nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

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	EDC system was used to collect clinical data. Single-cell RNA sequencing and multiplex immunofluorescence were conducted on patient pre-treatment tumor samples.

Data analysis	Statistical analyses and data visualization were performed using the following software and packages: * R (version 4.0.3, http://www.R-project.org/) * Python (version 3.7, https://www.python.org/) * GraphPad Prism (version 9.0.2, https://www.graphpad.com/) * CeleScope (version 1.14.0, Singleron Biotechnologies) * STAR aligner (version 2.6.1a) * FeatureCounts (version 2.0.1) * Scanpy (version 1.8.1, https://github.com/scverse/scanpy) * Harmony (version 1.0, https://github.com/immunogenomics/harmony) * Seurat (version 4.0.4, https://github.com/satijalab/seurat) * InferCNV (version 1.2.1, https://github.com/broadinstitute/inferCNV) * xgboost (version 1.7.7.1, https://github.com/dmlc/xgboost) * SHAPforxgboost (version 0.1.3, https://github.com/liuyanguu/SHAPforxgboost) * pROC (version 1.18.0, https://github.com/cran/pROC) * QuPath (version 0.4.3) * PASS (version 15.0, NCSS LLC, Kaysville, Utah, USA)

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

Patient data remains confidential in accordance with privacy protection protocols. The complete study protocol and statistical analysis plan are provided in the supplementary information. Researchers seeking access to anonymized participant datasets should direct their requests to the corresponding author, including a detailed justification. All requests will undergo evaluation by the principal investigator site and sponsoring organization to ensure adherence to confidentiality requirements, with responses provided within an 8-week timeframe. Before any data release, applicants must execute a formal data-sharing agreement with the study sponsor. The raw single-cell RNA-seq data generated in this study have been deposited in the Genome Sequence Archive (GSA) of the National Genomics Data Center (NGDC), Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences, under accession code HRA014481 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA014481). These data are available under controlled access due to privacy restrictions related to patient consent. Access is granted to principal investigators from non-profit research institutions for research purposes only. Access applications can be submitted via the GSA-human portal following their guidelines. Requests are typically reviewed and responded to within 1 month. Once access is granted, the data will be available for download for a period of 1 month. The key analysis data are available within the Article, Supplementary Information, or Source Data file. Source data are provided with this paper. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	We report the sex of patients (biological attribute). The sex classification was based on patient self-reporting and information from family members and confirmed through physician examination.
Reporting on race, ethnicity, or other socially relevant groupings	All participants included in this study were East Asians, having been born and raised in China. Other socially relevant groupings were not considered as variables in this research.
Population characteristics	This study included 40 patients, all diagnosed with NSCLC and showing disease progression to first-line ICI therapy. The median age of the participants was 59.5 years (range: 35–75), with 85% (n = 34) males. Most patients (75%, n = 30) were either current or former smokers, and all were diagnosed with clinical stage IV. Brain metastases were observed in 20% (n = 8) of the cohort, bone metastases in 35% (n = 14), and concurrent liver metastases in 17.5% (n = 7). Additionally, 82.5% (n = 33) had an ECOG performance status of 1. Squamous cell carcinoma was identified as the most frequent histological type, accounting for 52.5% (n = 21) of cases.
Recruitment	This study was conducted at Hunan Cancer Hospital. Patient recruitment followed strict inclusion and exclusion criteria as specified in the study protocol. Eligible subjects selected for this study must meet all of the following criteria: 1. Sign written informed consent before implementing any trial-related procedures; 2. Age ≥18 years old and ≤75 years old; 3. No limit on the gender/sex; 4. Histological or cytological confirmed locally advanced (IIIB-IIIC) or metastatic (stage IV) NSCLC (International Association for the Study of Lung Cancer and Joint Committee on the American Classification of Cancer, TNM Lung cancer stage 8) without EGFR gene sensitive mutations, ALK gene fusion or ROS1 gene fusion confirmed by histological specimens. 5. Relapse following the failure of first-line anti-PD-1/PD-L1 antibody therapy is defined as follows: Only one anti-PD-1/PD-L1 antibody, either as monotherapy or in combination therapy, is permitted during the advanced stage of the disease, with no other forms of immunotherapy allowed.

6. Previous anti-PD-1/L1 antibody monotherapy or combination therapy has the best curative effect (according to RECIST 1.1 criteria) as partial remission, complete remission, or stable disease for ≥6 months (defined as within 6 months from the first medication) No disease progression has occurred);
7. Disease progression confirmed by imaging studies occurred during or after the most recent treatment.
Exclusion Criteria:
1. Subjects who meet the following criteria cannot be selected for this study:
2. The pathology is small cell lung cancer (SCLC), including lung cancer mixed with SCLC and NSCLC;
3. There is imaging evidence of tumor voids, tumor inclusion or invasion of large vessels. In addition, the proximity of the tumor to the large blood duct should be considered and the risk of severe bleeding associated with tumor shrinkage/necrosis after lenvatinib treatment should be excluded. (The large vessels in the chest include the main pulmonary artery, left pulmonary artery, right pulmonary artery, 4 pulmonary veins, superior vena cava, inferior vena cava and aorta);
4. Subjects who have previously used anti-PD-1, PD-L1 or other immunotherapy and meet the following conditions:
1) The toxicity that caused permanent discontinuation occurred before the termination of immunotherapy;
2) Prior to the administration of the study drug, the toxicity of the previous immunotherapy has not recovered or has not recovered to level 0-1. Asymptomatic and stable control of endocrine toxicity level 2 with appropriate replacement therapy is allowed to enter the group;
3) Adverse events that require additional immunosuppressive agents in addition to corticosteroids, or adverse events that still recur in the use of corticosteroids during previous immunotherapy.
More details of patient inclusion and exclusion criteria are provided in the Supplementary Information.

Ethics oversight

This study complies with all relevant ethical regulations regarding research involving human participants. The study protocol was approved by the Institutional Review Board of Hunan Cancer Hospital (Ethics Approval No: CXSL1800119). The study design and conduct were performed in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines. Written informed consent was obtained from all participants prior to enrollment.

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

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	Historical data indicate that the ORR for chemotherapy as second-line therapy is approximately 12%. This trial aims to observe an ORR of 30%. Based on a power of 80%, an alpha of 0.05, and accounting for a 15% dropout rate, the required sample size is 40 patients. Sample size was calculated using PASS software (version 15.0, NCSS LLC, Kaysville, Utah, USA).
Data exclusions	No patients were excluded from the data analysis.
Replication	All experimental findings reported in this study are reproducible. Experimental procedures were performed on samples from multiple patients, yielding consistent conclusions. Both statistically significant and non-significant findings are reported.
Randomization	This is a single-arm Phase 2 clinical trial with no control group. Therefore, random allocation into experimental groups is not applicable. All eligible participants meeting the inclusion/exclusion criteria were enrolled into the single treatment arm.
Blinding	This was a single-arm phase 2 clinical trial without blinding of investigators and patients, as all participants received identical treatment. To minimize potential bias, we employed objective response criteria (RECIST 1.1) and conducted the trial and data analysis in accordance with the pre-specified study protocol and statistical protocols.

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
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

All antibodies were commercially purchased and included: anti-human CD4 (Abcam, Cat. no. ab133616, clone EPR6855, lot 1008803-27, 1:300), anti-human CD3 (Abcam, Cat. no. ab16669, clone SP7, lot 1027923-29, 1:200), anti-human PD-1 (Abcam, Cat. no. ab237728, clone CAL20, lot 1052719-8, 1:200), anti-human CD8 (CST, Cat. no. 70306S, monoclonal, lot 4, 1:200), anti-human BCL-6 (MAB, Cat. no. MAB0746, clone MX042, lot 2401170746a, working solution), anti-human CXCL13 (Proteintech, Cat. no. 10927-1-AP, polyclonal, lot 45136, 1:200), anti-human CD163 (Abcam, Cat. no. ab156769, clone DT12G12, lot 1038180-3, 1:150), anti-human CD68 (Abcam, Cat. no. ab192847, clone SP251, lot 1028484-23, 1:200), anti-human CXCL10 (Proteintech, Cat. no. 10937-1-AP, polyclonal, lot 130386, 1:400), anti-human PD-L1 (Abcam, Cat. no. ab237726, clone CAL10, lot 1028151-15, 1:200), anti-human CTLA4 (CST, Cat. no. 53560T, monoclonal, lot 3, 1:100), DAPI (Sigma, Cat. no. D9542-10MG, lot 320033, 1:1000) was used for nuclear staining. Application: mIF for all antibodies.

Validation

The development and validation of multiplex protocols adhered to standard operating procedures established in our pathology laboratory. For each antibody lot, we performed dilution optimization using the designated fluorescent probe, with standardized pathologist review and appropriate positive control tissues (documentation and protocols provided upon request). All antibodies were further validated within the full multiplex panel context.

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

Clinicaltrial.gov number: NCT04777084

Study protocol

Details about the study protocol are available in the Methods section of the manuscript, supplementary information and on the website https://clinicaltrials.gov/study/NCT04777084.

Data collection

This phase II prospective cohort study, conducted at a single center with an open-label and nonrandomized design, examined the efficacy and safety of IBI318 combined with lenvatinib in patients with advanced non-small cell lung cancer without actionable driver mutations who had developed acquired resistance to first-line PD-1/PD-L1 inhibitors. The study was registered on ClinicalTrials.gov on February 27, 2021 (NCT04777084; https://clinicaltrials.gov/search?cond=Lung%20Cancer&term=NCT04777084). All participants provided written informed consent before enrollment, which occurred from December 21, 2021 to June 21, 2023, including consent for publication of deidentified medical data.

Outcomes

The primary outcome was the objective response rate at 12 weeks, defined as the proportion of patients who achieved complete or partial response approximately 12 weeks after treatment initiation. Secondary outcomes included: (1) best overall response, defined as the highest level of response (complete or partial) recorded during the study; (2) progression-free survival, defined as the time from treatment initiation to radiologically confirmed progression or death; (3) overall survival, defined as the time from treatment start to death; (4) disease control rate, defined as the percentage of patients with complete response, partial response, or stable disease as their optimal outcome; (5) duration of response, defined as the interval from the first response to progression or death; and (6) safety profile, characterized by the incidence and severity of treatment-related adverse events according to NCI-CTCAE version 5.0.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A