Supplementary Methods

1. Tissue NGS Analysis

Targeted DNA and RNA sequencing were performed using TruSight Tumor 170 (Illumina, San Diego, CA). The TruSight Tumor 170 panel was designed to detect 170 cancer-related genes, including 151 genes with potential single nucleotide variants (SNVs) and indels, 59 genes with potential amplifications, and 55 genes with fusion and splice variants (S1 Table). Briefly, 40 ng of formalin-fixed paraffin-embedded (FFPE) tissue-derived DNA and RNA were extracted using QIAGEN AllPrep DNA/RNA FFPE Kit (Qiagen, Hilden, Germany). After hybridization capture-based target enrichment, paired-end sequencing (2×150 bp) was performed using a NextSeq sequencer (Illumina) according to the manufacturer's instructions. Variants with a total depth of at least 100× and variant allele frequency of at least 3% was included for analysis. Variant interpretation was based on recommendations from the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. Actionable genetic alterations were stratified into one of four levels based on OncoKB website (http://www.OncoKB.org). Tier 1 variant included level 1 and level 2 genetic alterations that are FDA-approved biomarkers and standard of care. Tier 2 variant included alterations with compelling clinical or preclinical evidence to drug response.

2. Blood NGS Analysis

1) Blood samples and ctDNA isolation and sequencing

Briefly, blood samples were collected in Streck™ tubes. Samples were shipped at room temperature overnight to Guardant Health (Redwood City, CA). On receipt, 10ml of blood was processed by centrifugation by 1,600g for 10 minutes at 4℃ to isolate plasma. cfDNA was then extracted from 1mL aliquots of plasma using the QIAamp circulating nucleic acid kit (Qiagen), concentrated and size selected using Agencourt Ampure XP beads (Beckman Coulter), and quantified by Qubit fluorometer (Life Technologies, Carlsbad, CA).

The G360 panel is a CLIA-certified, College of American Pathologists (CAP)-accredited, New York State Department of Health (NYSDOH)-approved test that detects SNV in 73 genes as well as copy number amplifications (CNA) in 18 genes, fusions in six genes, and insertions or deletions (indels) in 23 genes (S2 Table). Following cell-free DNA (cfDNA) isolation, 5-30ng of DNA underwent oligonucleotide barcoding for digital sequencing library preparation. This library was amplified and enriched for the target genes using biotinylated custom baits. Each of the cancer-related genes were pair-end sequenced on an Illumina HiSeq 2500. Each base pair had a 15,000× average coverage depth. After sequencing, algorithmic reconstruction of the digitized sequencing signals was used to reconstruct the cfDNA fragments.

The Illumina sequencing reads were mapped to the hg19/GRCh37, and cfDNA genomic alterations were identified from the sequencing data by Guardant Health's proprietary bioinformatics algorithms. The absolute number of unique DNA fragments at a given nucleotide position is quantified,

enabling a quantitative measurement of cfDNA as a percentage of the total cfDNA. The variant allele frequency for a given somatic alteration is calculated as the fraction of cfDNA molecules harboring the variant of interest divided by the total number of unique cfDNA molecules mapping to the variant position. The reportable range for SNV, indels, fusions, and CNAs in cfDNA by the G360 assay is ≥0.04%, ≥0.02%, ≥0.04%, and ≥2.12 copies, respectively. Plasma copy number of is reported by centiles with 2+ being between the 50th to 90th percentile in the Guardant Health database and 3+ being greater than the 90th percentile.