Supplementary

Materials and methods

IHC and analysis

IHC was performed to analyze the expression of PD-L1, PD-1, CD8, CD68, CD163 and Foxp3 in the primary and metastatic tumor tissues. Positive PD-L1 expression was scored using a CPS system based on the membrane-positive tumor cells and immune cells. CPS = [(PD-L1+ tumor cells + PD-L1+ immune cells)/ total number of tumor cells]×100. The average density (cells/ high power field) of each lymphocyte subset was quantitatively scored by pathologist. Nine high power fields (magnification ×400) in tumor areas were selected randomly for positive cells counting and the calculation of average number of cells. The antibodies used in the experiment were as follows: anti-PD-L1 (13684, Cell Signaling Technology), anti-CD8 (C8/144B, Ascend Biology), anti-PD-1 (ZM43, Ascend Biology), anti-Foxp3 (ab22510, mAbcam), anti-CD68 (ZM0464, ZSGB-BIO), anti-CD163 (ZM0428, ZSGB-BIO).

NGS

High-quality genomic DNA (gDNA) was extracted from the primary Formalin-fixed paraffin-embedded (FFPE) tumor tissue using commercial kits according to the manufacturer's instructions (Cat Qiagen, No. 56404). Libraries for Exome sequencing were prepared using the KAPA HyperPrep Kits (Roche, KK8504) and xGen® Exome Research Panel v1.0 (IDT). The final libraries were subjected to Illumina sequencing on the Illumina Novaseq6000 sequencer.

Targeted sequencing analyses

High-quality gDNA was extracted from the metastatic FFPE tumor tissues and peripheral blood mononuclear cells using DNeasy Blood & Tissue Kit (Qiagen, Inc.) according to the manufacturer’s instructions. The Panel used for targeted sequencing was HJiA_618G in Accel-NGS 2S DNA Library Kit (Swift Biosciences, Inc.). Samples underwent paired-end sequencing on an Illumina Novaseq platform (Illumina Inc) with a 150-bp read length. Mean coverage of 1260.5× and 223.6 × were achieved for tumor gDNA and peripheral blood mononuclear cells gDNA, respectively.

MiNiPDX

MiNiPDX was performed *in vivo* using OncoVee® MiNiPDX for screening clinically effective regimens for this patient. In the test, patient-derived tumor cells were arrayed within hollow fiber capsules, implanted subcutaneously into mice and cultured for 7 days. The administration was started on the first day of inoculation. There were three regimens as followed: erlotinib plus bevacizumab, everolimus plus bevacizumab and lenvatinib plus everolimus. The cellular activity morphology and pharmacokinetics were systematically evaluated according to ATP test. Reponses to drug were examined by tumor cell relative growth inhibition rate after taking out the MiNiPDX devices.

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