Supplementary Text for "Immuno-genomic atlas for immune checkpoint blockade-based cancer immunotherapy"

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Comparison of transcriptomic data before and after-batch correction

We compared transcriptomic data with and without batch effect correction in a sample subset with Metastatic Urothelial Cancer-Mariathasan (348 samples), Melanoma-Riaz (139 samples), and Melanoma-Gide (98 samples). The principal component analysis (PCA) was performed for uncorrected and batch corrected gene expression. We observed that batch correction mitigated variability associated with the study-of-origin and preserved gene expression variability that was associated with ICB response (Supplementary Figure S1A). We used one positive gene (CXCL13) and one negative gene (CDKL5) as examples. CXCL13 is one of the most consistent ICB response-associated genes across three data sets. The log2(Fold Change) and P-values from each original study were maintained in the batch corrected data (Supplementary Figure S1B). As another example, CDKL5 expression is unassociated with ICB response in any of the three data sets. The result of CDKL5 from the batch-corrected data reproduced those from uncorrected data as well (Supplementary Figure S1C). We ranked genes by their associations with ICB response in the pan-cancer batch-corrected data. GSEA analysis found that the 88 genes, whose expression were associated with ICB response in all three data sets (FDR<0.05), were extremely highly enriched in the top in the pan-cancer data (Supplementary Figure S1D). These results further demonstrated that their associations with ICB response in individual studies were preserved after batch correction.

Potential applications based on Cancer-Immu

Application-1: Signatures prioritization of all 3,652 samples in 16 tumors

The meta-analysis of all 3,652 samples in 16 tumors identified 162 genes, whose expression were significantly associated with ICB response (FDR<0.05) (Supplementary Figure S2A). Among these genes, PD-1 (PDCD1), CD8 (CD8A and CD8B), and IFNG are well-known to regulate efficacy of cancer immunotherapy. Other signatures may play important roles during immune activation although no direct evidence explained that they were the predictive features of ICB response. LAG3 expression is the strongest predictor of ICB response (FDR=1.3e-5, OR=1.56 [1.35-1.80]), followed by IFNG (FDR=1.3e-5, OR=1.59 [1.37-1.84]) and IL21 (FDR=1.2e-4, OR=1.51 [1.31-1.74]). LAG3 is expected as the foremost target next to PD-1/PD-L1 and CTLA-4 in the development of cancer immunotherapy (1). IFNG is a key cytokine produced by activated T cells. Its high expression and/or IFNG-centric signalling genes were associated with ICB-based immune response (2,3). Overexpressed of IL21 suppresses tumor growth through enhanced antitumor immunity and expands the pool of cytotoxic CD8+ T cells, NK cells and NKT cells (4,5). In addition, CXCL9 (FDR=0.045, OR=1.5 [1.21-1.85]), CXCL10 (FDR=1.8e-3, OR=1.5 [1.28-1.76]), CXCL11 (FDR=1.8e-3, OR=1.42 [1.23-1.62]), and CXCL13 (FDR=0.016, OR=1.52 [1.25-1.85]) expression were significantly upregulated in responders compared to non-responders. CXCL9 enhances recruitment of cytotoxic CD8+ T cells into tumor by binding to CXCR3 which is an important receptor on T cell. CXCL13 is a T-cell-intrinsic marker to reflect ICB sensitivity (6). Predominantly expressed by macrophages, CXCL10 and CXCL11 were identified as macrophage signatures (7). Consistently, M1 Macrophage was identified to be a strong ICB predictor (FDR=4.8e-5, OR=1.45 [1.24-1.69]). These 162 genes were highly enriched in immune-stimulated pathways, such as primary immunodeficiency in KEGG (FDR=0), T cell receptor signalling pathway (FDR=4.5e-11) and etc. (Supplementary Figure S2B), activation of immune

cells in biological processes of Gene Ontology (FDR=0 for adaptive immune response, FDR=0 for T cell activation, FDR=3.4e-10 for B cell activation and etc.) (Supplementary Figure S2C).

Application-2: TMB was used as an example to demonstrate the usage of specific signature assessment module of meta-analysis.

There were ten cohorts with TMB data available, which included Van Allen, Riaz, Snyder-Nathanson, Hugo and Liu cohorts for melanoma, Hira Rizvi and Naiyer Rizvi cohorts for non-small cell lung cancer, Le cohort for colorectal cancer, Yost for basal cell carcinoma, and Samstein cohort for multiple cancer types. Except Riaz, Hugo and Yost cohorts, all other cohorts showed that responders carried higher TMB than non-responders (left panel of Supplementary Figure S3). The meta-analysis on the ten cohorts demonstrated that TMB was a strong predictor of ICB response (P=1.1e-5, OR=1.54 [1.27-1.86]). For survival analysis, samples were split into two groups by a user defined cut-off (median-split by default). All cohorts showed that the overall survival (OS, P=5.6e-5, OR=1.50 [1.23-1.82]) or progression-free survival (PFS, P=1.3e-5, OR=2.80 [1.76-4.46]) of patients with high TMB were higher than those with low TMB, even though the results were not significant in one or two cohorts (middle and right panels of Supplementary Figure S3).

Application-3: The associations between DGKZ mutations and ICB responsiveness, OS and PFS based on pan-cancer analysis

DGKZ mutations were significantly enriched in responders in the aggregated cohort of all 3,652 samples, where 15 of 296 responders (5.07%) carry DGKZ mutations, compared to only 3 of 438 non-responders (0.68%) having DGKZ mutations (p=2.8e-4, FDR=0.039). In almost all individual cohorts (except Van Allen data set), no significant differences between responders and non-

responders were detected—perhaps because individual studies reported very few mutations (top panel of Supplementary Figure S4). DGKZ mutations were not observed in the other cohorts. Moreover, patients with DGKZ mutation had significantly higher OS and PFS rates in the aggregated cohort but not when each individual cohort was analysed separately (middle and bottom panel of Supplementary Figure S4). Consistently, knockout of DGKZ has been reported to improve antitumor activities of T cells (8). This example demonstrated that pan-cancer analysis provides the ability to reveal those potential signatures which were masked by cohort-dependent noise and thus could not be sorted out in small scale studies.

Application-4: Dynamic expression of MAPK4 and MET were associated with ICB responsiveness, OS and PFS.

To investigate the association of dynamic features with immunotherapy response, Cancer-Immu allows users to explore the alteration of signatures before and after treatment from the same patients in the specific signature assessment, including expression, expression sum and immune cell components. Using 47 matched samples that were measured before and after treatment in Riaz cohort as an example, the alteration of MAPK4 and MET expression were found to be associated with immunotherapy response. Overexpression of MAPK4 or MET promotes tumor progression by stimulating the PI3K/AKT pathway (9,10). Decreased MAPK4 or MET expression probably indicates inhibition of cancer cell proliferation after ICB-based therapy and could be a potential biomarker. We found responders tended to have decreased MAPK4 expression from pre- to on-treatment, while non-responders were the opposite ($p=0.0019$, top panel of Supplementary Figure S5A). Moreover, patients with decreased MAPK4 expression have higher OS and PFS rates than those with increased abundances ($p=0.20$ and 0.0064, middle and bottom panels of Supplementary Figure S5A). However, MAPK4 expression was not related to ICB responsiveness

in either pre-treatment or after-treatment patients (Supplementary Figure S5A). Compared with static abundance of MAPK4, the dynamic expression change of MAPK4 shows predictive performance for ICB-based clinical outcome. A similar phenomenon was observed on MET gene. Samples with decreased MET expression were more likely to be responders and had significantly improved OS and PFS. However, no significant associations were observed between ICB response and static abundance of MET regardless of before- or post-treatment (Supplementary Figure S5B). Dynamic features provide a different view to explore the tumor-immune interactions. With the ability of exploring dynamic features and accepting single or multiply time points data, Cancer-Immu is expected to stimulate interest in dynamic interactions between immune and cancer cells.

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Supplementary Figures

Supplementary Figure S1. The comparison of transcriptomic data with and without batch correction. **A**. PCA plots before and after batch correction in melanoma-Gide, melanoma-Riaz and metastatic urothelial cancer (UMC)-Mariathasan data sets. **B** and **C**. The associations of CXCL13 and CDKL5 expression with ICB response before and after batch correction. **D**. A pre-ranked GSEA analysis for common significant genes which were identified by each individual data set.

Supplementary Figure S2. The top significant genes in meta-analysis across all samples and functional enrichment analysis of those genes. A. Meta-analysis result of most significant genes and some well-known genes. **B** and **C**. Top significant enriched KEGG pathways and GO biological process.

Supplementary Figure S3. The associations of TMB with ICB therapeutic response, OS and PFS in ten cohorts using specific signature assessment of the meta-analysis module.

Supplementary Figure S4. The associations of DGKZ mutation with ICB therapeutic response, OS and PFS in the pan-cancer analysis across all genetic data-available samples. NSCLC denotes non-small cell lung cancer.

Supplementary Figure S5. Dynamic expression of MAPK4 and MET gene. A and **B**. The associations of dynamic and static expression of MAPK4 and MET with ICB therapeutic response, OS and PFS in Riaz cohort.