Supplementary Methods

Immunofluorescence histology

Detection and quantification of various immune cell infiltrates was carried out using a multiplexed approach with a combination of fluorescence and chromogenic chemistry. This allowed staining of up to six different antibodies and two different nuclear stains from the same FFPE section, and analysis of the different channels independently or together as overlays. For analysis of different immune cell infiltrates on biopsy samples, 3.5 µm sections were cut from FFPE tissues and processed for staining. The following monoclonal primary antibodies were used: mouse anti-CD8+ (cat. number: M7103, Clone C8/144B), rabbit anti-CD4+ (cat. number: 133616, Clone EPR6855), and rabbit anti-Granzyme B (cat. number: ab4059, polyclonal). Detection utilized either TSA 647 (for CD8+, Granzyme B), or TSA 750 (for CD4+). The stained biopsy samples were imaged with an automated whole-slide scanner (Panoramic 250 FLASH, 3DHISTECH Ltd or similar) using a Plan-Apochromat 20 × objective (numerical aperture 0.80). For quantitation, a color information-based image processing methodology was applied as developed at Institute for Molecular Medicine (Helsinki, Finland). Nuclei were visualized by staining with DAPI.

Next-generation sequencing analyses

DNA and RNA from tumor biopsies were extracted (Qiagen AllPrep DNA/RNA kit) and underwent next generation sequencing (Illumina NovaSeq 6000 Systems at Personalis Inc., CA, USA). For RNA sequencing analysis, the paired-end reads were initially trimmed using trim_galore (RRID:SCR_011847, version 0.6.5) with adaptor sequence 'AGATCGGAAGAGC'. Reads were mapped to the human genome (hg38) with the ONCOS-102 genome appended using STAR (RRID:SCR_015899, version 2.7.3a) and gene expression was quantified by featureCounts (RRID:SCR_012919, version 2.0.0) with gencode annotation (version 38). Differential gene expression analyses were performed with DESeq2 (RRID:SCR_015687) using default parameters. Coefficients were fitted for survival at Month 18, and patient id to allow a full-rank model matrix, and contrasts between deceased or live at Month 18 coefficients were extracted. DNA sequencing analysis was performed by Personalis Inc. (CA, USA) using their ImmunoID platform. Briefly, HaplotypeCaller (GATK) was used to generate the core set of singlenucleotide variant (SNV) calls and their accompanying quality metrics. The pipeline then used GATK's variant quality score recalibration module, which stratified SNVs by their likelihood of representing false-positive calls, and in-house SNV accuracy software, which incorporated both genomic context and sequence alignment information into a model that corrected miscalled variants. All calls were made on BAM files recalibrated by GATK's BAM processing tools. MuTect (RRID:SCR_000559) was used to call somatic SNVs and indels, with Vardict used for calling small somatic insertions or deletions (<50 bp). Somatic SNV and indel calls were combined and analyzed through a comprehensively tested set of filters based on (i) alignment metrics such as sequence coverage and read quality, (ii) positional features such as proximity to a gap region, and (iii) likelihood of presence in normal tissue.