Supplementary Methods

Whole-Exome Sequencing

DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens using the All Prep DNA/RNA FFPE kit on a QIAcube (Qiagen). Nucleic acids were quantified using Qbit and assayed by Fragment Analyzer (Agilent), followed by library preparation using the HyperPrep kit (Kapa Biosystems), multiplexing of 6-8 libraries, and exome capture using the xGen Exome v2 (Integrated DNA Technologies). Exomes were sequenced on an Illumina NextSeq. Reads were trimmed with cutadapt version 2.4, and sample quality was checked with fastqc (1). This analysis was performed in accordance to the GATK (RRID:SCR_001876) best practices for variant discovery workflow. BWA (RRID:SCR_010910) mem version 0.7.17 (2) was used to align reads to the reference genome GRCh38. Read duplicates were flagged using Picard tools MarkDuplicates (3) to ensure duplicated reads were not considered during variant calling. BaseRecalibrator and ApplyBQSR (4) were used to detect and correct for patterns of systematic errors in base quality scores that could result in false-positive variant calls. Mutect2 (5) from the GATK package version 4.1.8.0 was used to call SNVs and indels from mapped reads; a standard panel of normals was downloaded from GATK best practices google bucket. Variants were filtered for *read orientation bias* common in FFPE samples using the method proposed by GATK. Variant calls were optimized with FilterMutectCalls to balance sensitivity and precision of variants passing filtering (4,5). Lastly, Funcotator was used to annotate coding regions affected by variant callsets for each sample (4,5). Variants detected at ≥5% allelic frequency with ≥20x coverage (variant plus reference) at the genomic locus were considered tumor mutations.

Supplemental References Cited

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