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Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
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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	Microsoft Excel was used for data collection.
Data analysis	Data analyses were performed using SAS software version 9.4, R version 4.2.2, and GraphPad Prism v.9. All codes used in the analyses described in this study were based on existing tools and software, and no custom software, programs, or scripts were used. We have uploaded the code used in the analyses to Github, accessible at the following link: https://github.com/CancerImmunologyNCC/Bando_etal_2024_RNAseq.

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

All exome and transcriptome data have been deposited in the NBDC Human Database (https://humandbs.dbcls.jp/en/hum0294-v1). The accession numbers are JGAS000708 for the study and JGAD000841 for the dataset. These data are subject to restricted access and available only to researchers who have submitted a data usage application and have had their application approved. If one wishes to use this data, one must submit a data usage application through the application system. The procedure is described in the following website: https://humandbs.dbcls.jp/en/data-use. Use of the data is subject to the NBDC Human Data Sharing Guidelines and the NBDC Human Data Handling Security Guidelines (for data users). The hg38 dataset was accessed from the following link: https://www.gencodegenes.org/human/release_34.html. Source data for Fig. 2, 3, 4, and 5 and Extended Data for Fig. 2, 3, 4, 5, 6, 7, 8, and 9 are provided as Source Data files. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

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	Participants enrolled in this clinical trial includes 41 men and 9 women. No sex-based analyses have been performed because there have been no data regarding the difference of efficacies of IO and survival between male and female in ESCC. The sex / gender of participants was determined based on participants' self-reporting.
Reporting on race, ethnicity, or other socially relevant groupings	All of the participants enrolled in this clinical trial were Japanese.
Population characteristics	All patients had histologically confirmed unresectable locally advanced primary ESCC. In the primary cohort, median age was 64 years, and 82.5% (33/40) of the patients were male. A total of 95.0% (38/40) of patients had clinical T4 disease, and 92.5% (37/40) had lymph node metastasis. Furthermore, 25.0% (10/40) of the patients had extra-regional M1 lymph node metastasis.
Recruitment	Patients with unresectable locally advanced ESCC who were treated with 2 cycles of cisplatin/5-FU plus 60 Gy of radiation were enrolled. We sequentially registered cases that met the eligibility criteria from each clinical trial site, and we believe that there was no self-selection bias or other significant recruitment biases.
Ethics oversight	This study was approved by the ethics committees at each institution (National Cancer Center Institutional Review Board, Aichi Cancer Center Institutional Review Board, Saitama Cancer Center Review Board, Shizuoka Cancer Center Review Board, and the Cancer Institute Hospital of Japanese Foundation for Cancer Research Review Board). All patients were required to sign written informed consent forms.

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	We calculated the sample size of the primary locally advanced ESCC cohort at 38 with a cCR of 40% deemed promising and one of 20% deemed unacceptable (one-sided α, 0.05; β, 0.2).
Data exclusions	All the data aquired in this clinical trial were analyzed.
Replication	ince this was a single arm hase study, the specimens were collected from patients only once at each time point, and the amount of each specimen collected was very small. Thus, it was not possible to repeat the validation e periments.
Randomization	Randomization was not performed. The study focused on a limited group of patients with unresectable locally advanced esophageal squamous cell carcinoma, and it was challenging to secure a sufficient number of cases for randomization to evaluate the efficacy and safety of the trial treatment.
Blinding	Blinding was not performed. This was a non-randomised study without blinding of outcome assessments.

Behavioural & social sciences study design

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

Study description	N/A
Research sample	N/A
Sampling strategy	N/A
Data collection	N/A
Timing	N/A
Data exclusions	N/A
Non-participation	N/A
Randomization	N/A

Ecological, evolutionary & environmental sciences study design

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

Study description	N/A
Research sample	N/A
Sampling strategy	N/A
Data collection	N/A
Timing and spatial scale	N/A
Data exclusions	N/A
Reproducibility	N/A
Randomization	N/A
Blinding	N/A

Did the study involve field work? ☐ Yes ☑ No

Field work, collection and transport

Field conditions	N/A
Location	N/A
Access & import/export	N/A
Disturbance	N/A

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	Cells (PBMCs and TILs) were washed using washing solution and subjected to staining with surface antibodies, including the anti-CTLA-4 antibody (1:50 dilution). The antibodies used in this study were described in the online methods section of the manuscript and supplementary table 7. Cells were stained with antibodies against CD3 (clone: SP7, ab16669, Abcam, Cambridge, UK, 1:200), CD8 (clone: C8/144B, M710301-2, Agilent, Santa Clara, CA, 1:200), FOXP3 (clone: 236A/E7, ab96048, Abcam, 1:100), Cytokeratin (clone: AE1 + AE3, ARG56129, Arigo Biolaboratories, Hsinchu, Taiwan, 1:200), and PD-1 (clone: EPR4877(2), ab137132, Abcam, 1:50). Anti-CD11c antibody was diluted to 1:50. For the FCM analyses, the following target molecules were involved: EOMES (clone: WD1928, eBioscience, 1:50), Ki67 (clone: B56, BD, 1:50), TCF1 (clone: 7F11A10, BioLegend, 1:50), HLA-DR (clone: L243, BioLegend, 1:50), PD1 (clone: MIH4, BD, 1:50), CD80 (clone: 2D10, BioLegend, 1:50), CD19 (clone: HIB19, BioLegend, 1:50), CCR7 (clone: G043H7, BioLegend, 1:50), CD3 (clone: UCHT-1, BD, 1:50), CD45RA (clone: HI100, BioLegend, 1:50), CD8a (clone: RPA-T8, BioLegend, 1:50), CD14 (clone: M5E2, BD, 1:50), CD4 (clone: SK3, BD, 1:50), CD56 (clone: NCAM16.2, BD, 1:50), PD-L1 (clone: MIH1, BD, 1:50), CD11c (clone: B-ly6, BD, 1:50), CD45 (clone: HI30, BD, 1:50), FOXP3 (clone: 236A/E7, eBioscience, 1:50), CTLA-4 (clone: L3D10, BioLegend, 1:50), CD86 (clone: 2331 (FUN-1), BD, 1:50), Tbet (clone: eBio4B10, eBioscience, 1:50).
Validation	We previously validated antibodies used in this study in our lab. The antibodies commercially available were validated by the manufacturers and used according to the manufacturer's instructions.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	N/A
Authentication	N/A
Mycoplasma contamination	N/A
Commonly misidentified lines (See ICLAC register)	N/A

Palaeontology and Archaeology

Specimen provenance	N/A
Specimen deposition	N/A
Dating methods	N/A

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	N/A

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

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	N/A
Wild animals	N/A
Reporting on sex	N/A
Field-collected samples	N/A
Ethics oversight	N/A

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	We registered the study at the University Hospital Medical Information Network (clinical trial information number: UMIN000034373).
Study protocol	We briefly described the protocol of this study in the section of "Study design and treatments". In addition, the full study protocol has been provided.
Data collection	The data center of the Clinical Research Support Office, National Cancer Center Hospital East (NCCHE-OCRS), confirmed patient eligibility; data collection, analyses, and interpretation were also performed by the NCCHE-OCRS. Seven institutions including National Cancer Center Hospital East, National Cancer Center Hospital, Saitama Cancer Center, Cancer Institute Hospital of JFCR, Shizuoka Cancer Center, and Aichi Cancer Center participated in this study. The enrolment period was from December 2018 to March 2021, and the study was completed in January 2022.
Outcomes	Primary outcome: In patients with unresectable locally advanced esophageal squamous cell carcinoma, achieving a complete response (CR) after chemoradiotherapy has been clearly associated with long-term prognosis, and the CR rate is considered a surrogate endpoint for long-term prognosis. Secondary outcomes: We evaluated progression-free survival and overall response rate as alternative endpoints to CR, as well as overall survival as the true endpoint. Safety was also assessed.

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☑	☐	Public health
☑	☐	National security
☑	☐	Crops and/or livestock
☑	☐	Ecosystems
☑	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☑	☐	Demonstrate how to render a vaccine ineffective
☑	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☑	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☑	☐	Increase transmissibility of a pathogen
☑	☐	Alter the host range of a pathogen
☑	☐	Enable evasion of diagnostic/detection modalities
☑	☐	Enable the weaponization of a biological agent or toxin
☑	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	N/A
Files in database submission	N/A
Genome browser session (e.g. UCSC)	N/A

Methodology

Replicates	N/A
Sequencing depth	N/A
Antibodies	N/A
Peak calling parameters	N/A
Data quality	N/A
Software	N/A

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	Cells (PBMCs and TILs) were washed using washing solution and subjected to staining with surface antibodies (1:50 dilution). Intracellular staining of some molecules was performed with the corresponding antibodies and the Foxp3/Transcription Factor Staining Buffer Set.
Instrument	Cells were analyzed with a FACSymphony (BD Biosciences).
Software	FlowJo software (Treestar, Ashland, OR) was used.
Cell population abundance	All the cells obtained from PBMCs and TILs were analyzed.
Gating strategy	The gating strategy used in this study is shown in Extended Data Fig. 9

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

Magnetic resonance imaging

Experimental design

Design type	N/A
Design specifications	N/A
Behavioral performance measures	N/A
Imaging type(s)	N/A
Field strength	N/A
Sequence & imaging parameters	N/A
Area of acquisition	N/A

Diffusion MRI ☐ Used ☑ Not used

Preprocessing

Preprocessing software	N/A
Normalization	N/A
Normalization template	N/A
Noise and artifact removal	N/A
Volume censoring	N/A

Statistical modeling & inference

Model type and settings	N/A
Effect(s) tested	N/A

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

(See Eklund et al. 2016)

Correction N/A

Models & analysis

n/a	Involved in the study
☑	☐ Functional and/or effective connectivity
☑	☐ Graph analysis
☑	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity N/A

Graph analysis N/A

Multivariate modeling and predictive analysis N/A