Supplementary Methods

Patient population

This multicenter retrospective analysis included all consecutive patients with documented stage III NSCLC (AJCC 8th Edition) treated with platinum-based chemotherapy concurrently with definitive radiation therapy and received at least one dose of consolidation durvalumab between November 2017 to July 2022. Clinicopathologic and genomic data were obtained from Dana-Farber Cancer Institute (DFCI) and Memorial Sloan Kettering Cancer Center (MSKCC) cohorts. Patients were included if they had consented to each institution's institutional review board-approved medical review protocols. The patient studies were conducted according to the ethical guidelines of the Declaration of Helsinki. A total of 197 patients at DFCI (N=98) and MSKCC (N=99) were identified. Data analysis was performed from November 2022 to April 2023.

Specific Site

Dana-Farber Cancer Institute cohort

Patients at the Dana-Farber Cancer Institute who consented to institutional review board-approved protocols DF/HCC 02-180, 11-104, 13-364, and/or 17-000 which allowed for conducting translational research and multiplexed immunofluorescence, respectively, were also included.

Programmed death ligand 1 immunohistochemistry

The PD-L1 tumor proportion score (TPS) was determined by immunohistochemistry using validated anti-PD-L1 antibodies: E1L3N (Cell Signaling Technology, Danvers, MA), 22C3 (Dako North America Inc, Carpinteria, CA), and SP263 [Roche Tissue Diagnostics, Oro Valley, AZ]) depending on local institutional practice. PD-L1 scores were performed on pre-treated tissue.

Next Generation Sequencing (NGS)

Non-small cell lung cancers were sequenced by targeted NGS using OncoPanel[1,2] (DFCI) and MSK-IMPACT[3] (MSKCC) platforms. TMB distributions were harmonized between the two platforms as previously described[4]. The tumor content was determined by pathologist assessment at DFCI or by molecular assessment at MSKCC for each sample[3].

Quantification of aneuploidy using ASCETS

To quantify aneuploidy levels, targeted sequencing data were analyzed using ASCETS (Arm-level Somatic Copy-number Events in Targeted Sequencing), as previously described[5]; this method utilizes segmentation files and log2 copy ratios (LCRs) for all interrogated genomic loci. LCRs are used to determine the noise per segment by separating alternating LCRs within a given segment into two groups and calculating their difference in means. These differences are compiled across all segments and samples to determine a noise threshold representing one standard deviation above the mean. This threshold and its additive inverse are taken as amplification and deletion thresholds, respectively. After stratifying amplified and deleted segments, ASCETS computes the fraction of chromosome arm encompassed by copy-number segments of each arm corresponding to each segment class (amplified, deleted, or neutral). A chromosomal arm was considered altered if at least 70% of its territory was either amplified or deleted. Samples with >20% tumor purity and chromosomal arms with adequate coverage were included to determine arm-level events. The number of altered chromosome arms for each tumor was calculated as the sum total of altered arms, ranging from 0 (no arm alterations) to 39 (all arms altered); the chromosome arms included in this calculation included the long and short arms for

chromosomes 1-12 and 16-20, as well as the long arms for the acrocentric chromosomes 13-15 and 21-22[6]. The fraction of chromosomal arm alterations is defined as the number of altered chromosome arms divided by the number of chromosome arms assessed for each sample. Because of the relationship between fraction of chromosomal arm alterations and tumor content[5], we multiplied the fraction of chromosomal arm alterations by [1 - (Tumor content/100)][7,8] to calculate the adjusted fraction of chromosomal arm alterations, hereafter referred to as FAA. The FAA distributions were harmonized across the different NGS versions and the two different platforms (DFCI OncoPanel and MSK-IMPACT) by applying a normal transformation followed by standardization to FAA Z-scores, as previously described for TMB harmonization across different platforms[4]. We designated aneuploidy as high and low, considering the median FAA value as a boundary.

Specific Site

Memorial Sloan Kettering Cancer Center

The copy number profile for each tumor in the MSKCC cohort found using FACETS (Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing) v0.5.14[9] compared to its matched normal. The segmentation profile was compiled with FACETS-suite and used to determine aneuploidy with ASCETS, as described above.

Tumor mutational burden assessment and harmonization

Tumor mutational burden (TMB), defined as the number of somatic, coding, base substitution, and indel mutations per megabase (Mb) of genome examined, was determined using the OncoPanel and MSK-IMPACT NGS platforms. DFCI mutation counts were divided by the number of bases covered in each OncoPanel version: v1, 0.753334 Mb; v2, 0.826167 Mb; and v3, 1.315078 Mb. For MSKCC samples, the mutation count was divided by 0.896665, 1.016478, and 1.139322 Mb for the 341-, 410-, and 468-gene panels, respectively. Because TMB was determined using two different platforms, TMB distributions were harmonized across institutions by applying a normal transformation followed by standardization to Z-scores, as previously described[4]. Power transformations were used to normalize cohort-specific TMB distributions, and Tukey's ladder of powers in the rcompanion package was used to identify the optimal transformation coefficient. Normalized distributions were then standardized into Z-scores by subtracting the transformed distribution mean and dividing by the standard deviation.

Multiplexed immunofluorescence (ImmunoProfile)

Multiplexed immunofluorescence (mIF) was performed on a separate cohort of NSCLCs from the DFCI to determine the immunophenotype-associated subgroups by staining 5-micron formalin-fixed, paraffin-embedded (FFPE) whole tissue sections with standard, primary antibodies sequentially and paired with a unique fluorochrome followed by staining with nuclear counterstain/4′,6-diamidino-2-phenylindole (DAPI)[10,11]. All samples were stained for PD-L1 (clone E1L3N), PD-1 (clone EPR4877[2]), CD8 (clone 4B11), and FOXP3 (clone D608R). Each sample had a single slide stained and scanned at 20x resolution by a Vectra Polaris imaging platform. Regions of Interest (ROIs) were defined for each image, and only these regions were used for quantitative image analysis. Within each ROI, InForm Image Analysis software (Perkin Elmer/ Akoya) was run to phenotype and score cells based on biomarker expression. A custom script quantified the number/ percentage of positive cells for relevant biomarkers in the intra-tumoral region, defined as the region of the slide consisting of tumors beyond the tumor-stroma interface. Cell count was calculated per ROI and averaged (unweighted) across ROIs, reported as count per millimeter squared +/- standard error. Statistical significance of differential cell type enrichment between groups was estimated with the Wilcox/Kruskal Rank Sum test.

Statistical Analysis

Categorical and continuous variables were summarized descriptively using percentages and medians. The Wilcoxon-Rank Sum test and Kruskal-Wallis test were used to test for differences between continuous variables, and Fisher's exact test was used to test for associations between categorical variables. The clinical follow-up schedule followed standard treatment and included history, physical, and chest CT every 3-4 months for the first 2 years. Event-time distributions were estimated using Kaplan-Meier methodology. Log-rank tests were used to test for differences in event-time distributions, and Cox proportional hazards models were used to estimate hazard ratios in univariable and multivariable models for PFS and OS. We first discovered the effect of baseline clinicopathological factors of tumor stage, age, sex, smoking status, histology, Eastern Cooperative Oncology Group performance status (ECOG PS), aneuploidy level (low vs high), TMB (lower vs middle vs upper tertile), and PD-L1 TPS levels (<90% vs ≥90%) on clinical outcomes in univariable analyses. Multivariable models included factors found to be predictive from a complementary study using this patient population[12] and included the variables with P-value <0.1 into the multivariable analysis to control for the potential confounding effects. All P-values are 2-sided and confidence intervals are at the 95% level, with significance pre-defined to be at the two-sided 0.05-level. Relevant co-variants were analyzed for interaction. All statistical analyses were performed using R version 3.6.3.

Supplementary References

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