Supplemental methods:

Nucleic acid preparation for exome capture and RNA sequencing:

DNA and RNA were isolated from a single core needle biopsy from each tumor sample using the Qiagen DNA/RNA AllPrep Micro kit. DNA was isolated from peripheral blood mononuclear cells (PBMC) at different timepoints using the DNEasy kit (Qiagen). Genomic DNA concentration was quantified on an Invitrogen Qubit® 2.0 Fluorometer (Life Technologies-Invitrogen, Carlsbad, CA, USA) and Trinean DropSense96 spectrophotometer (Caliper Life Sciences, Hopkinton, MA).

Whole exome sequencing:

Exome sequencing libraries were prepared using the Agilent SureSelectXT Reagent Kit and exon targets isolated using the Agilent All Human Exon v6 (Agilent Technologies, Santa Clara, CA, USA). 200 ng of genomic DNA was fragmented using a Covaris LE220 focused-ultrasonicator (Covaris, Inc., Woburn, MA, USA) and libraries prepared and captured on a Sciclone NGSx Workstation (PerkinElmer, Waltham, MA, USA). Library size distributions were validated using an Agilent 2200 TapeStation. Additional library QC, blending of pooled indexed libraries, and cluster optimization was performed using Life Technologies' Invitrogen Qubit® 2.0 Fluorometer.

The resulting libraries were sequenced on an Illumina HiSeq 2500 using a paired-end 100bp (PE100) strategy. Image analysis and base calling was performed using Illumina's Real Time Analysis v1.18 software, followed by "demultiplexing" of indexed reads and generation of

FASTQ files using Illumina's bcl2fastq Conversion Software v1.8.4 (http://support.illumina.com/downloads/bcl2fastq_conversion_software_184.html). Read pairs passing standard Illumina quality filters were retained for further analysis, yielding an average of 65.2M read pairs for the tumors and 64.4M read pairs for the normals among samples reported here. Paired reads were aligned to the human genome reference (GRCh37/hg19) with the BWA-MEM short-read aligner [1 2]. The resulting alignment files, in standard BAM format, were processed by Picard 2.0.1 and GATK 3.5 [3] for quality score recalibration, indel realignment, and duplicate removal according to recommended best practices [4].

To call somatic mutations from the analysis-ready tumor and normal BAM files, we used three independent software packages: MuTect 1.1.7 [5] and Strelka 1.0.14 [6]. Variant calls from both tools, in VCF format, were annotated with Oncotator [7]. Annotated missense somatic variants were combined into a single summary for each sample as follows. First, any mutation annotated as “somatic” but present in dbSNP was removed if it was not also present in COSMIC or its minor allele frequency was greater than 1% (according to the UCSC Genome Browser snp150Common table). Variants supported by both variant callers were retained, and those supported by only one variant caller were subject to manual inspection.

RNA-Seq data processing:

An RNA-seq library was prepared from total RNA using the TruSeq RNA Sample Prep v2 Kit (Illumina, Inc., San Diego, CA, USA) and a Sciclone NGSx Workstation (PerkinElmer, Waltham, MA, USA). Library size distributions were validated using an Agilent 2200 TapeStation (Agilent

Technologies, Santa Clara, CA, USA). Additional library QC, blending of pooled indexed libraries, and cluster optimization was performed using Life Technologies' Invitrogen Qubit® 2.0 Fluorometer (Life Technologies-Invitrogen, Carlsbad, CA, USA). The library was sequenced on an Illumina HiSeq 2500 to generate 61M read pairs (two 50nt reads per pair). Remaining reads were aligned to a human RefSeq derived reference transcriptome with RSEM 1.2.19[8] to derive abundances for each gene in transcript-per-million (TPM) units.

Antigen presenting cells

Autologous B cells were isolated from PBMC using positive selection with magnetic beads coated with antibodies recognizing CD19 (Miltenyi, cat 130-050-301) according to the manufacturer's instructions (Miltenyi). B cells were cultured for 7 days in B-cell media comprised of IMDM media (Life Technologies) supplemented with 10% human serum (in-house), 100 U/ml penicillin and 100 µg/ml streptomycin (Life Technologies), 2 mM L-glutamine (Life Technologies), and 200 U/ml IL-4 (Peprotech) in the presence of 3T3 cells expressing human CD40L as described [9]. B cells were then re-stimulated with irradiated (5000 Gy) 3T3 expressing human CD40L cells and fresh medium containing IL-4 was added every 3 days. B cells were used in assays at day +3 after stimulation.

Peptides and T cell culture

To discover which mutations had detectable T cell responses, PBMC were initially stimulated with overlapping 20-mer peptides (Elim Biopharma). Two crude peptides spanning each mutation with the mutated residue at position +7 or +13 of the 20 amino acid sequence were used for stimulation. Initial peptide stimulation for detecting mutations was performed with a

pool of all 90 peptide encompassing the 45 mutations being screened in RPMI media with L-glutamine and HEPES (Gibco) supplemented with 10% human serum (produced in house), 50 μM beta-mercaptoethanol, 100 U/ml penicillin and 100U/ml streptomycin, 4 mM L-glutamine (termed CTL media) supplemented starting on day +2 with 10U/ml recombinant IL-2 (peprotech), with fresh media and cytokines added every 3 days, with Elispot assay conducted at day 13.

T cell culture with defined antigens to assess expansion of antigen specific clones by sequencing were conducted using >80% pure 27-mer peptides for the neoantigens, and overlapping peptide pools (Peptivator, Milltenyi) for the self-antigens, with peptides at 2μg/ml in CTL supplemented with IL-21 (30 ng/ml), IL-7 (5 ng/ml), IL-15 (1 ng/ml) and IL-2 12 U/ml for 13 days, with cytokines supplemented every 3 days. At day +13 DNA was extracted for TCRVb sequencing from a portion of the culture, and the remainder was re-stimulated with autologous B cells pulsed with a single 20 μg/ml 27-mer peptide or 20ug/ml self-peptide pool for 4 hours, followed by staining and sorting live cells IFN-γ secreting T cells (Interferon secretion kit, Milltenyi) on a FACS Aria2.

Sorted T cells (3 or 10 cells per well) were expanded at limiting dilution in a 96-well plate in the presence of $1.0x10^5$ irradiated allogeneic PBMCs, 2 μg/ml phytohemagglutinin (Sigma), and IL-2 (100 U/ml). After expansion, T cell lines were incubated with autologous B cells pulsed with mutant peptides and IFN-γ production was measured by ELISA to identify those with antigen specificity. Reactive lines were then expanded using a rapid expansion protocol described previously and cryopreserved (21). Cryopreserved cells were thawed and rested overnight in CTL media supplemented with 10 U/ml IL-2 prior to ELISA assay.

Elispot and ELISA assays

Following peptide stimulation, CD8+ T cells were enriched using the CD8 positive selection kit (Stemcell) and 100,000 CD8 enriched or CD8 depleted (CD4 predominant) cells were incubated with 100,000 autologous B cells in the presence of a pair of 20-mer crude peptides encompassing an individual candidate mutation using the human Interferon gamma elispot kit (Mabtech). For peptides with a signal qualitatively above baseline in the assay with crude peptides, the same stimulated culture was then assayed with >80% pure 27-mer peptides with either the mutated or wildtype amino acid at position +13.

Elisa assays were conducted overnight in CTL medium supplemented with the indicated amounts of peptide and 50,000 T cells and 100,000 B cells in a 96 well plate. IFN-γ was quantitated using the human IFN-γ ELISA kit (thermo Fisher)

Supplemental tables:

Table S1: Mutations detected in the patient by exome sequencing

Table S2: Expression of genes in the pre-treatment and on treatment tumor biopsies

Table S3: Frequency of TCRVβ clonotypes in different samples

Supplemental figures:

Figure S1. Variant allele frequency distribution of mutations detected by whole exome sequencing

Figure S2. Initial screening of candidate mutations

PBMC were stimulated for 13 days with a single pool of peptides encompassing 45 different mutations (A) (2μg/ml) and CD8+ T cell were then enriched by positive immunomagnetic selection. The CD8+ depleted (B) and CD8+ enriched (C) fraction were then incubated 100,000 cells with 100,000 autologous B cells and each individual peptide, and IFN-γ secretion was measured by EliSpot assay in technical duplicate. Selection of candidates for verification are shown in A.

Figure S3. Nonspecific expansion of TCRVβ clones during culture without peptide.

PBMC (5e6 cells per culture) were stimulated with peptide pools or a negative control and following 2 weeks of culture subjected to TCRVβ deep sequencing. A) TCRVβ clonotype frequency in two independent negative control stimulations were compared and plotted to illustrate variability of replicates. B) Multiple TCRVβ clonotypes expanded in a single negative control stimulation but not seen in any other stimulation.

Figure S4. Similar clonal composition of tumor before and during treatment.

TCRVβ clonal frequency before and during treatment were plotted with known neoantigen-specific clones in blue and others in red

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