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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	- All experimental pre-clinical data was collected and stored according to The University of Texas MD Anderson Cancer Center intellectual property protection policy. Statistical analysis was executed using GraphPad Prism Software v 10.0.3. - Clinical data from the retrospective multi-institutional non-squamous NSCLC cohort was obtained from electronic medical records review. POSEIDON (ClinicalTrials.gov identifier: NCT03164616) data was obtained in accordance with the protocol approved by relevant ethics committees and regulatory authorities and AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure. Statistical analysis was performed on IBM SPSS Statistics v.24.0 (Armonk, NY, USA), R version 4.1.2 (2021-11-01) and SAS 9.4 (SAS, Cary, NC, USA). - Single-cell RNAseq was performed at the ATGC core at MD Anderson Cancer Center, utilizing a NovaSeq6000 sequencer. Raw scRNA-seq data were pre-processed (demultiplex cellular barcodes, read alignment and generation of gene count matrix) using Cell Ranger Single Cell pipeline provided by 10X Genomics, utilizing the mouse reference transcriptome, GRCm38 (mm10). Data merging, filtering, doublets removal, batch-effect evaluation and data normalization were performed following standard protocols as previously described83. Seurat R package (v4.3) was used to analyze the normalized gene-cell matrix and "Harmony" (v0.1.1) was applied for batch effect correction. Each cluster was determined according to high variable genes identified applying FindClusters Seurat function and validated with "SingleR" (v2.2.0). - Flow cytometry data was acquired on LSR Fortessa flow cytometer (BD Biosciences) and analyzed using FlowJo 10.8.1 software (BD Biosciences).
Data analysis	All analysis were performed using standard protocols with previously described computational tools. No custom code was used in this study. Statistical analysis was performed on IBM SPSS Statistics v.24.0 (Armonk, NY, USA), R version 4.1.2 (2021-11-01), SAS 9.4 (SAS, Cary, NC, USA) and GraphPad Prism (v9.0). .Flow cytometry data was analyzed using FlowJo (v10.8.1).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Data generated in this study are available within the article and its supplementary files. scRNA-seq raw and processed data reported in this article are available upon request and will be have been deposited to NCBI Gene Expression Omnibus (GEO accession numbr: GSE267321) deposited at the NCBI sequence read archive (SRA) in accordance with Nature Research Data policies. Data underlying the clinical trial findings may be requested in accordance with AstraZeneca's data sharing policy, described in further detail at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure. All other individual de-identified participant data supporting the retrospective clinical data results reported in this article would be available on request according to General Data Protection Regulation (GDPR) standards. Only summary clinical data may be shared, patient-level image or genetic data is not available for access in our repository in the interest of protecting patient privacy. Materials, reagents or other experimental data are available upon reasonable request from the corresponding authors. Source data for experiments presenting data from animal models have been provided for main and extended data figures.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Poseidon study planned to randomly assign approximately 1,000 patients to obtain approximately 497 PFS events and 532 OS events across the D + CT and CT arms for the final (primary) analyses of PFS and OS, planned at approximately 75% and 80% maturity, respectively. The final intention to treat population included in this study comprised 1,013 patients The retrospective cohort included 871 patients consecutive patients under a convenience sampling strategy in the pre-specified time frame. Animal cohort sample size was determined according to previous studies from our group evaluating anti-tumor efficacy, according to the current Institutional Animal Care and Use Committee (IACUC) approved protocol.
Data exclusions	No data was excluded.
Replication	Translational data presented in the manuscript included experiments performed in three independent laboratories and major findings were validated in models bearing LKB1 and/or KEAP1 mutations across different backgrounds.
Randomization	The POSEIDON study is a phase III, global, randomized, open-label study with a three-arm design where patients were randomly assigned (1:1:1) with stratification by PD-L1 expression (≥ 50% v < 50% of TCs), disease stage (IVA v IVB, per International Association for the Study of Lung Cancer Staging Manual in Thoracic Oncology version 8),21 and histology (squamous v nonsquamous) to tremelimumab 75 mg plus durvalumab 1,500 mg and chemotherapy for up to four 21-day cycles, followed by durvalumab 1,500 mg once every 4 weeks until disease progression (PD), with one additional tremelimumab dose after chemotherapy at week 16/cycle 6 (fifth dose); durvalumab 1,500 mg plus chemotherapy for up to four 21-day cycles, followed by durvalumab 1,500 mg once every 4 weeks until PD; or chemotherapy for up to six 21-day cycles. The retrospective multicentric clinical cohort with patients treated with immune checkpoint inhibitors included non-randomized clinical data from consecutive patients. In vivo experimental studies were performed with random allocation of mice to the different treatment arms described in each experiment once they reached the pre-determined tumor volume and/or designated time post tumor injection.
Blinding	Progression-free survival was evaluated by blinded independent central review in the POSEIDON trial. Investigators were not blinded to the treatment groups or genotypes in the experimental studies (in vivo and in vitro) reported in this manuscript.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The following antibodies were used in the in vivo pre-clinical theurapeutic experiments:
Anti-PD-1 (clone 29F.1A12, BioXCell); anti-CTLA-4 200 µg (clone 9H10, BioXCell); Anti-CD4 (clone GK1.5, BioXCell); anti-CD8 (clone 2.43, BioXCell), αLy6G antibody (clone 1A8, BioXCell), αCCL2 antibody (clone 2H5, BioXCell) and corresponding isotype IgG controls (BioXCell catalog numbers BE0089 and BE0087).

Antibodies used in the flow cytometry analysis:
Fluorochrome-conjugated monoclonal antibodies were purchased from Biolegend: Pacific blueTM-anti-CD45 (Cat# 103126, 30-F11), PE/DazzleTM 594 anti-CD3 (Cat# 100246, 17A2), APC/Cy7 anti-CD4 (Cat# 100526, RM4-5), PE/Cy7-anti-CD8 (Cat# 100721, 53-6.7), APC-anti-T-bet (Cat# 644814, 4B10), PE-anti-ICOS (Cat# 117405, 7E.17G9), BV711TM-anti-CD44 (Cat# 103057, IM7), BV605TM-anti-PD-1 (Cat# 135220, 29F.1A12), BV 711TM-anti-Gr-1 (Cat# 108443, RB6-8C5), BV 785TM-anti-CD11c (Cat# 117336, N418), BV 650TM-anti-CD11b (Cat# 101259, M1/70), PE/Cy7-anti-I-A/I-E (MHC class II, Cat# 107629, M5/114.15.2), BV 605TM-anti-Ly6C (Cat# 128035, HK1.4), PerCP/Cy5.5-anti-Ly6G (Cat# 127615, 1A8); from BD Biosciences: BUV395-anti-CD25 (Cat# 564022, PC61), BV605-anti-PD-1 (Cat# 748267, RMP1-30); from Life Technologies: PerCP-Cy5.5-anti-FoxP3 (Cat# 45-5773-82, FJK-16s); from Invitrogen PE-anti-iNOS (Cat# 12-5920-80, CXNFT); and from Tonbo Bioscience FITC-anti-CD62L (Cat# 35-0621-U500, MEL-14), APC-anti-F4/80 (Cat# 20-4801-U100, BM8.1).

Validation

Validation of the antibodies was provided according to the manufacturer`s website/datasheet data; prior publications and supplementary data provided in the manuscript.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

The KL5 and KL2 polyclonal KrasG12C-mutant Stk11-deficient lung adenocarcinoma cell lines were established from autochthonous lung tumors in compound conditional KrasLSL-G12C+/wt;Stk11/Lkb1Fl/Fl mice (on a C57Bl/6 genetic background), following lung tumor induction with instranasal instillation of ofAdenoviral Cre recombinase (University of Iowa Viral Vector Core) as previously described85 and were used to establish subcutaneous allograft tumors in syngeneic recipient C57Bl/6 mice.
LKR10 and LKR13 KrasG12D-mutant LUAD cells (on a 129Sv genetic background, previously generated in Dr Tyler Jacks' laboratory) were transiently transfected with Keap1 CRISPR/Cas9 KO plasmid (sc-424513-KO-2) or Stk11/Lkb1 CRISPR/Cas9 KO plasmid (sc-423192) from Santa Cruz Biotechnology (LKR10 derivative models) or pSpCas9(BB)-2A-GFP (PX458) plasmid with specific sgRNAs (LKR13 derivative models). The plasmid vectors for CRISPR-Cas9 mediated Keap1 and Stk11/Lkb1 KO were verified by DNA sequencing.

Authentication

Only mouse lines were utilized. None of the cell lines were independently authenticated.

Mycoplasma contamination

Mycoplasma testing was performed routinely and cells were found to be free from mycoplasma contamitantion

Commonly misidentified lines
(See ICLAC register)

None

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Mice were housed in the Research Animal Support Facility at the University of Texas MD Anderson Cancer Center. All animal studies described here were conducted according to protocols approved by the University of Texas MD Anderson Cancer Center Institutional Animal Care and Use Committee (IACUC).
Six to twelve week-old male C57BL/6 mice (for experiments with KL2 and KL5 cell lines) and 129/Sv mice (for experiments with LKR10 and LKR13-derived isogenic cell lines) were injected with cells of the indicated genotypes.

Wild animals

No wild animals were used in this study.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight	Animal experiments were performed in accordance with protocols approved by the UT MD Anderson Cancer Center Institutional Animal Care and Use Commitee.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	A comprehensive description of the study population characteristics of the clinical cohorts is provided in the Data Supplement
Recruitment	Briefly, patients age ≥ 18 years with stage IV NSCLC21 were eligible for inclusion, provided they had not previously received systemic therapy for mNSCLC; had Eastern Cooperative Oncology Group performance status 0 or 1; and had measurable disease according to RECIST v1.1.22 The patients' tumors were to have no sensitizing EGFR mutations or ALK rearrangements (by local assessment) and PD-L1 expression status that was assessed at a central laboratory using the VENTANA PD-L1 (SP263) immunohistochemistry assay (Ventana Medical Systems, Tucson, AZ)23 before random assignment. Patients with treated and stable brain metastases were eligible
Ethics oversight	The clinical studies were performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. The protocol and all modifications were approved by relevant ethics committees and regulatory authorities. All patients provided written informed consent

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	POSEIDON study was registered under ClinicalTrials.gov identifier: NCT03164616
Study protocol	Full protocol is available at https://ascopubs.org/doi/suppl/10.1200/JCO.22.00975/suppl_file/protocol_JCO.22.00975.pdf
Data collection	Between June 27, 2017, and September 19, 2018, 1,013 patients from 142 sites in 18 countries were randomly assigned to T + D + CT (n = 338), D + CT (n = 338), or CT (n = 337)
Outcomes	The primary end points were progression-free survival (PFS), evaluated by blinded independent central review (BICR) per RECIST v1.1, and overall survival (OS) for D + CT versus CT. PFS was defined as the time from random assignment to objective PD or death from any cause in the absence of progression and OS as the time from random assignment to death from any cause. Key alpha-controlled secondary end points were PFS and OS for T + D + CT versus CT.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For studies of immune modulation, treatment with single or dual ICB commenced on day 5 post subcutaneous tumor implantation and followed the same dosing regimen as previously described for the efficacy experiments. Tumor samples were harvested after five doses of single or dual ICB, on day 18. For chemo-immunotherapy experiments with the K and KLK (clone 17) models, mice received a total of 3 doses of chemotherapy and/or immunotherapy (D1: Carboplatin+Paclitaxel +ICB); D4 (Paclitaxel +ICB); D7 (Carboplatin+Paclitaxel+ICB), and tumors were collected for FACS-based immune profiling 48 hours later. Harvested tumors were immediately processed into 2-4 mm3 pieces and transferred to a gentleMACS Octo Dissociator tube containing a solution with liberase (25 ug/ml), Dnase 1 type 1 (30 U/ml) and Hyaluronidase (0.01%) in serum-free RPMI-1640 medium and incubated for 45 minutes at 37oC. Enzyme reactions were stopped by addition of cold RPMI-1640 (10% FBS) and suspensions were dispersed through a 70-µm cell strainer twice. After red blood cell lysis (using RBC lysis buffer, Biolegend), single-cell suspensions (~1 x 106 cells in 50 µl total volume) were incubated with FcR-blocking reagent rat anti-mouse CD16/32 (2.4G2, BD Biosciences) for 15 min on ice. For extracellular staining, cells were stained with a mixture of conjugated antibodies in FACS buffer, including ghost dye violet 510 (Fisher Scientific) for 1 hour at RT in the dark. For intracellular cytokine and transcription factor staining, single-cell suspensions were fixed and permeabilized using eBioescience FoxP3/Transcription Factor Staining kit (Life Technologies) according to manufacturer's instructions. Fluorochrome-conjugated monoclonal antibodies were purchased from Biolegend: Pacific blueTM-anti-CD45 (30-F11), PE/DazzleTM 594 anti-CD3 (17A2), APC/Cy7 anti-CD4 (RM4-5), PE/Cy7-anti-CD8 (53-6.7), APC-anti-T-bet (4B10), PE-anti-ICOS

	(7E.17G9), BV711TM-anti-CD44 (IM7), BV605TM-anti-PD-1 (29F.1A12), BV 711TM-anti-Gr-1 (RB6-8C5), BV 785TM-anti-CD11c (N418), BV 650TM-anti-CD11b (M1/70), PE/Cy7-anti-I-A/I-E (MHC class II, M5/114.15.2), BV 605TM-anti-Ly6C (HK1.4), PerCP/Cy5.5-anti-Ly6G (1A8); from BD Biosciences: BUV395-anti-CD25 (PC61), BV605-anti-PD-1 (RMP1-30); from Life Technologies: PerCP-Cy5.5-anti-FoxP3 (FJK-16s); from Invitrogen PE-anti-iNOS (CXNFT); and from Tonbo Bioscience FITC-anti-CD62L (MEL-14), APC-anti-F4/80 (BM8.1).
Instrument	LSR Fortessa Flow Cytometer (BD Biosciences) .
Software	FlowJo (v10.8.1).
Cell population abundance	Studies were performed on bulk tumor consisting of tumor cells and immune cells.
Gating strategy	FSC-A/SSC-A gates were used to select mononuclear cells. FSC-A/FSC-H gates were then used to gate single cells. Live cells were then gated based on ghost dye violet 510. CD45+ leukocytes were further gated into different populations of immune cells based on their co-expression of distinct markers. When needed, fluorescence minus one control was used to define positive/negative cell populations.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.