SUPPLEMENTARY METHODS

Multiplex Immunofluorescence

Select specimens were assessed with multispectral immunofluorescence as previously described [1] with minor modifications as follows. Tumors were stained for simultaneous detection and quantitation of cytokeratin (tumor cells), CD8 (cytotoxic T cells), FoxP3 (regulatory T cells), CD163 (macrophages), PD-1, and PD-L1 as outlined in table below. Nuclei were visualized by DAPI staining (Perkin Elmer Opal 7-color kit). Multiplexed slides were scanned using the PerkinElmer Vectra3.0 (Perkin Elmer, Hopkington, MA) multispectral microscope. PD-L1 expression on tumor cells for Johns Hopkins University (JHU) patients was manually interpreted on whole slide scans consistent with interpretation guidelines for PD-L1 immunohistochemistry using the SP142 clone. Immunohistochemistry using the E1L3N clone was utilized to quantify PD-L1 expression on pre-treatment tumor cells for Memorial Sloan Kettering Cancer Center (MSKCC) patients.

Position	Antibody	Clone (host)/Company	Final Concentration	Incubation	TSA dyes
1	FoxP3	236A/E7 (mouse)/Affymetrix	5.00 µg/mL	30 min	570
2	CD8	4B11 (mouse)/AbD Ser	1:800	30 min	540
3	AE1/AE3	M3515 (mouse) DAKO	1:500	30 min	620
4	PD-1	EPR4877 (rabbit)/AbCam	0.5 µg/mL	30 min	650
5	PD-L1	SP142 (rabbit)/Spring Bio.	0.19 µg/mL	60 min	520
6	CD163	10D6 (mouse)/Leica Bio.	0.49 µg/mL	120 min	520
7	DAPI*	Perkin Elmer Opal 7-color kit	2 drops/ml	5 min	

*Technically non-antibody fluorescent dye that binds minor groove of DNA and allows nuclei visualization.

Whole exome sequencing and bioinformatics analysis

Whole exome sequencing was performed on pre-treatment tumor and matched normal samples for the 5 Johns Hopkins University (JHU) cases with sufficient tissue (Supplemental Table 2). Formalin-fixed paraffin-embedded (FFPE) tumor samples underwent pathological review for confirmation of diagnosis and assessment of tumor purity. Tissue sections from each FFPE block were macrodissected to remove normal tissue. Matched normal samples were provided as peripheral blood. DNA was extracted from

patients' tumors and matched peripheral blood using the Qiagen DNA FFPE and Qiagen DNA blood mini kit, respectively (Qiagen, CA). Fragmented genomic DNA from tumor and normal samples was used for library construction and exonic regions were captured in solution using the Agilent SureSelect v.4 kit (Agilent, Santa Clara, CA) according to the manufacturers' instructions as previously described [2]. Paired-end sequencing, resulting in 100 bases from each end of the fragments for the exome libraries was performed using Illumina HiSeq 2500 instrumentation (Illumina, San Diego, CA). The mean depth of total coverage for the JHU tumor samples was 217x (Supplementary Table 2). Somatic mutations, consisting of point mutations, insertions, and deletions across the whole exome were identified using the VariantDx custom software for identifying mutations in matched tumor and normal samples as previously described [2]. Somatic sequence alteration calls are listed in Supplementary Table 3.

For the 3 MSKCC cases, the MSK-IMPACT targeted next-generation sequencing (NGS) assay was utilized to identify tumor-derived mutation in 468 genes [3]. Sequencing metrics and variants identified are summarized in Supplementary Tables 1-3.

Normalized tumor mutation burden conversion

Tumor mutation burden (TMB) values from whole exome sequencing and targeted NGS were normalized to provide comparability of samples across platforms as follows: the regions of interest (ROI) from each panel were applied to an *in silico* evaluation of exome-based mutation burden and panel-specific mutation burden. Exome-based somatic mutations identified by the TCGA PanCancer Atlas MC3 project [4] from 9,041 patients were collected, requiring a minimum of 4 supporting reads and 5% mutant allele frequency. Mutations present within each panel's ROI were aggregated to compute *in silico* panel tumor mutation loads for each patient. For each ROI, we computed moving quantile values (5^{th}, 10^{th}, 25^{th} and 50^{th} percentiles) of total mutational load across a sliding window of log-transformed

panel loads. We employed the moving median values (50th percentile) to generate an estimate for expected total mutational burden given a specific panel load (Supplementary Tables 1-2).

Feasibility & Safety Stopping Rules

Feasibility

The feasibility of neoadjuvant nivolumab plus ipilimumab was based on patients proceeding to surgery without extended treatment-related delay, defined as greater than 24 days following the initially planned surgery date. A probability-based decision rule was used to decide if the probability of successfully proceeding to surgery as planned was convincingly less than .90.

Previously we expected*, a priori*, the feasibility of neoadjuvant nivolumab to be high and that 90% of patients would not have surgery delayed. Based on results of our study of neoadjuvant nivolumab in resectable NSCLC,[5] where all 19 patients proceeded to surgery without delay, we expected this would be true for the nivolumab plus ipilimumab arm as well. The monitoring rule for the nivolumab plus ipilimumab arm therefore used an *a priori* optimistic Beta(9,1) prior distribution. This distribution corresponds to an assumption that 9 out of 10 patients will proceed to surgery as planned and 90% certainty that feasibility is between .715 and .994. The stopping rule would hold enrollment if, given the data, there is at least 90% probability that fewer than 90% of patients could continue to surgery without treatment related delays (see table below).

Stopping Rule for Feasibility

No. patients for whom the regimen is feasible	0	1	2	3	4	5	6	7
No. of patients	2	4	5	6	8	10	12	14

Safety

Neoadjuvant nivolumab, 3 mg/kg IV, on days -28 and -14 prior to surgery had been tested for safety and feasibility in 19 patients in our previous study of neoadjuvant nivolumab in resectable NSCLC.[5] Eighteen of 19 patients received all doses of neoadjuvant therapy.

The primary dose-limiting toxicities (DLTs) of concern for safety monitoring in this study arm of neoadjuvant nivolumab plus ipilimumab included grade 3-4 toxicities of liver, GI, renal, lung parenchyma and any other grade 3-4 toxicity, defined according to CTCAE v4.0, that in the opinion of the investigator significantly interfered with the subjects' optimal perioperative management. They were monitored continuously through day 100 following the last dose of study treatment (or day 30 post surgery, whichever was longer).

For our study of neoadjuvant nivolumab in resectable NSCLC, we assumed that the risk of grade 3-4 toxicities in advanced NSCLC and other solid tumors was 25% and used a Beta prior distribution with parameters 1 and 3.[5] With this prior, there is 90% probability that the proportion of these toxicities is between 1.7% and 53.6%. The safety stopping rule for our study arm of nivolumab plus ipilimumab applied this prior distribution to the observed number of patients experiencing DLTs with computation of the resulting probability of DLT. If the posterior probability of risk >.25, based on Bayes rule and the assumption implied by the prior, was 70% or higher, the study would stop (see table below).

In the first six patients enrolled, there were two modifications to the above stopping rule:

1. If the first patient on study experienced a DLT, we would not stop, but treat one additional patient before making a decision.

2. In the first six patients, if there had been one DLT and a second DLT was seen in the fifth or sixth patient, the study would be paused for an additional safety review and may or may not continue.

Stopping rule for safety

Stop if DLTs in	2	3	4	5
And N total patients	2-4	5-8	9-11	12-15

SUPPLEMENTAL METHODS REFERENCES

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3. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. *J Mol Diagn*. 2015;17(3):251-64.
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