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	Clinical data collection via 'eCRF' (Medidata Rave EDC, v2020.1). IHC/IF image acquisiton was performed using Scanscope (console v102.0.7.5 and controller v102.0.8.60), Zen Blue (v3.1), PhenoImager HT imaging system (v1.1), Visiopharm (v2020.01.1, v2020.08.4) and inForm software (v2.5).
Data analysis	Graph plotting and statistical analyis were performed with GraphPad Prism v10. Calculations, correlations and visualization analyses of TCGA and Nanostring data was performed using the the statistical software environment R (versions 3.3, 3.6, 4.0, 4.3; R Core Team, R Foundation for Statistical Computing, Vienna, Austria) including various R/ Bioconductor packages (pheatmap, ggplot2, grid, DESeq2, multtest, data.table, DT, stringr, maftools, devtools, ggpubr, tidyverse, survival, NanoStringNorm, limma, EnhancedVolcano). To retrieve clincial information on patients from TCGA (https://portal.gdc.cancer.gov/) the packages RTCGA.clinical and TCGAbiolinks together with supplementary information from original TCGA publications were used. The corresponding source code for analyzing the TCGA data and creating the figures was deposited on GitHub (https://github.com/icbi-lab/GDF15_TCGA). For deconvolution of immune cells based on RNA sequencing data (TPM) quanTIseq (v2.0) was used (https://icbi.at/software/quantiseq/, https://github.com/omnideconv/). To infer tumor purity as well as the fraction of stromal and immune cells ESTIMATE (v1.0.13) was used (https://bioinformatics.mdanderson.org/estimate/). For gene set enrichment analysis GSEA v4.1 (http://software.broadinstitute.org/gsea/) and for single sample gene set enrichment analysis ssGSEA v2.0 (https://github.com/broadinstitute/ssGSEA2.0) was used. For PK/PD modeling Monolix (v2019R1) software based on the SEAM (stochastic approximation expectation-maximization) algorithm was used.

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 responses, demographic information, and safety outcomes, as well as IHC quantifications, serum analysis data and RNAseq signature scores are available within the paper, its supplementary information and provided as source data.
The results shown regarding the indication selection are in part based upon data generated by the TCGA Research Network (https://www.cancer.gov/tcga) and available at the GDC data protal (https://portal.gdc.cancer.gov/).
Normalized RNA sequencing data (TPM) from pan-cancer analyses of TCGA were obtained from http://firebrowse.org and together with related immune signatures, estimated immune cell fractions, as well as detailed information of selected indications (LUAD, LUSC, BLCA phenotypes) are available at zenodo (https://doi.org/10.5281/zenodo.13716226). Pathway analysis of gene expression data in patients is based on (Reactome) pathway information from ConsensusPathDB (http://cpdb.molgen.mpg.de/CPDB).

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	Demographic tables display information on sex of participants and are calculated as percentage of full analysis set of each cohort.
Reporting on race, ethnicity, or other socially relevant groupings	Demographic tables display information on race of participants and are calculated as percentage of full analysis set of each cohort.
Population characteristics	Population characteristics and demographics are fully described in Supplementary Tables 1, 3, 6, 10, 12 and 14 of the manuscript.
Recruitment	Patients were enrolled according to their diagnosis and disease stage and were required to fulfill all inclusion criteria and not meet any exclusion criteria accordingly. At the following highly experienced and specialized solid tumor clinical trial sites across Europe, patients were recruited and enrolled: - Clinica Universidad de Navarra, Pamplona, Spain - Hospital Universitario HM Sanchinarro START, Madrid, Spain - Hospital Universitari Vall d'Hebron, Barcelona, Spain - NEXT Oncology, Phase 1 Unit, Barcelona, Spain - Catalan Institute of Oncology (ICO), Barcelona, Spain - Hospital 12 de Octubre, Madrid, Spain - Hospital Clínic de Barcelona (Hospital Clinic i Provincial), Barcelona, Spain - University Hospital Zurich, Zurich, Switzerland - Cantonal Hospital St. Gallen, St. Gallen, Switzerland - University Hospital Basel, Basel, Switzerland - University Hospital Wuerzburg, Interdisciplinary Study Center (ISZ) with ECTU; Wuerzburg, Germany - University Hospital Essen; Internal Clinic (Tumor Research), West German Tumor Center Essen, Essen, Germany - University Hospital Frankfurt, Center of Internal Medicine, Frankfurt, Germany For the recruiting sites, competitive recruitment applied and enrolment numbers varied between sites depending on availability of eligible patients. Due to the clearly defined inclusion and exclusion criteria together with the large number of clinical trial sites across Europe, no selection bias is evident. Patients who were offered participation in the clinical trial were carefully informed about alternative treatment options. Patients were provided copies of the study informed consent document and were fully aware of risks prior to trial enrollment. As patients needed to fulfill eligibility criteria of the trial, this could have caused selection bias.
Ethics oversight	Regulatory authorities in Germany (Paul-Ehrlich-Institut [PEI), Spain (Agencia Española de Medicamentos y Productos Sanitarios [AEMPS]) and Switzerland (Swissmedic) and the local ethics committee in charge of the clinical trial site approved the study following applicable rules and regulations: • Comité de Ética de la Investigación con Medicamentos (CEIm), Pamplona, Spain • Swissethics, Cantonal Ethics Committee, Zurich, Switzerland • Ethics Committee of the University of Würzburg, Würzburg, Germany

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	The study was conducted as a combined phase 1/2a trial. Phase 1 was conducted as a 3+3 dose escalation trial , therefore sample sizes for phase 1 were defined by the dose-escalation scheme and were not sized to obtain statistical power for correlative analysis. As such no statistical methods were used to predetermine statistical sample size. Statistical design of phase 2a is described in the methods section of the manuscript accordingly. In the exploratory analysis, sample sizes for IHC, serum analysis and RNA sequencing were dictated by the availability of high-quality tissues for staining/RNA extraction and serum quality for PK/PD assessment.
Data exclusions	No data exclusions occurred.
Replication	Clinical trial results can not be replicated.
Randomization	The reported study is a combined phase 1/2a study and was conducted in a single-arm design. Therefore, randomization is not part of the study and reflects the state-of-the-art design for early clinical development investigating safety, definition of the recommended phase 2 dose and initial signals for clinical activity.
Blinding	The reported study is a combined phase 1/2a study and was conducted in a single-arm design. Therefore, blinding is not applicable as no comparator arm existed.

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

Primary antibodies for IHC:
anti-GDF-15 rabbit polyclonal antibody (1:50 and 1:100; Sigma Aldrich, ref: HPA011191; lots: 000017158, 000018018, 000024410, 000025267,000039803,000041678) https://www.sigmaaldrich.com/DE/de/product/sigma/hpa011191
anti-aSMA mouse monoclonal (1A4) antibody (Sigma, ref: A2547) https://www.sigmaaldrich.com/DE/en/product/sigma/a2547
anti-PD-1 rabbit polyclonal antibody (Sigma, ref: PRS4065)
anti-PD-Ll rabbit monoclonal (SP263) antibody (Roche Diagnostics, ref: 790-4905)
Rabbit monoclonal negative control Ig (Roche Diagnostics, ref: 790-4795)
https://diagnostics.roche.com/global/en/products/lab/pd-l1-sp263-ventana-rtd001236.html#productSpecs
anti-pan-Keratin mouse monoclonal (AE1/AE3/PCK6) antibody (Roche Diagnostics, ref: 05267145001) https://diagnostics.roche.com/global/en/products/lab/pan-keratin-ae1-ae3-pck26-250-tests-rtd000684.html
anti-SOXl0 rabbit monoclonal (SP267) antibody (Roche Diagnostics, ref: 760-4968) https://diagnostics.roche.com/global/en/products/lab/sox-10-sp267-rtd001132.html
anti-CD3, anti-Granzyme B, anti-Ki67, anti-panCK, anti-SOXl0 in UltiMapper 1/0 T-act kit (Ultivue, ref: ULT20104 and ULT20110) https://ultivue.com/t-act-fixvue-panel/
anti-CD8 mouse monoclonal (C8/144B) antibody (Agilent Technologies, ref: M710301-2) https://www.agilent.com/store/productDetail.jsp?catalogId=M710301-2
anti-CD8 mouse monoclonal (C8/144B) antibody (Dako/Agilent, ref: GA62361-2) https://www.agilent.com/store/productDetail.jsp?catalogId=GA62361-2

anti-FoxP3 rabbit monoclonal (SP97) antibody (Abcam, ref: Ab99963) https://www.abcam.com/en-de/products/primary-antibodies/foxp3-antibody-sp97-ab99963
anti-panCK mouse monoclonal (C-11) antibody (abcam, ref: ab7753) https://www.abcam.com/en-de/products/primary-antibodies/pan-cytokeratin-antibody-c-11-ab7753
anti-CD45 rabbit polyclonal antibody (abcam, ref: ab10558) https://www.abcam.com/en-de/products/primary-antibodies/cd45-antibody-ab10558
anti-PD-Ll rabbit monoclonal (SP142) antibody (abcam, ref: ab228462) https://www.abcam.com/en-de/products/primary-antibodies/pd-l1-antibody-sp142-c-terminal-ab228462
anti-Ki67 rabbit monoclonal (SP6) antibody (abcam, ref: ab16667) https://www.abcam.com/en-de/products/primary-antibodies/ki67-antibody-sp6-ab16667
anti-Vimentin rabbit monoclonal (EPR3776) antibody (abcam, ref: ab92547) https://www.abcam.com/en-de/products/primary-antibodies/vimentin-antibody-epr3776-cytoskeleton-marker-ab92547
anti-CD4 rabbit monoclonal (EPR6855) antibody (abcam, ref: ab133616) https://www.abcam.com/en-de/products/primary-antibodies/cd4-antibody-epr6855-ab133616
anti-CD163 rabbit monoclonal (EPR19518) antibody (abcam, ref: ab182422) https://www.abcam.com/en-de/products/primary-antibodies/cd163-antibody-epr19518-ab182422
anti-FoxP3 rabbit monoclonal (236A/E7) antibody (abcam, ref: ab20034) https://www.abcam.com/en-de/products/primary-antibodies/foxp3-antibody-236a-e7-ab20034
anti-CD4 rabbit monoclonal (SP35) antibody (CellMarque, ref: 104R) https://www.cellmarque.com/antibodies/CM/2030/CD4_SP35
anti-CD3 rabbit monoclonal (D7A6E) antibody (CellSignaling, ref: 85061) https://www.cellsignal.com/products/primary-antibodies/cd3e-d7a6e-xp-rabbit-mab/85061
Secondary antibodies for IHC :
goat anti-mouse IgG, goat anti-mouse IgM and goat anti-rabbit HQ labelled antibody in OptiView DAB IHC Detection Kit (Roche Diagnostics, ref: 760-700) https://diagnostics.roche.com/global/en/products/lab/optiview-dab-ihc-detection-kit-rtd001078.html
anti-mouse HQ-conjugated secondary antibody (Roche Diagnostics, ref: 760-4814) https://diagnostics.roche.com/global/en/products/lab/mouse-hq-discovery-rtd000837.html
anti-rabbit HQ-conjugated secondary antibody (Roche Diagnostics, ref: 760-4815) https://diagnostics.roche.com/global/en/products/lab/rabbit-hq-discovery-rtd000840.html
goat anti-rabbit nitropyrazole (NP)-conjugated secondary antibody (Roche Diagnostics, ref: 760-4817) https://diagnostics.roche.com/global/en/products/lab/rabbit-np-discovery-rtd000841.html

Validation

All antibodies were listed by the respective vendor as validated for immunohistochemistry.

Antibody validation information provided by Sigma-Aldrich:
Additional Validation Data to Ensure Antibody Reproducibility, Specificity and Performance
orthogonal RNAseq: Orthogonal Validation Using RNA-seq - Demonstrating antibody specificity through an antibody-dependent method correlated with an antibody-independent method (RNA-seq).
RNAi knockdown: Genetic Strategies – Demonstrating antibody specificity through knockout/knockdown method. Expected Results: Diminished or absence of band in Western blotting in knockdown/knockout validation.

Antibody validation information provided by Roche Diagnostics:
Performance characteristics are evaluated by conducting staining tests for sensitivity, specificity and precision and results are reported in the respective method sheets which can be accessed through the links provided with each antibody.
Precision studies are completed to demonstrate:
- lot-to-lot precision of the antibody
- within run and between day precision
- across instruments and platform precision

Antibody validation information provided by DAKO/Agilent:
Primary Antibodies for Dako Omnis ensure accurate and reliable IHC Results
The FLEX RTU Solution offers dedicated series of high-quality, pre-diluted, ready-to-use (RTU) primary antibodies that are accompanied by appropriate validated protocols to support your lab in providing reliable and reproducible diagnostic results.
The robust IHC tests are calibrated and validated for reliable diagnostic use, ensuring that the antigen is correctly demonstrated at both high and low expression levels in tissue to support your lab in reducing the risk of false negative and false positive results.

Antibody validation information provided by abcam:
Our recombinantly manufactured antibodies provide:
- Unrivaled batch-to-batch consistency
- Confirmed specificity due to industry-leading validation
- Ease of scalability and long-term supply
- High-throughput in vitro manufacture of recombinant antibodies
To ensure specificity, our recombinant antibodies undergo industry-leading validation. This includes over 3,600 with knock-out validation, so you can move your research forward faster. And because the selection process for the desired clone occurs at both the hybridoma and recombinant cloning stages, we can select antibodies with the most favorable qualities for you. Our recombinant antibodies have a remarkably high affinity, with KD values in the picomolar range. High-affinity antibodies allow greater sensitivity in assays as they bind strongly to the antigen and maintain this bond better under challenging conditions compared to low-affinity antibodies.

Antibody validation information provided by CellMarque (part of Merck Millipore):
Before releasing the antibody to manufacturing, we ensure that our antibodies meet our strictest specifications. Only antibodies that pass our stringent review are made available to customers. Once produced and released for sale, we support customers' research efforts with a highly specialized team of technical support scientists and field engineers. To support our multi-step, multi-application validation process, we have a tissue and blot library with over 1300 lysates, allowing us to precisely determine each antibody's specificity. At Merck Millipore, we have the advantage of having an entire cell analysis technology development team in-house. Using confocal microscopes and high-throughput IHC instruments, we can obtain accurate data faster than manual imaging. Further

scientific review determines whether staining patterns conform to published subcellular expression. For immunohistochemistry, we include negative controls, to confirm the signal.

Antibody validation information provided by CellSignaling:
We guarantee that our antibodies are fit for purpose by carefully tailoring the combination of validation strategies applied to each product. This means customizing our validation process according to the biological role of the target, while considering the sensitivity requirements of the downstream assay, the availability of appropriate testing models, and the relevance of each method to target investigation. Immunohistochemistry (IHC) involves the labeling of cellular proteins in tissue samples with a specific primary antibody and visualization of the target with detection reagents. The determination of target specificity in immunohistochemical analysis requires multiple validation steps. CST scientists use a variety of methods, as appropriate, to validate each IHC-recommended antibody, ensuring that the staining you observe with each CST antibody is specific and believable.
Validation Steps Include:
• Western blot analysis is performed to demonstrate specific bands of the appropriate molecular weight(s), with minimal cross-reacting bands.
• Paraffin-embedded cell pellets of known target expression levels are used to verify target specificity.
• Antibody performance is assessed in relevant mouse models of cancer.
• Xenografts generated from cell lines with known target expression levels help verify target specificity.
• Human cancer tissue arrays are used to demonstrate antibody performance over a broad spectrum of tissue types.
• Staining on fresh frozen tissues is performed when appropriate.
• The use of blocking peptides verifies specificity and rules out Fc-mediated binding and other non-specific staining.
• Thorough lot testing ensures the reproducibility necessary for accurate IHC results.
• Dilutions and protocols are predetermined and specified; control reagents are also available.

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	NCT04725474; EudraCT Number 2020-002103-19; CTR Number 2024-512575-12
Study protocol	Study protocol can be accessed at https://euclinicaltrials.eu/search-for-clinical-trials/?lang=en&EUCT=2024-512575-12-00 and was provided as redacted version to Nature.
Data collection	Data were collected at the following 13 clinical trial sites in three countries (Spain, Switzerland and Germany): • Clinica Universidad de Navarr, Pamplona, Spain • Hospital Universitario HM Sanchinarro START, Madrid, Spain • Hospital Universitari Vall d'Hebron, Barcelona, Spain • NEXT Oncology, Phase 1 Unit, Barcelona, Spain • Catalan Institute of Oncology (ICO), Barcelona, Spain • Hospital 12 de Octubre, Madrid, Spain • Hospital Clínic de Barcelona (Hospital Clinic i Provincial), Barcelona, Spain • University Hospital Zurich, Zurich, Switzerland • Cantonal Hospital St. Gallen, St. Gallen, Switzerland • University Hospital Basel, Basel, Switzerland • University Hospital Wuerzburg, Interdisciplinary Study Center (ISZ) with ECTU; Wuerzburg, Germany • University Hospital Essen; Internal Clinic (Tumor Research), West German Tumor Center Essen, Essen, Germany • University Hospital Frankfurt, Center of Internal Medicine, Frankfurt, Germany Period times for recruitment were from 12/2020 to 04/2024 Data collection started in 12/2020 and is ongoing.
Outcomes	Outcomes are defined in the clinical study protocol (definition of AE/SAE, DLTs and MTD) and in the methods section of the manuscript for response assessment. Assessment of additional secondary endpoints including pharmaco-kinetics and -dynamics are described in the methods section of the manuscript.

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.*