Supplementary Methods

1. DNA extraction, library preparation, targeted capture, and sequencing

Cell-free DNA (cfDNA) was extracted using the QiAmp Circulating Nucleic Acid Kit (Qiagen) following the manufacturer's instructions. Genomic DNA (gDNA) from white blood cells was extracted using the QIAamp DNA Mini Kit (Qiagen). DNA concentration was quantified with the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). For formalin-fixed paraffin-embedded (FFPE) tissue, only samples with a tumor content of ≥ 20% were eligible for analyses. Genomic DNA was isolated from tissue samples using the ReliaPrep FFPE gDNA Miniprep System (Promega) and quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) following the manufacturer's instructions.

DNA libraries were prepared using the KAPA Hyper Prep Kit (KAPA Biosystems) following the manufacturer's protocol. Cell-free DNA libraries were individually barcoded with unique molecular identifiers (UMI). In brief, 30-60 ng of cfDNA were subjected to end-repairing, A-tailing and ligation with indexed adapters. The libraries were then polymerase chain reaction–amplified and purified for target enrichment. The concentration and size distribution of each library were determined using a Qubit 3.0 fluorometer (Thermo Fisher Scientific) and a LabChip GX Touch HT Analyzer (PerkinElmer), respectively.

For targeted capture, indexed libraries were subjected to probe-based hybridization with a customized next generation sequencing (NGS) panel covering the whole exons of 733 cancer-related genes. The probe baits were individually synthesized 5′ biotinylated 120 bp DNA oligonucleotides (IDT, Coralville, IA). Repetitive elements were filtered out from intronic baits according to the annotation by UCSC Genome RepeatMasker. The xGen Hybridization and Wash Kit (IDT) were employed for hybridization enrichment. Briefly, 500 ng indexed DNA libraries were pooled to obtain 2 μg of DNA. The pooled DNA sample was then mixed with Human Cot-1 DNA and xGen Universal Blockers-TS Mix and dried down in a SpeedVac system. The Hybridization Master Mix was added to the samples and incubated in a thermal cycler at 95℃ for 10 minutes, before being mixed and incubated with 4 μL of probes at 65℃ overnight. Target regions were captured following the manufacturer's instructions. The concentration and fragment size distribution of the final library were determined using a Qubit 3.0 fluorometer (Thermo Fisher Scientific) and a LabChip GX Touch HT Analyzer (PerkinElmer), respectively. The captured libraries were loaded onto a NovaSeq 6000 platform (Illumina) for 100 bp paired-end sequencing.

2. NGS data processing, and variant calling

Raw data were mapped to the reference human genome hg19 using the Burrows-Wheeler Aligner. An in-house developed software was used to generate duplex consensus sequences based on dual UMI integrated at the end of the DNA fragments. To improve specificity, especially for variants with low allele frequency in the circulating tumor DNA, an in-house loci specific variant detection model based on binomial test was applied. The variants were subsequently filtered by their supporting count, strand bias status, base quality, and mapping quality. In addition, variant calling was also optimized to detect variants at short tandem repeat regions. Single-nucleotide polymorphism and indels were annotated by ANNOVAR against the following databases: dbSNP (v138), 1000Genome, and ESP6500 (population frequency > 0.015). Only missense, stopgain, frameshift and non-frameshift indel mutations were kept. Copy number variations (CNVs) and gene rearrangements were detected as described previously. For *ERBB2* copy number stratification, the bottom1/3 was defined as "low ERBB2 CNV", the top 2/3 was defined as "high ERBB2 CNV". Blood tumor mutation burden was detected on the 733-gene panel using validated methods. Only pathogenic and likely pathogenic mutations were considered for the oncoplot and resistance analysis.