Data S1. Details of targeted next-generation sequencing panels and bioinformatics procedures.

Targeted 14-gene RNA-based sequencing (RNASeq) panel. RNASeq was performed on formalin-fixed and paraffin-embedded (FFPE) tumor tissues using the FusionPlex Lung Panel (Integrated DNA Technologies, Inc.), as previously described (23). This commercial targeted panel allows for the detection of single nucleotide variations (SNVs) and small insertions-deletions (indels) in *ALK*, *BRAF*, *KRAS*, *EGFR*, *RET* and *ROS1* genes, as well as fusions and/or exon skipping in *ALK*, *BRAF*, *EGFR*, *FGFR1*, *FGFR2*, *FGFR3*, *MET*, *NRG1*, *NTRK1*, *NTRK2*, *NTRK3*, *RET* and *ROS1* genes, together with assessment of their RNA expression levels (see Table SI for details). FFPE tumor sections were required to contain ≥10% tumor cells, and a minimum of 20 ng of extracted RNA was used as input material for library preparation. The assay demonstrated a sensitivity of 95%, with a minimum sequencing depth of 10X for control genes. Next-generation sequencing data were analyzed using Archer Analysis Software version 6.2.7 (Integrated DNA Technologies, Inc.).

Targeted 51-gene DNA-based sequencing panel. The custom Solid Tumor Solution panel (SOPHiA GENETICS) is a DNA capture-based targeted sequencing assay covering regions of interest across 51 cancer-associated genes (see Table SII for details). Library preparation and sequencing were performed according to the manufacturer's recommendations and as previously described (9). Briefly, 10-100 ng of genomic DNA was used as input for library preparation. Raw sequencing data were analyzed using the Sophia DDM software (pipeline versions 5.5.84, 5.5.93 and 5.5.98; SOPHiA GENETICS). Variant calling required a minimum sequencing depth of 300X and ≥95% target region coverage. The limit of detection was defined as a variant allele frequency of 5% at a read depth of 600X.

Copy number variations (CNVs) were also assessed for 24 genes, including *ALK*, *BRAF*, *CDK4*,*CDKN2A*, *EGFR*, *ERBB2*, *FBXW7*, *FGFR1*, *FGFR2*, *FGFR3*, *HRAS*, *KIT*, *KRAS*, *MET*, *MYOD1*, *NRAS*, *PDGFRA*, *PIK3CA*, *RAF1*, *RET*, *ROS1*, *SF3B1*, *TERT* and *TP53* (Table SII). CNV detection was performed using the CNV module of the Sophia DDM software (SOPHiA GENETICS), which identifies gene amplification events by normalizing coverage levels across target regions within individual samples and across samples from the same sequencing batch, followed by estimation of the average copy number per region. Copy numbers exceeding 3.25 were reported by the algorithm. Samples exhibiting high coverage noise or coverage patterns deviating from those of other samples within the same batch were excluded from CNV analysis.

In addition, the microsatellite instability (MSI) status was determined using the Sophia DDM software by analyzing six unique microsatellite loci and comparing their length distributions to both run-specific reference profiles and a global reference dataset derived from >400 clinical samples.

Hedera profiling 2 (HP2) circulating tumor DNA (ctDNA) panel. The HP2 ctDNA panel (Hedera Dx) was used for the analysis of liquid biopsy samples, as previously described (22). This targeted capture-based DNA sequencing assay enables the detection of SNVs and indels in 32 genes, CNVs in 14 genes, fusions in 9 genes, exon skipping in 2 genes and MSI through the analysis of 36 microsatellite regions (see details in Table SIII). The assay incorporates unique molecular identifiers (UMIs) to enhance the sensitivity for the detection of genomic alterations at low variant allele frequencies, making it particularly suitable for liquid biopsy applications. A minimum input of 30 ng extracted DNA was used for the HP2 assay. Bioinformatics analysis of raw sequencing data was performed using Hedera Prime software version 2.7.0 (Hedera Dx). The analytical modules for the detection of SNVs, indels, fusions, CNVs and MSI have been previously described by Lescuyer *et al* (22). The general limit of detection was defined as a 0.5% allele frequency, with a minimum read depth of 800X after deduplication and UMI grouping.