Supplementary Methods

Estimation of immunogenomic indicators

Tumor purity and immune score were calculated by the ESTIMATE algorithm provided in the R package "estimate".[1] The tumor purity was also evaluated using the ASCAT algorithm, which is based on the evaluation of somatic copy number variations (SCNAs). Besides, the abundances of immune and stromal cells were quantified using the Microenvironment Cell Populations-counter (MCP-counter) algorithm.[2] The cytolytic activity (CYT) score, which reflects the anti-tumor immune activity of CD8+ cytotoxic T cells and macrophages, is defined as the geometric mean of RNA level of granzyme A (GZMA) and perforin (PRF1).[3] TMB is defined as the total number of non-synonymous somatic mutations per million bases, with 50 Mb as the estimate of exome size. T cell receptor (TCR) and B cell receptor (BCR) profiling data were obtained from a previous publication.[4]

GO analysis/GSEA/ssGSEA

Gene Ontology (GO) term enrichment analysis was performed using the Metascape website (https://metascape.org/). Up-regulated and down-regulated pathways between pCR and RD samples were identified by running a gene set enrichment analysis (GSEA) function embedded in the "clusterProfiler" R package.[5] Reference gene sets of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were downloaded from https://www.genome.jp/kegg/, and that of Reactome and HALLMARK were obtained from the Human MSigDB Collections (https://www.gsea-msigdb.org/gsea/index.jsp). ssGSEA was performed to calculate the enrichment scores of cGAS-STING and NLRP3 gene sets using the R package "GSVA".[6]

Immunohistochemistry (IHC) staining and scoring

The 4-μm-thick formalin-fixed and paraffin-embedded (FFPE) tissue slices were placed in an oven at 65°C for 4h, and then deparaffinized in xylene and rehydrated in 100%, 95%, 85%, 75% alcohol successively. Heat-induced epitope retrieval was performed with Tris-EDTA buffer (pH 9.0) or sodium citrate epitope retrieval solution (pH 6.0) by microwave treatment (MWT). Then, the slides were treated with 3% hydrogen peroxide for 15 min and incubated overnight at 4°C with relevant primary antibody of MHC-I (Abcam, Cat#ab134189), CD4 (Abcam, Cat#ab133616), CD8 (Cell Signaling Technology [CST], Cat#70306), CD20 (Abcam, Cat#ab78237), CD31 (CST, Cat#3528), CA9 (CST, Cat#5649), and CDH2 (CST, Cat#13116), respectively. Next, the slides were incubated with horseradish peroxidase-conjugated antibody for 1h at 37°C and treated with diaminobenzidine for color visualization. The nuclei were counterstained with hematoxylin for 10 seconds. Positive cells were counted for CD4, CD8, CD20 and CD31, and mean optical density was assessed for MHC-I, CA9 and CDH2. Image-Pro Plus version 6.0 software (Media Cybernetics, Inc.. Rockville, MD, USA) was used to evaluate the IHC results of slides. For every slide, three tissue fields at 10× magnification were selected randomly for analysis. Finally, the mean score was regarded as the value of each sample and subjected to statistical analysis.

Multiplex immunohistochemistry (mIHC)

mIHC was conducted using the Opal 6-Plex Manual Detection Kit (Akoya Bioscience, NEL861001KT) following the manufacturer's protocol. The relative markers CD8 (CST, Cat#70306, Opal 520), GZMB (CST, Cat#46890, Opal 570), TCF1/7 (CST, Cat#2203, Opal 690), and PanCK (Abcam, Cat#ab7753, Opal 480) were evaluated via preliminary IHC. Briefly, the 4-μm-thick FFPE tissue sections were deparaffinized, rehydrated, and epitope-retrieved like IHC. Slides were incubated with primary antibody at room temperature for 1 h, followed by Opal Polymer HRP Ms+Rb at 37 °C for 10min and tyramide signal amplification reagents

at room temperature for 10min (Opal 480, 520, 570 and 690, Akoya Bioscience, 1:100). Antibody stripping and epitope retrieval were performed round by round until all markers were stained. Nuclear staining was performed using a DAPI working solution at room temperature for 5 min, and then they were stored at 4°C until image acquisition. Slides were scanned using the Vectra Polaris Quantitative Pathology lmaging System (PerkinElmer). Multispectral image unmixing and positively stained cells were performed using QuPath software (version 0.4.3).

RNA-seq

Briefly, library was constructed by the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® according to the manufacturer's instructions. After the library was qualified, it was sequenced using the Illumina NovaSeq 6000 platform with a double-ending of 150 nt. The FASTQ data was filtered and mapped to the human genome (hg38) using Hisat2 (v2.0.5), and featureCounts (v1.5.0-p3) was used to count the reads numbers mapped to each gene.

Supplementary References

1. Yoshihara K, Shahmoradgoli M, Martínez E, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. Nat Commun. 2013;4:2612.

2. Becht E, Giraldo NA, Lacroix L, et al. Estimating the population abundance of tissue-infiltrating immune and stromal cell populations using gene expression. Genome Biol. 2016;17(1):218.

3. Rooney MS, Shukla SA, Wu CJ, et al. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell. 2015;160(1-2):48-61.

4. Bagaev A, Kotlov N, Nomie K, et al. Conserved pan-cancer microenvironment subtypes predict response to immunotherapy. Cancer Cell. 2021;39(6).

5. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16(5):284-287.

6. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics. 2013;14:7.