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

Plate reader: BioTek Gen 5 version 3.14 (Agilent)
WB scanner: LI-COR acquisition software version 2.0 (LICOR)
HPLC: LauraTM radiochromatography data, collection and analysis software version 6.5.1.19 (LabLogic)
Confocal microscopy: Zen microcopy software version 3.6 (Zeiss)
Fluorescence microscopy: EVOSTM M 5000 Imaging System (Invitrogen)
Live cell imaging: Incucyte® S3 Live-Cell Analysis Sytem (SARTORIUS)
PET/CT imaging: NuclineTM acquisition software (Mediso)
Cell counter: Countess 3 Automated Cell Counter (Invitrogen)
TEM images: JEM-1400 Plus Electron Microscopy (JEOL)
Toxicology studies: ACE AXCEL® clinical chemistry system (Alfa Wassermann Diagnostic Tenchnologies, LLC)
MALDI: FlexAnalysis version 3.4 (Bruker Daltonics)
iTLC: WinScan software version 3.0 (Bioscan)
IHC: Mirax Digital Slide Scanner, SlideViewer version 2.5.0.143918, and Aperio GT 450DX Slide Scanner 40X, Histowiz app version cc833f1
Gamma counter: 2480-0010 WIZARD2 automatic gamma counter (Perkin Elmer)

Data analysis

All software used in this study for data analyses are described in the methods section and include:
PET/CT analysis: Imalytics Preclinical software version 3.1 (Gremse-IT)
Fluorescence images: FIJI version 1.8.0_322 (Image J; Java)
WB images: ImageJ 1.52a version 1.8.0_112 (Java) and Empiria Studio version 3.2
Live cell imaging analysis: Incucyte® S3 Live-Cell Analysis Sytem (SARTORIUS)
2D TEM reconstruction: Relion version 3.1 (MRC laboratory Molecular Biology; PMID: 30412051)

Toxicology analysis: ACE AXCEL® clinical chemistry system (Alfa Wassermann Diagnostic Tenchnologies, LLC)
HPLC analysis: LauraTM radiochromatography data, collection and analysis software version 6.5.1.19 (LabLogic)
iTLC analysis: WinScan software version 3.0 (Bioscan)
Statistical analysis and graph plotting: Prism version 10.5.0 (GraphPad), R 4.4.0 (2024-04-24) and RStudio 2026.01.1+403 (R packages: emmeans 1.10.7, survival 3.5, nlme 3.1-166, tidyverse 2.0.0 and ggplot2 4.0.2)
Co-registration: Python v. 3.12.12 (Anaconda), NumPy v.2.3.5, OpenCV v.4.13.0, Matplotlib v. 3.10.8, scikit-image v. 0.26.0, pandas v. 2.3.3, pystackreg v. 0.2.8

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 relevant data are provided in this Article and its Supplementary Information, and Source data are provided with this paper. The code for data processing and analysis of EGFR and HER2 IHC co-registration is fully referenced and described in the Methods section and Supplementary information. For further assistance, please contact the corresponding author.

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	Not applicable.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable.
Population characteristics	Not applicable.
Recruitment	Not applicable.
Ethics oversight	Not applicable.

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 imaging/biodistribution studies used at least 3 mice per group. N=3 per group allows 80% power to detect group mean difference effect size of 1.8 by a 2-sided 2-sample t-test at alpha=5%. The therapy studies used at least 7 mice per group. N=7 per group allows 80% power to detect a group mean difference effect size of 1.32 by a 2-sided 2-sample t-test at alpha=5%. N=4 per group for tumor tissue markers allows 80% power to detect a group mean difference effect size of 2.38 by a 2-sided 2-sample t-test at alpha=5%. For in vitro studies, no calculations were performed prior to determine the sample size; the sample size was determined based on similar in vitro studies in the field (PMID: 41212147, PMID: 32213539).
Data exclusions	Nude mice (athymic, immunodeficient) are highly susceptible to skin infections, C. bovis. Animals that developed severe C. bovis infection following transfer from housing to the PCIF facility were excluded from analysis.
Replication	Independent biological replicates were performed to ensure reproducibility. Antibody conjugations and radiolabeling were performed in technical replicates. In vitro data were collected and analyzed in technical triplicates, and in different cancer cell lines. SDS-PAGE and Western blot analysis and immunofluorescence was performed in technical replicates, and one representative replicate presented for illustration with additional images in SI.

Radio TLC, HPLC, MALDI analysis was performed in replicates.
In vivo PET/CT and biodistribution, and therapy were performed in replicates and in different tumor models, and replication performed in both female and male mice.
Experiments were replicated at least twice by 2-3 independent personnel, and attempts at replication were successful.
All n replicates are reported in the manuscript and on which statistics are based are biological replicates.

Randomization

For all experiments, stratified random sampling was employed to assign the mice randomly to experimental or controls groups. Mice were randomized before treatments when tumor volumes reached 100-300 mm3 to ensure comparable tumor sizes between groups at baseline.
For in vitro experiments, samples were allocated into experimental groups randomly.
All mice used in the study were gender- and age-matched.

Blinding

Therapy studies: personnel involved in the tumor measurements, mouse weight, or toxicology studies were blinded to the treatment groups, except for the T-DXd resistant studies. For those studies, it was not possible to blind because it was a single treatment.
Imaging studies: personnel involved in the ex vivo biodistribution were blinded to radiotracer administration.
Personnel involved in the acquisition of the MALDI and TEM were blinded to the sample group.
Personnel involved in the statistical analyses were blinded to the treatment groups.
In vitro experiments were not blinded.

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

1. Herceptin, Trastuzumab
2. Vectibix, Panitumumab
3. Perjeta, Pertuzumab
4. Ado-trastuzumab, T-DM1
5. Enhertu, T-DXd
6. rabbit anti-HER2 (ab131490; Abcam); https://www.abcam.com/en-us/products/primary-antibodies/erbb2-her2-antibody-ab131490
7. rabbit anti-EGFR (ab52894; Abcam); clone EP38Y; https://www.abcam.com/en-us/products/primary-antibodies/egfr-antibody-ep38y-ab52894
8. mouse anti-β-actin (A1978; Sigma); clone AC-15, monoclonal; https://www.sigmaaldrich.com/US/en/product/sigma/a1978
9. DM1 monoclonal antibody (Invitrogen, MA5-42528); clone A4G3; https://www.thermofisher.com/antibody/product/DM1-Antibody-clone-A4G3-Monoclonal/MA5-42528
10. anti-trastuzumab antibody (Biotechne, MAB95471-SP); clone 2261E, monoclonal; https://www.bio-techne.com/p/antibodies/anti-trastuzumab-anti-idiotype-antibody-2261e_mab95471
11. anti-rabbit goat IgG conjugated with Alexa Fluor 680 (Invitrogen, A-21076); https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21076
12. anti-mouse goat IgG conjugated with Alexa Fluor 800 (Invitrogen, A32730); https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32730
13. anti-human goat IgG conjugated with Alexa Fluor 680 (ThermoFisher, SA000069); https://www.thermofisher.com/antibody/product/Goat-anti-Human-IgG-H-L-SRM-Secondary-Antibody-Polyclonal/SA000069
14. Human IgG Isotype Control (Invitrogen, 31154); https://www.thermofisher.com/antibody/product/Human-IgG-Isotype-Control/31154
15. CoraLite® Plus 647 Anti-Human CD107a / LAMP1 (Proteintech, CL647-65051); H4A3, monoclonal; https://www.ptglab.com/products/CD107a-Antibody-CL647-65051.htm?srsltid=AfmBOooYWrf3nRq6sAlYjhGzB4oQqbtx544MFgtIsQlF2KKpuf_zmyhn
16. BSCFV-155 (Creative Biolabs; anti-EGFR based on clone C225 and anti-HER2 based on clone 4D5)
17. C-erbB-2 (human) clonal antibody (A24-V) ALX-810-227, Enzo; https://www.fishersci.com/shop/products/c-erbb-2-h-cab-a24-v-1ml/502010086
18. Bavencio, Avelumab
19. InVivoMAb anti-human/mouse/rat/canine/swine PSMA (Catalog #BE0444), Clone 3F11, Bioxcell; https://bioxcell.com/invivomab-anti-human-mouse-rat-canine-swine-psma-be0444
20. Avastin, bevacizumab

Validation	Antibodies used in the WB studies were validated according to the manufacturer's statement. Validation data is available on the vendor's website. All antibodies purchased in this study have valid citations. 1. rabbit anti-HER2 (ab131490; Abcam); https://www.abcam.com/en-us/products/primary-antibodies/erbb2-her2-antibody-ab131490 2. rabbit anti-EGFR (ab52894; Abcam); clone EP38Y; https://www.abcam.com/en-us/products/primary-antibodies/egfr-antibody-ep38y-ab52894 3. mouse anti-β-actin (A1978; Sigma); clone AC-15, monoclonal; https://www.sigmaaldrich.com/US/en/product/sigma/a1978 4. DM1 monoclonal antibody (Invitrogen, MA5-42528); clone A4G3; https://www.thermofisher.com/antibody/product/DM1-Antibody-clone-A4G3-Monoclonal/MA5-42528 5. anti-trastuzumab antibody (Biotechne, MAB95471-SP); https://www.bio-techne.com/p/antibodies/anti-trastuzumab-anti-idiotype-antibody-2261e_mab95471 6. anti-rabbit goat IgG conjugated with Alexa Fluor 680 (Invitrogen, A-21076); https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21076 7. anti-mouse goat IgG conjugated with Alexa Fluor 800 (Invitrogen, A32730); https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32730 8. anti-human goat IgG conjugated with Alexa Fluor 680 (ThermoFisher, SA000069); https://www.thermofisher.com/antibody/product/Goat-anti-Human-IgG-H-L-SRM-Secondary-Antibody-Polyclonal/SA000069 9. anti-trastuzumab (Biotechne, MAB95471-SP); https://www.bio-techne.com/p/antibodies/anti-trastuzumab-anti-idiotype-antibody-2261e_mab95471 For the IHC staining, appropriate controls were used to validate the specificity of the antibodies used. A positive and a negative control were included in the studies. Antibody conjugates and radiolabeling were prepared in-house and those were validated via SDS-PAGE analyses, and their binding and internalization was further validated through biochemical assays in cancer cells using positive and negative controls. Dose-response curves were generated.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Human cancer cell lines NCIN87 (gastric), A431 (epidermoid), BT474 (breast), CT26 (colorectal), MDA-MB-231 (breast), MIAPaCa-2, (pancreatic) and JIMT1 (breast) were purchased from the American Type Culture Collection (ATCC)
Authentication	Commercial cell lines were authenticated through STR analyses from ATCC or WUSTL, and their morphology and doubling times were regularly checked. CT26.hHER2 were validated for their expression of human HER2 by Western blot, IHC, and PET studies.
Mycoplasma contamination	All cells were tested bi-weekly/monthly, and re-tested before animal studies, and confirmed negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

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	Six- to 8-week-old female or male nu/nu mice; 4 to 6-week-old female or male BALB/c obtained from Charles River Laboratories. Animal studies were performed in the Washington University School of Medicine in compliance with institutional guidelines and under Institutional Animal Care and Use Committee (IACUC) approved protocols (Animal Protocol 21-0087, 24-0274; IBC protocol 13652, PI: Pereira). Animals were housed at temperatures between 68-79F, humidity between 30% to 70%, a light cycle ON at 6:00 AM and OFF at 6:00 PM, with a negative pressure room.
Wild animals	No wild animals were used in the study.
Reporting on sex	The mice included in this study were matched for sex to minimize potential confounding variables when possible to ensure that sex and age were not significant sources of bias in our analysis. Both female and male mice were used, except in the breast cancer xenografts where only female mice were used due to the problems of growing breast cancer cells in male mice and the prevalence of breast cancer in females. No sex-based analyses was done.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	Animal studies were performed in the Washington University School of Medicine in compliance with institutional guidelines and under Institutional Animal Care and Use Committee (IACUC) approved protocols (Animal Protocol 21-0087, 24-0274; IBC protocol 13652, PI: Pereira).

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

Plants

Seed stocks	No seed stocks were used in this study.
Novel plant genotypes	No novel plant genotypes were applicable in this study.
Authentication	Not applicable.