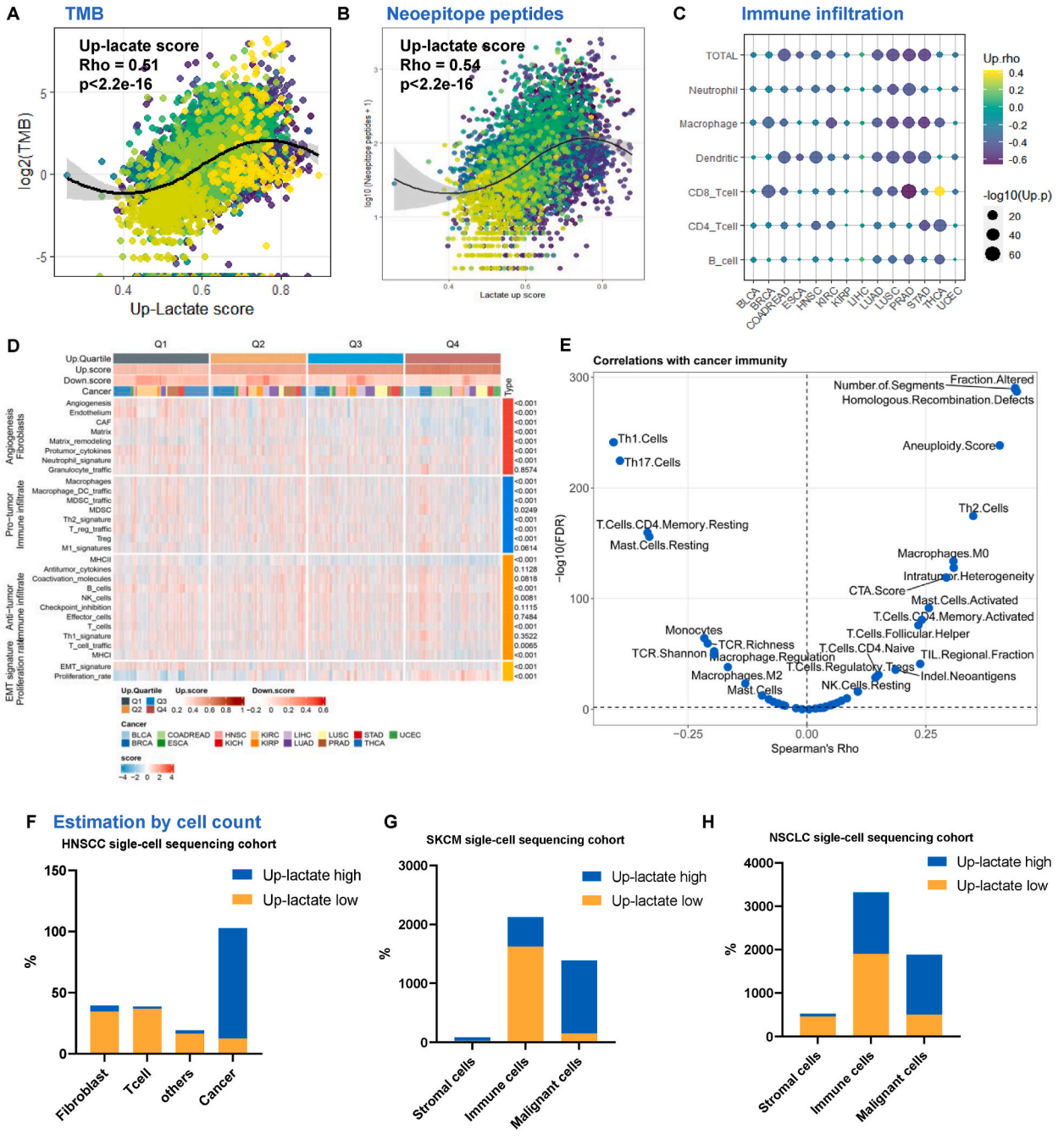

Fig. 4. Up-regulated lactate signature was corrected with immune cold tumor.
(A–B) Positive correlations between up-lactate scores and log transformed TMB and predicted neoepitope peptides. Different cancer types were marked by different colors.
(C) Bubble plots illustrating the positive correlations between up-lactate scores and immune cells infiltration estimated by TIMER across different cancer types in the TCGA cohort.
(D) Heatmap depicting the different signatures and phenotypes (e.g., angiogenesis, fibroblasts, pro-tumor immune infiltrate, anti-tumor immune infiltrate, EMT signature, proliferation, defined by Bagaev et al. [22]) in 1st-3rd quartile (Q1-Q3) groups of up-lactate score.
(E) Scatter plots illustrating the positive or inverse correlations of up-lactate scores and immunity signatures defined previously [23].
(F–H) The cell counts of immune cells, stromal cells, and malignant cells in the 1st-3rd quartile (Q1-Q3) groups of up-lactate score in the HNSCC, SKCM, and NSCLC single-cell sequencing datasets. P values were calculated by Fisher exact test.

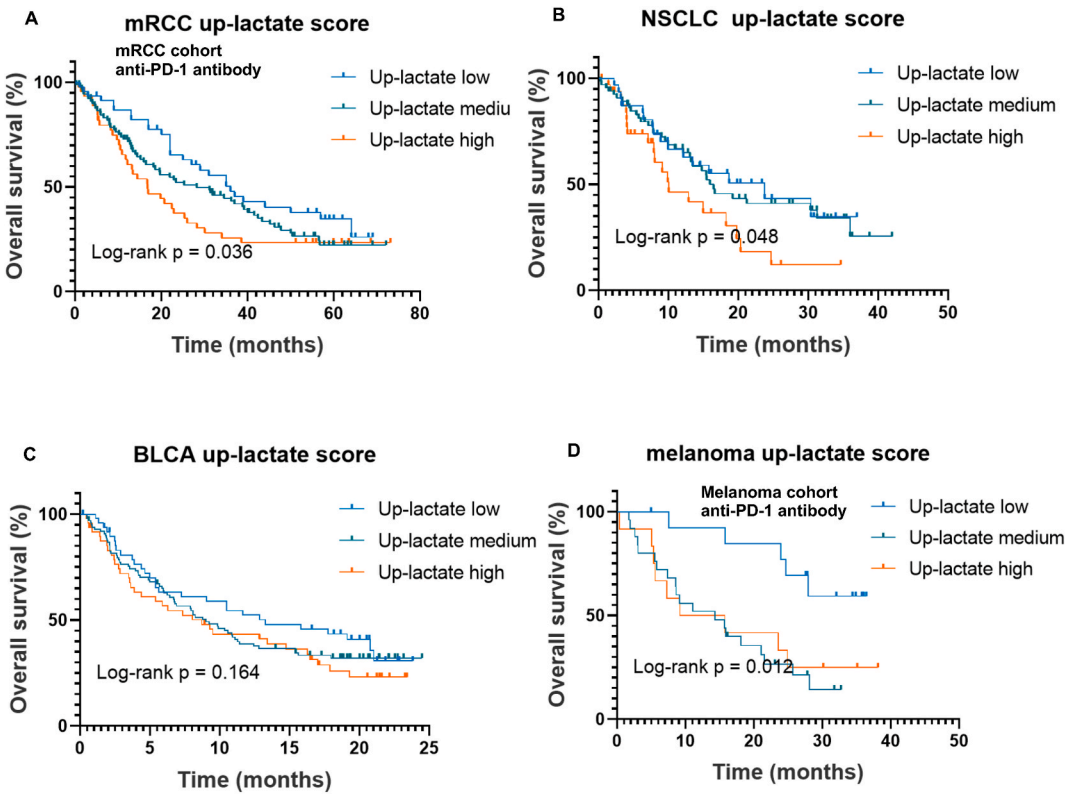

Fig. 5. Up-regulated lactate signature was associated with immunotherapy failure.
(A–D) Kaplan-Meier curves showing survival by the 1st-3rd quartile (Q1-Q3) of up-lactate score in the mRCC, NSCLC, BLCA, and melanoma patients who received anti-PD1 regimens.

3.6. Association between the pan-cancer up-lactate signature and cancer pathways

To further uncover the mechanism of intrinsic immunologic resistance, we endeavored to find putative pathway regulation mediated by lactate dysregulation. The enrichment of mRNA and protein levels highlighted similar modules, where the glycolysis signaling, G2M checkpoint, DNA repair, E2F target, MYC signaling was significantly correlated with up-lactate score in both the TCGA and CPTAC cohorts (Figs. S7A–7B), as well as in the immunotherapeutic cohorts (Figs. S7C–S7D).

Notably, mTOR signaling was positively correlated with up-lactate signature regardless of tumor types or therapeutic modality (Figs. S7A–7D). We then used high-throughput spatial transcriptomic data from prostate cancer to locate the spatial distribution of the up-lactate signature and mTOR signaling. We observed a similar expression pattern of the up-lactate signature and mTOR signaling in HE-stained FFPE samples of prostate cancer (higher ssGSEA scores of the up lactate and mTOR signaling in the cluster 0, 2, 3, 6, 7, 9, 12, 13 compared with those in the cluster 1, 5, 8, 10, 14 (Figs. S7E–S7h);). Meanwhile, mutually exclusive expression pattern of up-lactate and down-lactate signatures in spatial partitioning further validated the above speculation, that the up-lactate signature, rather than the down-lactate signature, originated from cancer cells, and the up- and down-lactate signatures were mutually exclusive.

3.7. The validation of immunotherapeutic efficacy of up-lactate signature with in-house cohort

To further validate the immunotherapeutic efficacy of up-lactate signature, we retrospectively analyzed the patients with advanced NSCLC, who received anti-PD-1 antibody regimen. The baseline characteristic was shown in Table S7. The ORR was 31.3 %, and the median PFS was 4.3 months. We observed a gradually increase in survival from the bottom quartile (Q1) to the top quartile (Q3) of the up-lactate scores (log-rank $p = 0.05$; Fig. 7A), whereas the down-lactate scores did not predict immunotherapeutic efficacy (log-rank $p = 0.35$**)**. Further mechanism analysis revealed consistent signaling pathway activation, similar tothe TCGA and CPTAC cohorts, that glycolysis signaling, G2M checkpoint, DNA repair, E2F target, MYC signaling were significantly positively correlated with the up-lactate score (Fig. S8A). Additionally, the up-lactate score was negatively correlated with immune cells infiltration, including $CD8^+$ T cells, B cells, and Monocyte etc ($p < 0.05$; Fig. 7B). mTOR signaling was positively correlated with the up-lactate score (rho = 0.46, $p < 0.05$; Fig. 7C), further supporting that the up-lactate score is associated with mTOR signaling and contributes to the immune "cold" tumor.

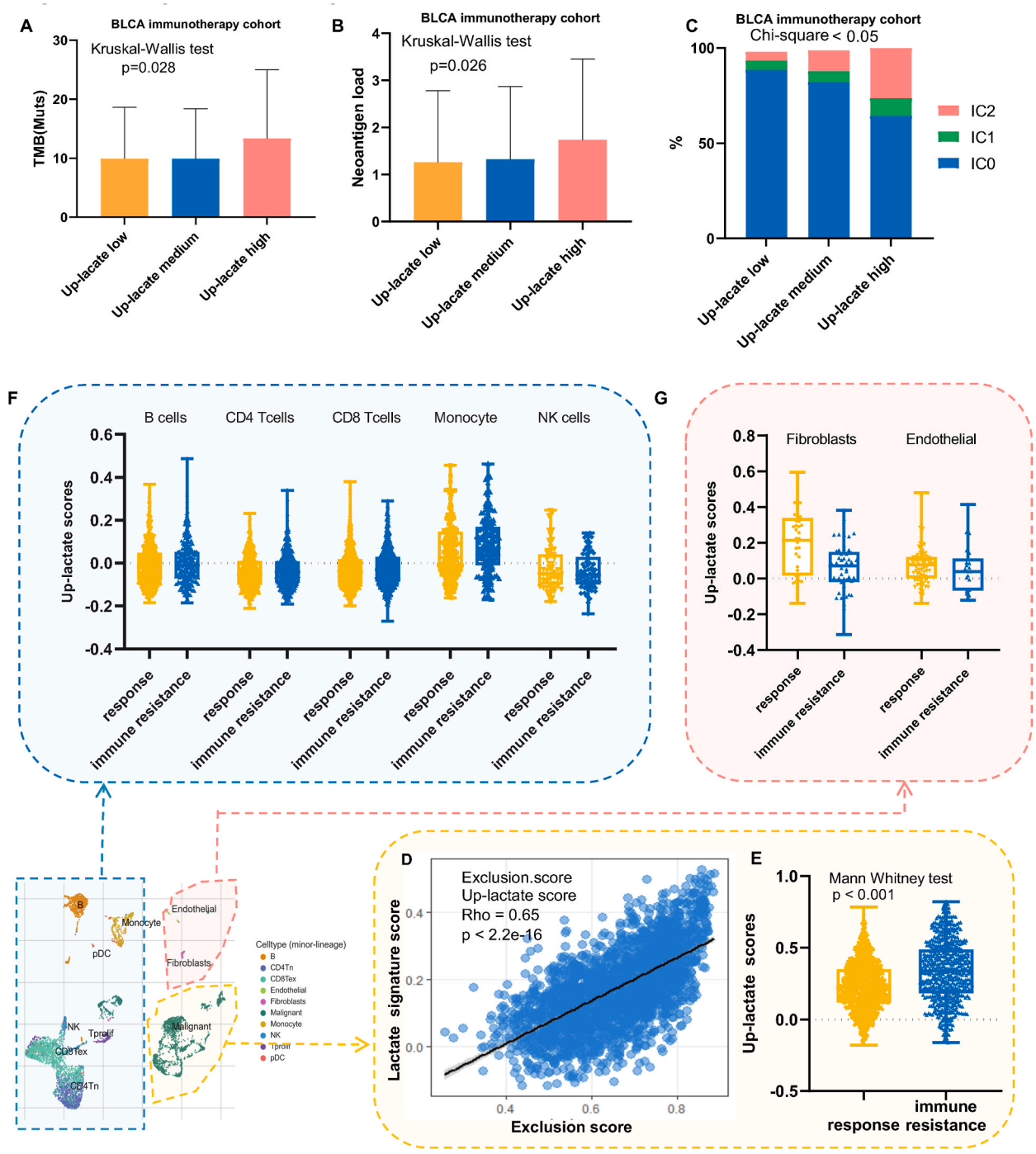

Fig. 6. Up-regulated lactate signature of malignant cells was associated with intrinsic immune resistance.
(A–B) Bar plots representing the tumor mutational burden (Muts) in different groups stratified by the 1st-3rd quartile (Q1-Q3) of up-lactate scores in the BLCA immunotherapy cohort, with error bars to indicate s.d.
(C) The frequency of PD-L1 expression (negative: IC0, moderate: IC1, strong positive: IC2) in different groups stratified by the 1st-3rd quartile (Q1-Q3) of up-lactate scores in the BLCA immunotherapy cohort.
(D) The correlations between up-lactate scores and T cell exclusion scores in the malignant cells of the SKCM cohort. P values were compared by Spearman's correlation.
(E) The comparisons of up-lactate scores between the immune response and immune resistance group in the malignant cells. P values were compared by Mann-Whitney test.
(F–G) The comparisons of up-lactate scores between immune response group and immune resistance group in the different immune cells, and stromal cells. P values were compared by Mann-Whitney test.

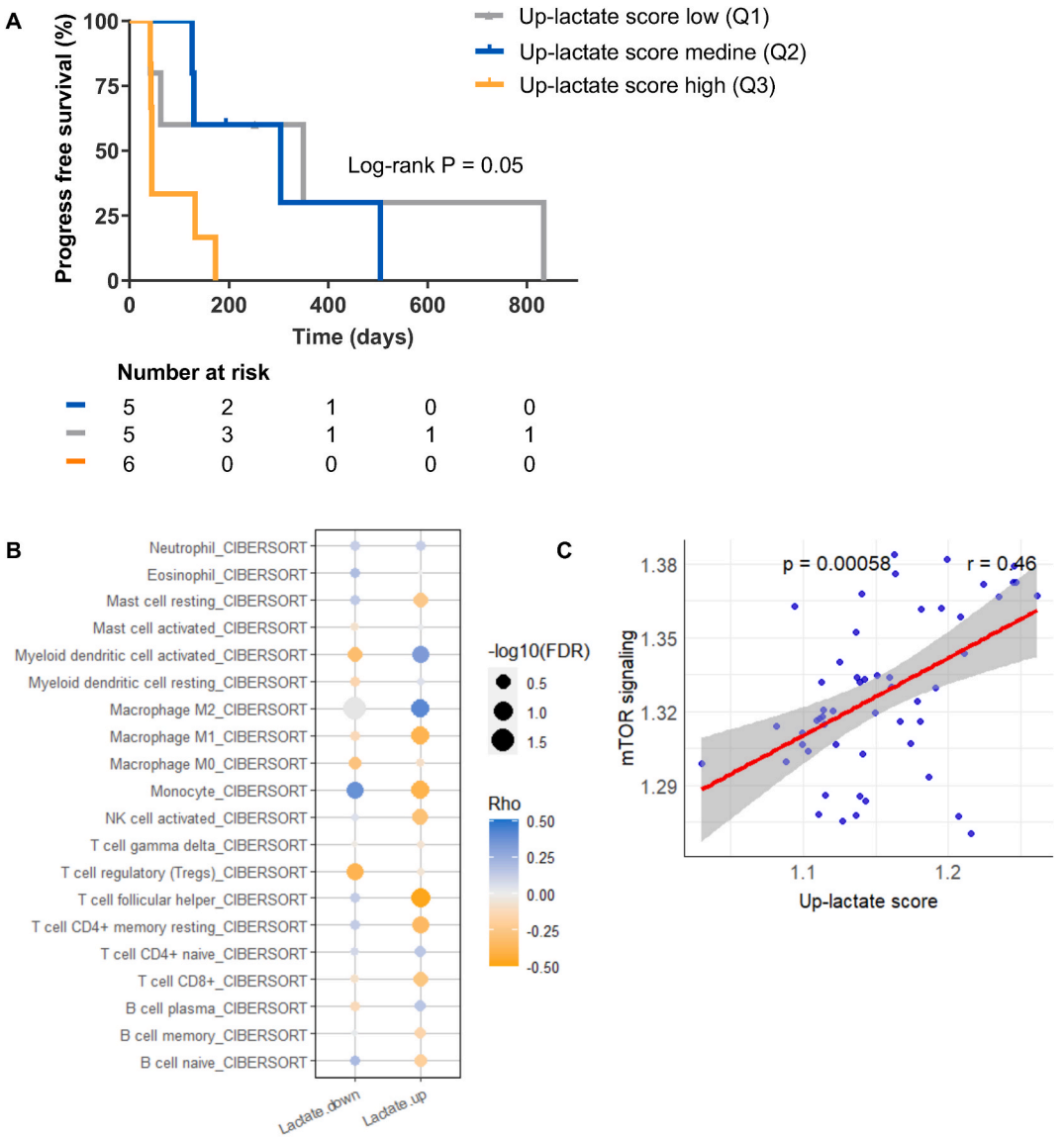

Fig. 7. Up-lactate score was associated with immune resistance in in-house cohort.
(A) Kaplan-Meier curves showing survival by the 1st-3rd quartile (Q1-Q3) of up-lactate score in NSCLC patients who received anti-PD1 regimens.
(B) Bubble plots illustrating the positive correlations between up-lactate scores and immune cells infiltration estimated by CIBERSORT.
(C) The correlations between up-lactate scores and mTOR signaling score. P values were compared by Spearman's correlation.

4. Discussion

Specifically, we defined a pan-cancer lactate score by integrating up-or down-regulated lactate genes through comparison between tumor and adjacent normal tissues. This score was extensively expressed across multiple cancers, and played opposite roles in prognosis, immune regulation, and pathway activation. Our results demonstrated that the pan-cancer up-lactate signature, rather than the down-lactate signature, was an intrinsic property of tumor cells and associated with immune "cold" tumor. Up-lactate score was associated with immunotherapeutic resistance in multiple cancers. Pathway analysis further suggested that up-lactate signature may regulate mTOR signaling in various cancer types. The immunotherapy resistance and pathway regulation of up-lactate score was validated by in-house cohort.

Our results suggested that the up-lactate signature, not the down-lactate signature, was associated with decreased immune cell infiltration at the tumor margin and center, representing an immune "cold" tumor phenotype [27,28]. Given the higher immunogenicity but limited immune cell infiltration in tumors with high up-lactate score, it may be associated with primary ICI resistance. The cancer cell-intrinsic contribution of the up-lactate signature to ICI resistance was further validated through analysis of cancer-specific cells [12]. Results showed that the malignant cells of ICI-non-responders had higher up-lactate scores than ICI-responders, indicating

that the immunosuppression was not confounded by stromal or immune components. Since cancer-intrinsic immune resistance significantly contributes to the inefficacy of anti-PD-1/anti-PD-L1 regimens, high lactate levels were associated with poor survival in multiple cancers, including mRCC, BLCA, NSCLC, and melanoma, in cohorts of patients receiving anti-PD-1/PD-L1 treatment. In this study, we conducted an integrative analysis across more than 10,000 patients from pan-cancer cohorts to identify a cancer-specific up-lactate signature. This signature is associated with T cell exclusion and serves as a predictive marker for immune checkpoint inhibitor (ICI) resistance. Our findings hold potential for several clinical applications: (i) Lactate signature predicts immune resistance in patients who received anti-PD(L)-1 regimen. We observed a gradual survival benefit from the low to high up-lactate scores, consistently in patients with mRCC, NSCLC, BLCA, and melanoma who received anti-PD-1 regimens. This further suggests the a high lactate score is associated with resistance to anti-PD-1 antibody. To further validate the immunotherapy resistance of up-lactate score, we respectively analyzed advance-stage NSCLC patients who had received an-PD-1 antibody. In the in-house NSCLC cohort, a high up-lactate score was associated with a poor immunotherapeutic efficacy.

(ii) A combination therapeutic strategy, such as lactate blockade combined with anti-PD-1 antibody, holds promise for overcoming the primary immune resistance. Hermans et al. [29] employed inhibitors of LDHA and successfully reversed resistance to ICIs in murine models [3]. Correspondingly, our study highlights the rationale for new combination regimens consisting of ICIs and lactate blockade to overcome the primary immune resistance.

(iii) Pathway regulation target screening will help identify more cancer-intrinsic resistant mechanisms. In this study, we found that the up-lactate score was positively correlated with glycolysis signaling, G2M checkpoint, DNA repair, and MYC signaling in TCGA and CPTAC cohorts, as well as in immunotherapeutic cohorts. Pathway activation was further validated in our in-house cohort. Moreover, the positive correlation of the up-lactate signature with mTOR signaling was observed in the pan-cancer analysis, regardless of tumor type and therapeutic modalities. High-throughput spatial transcriptomic data of prostate cancer revealed the same spatial distribution of up-lactate and mTOR signaling in situ, further indicating the co-concurrence of these two pathways. However, further investigation is warranted to explore the signaling crosstalk occurring in cancer-intrinsic cells.

Previous studies have seldom defined the cancer specific signature based on tissue bulk RNA sequencing, and the robustness of the signatures or classification models for the prognostication or therapeutic response varied due to factors like tumor purity, immune cell infiltration or stromal context, cancer heterogenicity, thus making them less reproducible in clinical practice. Single-cell sequencing provides a high-resolution approach to distinguish cancer cells from stromal or immune cells, making it possible to identify the cancer-intrinsic signatures to predict prognosis or therapeutic response [12]. However, the high cost and the complexity of reproducing the data limit its utility in cancer cohorts with large sample size nowadays. Our study provides a framework for identifying the cancer-intrinsic program linking malignant cell states to T cell infiltration levels.

4.1. Limitations of the study

There are several limitations to this study that warrant consideration. First, while our study demonstrates a strong association between high up-lactate score and immune resistance, further tissue-agnostic, biomarker-driven clinical trials are essential to validate this hypothesis. Second, although we observed a trend of increasing survival benefit with decreasing up-lactate score, overlap between certain groups, particularly the high and medium lactate score groups in melanoma, complicates interpretatin The optimal cutoff value for different tumor types needs further validation in prospective, randomized controlled trails. Finally, our findings linking mTOR, the up-lactate signature and the immunosuppression of 'immune cold' tumors are correlative in nature, and additional mechanistic studies are needed to establish a causal relationship.

5. Conclusion

Collectively, we defined a pan-cancer up-lactate signature, by leveraging pan-cancer cohorts, and mapped malignant cell states associated with ICI resistance, revealing a coherent program that has prognostic and predictive value and may be therapeutically targeted.

CRediT authorship contribution statement

Shuiting Fu: Writing – original draft, Conceptualization. **Jiachen Xu:** Writing – original draft, Funding acquisition, Data curation, Conceptualization. **Chunming Wang:** Writing – original draft, Data curation. **Cheng Zhang:** Writing – original draft, Software, Methodology. **Chengcheng Li:** Writing – original draft, Formal analysis, Data curation. **Wenchuan Xie:** Visualization, Software, Methodology, Formal analysis. **Guoqiang Wang:** Visualization, Methodology, Formal analysis. **Xin Zhu:** Writing – original draft. **Yuyan Xu:** Data curation. **Yaohong Wen:** Data curation. **Jingyuan Pei:** Data curation. **Jun Yang:** Data curation. **Mingyang Tang:** Data curation. **Hongkun Tan:** Formal analysis. **Shangli Cai:** Writing – review & editing, Visualization. **Lei Cai:** Writing – review & editing, Project administration. **Mingxin Pan:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

All datasets used in this study have been previously published.

Consent for publication

Not applicable.

Availability of data and materials

All data used in this study are publicly available as described in the Method section. The web links or unique identifiers for public cohorts/datasets are described in the paper. All the datasets used in current study were publicly available through the TCGA and CPTAC data portal, as well as the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) with corresponding accession number and published papers. Sample annotation, processed, and normalized data of single cell sequencing are available in the Tumor Immune Single-cell Hub (TISCH) (http://tisch.comp-genomics.org/search-gene/). All analysis scripts are available on reasonable request. Software and code used in this study are referenced in their corresponding **Method** sections.

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Declaration of competing interest

The authors declare that they do not have any competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39491.

References

[1] T.P. Brown, et al., The lactate receptor GPR81 promotes breast cancer growth via a paracrine mechanism involving antigen-presenting cells in the tumor microenvironment, Oncogene 39 (2020) 3292–3304, https://doi.org/10.1038/s41388-020-1216-5.
[2] L. Ippolito, A. Morandi, E. Giannoni, P. Lactate Chiarugi, A metabolic driver in the tumour landscape, Trends Biochem. Sci. 44 (2019) 153–166, https://doi.org/10.1016/j.tibs.2018.10.011.
[3] MM de Jonge, A Auguste, LM van Wijk, PC Schouten, M Meijers, NT Ter Haar, VTHBM Smit, RA Nout, MA Glaire, DN Church, H Vrieling, B Job, Y Boursin, CD de Kroon, E Rouleau, A Leary, MPG Vreeswijk, T Bosse, Frequent Homologous Recombination Deficiency in High-grade Endometrial Carcinomas, Clin Cancer Res 25 (3) (2019 Feb 1) 1087–1097.
[4] M. Certo, C.H. Tsai, V. Pucino, P.C. Ho, C. Mauro, Lactate modulation of immune responses in inflammatory versus tumour microenvironments, Nat. Rev. Immunol. 21 (2021) 151–161, https://doi.org/10.1038/s41577-020-0406-2.
[5] Z.H. Wang, W.B. Peng, P. Zhang, X.P. Yang, Q. Zhou, Lactate in the tumour microenvironment: from immune modulation to therapy, EBioMedicine 73 (2021) 103627, https://doi.org/10.1016/j.ebiom.2021.103627.
[6] G.A. Brooks, The science and translation of lactate shuttle theory, Cell Metabol. 27 (2018) 757–785, https://doi.org/10.1016/j.cmet.2018.03.008.
[7] N.D.L. Owens, E. De Domenico, M.J. Gilchrist, An RNA-seq protocol for differential expression analysis, Cold Spring Harbor protocols 2019 (2019), https://doi.org/10.1101/pdb.prot098368.
[8] Q. Song, et al., Dissecting intratumoral myeloid cell plasticity by single cell RNA-seq, Cancer Med. 8 (2019) 3072–3085, https://doi.org/10.1002/cam4.2113.
[9] L. Ma, et al., Tumor cell biodiversity drives microenvironmental reprogramming in liver cancer, Cancer Cell 36 (2019) 418–430.e416, https://doi.org/10.1016/j.ccell.2019.08.007.
[10] K.G. Paulson, et al., Acquired cancer resistance to combination immunotherapy from transcriptional loss of class I HLA, Nat. Commun. 9 (2018) 3868, https://doi.org/10.1038/s41467-018-06300-3.
[11] A.J. Shih, et al., Identification of grade and origin specific cell populations in serous epithelial ovarian cancer by single cell RNA-seq, PLoS One 13 (2018) e0206785, https://doi.org/10.1371/journal.pone.0206785.

[12] L. Jerby-Arnon, et al., A cancer cell program promotes T cell exclusion and resistance to checkpoint blockade, Cell 175 (2018) 984–997.e924, https://doi.org/10.1016/j.cell.2018.09.006.
[13] P. Zhang, et al., Dissecting the single-cell transcriptome Network underlying gastric premalignant lesions and early gastric cancer, Cell Rep. 27 (2019) 1934–1947.e1935, https://doi.org/10.1016/j.celrep.2019.04.052.
[14] D. Sun, et al., TISCH: a comprehensive web resource enabling interactive single-cell transcriptome visualization of tumor microenvironment, Nucleic Acids Res. 49 (2021) D1420–D1430, https://doi.org/10.1093/nar/gkaa1020.
[15] S.V. Puram, et al., Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer, Cell 171 (2017) 1611–1624, https://doi.org/10.1016/j.cell.2017.10.044, e1624.
[16] A. Liberzon, et al., The Molecular Signatures Database (MSigDB) hallmark gene set collection, Cell systems 1 (2015) 417–425, https://doi.org/10.1016/j.cels.2015.12.004.
[17] S.L. Carter, et al., Absolute quantification of somatic DNA alterations in human cancer, Nat. Biotechnol. 30 (2012) 413–421, https://doi.org/10.1038/nbt.2203.
[18] F. Favero, et al., Sequenza: allele-specific copy number and mutation profiles from tumor sequencing data, Ann. Oncol. : official journal of the European Society for Medical Oncology 26 (2015) 64–70, https://doi.org/10.1093/annonc/mdu479.
[19] L. Bao, M. Pu, K. Messer, AbsCN-seq: a statistical method to estimate tumor purity, ploidy and absolute copy numbers from next-generation sequencing data, Bioinformatics 30 (2014) 1056–1063, https://doi.org/10.1093/bioinformatics/btt759.
[20] N.B. Larson, B.L. Fridley, PurBayes: estimating tumor cellularity and subclonality in next-generation sequencing data, Bioinformatics 29 (2013) 1888–1889, https://doi.org/10.1093/bioinformatics/btt293.
[21] A. Chakravarthy, L. Khan, N.P. Bensler, P. Bose, D.D. De Carvalho, TGF-β-associated extracellular matrix genes link cancer-associated fibroblasts to immune evasion and immunotherapy failure, Nat. Commun. 9 (2018) 4692, https://doi.org/10.1038/s41467-018-06654-8.
[22] A. Bagaev, et al., Conserved pan-cancer microenvironment subtypes predict response to immunotherapy, Cancer Cell 39 (2021) 845–865.e847, https://doi.org/10.1016/j.ccell.2021.04.014.
[23] V. Thorsson, et al., The immune landscape of cancer, Immunity 48 (2018) 812–830.e814, https://doi.org/10.1016/j.immuni.2018.03.023.
[24] M. Binnewies, et al., Understanding the tumor immune microenvironment (TIME) for effective therapy, Nature medicine 24 (2018) 541–550, https://doi.org/10.1038/s41591-018-0014-x.
[25] S.C. Wei, et al., Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade, Cell 170 (2017) 1120–1133.e1117, https://doi.org/10.1016/j.cell.2017.07.024.
[26] E. Koustas, P. Sarantis, A.G. Papavassiliou, M.V. Karamouzis, The resistance mechanisms of checkpoint inhibitors in solid tumors, Biomolecules 10 (2020), https://doi.org/10.3390/biom10050666.
[27] J. Galon, D. Bruni, Approaches to treat immune hot, altered and cold tumours with combination immunotherapies, Nat. Rev. Drug Discov. 18 (2019) 197–218, https://doi.org/10.1038/s41573-018-0007-y.
[28] M.D. Vesely, T. Zhang, L. Chen, Resistance mechanisms to anti-PD cancer immunotherapy, Annu. Rev. Immunol. 40 (2022) 45–74, https://doi.org/10.1146/annurev-immunol-070621-030155.
[29] D. Hermans, et al., Lactate dehydrogenase inhibition synergizes with IL-21 to promote CD8+ T cell stemness and antitumor immunity, Proc. Natl. Acad. Sci. USA 117 (2020) 6047–6055.