Supplementary methods

Hematoxylin and eosin staining. Tissue samples were sliced and submerged in 10% neutral buffered formalin. The fixation occurred at 25˚C for 3-6 h. After fixation, the tissue samples were dehydrated, embedded in paraffin, and tissue sections were 4 microns thick. The paraffin sections were immersed in xylene for 10 min, xylene changed and soaked for another 10 min to dissolve the wax. Samples were decaxified using a gradient of ethanol concentrations (anhydrous ethanol, 95%, 85%; 70% ethanol), each immersion lasting 5 min. The hydrated tissue sections were cleaned by immersion in PBS solution, each immersion lasting 5 min, repeated three times. Subsequently, tissues were stained in hematoxylin at room temperature for 10 min. Afterwards, excess hematoxylin stain was rinsed with distilled water. The samples were differentiated using 1% hydrochloric acid in ethanol, and the sections were rinsed thoroughly with distilled water. The bluing process was completed using 0.6% ammonia water, rinsing with clean water, and then rinsing the sections thoroughly with distilled water. The sections were immersed in eosin dye at room temperature for 1 min. The sections were thoroughly rinsed with distilled water, then dehydrated using a gradient of 80% ethanol for 5 sec, 95% ethanol for 2 min and anhydrous ethanol for 2 min. The dehydrated tissue sections were immersed in in xylene twice, each immersion lasting 4 min. Finally, tissue sections were dried and sealed with neutral resin. Images were captured with the Olympus BX43 light microscope (Olympus Corporation).

IHC staining. Tissues are embedded in paraffin and fixed in 10% formalin at room temperature for 24 h. Paraffin sections were cut to 3 μm-thick and baked at 72˚C for 1 h. Dewaxing was performed with xylene in 2-3 changes, 10 min per change, followed by a graded alcohol series of 100, 100, 95 and 75%, 2 min/change. The sections were washed 5 times with PBS, 2 min/wash, with 1% Tween-20 added to the PBS. High-pressure antigen retrieval was performed at 100˚C for 2.5 min, followed by cooling to room temperature under cold water. The sections were washed 3-5 times with PBS, 2 min/wash, with 1% Tween-20 added to the PBS. After which, sections were incubated with 3% hydrogen peroxide at room temperature for 10 min and rinsed thoroughly with distilled water. The sections were washed 3-5 times with PBS, 2 min/wash, with 1% Tween-20 added. An appropriate amount of the primary antibody for each IHC index (CD3, cat. no. TA506064; Vimentin, cat. no. ZM-0260; CD5, cat. no. TA501335; CD10, cat. no. TA808300; BCL-6, cat. no. TA804186; multiple myeloma 1, cat. no. ZM-0401; BCL-2, cat. no. TA803003; C-Myc, cat. no. TA500002; p53, cat. no. TA502780; CD20, cat. no. TA800385; CD30, cat. no. TA801630; CD21 cat. no. TA327627; Ki-67 cat. no. TA500265; all OriGene Technologies, Inc.) was added according to the manufacturer's instructions, and the sections were incubated at 37˚C for 1 h. The sections were then washed 3-5 times with PBS, 2 min/wash, with 1% Tween-20 added. An appropriate amount of the secondary antibody stock solution (ultra-sensitive enzyme-labeled goat anti-mouse/rabbit IgG polymer; cat. no. PV-8000; Technologies, Inc.) was added according to the manufacturer's instructions, and the sections were incubated at 37˚C for 30 min. The sections were washed 3-5 times with PBS, 2 min/wash. DAB (cat. no. ZLI-9018; OriGene Technologies, Inc.) was used for color development, prepared fresh, for 6-8 min, and the reaction was stopped and rinsed with tap water for 3-5 min. Hematoxylin restaining was performed at room temperature for 3 min, followed by washing with tap water and differentiation in hydrochloric acid alcohol (time adjusted based on the outcome of restaining). The sections were dehydrated through a graded series of alcohols, cleared in xylene and mounted with neutral balsam. Images were captured with the Olympus BX43 light microscope (Olympus Corporation).

PD-L1 testing. The PD-L1 procedure was performed using the Dako Autostainer Link 48 platform, following a standardized protocol validated for the PD-L1 IHC 22C3 pharmDx assay (1). Deparaffinization, rehydration and target retrieval was conducted in the PT Link (Dako PT100; Agilent Technologies Inc.). The monoclonal mouse anti-human PD-L1 clone 22C3 (cat. no. M3653; Dako; Agilent Technologies Inc.) stock solution was diluted 50 times with 1X PBS. The samples were treated with the monoclonal mouse anti-human PD-L1 clone 22C3 or a control agent at 4˚C for 16-18 h, following which they were incubated with the MaxVision TM HRP-Polymer anti-Mouse/Rabbit IHC Kit (cat. no. KIT-5020; MXB Biotechnologies Inc.) at room temperature for 20 min according to the manufacturer's instructions, that matched the primary antibody's host species. Following this stage, the samples were treated with 20 μl DAB visualization reagent (cat. no. DAB-2031; MXB Biotechnologies Inc.) at room temperature for 4 min that contained secondary antibody molecules and horseradish peroxidase molecules linked to a dextran polymer backbone. The addition of 3,3'-diaminobenzidine tetrahydrochloride chromogen and enhancer led to the formation of a visible antigen reaction product. The samples were then stained with hematoxylin at room temperature for 4 min and prepared with coverslips. Images were captured with the Olympus BX43 light microscope (Olympus Corporation).

DNA extraction and targeted enrichment. FFPE genomic DNA was purified using the QIAamp DNA FFPE Tissue Kit (Qiagen). cfDNA was extracted using the NucleoSpin Plasma XS kit (Macherey Nagel) with optimized manufacturer's protocols. Fresh tissue DNA and whole blood DNA were extracted using the DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's protocols. The DNA was quantified using the dsDNA HS Assay Kit on a Qubit Fluorometer (Life Technologies). Sequencing libraries were prepared using the KAPA Hyper Prep Kit (KAPA Biosystems), as described previously (2). Indexed DNA libraries were pooled together for probe-based hybridization (3) capture of the targeted gene regions covering 437 cancer-related genes. The finial libraries were quantified by qPCR using the KAPA Library Quantification Kit (KAPA Biosystems; Roche Diagnostics) for sequencing.

Sequencing data processing. Paired-end sequencing of the 300 bp amplicon was performed using the Illumina HiSeq4000 platform, followed by data analysis as previously described (4). The mean coverage depth was >100x for the whole blood control samples, and >300x for tumor tissues after removing PCR duplicates. For cfDNA samples, the original targeted sequencing depth was >3,000x.

The final concentration of the library was determined based on the sample throughput and sample quality. In brief, sequencing data were analyzed by Trimmomatic (5) to remove low-quality (quality <15) or N bases, and were then mapped to the human reference genome, hg19, using the Burrows-Wheeler Aligner (BWA-mem, v0.7.12; https://github.com/lh3/bwa/tree/master/bwakit). PCR duplicates were removed by Picard (available at https://broadinstitute.github.io/picard/). The Genome Analysis Toolkit (GATK 3.4.0; https://software.broadinstitute.org/gatk/) was used to perform local realignments around indels and base quality reassurance. Gene fusions were identified by FACTERA (6). Somatic SNPs and indels were analyzed by VarScan2 (7) and Mutect2, with the mutant allele frequency cutoff at 2% for tissue samples and a minimum of three unique mutant reads. Common SNPs were excluded using dbSNP (v137) if they were present in >1% population frequency in the 1000 Genomes Project or the Exome Aggregation Consortium (ExAC) 65,000 exomes database. The resulting mutation list was further filtered by an in-house list of recurrent artifacts based on a normal pool of whole blood samples.

References

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