nature portfolio

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Last updated by author(s): Dec 19, 2025

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	Data collection was performed in Microsoft Office Excel 2019.
Data analysis	Data analysis was performed in IBM SPSS Statistics 27.0, and Graphpad Prism 9.1.2

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

The trial protocol is available a Supplementary Note in the Supplementary Information file. Deidentified clinical data of each patient including clinical demographics, tumor characteristics, safety assessments, and multiplex immunofluorescence data are available in the manuscript or additional files. Deidentified radiograpic and pathological data, and blood test results of each patient were available in the figure 2A, 2B, 4C, 4D of Source Data file, Supplementary Data 1 and Supplementary Data 2. The raw NGS sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences

(GSA-Human: HRA013397) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human/browse/HRA013397. The raw sequencing data contain information unique to individuals are available under restricted access, authorization by the Clinical Research Ethics Board is required as per institutional policy prior to any disclosure of participants' genomic data and individual de-identified participant clinical data, which will be made available for scholarly research objectives upon submission of a justified application to the corresponding author via huyayi@shsmu.edu.cn. The remaining data are available within the Article, Supplementary Information or Source Data file.

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	Sex was not limited in this study. Sex of participants was determined based on self-report.
Reporting on race, ethnicity, or other socially relevant groupings	The study was not involved with the constructs of race and/or ethnicity.
Population characteristics	The eligible participants were aged 18-75 years and had histologically confirmed R/M HNSCC; had received at least one cycle of anti-PD-1 therapy in the R/M setting; had at least one measurable lesion according to the Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1;9 had radiographic confirmation of disease progression or clinical judgment indicating no further benefit from anti-PD-1 therapy; had confirmed HPV-negative disease by immunohistochemistry (IHC); had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and had acceptable bone marrow and organ function. Participants received cetuximab intravenously at an initial dose of 400 mg/m² followed by weekly maintenance dose of 250 mg/m2. Dalpiciclib was administered orally at a dose of 150 mg once daily for 21 consecutive days, followed by a 7-day break. Each 28 days were deemed as a treatment cycle. Treatment was continued until disease progression, unacceptable toxicity, or patient withdrawal, whichever occurred first.
Recruitment	Patients were recruited and screened at Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. Briefly, patients that presented with diagnosis of R/M HNSCC progressed from prior anti-PD-1 therapy in the R/M setting at our center, and who were potentially eligible for this study were were informed of this and other studies for which they were eligible. Patients who were deemed eligible were informed about the aims of the study, the possible adverse events, and the procedures and possible hazards to which he/she would be exposed. The utilization of single-arm,single center design might lead to selection bias, the recruited patients might be concentrated in specific geographical regions and patient populations (the hospital's specialized clinical focus might attract certain types of HNSCC patients). Additionally, all diagnostic and therapeutic procedures were performed by the medical team of a single center, lacking heterogeneous verification across multiple centers.
Ethics oversight	The study was approved and monitored by the Ethics Committee and the Clinical Trial Center of Shanghai Ninth People's Hospital affiliated to Shanghai Jiao Tong University School of Medicine.In our trial, dalpiciclib and imaging examinations were provided free of charge for all enrolled patients.

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 showed that the ORR for cetuximab monotherapy as second-line treatment was 13%. The study adopted a Simon's two-stage design to minimize the number of subjects exposed to ineffective treatment. Based on the expected ORR of 33% for dalpiciclib combined with cetuximab, assuming α of 0.05 (two-sided) and power of 80%, we anticipated to enroll 14 participants in the first stage. If the number of responders (CR+PR) was no more than 1, the study would be terminated due to insufficient efficacy. If more than one participant obtained CR or PR, the study would additionally recruit another 12 participants in the second stage. A total of 26 participants were anticipated to enroll. If the total number of responders (CR + PR) across both stages was more than 6, the combination therapy would be considered effective.
Data exclusions	31 patients were screened, one patient did not meet the inclusion criteria and two patients withdrew contents before the treatment. these three patients were excluded from the analysis, and 28 patients were finally enrolled in this study.
Replication	Since this study is a single-arm study, the clinical findings cannot be directly replicated.
Randomization	No randomization or stratification was applied because of the single-arm study design.
Blinding	No blinding was applied because of the open-label study design. At the same time, the primary investigators were the treating physicians and they were not blinded for outcome.

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

Multiplex immunofluorescence:
DAPI (Servicebio, cat. no.G1012-100ML; ready-to-use, no dilution required), rabbit anti-human CD4 (Servicebio, cat. no. GB150062; 1:1000), mouse anti-human CD8 (Servicebio, cat. no. GB12068; 1:500), rabbit anti-human CD56 (Servicebio, cat. no. GB112671; 1:500), and rabbit anti-human FOXP3 (Servicebio, cat. no. GB152325; 1:500).

Validation

The validation of all of the antibodies depends on product data sheet and published literature.
Antibodies used in multiplex immunfluorescence staining for tumor in patients:
1. rabbit anti-human CD4 (Servicebio, cat. no. GB150062; 1:1000),https://www.service-bio.com/goodsdetail?id=24253
2. mouse anti-human CD8 (Servicebio, cat. no. GB12068; 1:500),https://www.service-bio.com/goodsdetail?id=7235
3. rabbit anti-human CD56 (Servicebio, cat. no. GB112671; 1:500),https://www.service-bio.com/goodsdetail?id=5765
4. rabbit anti-human FOXP3 (Servicebio, cat. no. GB152325; 1:500),https://www.service-bio.com/goodsdetail?id=24287
5. DAPI (Servicebio, cat. no.G1012-100ML; ready-to-use, no dilution required), https://www.service-bio.com/goodsdetail?id=1762

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

NCT05721443

Study protocol

The original clinical trial protocal was included in Supplementary Materials.

Data collection

Between March 7th, 2023 and November 1st, 2024, 31 patients were screened and 28 patients were enrolled at Shanghai Ninth People's Hospital. And the follow-up time is between March 7th, 2023 and March 3rd, 2025.

Outcomes

The primary endpoint was ORR, defined as the proportion of patients with partial response (PR) or complete response (CR) according to RECIST 1.1. Secondary endpoints were OS (defined as time from enrollment to death from any cause), PFS (defined as time from enrollment to the first disease progression or death from any cause, whichever occurred first), duration of response (DOR; defined as interval from the first time that patients achieved CR or PR to the first disease progression or death from any cause, whichever occurred first), and safety. Exploratory endpoints included ORR, PFS, and OS in populations with PD-L1 CPS ≥1 and 20, and ORR, PFS, and OS in individuals with abnormal CDK4 related genes (CDK4 amplification, CCND1 amplification, or CDKN2A deletion).

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.