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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	Gene expression profiles: a) CCLE cancer cell lines were downloaded from the DepMap (Public 19Q1); b) Transgenic mouse models of breast cancer were collected from GSE42640; c) TCGA patient data were extracted from QIAGEN OncoLand (201905); d) METABRIC patient data were downloaded from Synapse (https://www.synapse.org/#!Synapse:syn1757063); e) Inflammatory breast cancer patient data were collected from GSE23720; f) Breast cancer patients treated by Paclitaxel only were collected from GSE25066; g) RNA-seq data of clinical trial samples were generated by Illumina HiSeq 2000 at Genomics Core Faility at Mount Sinai; h) RNA-seq data of cell lines (MDA-MB-453, SK-BR-3, MDA-MB-436 and MDA-MB-468) were generated by Illumina NovaSeq 6000 in the CMPB Genomics Laboratory at St Jude Children's Research Hospital; i) Microarray data of MDA-MB-453, SK-BR-3, SUM-19 and MMTV-Neu mouse model were generated by DU-640 UV Spectrophotometer; j) The acetylomics data was generated by Thermo Fisher Q Exactive HF Orbitrap MS.
Data analysis	The RNA-seq data were processed using open source software tools, including FastQC (v-0.11.5), cutadapt (v-2.10), Salmon (v-0.9.1). The microarray data were analyzed by limma R package (v-3.42.2). The proteomics data were analyzed by open sourced tools, including SEQUEST for database search, JUMP software suite for quantification and normalization, limma R package for differential analysis.

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 gene expression profile data is available at GEO under GSE180607. It includes two subseries, one for the RNA-seq data of clinical trial samples and 4 breast cancer cell lines (GSE128623), and another one for the microarray data of three cell lines and one mouse model (GSE180606). The acetylomics data, includiing Raw files and pepXML files for each sample, can be accessed at PRIDE under the accession number of PXD026010. The codes for the HDAC6 score calculation and other analyses are freely available at https://github.com/jyyulab/HDAC6-score.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	In this trial, patients received ricolinostat orally (liquid) for 21 consecutive days of each 28-day cycle with nab-paclitaxel dosed at 100 mg/m2 on days 1, 8, and 15 until progression of the disease or unacceptable toxicity. Entry criteria included men or women with any metastatic breast cancer subtypes. Seventeen patients were accrued between March 2016 and February 2018 (14 were females). No separation between sexes was used in the study because the number of males was to low to paerform any statistically significant study.
Population characteristics	Seventeen patients were accrued between March 2016 and February 2018. Of these, 16 patients had an evaluable disease, as one patient dropped out at cycle 2 due to no longer wishing to participate in the trial and in the absence of any related toxicity. In the 16 evaluable patients, the median age was 57.5 years (range: 41-78), 14 were female (87.5%), 3 had triple-negative MBC, and 13 were HR+/HER2- MBC. The median number of prior lines was 3 (range: 0-10). Detailed information of each patient is given in extended data tables and ource data.
Recruitment	Ages Eligible for Study: 18 Years and older (Adult, Older Adult) Sexes Eligible for Study: All Accepts Healthy Volunteers: No Inclusion Criteria: Patients were recruited by personal interview with the oncologist. Subjects have histologically confirmed adenocarcinoma of the breast -- all breast cancer subtypes are allowed. Unresectable or metastatic breast cancer. Locally recurrent disease must not be amenable to any local treatment with curative intent. Metastatic disease must be demonstrated either radiographically or histologically. Patients may have measurable disease only, non-measurable disease only, or both (RECIST 1.1). ECOG performance status of 0-1. Must have recovered from the acute toxic effects of all prior therapy prior to registration for this study to grade 1 or less. Women and men of all races and ethnic groups are eligible for this trial. Minimum number of prior treatments required given standard nab-paclitaxel dosing: If HER2 negative: none If HER2 positive: two prior regimens containing HER2 targeted therapies in the inoperable locally advanced and/or metastatic setting. Prior therapy for inoperable locally advanced/metastatic disease should include trastuzumab plus pertuzumab as well as ado-trastuzumab. Pertuzumab and ado-trastuzumab must have been previously used, unless for reasons that include, but are not limited, to the following: intolerance to pertuzumab and/or ado-trastuzumab, medical contraindication, regimen declined by patient, treating investigator discretion, or medical insurance coverage issues which prevented administration of pertuzumab or ado-trastuzumab. These reasons must be reviewed with the study chairs and documented in the medical record and care report form. Patients who relapse within 12 months of completing neoadjuvant/adjuvant pertuzumab or ado-trastuzumab would be considered as having progressed on that regimen. There is no maximum number of prior treatments allowed in the metastatic setting. Age >18 years. Because breast carcinoma is a disease of adults that rarely occurs in children, children are excluded from this study. Patients must have normal organ and marrow function as defined below: leukocytes ≥3,000/mcL absolute neutrophil count ≥1,500/mcL platelets ≥100,000/mcL hemoglobin ≥9 g/dL total bilirubin ≤ 1.5 × the upper limit of normal AST(SGOT)/ALT(SGPT) ≤2.5 × institutional upper limit of normal Serum creatinine ≤ 1.5 × the upper limit of normal or calculated creatinine clearance ≥ 60 mL/min Subject is capable of understanding the informed consent process. The effects of ACY-1215 on the developing human fetus are unknown. For this reason and because the effects of chemotherapy are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study

participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 2 weeks after completion of ACY-1215 administration.
Exclusion Criteria:

Patients who have had chemotherapy, hormonal therapy, or radiotherapy within 2 weeks prior to entering the study or those who have not recovered from adverse events due to agents administered more than 2 weeks earlier. Concomitant treatment with bone-targeted therapies such as RANKL inhibitors or bisphosphonates is allowed.
Patients who are receiving any other investigational agents concurrently or have received investigational agents within 2 weeks or 5 half-lives of the compound or active metabolites, whichever is longer before the first dose of the study treatment.
Patients who have received HDAC inhibitors (including valproic acid, entinostat, vorinostat) are excluded
Subject is pregnant or nursing. Pregnant women are excluded from this study because ACY-1215 is an investigational therapy with unknown potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with ACY-1215, breastfeeding should be discontinued if the mother is treated with ACY-1215.
Symptomatic or unstable brain metastases. (Note: Asymptomatic patients with metastatic brain disease who have been on a stable dose of corticosteroids for treatment of brain metastases for at least 14 days prior to registration are eligible to participate in the study).
HIV+ with a CD4 count <200 are ineligible because these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.
Patients receiving any medications or substances that are strong inhibitors of CYP450 3A4 isoenzyme.
History of allergic reactions attributed to compounds of similar chemical or biologic composition to nab-paclitaxel.
Uncontrolled intercurrent illness including but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
Corrected QT interval (QTc) value > 480 msec at screening; family or personal history of long QTc syndrome or ventricular arrhythmias including ventricular bigeminy at screening; previous history of drug-induced QTc prolongation or the need for treatment with medications known or suspected of producing prolonged QTc intervals on electrocardiogram (EKG). If QTc prolongation on screening ECG is felt to be related to electrolyte imbalance, an EKG can be repeated after correction of electrolytes.

Ethics oversight	This study was approved by the Columbia Institutional Review Board (IRB-Q3709). Informed consent was obtained from all patients in the study.

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	No statistical method was used to predetermine sample size.
Data exclusions	We excluded two samples (T60 and T61), which were identified as outliers in the quality assessment, from the inflammatory breast cancer cohort. No other sample was excluded except when otherwise described in the text (e.g. tumor samples with low tumor content). No data was excluded in other analysis.
Replication	All experiments were replicated independently a minimum of 3 independent times if not otherwise described in the legends. All attempts at replication were successful.
Randomization	No randomization was required as the study was based on molecular and cellular biology techniques and did not involve allocation of experimental units across different treatment groups.
Blinding	Blinding was not relevant to the study as all results were derived from objective quantitative methods. No subjective measurements were taken.

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

Methods

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

Antibodies

Antibodies used

Immunohistochemistry: It was performed on formalin-fixed paraffin-embedded (FFPE) tumor tissue sections by the Neuropathology Brain Bank Core at Mount Sinai. All Slides were sectioned, mounted, and stained for hematoxylin and eosin, Ki-67(Abcam#15580, clone SP6; 1:200) and c-caspase 3(Cell signaling #9664s clone 5A1E at 1:50).

Proteomics:Each of the fractions was subjected to enrichment by PTMScan Acetyl-Lysine Motif (Cell Signaling Technology) antibody.

WT-blot: Proteins were resolved by SDS-PAGE, transferred to either nitrocellulose or PVDF membranes and analyzed with the following antibodies (clone ID is provided when available by the vendor): HDAC6 (rabbit polyclonal, Santa Cruz sc-11420, clone H-300) was used at 1:1000, α-tubulin(rabbit polyclonal, Cell Signaling #2144) used at 1:1000, acetylated α-tubulin used at 1:5000(mouse monoclonal, Sigma T7451, clone 6-11B-1), acetylated H3K27 at 1:1000 (rabbitpolyclonal, Abcam #4729), and MYC (rabbit polyclonal, clone Y69; Abcam #32072) used at 1:3000. K148 Ace-myc (rabbit polyclonal, Sigma, Cat# ABE25) used at 1:2000.

IP: The antibodies used were the same as per WT-Blot but 10X concentrated except HDAC6 which was obtained from Proteintech HDAC6 Cat#12834-1-AP.

Validation

HDAC6 antibody was validated using RNAi. The rest of the antibodies were validated based on the vendors' data.
-Cell signaling #9664s and Abcam#15580 were validated by proper IH staining in proliferating and apoptotic cells as well as propoer molecular size staining in Wt-blot.
- PTMScan Acetyl-Lysine Motif was validated by enrichment of Ac-Lys peptides.
- Cell Signaling #2144; Sigma T7451; Abcam #4729; Abcam #32072 and Sigma, Cat# ABE25 were validated by molecular size staining inWt-blot.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

All cell lines used (HEK-293T; MDA-MB-453; SKBR3; BT-474; ZR-75-1; MDA-MB-361; MDA-MB-231; T-47D; Hs-578T; MCF-7; BT-549; HCC-38; HCC1937; MDA-MB-468 and MDA-MB-436) were obtained from American Type Culture Collection (ATCC).

Authentication

Authentication was performed by ATCC. No additional authentication was performed.

Mycoplasma contamination

All lines tested negative by Mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

N/A

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 eight-week-old NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ female mice were also obtained from Jackson laboratory. Mice were subcutaneously injected under each flank at 3 month old with 10x10^6 of the corresponding cell lines described in the text. The animals were hosted in standard light cycles (12 light/12 dark), temperature (65-75°F)and humidity (40-60%).

Wild animals

This study didn't involve any wild animals.

Reporting on sex

All animals used were females.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All mouse experiments were conducted using protocols approved by the Institutional Animal Care and Use Committee (IUCAC) at the Icahn School of Medicine at Mount Sinai.

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	NCT02632071
Study protocol	full study can be access at https://www.clinicaltrials.gov/ct2/show/NCT02632071?term=ricolinostat&draw=2&rank=5
Data collection	Individual patient data collection was performed at Columbia medical center between March 2016 and February 2018 and compiled and evaluated by Dr. Codruta Chiuzan in the department of biostatistics at Columbia University.
Outcomes	Primary Outcome Measures : Maximum tolerated dose (MTD) of ACY-1215 (Ricolinostat) [Time Frame: 28 days] The maximum tolerated dose (MTD) combination is defined as the dose combination associated with a target probability of dose limiting toxicity (DLT) of 0.25. A dose-limiting toxicity is defined as the MTD with DLTs defined as any grade 3 non-hematologic toxicities despite maximal supportive care or any grade 4 hematologic toxicity. The MTD will be estimated using the time to event continual reassessment method (TITE-CRM). The TITE-CRM will use an empirical dose-toxicity model, with a sample size of 24. The dose-toxicity model is calibrated such that the method will eventually select a dose that yields between 17% and 33% DLT, which will be the recommended phase II dose (RP2D). Secondary Outcome Measures : Number of adverse events related to ACY-1215 (Ricolinostat) [Time Frame: up to 14 days following the last administration of study treatment] All patients will be evaluable for toxicity from the time of their first treatment with the study drug. Toxicities will be graded based upon CTCAE v4.0.2.

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	Standard tripsinization, PBS wash and single cell mash filtration was performed to prepare all tissue culture cells for Flow cytometry analysis. Cells were analyzed for phosphatidylserine exposure by annexin-V FITC / propidium iodide double staining using BD FITC Annexin V Apoptosis Detection Kit (Cat# 556547) according to the manufacturer's instructions.
Instrument	LSRFortessa X-20
Software	BD FACSDiva™ software
Cell population abundance	Flow C. was used to characterized the number of apoptotic and viable cells. Different abundance was observed depending on the cell line and treatment and these are shown in the main figures. Cells were analyzed for phosphatidylserine exposure by annexin-V FITC / propidium iodide double staining using BD FITC Annexin V Apoptosis Detection Kit (Cat# 556547) according to the manufacturer's instructions.
Gating strategy	Standard FSC and SSC gating was used to separate any potential cell debris, singlets and other cell aggregates. After that, the gating strategy shown in figure 1 using phosphatidylserine exposure by annexin-V FITC / propidium iodide double staining was used to identify viable and apoptotic cells.

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