Supplemental Methods

Whole exome sequencing was performed at the Broad Institute on 17 RNAlater tumor samples (11 at baseline on trial and 6 on treatment) using Illumina's ICE hybrid-capture bait set as previously described. (1-3) Germline DNA was obtained from peripheral blood mononuclear cells. Exome sequencing data alignment and initial processing were performed using the Broad Institute Picard pipeline. BAM files were uploaded into FireCloud (https://software.broadinstitute.org/firecloud/). Sequencing data were passed through additional quality control and processing methods in FireCloud. Quality-control cutoffs were mean target coverage > 100X (tumor) and > 50X (matched normal; GATK Depth of Coverage (4), cross-contamination of samples estimation (ContEst(5)) < 5%, tumor purity (ABSOLUTE (6), FACETS (7)) ≥ 10%, and tumor-in-normal contamination (deTIN (8)) < 10%. Two tumors were removed for purity < 10%.

An adaptation of the Getz Lab Cancer Genome Analysis WES pipeline (https://docs.google.com/document/d/1VO2kX_fgfUd0x3mBS9NjLUWGZu794WbTepBel3cBg08) developed at the Broad Institute was used to call, filter and annotate somatic mutations with modifications to enhance variant classification. For variant calling, the MuTect method (9) was employed to identify somatic single-nucleotide variants with computational filtering of artifacts introduced by DNA fixation procedures (4) and DNA oxidation during sequencing. (10) Strelka was used to identify small insertions or deletions, (11) and a panel of normal filtering was utilized for rare artifacts specific to the bait set used. (9) Oncotator was applied to annotate identified alterations. (12)

Only somatic non-synonymous mutations (i.e., missense, nonsense, indel, splice site) were included to enrich for functional genomic effects. Tumor mutation burden (TMB) was

defined as the nonsynonymous mutational burden normalized by megabases covered at adequate depth to detect variants with 80% power using MuTect given estimated tumor purity by ABSOLUTE. (2) The number of bases covered at a given depth threshold in the tumor was determined using the GATK DepthOfCoverage method. (4) Tumor purity and ploidy were estimated using ABSOLUTE (26) and FACETS. (7) The total number of copy number alterations for each tumor was calculated using an adapted binary segmentation method (CapSeg) (13) and genes were annotated with Oncotator. (12) Allelic copy number alterations were identified by incorporating heterozygous single-nucleotide polymorphisms into the binary segmentation method (Allelic CapSeg). Allelic segments were adjusted for tumor purity and ploidy. Allelic amplifications and deletions were called, integrating the purity- and ploidy-corrected allelic copy number, and then separated into gene-level copy number alterations. (6)

Whole transcriptome sequencing was performed at the Broad Institute on 17 RNAlater tumor samples (11 at baseline on trial and 6 on treatment) using established methods. (1, 3) RNA sequencing results were aligned using STAR and then quantified with RSEM to yield gene-level expression in transcripts per million (TPM).(14, 15) The following alignment metrics were considered: percentage of uniquely mapped reads, average mapped read length, number of splices, mismatch rate per base, percentage of multi-mapped reads, percentage of reads mapped to too many locations, percentage of unmapped reads due to too many mismatches, percentage of unmapped reads due to reads being too short, and percentage of unmapped reads due to other reasons. Samples were clustered across these quality-control metrics using principal-component analysis, which revealed no outlier samples.

For whole transcriptome sequencing analyses, differential gene pathway expression was evaluated with gene set enrichment analysis (GSEA) using the cancer hallmark gene sets from

the Molecular Signatures Database (16) at https://cloud.genepattern.org/ with upper quantile normalized TPM values and 1,000 gene set permutations. In addition, single-sample GSEA (ssGSEA) (17) was performed to generate nonparametric gene set scores for individual samples. Tumor immune cell composition was determined with the CIBERSORTx deconvolution algorithm, (18) inputting the RNA-seq TPM matrix for the cohort and using relative mode on the LM22 gene set with quantile normalization disabled, 1,000 permutations, and B mode batch correction to correct for the batch differences between the RNA-seq data in this study and the LM22 signature, which was derived from microarray data. Tumor infiltrating lymphocytes (TILs) were defined as T cells, NK cells, B cells, and plasma cells identified by CIBERSORTx. (18)

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