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	All data were collected using commercially available kits or previously published methods. Descriptions of each method and references are provided in the Methods section. Publicly available data were obtained from BEAT-AML: http://www.vizome.org/aml2, accessed September 2023; TCGA 29: https://portal.gdc.cancer.gov/projects/TCGA-LAML, accessed July 2023; STRING: https://string-db.org/, accessed via the stringdb R package (v. 2.4.2)
Data analysis	Computational analysis was performed using standard tools mplemented in Matlab R2021a or R version 4.1.0 with Bioconductor version 3.13. A detailed descrition of the packages that were used is provided in the Methods section.

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 publicly available data used in this study are available from BEAT-AML under http://www.vizome.org/aml2 and https://biodev.github.io/BeatAML2/ 11; TCGA

under https://portal.gdc.cancer.gov/projects/TCGA-LAML 29; and the STRING database under https://string-db.org/ or via the stringdb R package (v. 2.4.2) 95. The raw sequencing data (bulk- and single cell RNA-seq, single-cell DNA-seq) generated in this study have been deposited in the European Genome-Phenome Archive (EGA) under the accession codes EGAD50000000822, EGAD50000000823 and EGAD50000000824. The data is available under restricted access due to patient privacy concerns. Access can be requested by contacting the Tumor Profiler Center (TPC) leadership (https://tumorprofilercenter.ch/contacts). Data access will be granted to registered users listed on the with the TPC within four weeks of receipt of the Data Access Agreement, provided that the applicant provides all necessary ethics committee approval and supporting documents needed to meet the requirements of the agreement. The user institution agrees to destroy or discard the data once it is no longer used for the project, and in cases where data must be archived, it must be deleted within 10 years of the project's completion. If data has not been archived, it must be deleted no later than 2 years following the completion of the project. An extension to this period can be provided upon request to the TPC leadership. The raw proteomics data generated in this study have been deposited in MassIVE under the accession MSV000092970. The processed data for scRNA-seq and CyTOF are available from Zenodo under https://dx.doi.org/10.5281/zenodo.13837019. 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	Recruitment in this cohort was independent of sex, since any patient with relapsed or refractory AML was eligible. The final study population includes 10 female and 11 male patients. Throughout this manuscript, we use the term "sex" to describe biological sex as reported in the medical record of the patient. Due to the limited size of the cohort, we did not perform any analyses stratified by sex.
Reporting on race, ethnicity, or other socially relevant groupings	Information on race, ethnicity or other socio-economical parameters were not collected for this cohort.
Population characteristics	All participants in this study were patients with relapsed or refractory acute myeloid leukemia according to WHO 2016. Patients were eligible for the study if they were 18 years or older and provided written informed consent according to national legal and regulatory requirements prior to any project specific procedures. The detailed characteristics of the study population are provided in Supplementary Data 1.
Recruitment	Any patient matching the inclusion criteria described above was recruited for this study, therefore we do not expect any self-selection or other bias.
Ethics oversight	This study was approved by the ethics committee of the canton Zurich (CEC Zurich, BASEC-Nr: 2019-01326)

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 final cohort included 57 samples form 21 patients. Due to the exploratory nature of this analysis, no sample size calculation was performed for this study.
Data exclusions	Due to limited availability of samples, not all samples were measured by all technologies. Supplementary Data 1 provides detailed information on technology coverage and technology-specific exclusion of samples. In addition, when analyzing molecular correlates of venetoclax response (Figures 2-4), we excluded samples with < 5% blasts (column "Flagged for low blast content" in Supplementary Data 1). For comparisons between innate and treatment related resistance, we further excluded samples from patients who received venetoclax as part of their prior treatment (column "Venetoclax in previous lines" in Supplementary Data 1).
Replication	Key findings in this study were confirmed by exisiting literature and / or reanalysis of previously published work.
Randomization	This is an observational study and focuses on 1) feasibility and 2) exploratory analysis, therefore there was no need for randomization.
Blinding	This is an observational study and focuses on 1) feasibility and 2) exploratory analysis, therefore there was no need for blinding.

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

Antibodies for pharmacoscopy (PCY):
Alexa Fluor® 488 anti-human CD117 (c-kit) [104D2] 100 tests, BioLegend 313234 1/300
PE anti-human CD34 [581] 100 tests, BioLegend 343506 1/300
Alexa Fluor® 647 anti-human CD33 [P67.6] Antibody (100 tests), BioLegend 366626 1/300

Antibodies used for 4i DRP:
Epitope Catalogue # Manufacturer Species Concentration
CD33 (WM53) 303402 Biolegend Mouse 1/800
CD34(581) 343502 Biolegend Mouse 1/800
Anti-phospho-p70 S6 Kinase (Thr389) MABS82 Millipore Mouse 1/800
P-p44/42 MAPK (T202/Y204) 9101S Cell Signalling Rabbit 1/500
c-Kit (CD117) 3308 Cell Signalling Mouse 1/400
P-Akt (473)(D9E) 4060L Cell Signalling Rabbit 1/600
CD45 [2B11] ab187271 Abcam Mouse 1/500
Monoclonal Anti-Splicing Factor SC-35 antibody (SRSF2) S4045 Sigma Aldrich Mouse 1/1200
Anti-Histone H3 (trimethyl K4) antibody ab8580 Abcam Rabbit 1/2000
PCNA (D3H8P) 13110S Cell Signalling Rabbit 1/800
PCNA (polyclonal) ab139696 Abcam Chicken 1/2000
alpha tubulin [DM1A] ab64503 Abcam Mouse 1/4000
Anti-Tubulin (YL1/2) MAB1864 Merk Rat 1/8000
CD45 (polyclonal) PA1-27619 Invitrogen Chicken 1/2000
Cleaved Caspase-3 (D175) (5A1E) 9664S Cell Signalling Rabbit 1/600
Alexa Fluor 488 goat anti-mouse IgG (H+L) A-11029 Thermo Fisher Scientific Goat 1/500
Alexa Fluor 568 goat anti-rabbit A-11036 Thermo Fisher Scientific goat 1/500
Alexa Fluor 555 Rat IgG (H+L) Cross-Adsorbed A-21434 Thermo Fisher Scientific Rat 1/400
Alexa Fluor 555 anti chicken IgY (H+L) A-32932 Thermo Fisher Scientific Chicken 1/400
Alexa Fluor® 647 Goat Anti-Rabbit IgG (H+L) A-21245 Invitrogen Goat 1/400
c-Myc AB3252 Millipore Chicken 1/400
phospho-Stat3 (Tyr705) (D3A7) 9145S Cell Signalling Rabbit 1/300

Antibodies used for CyTOF are provided in Supplementary Data 14

Validation

All used antibodies are quality control tested by manufacturer using immunofluorescent staining with flow cytometric analysis.

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

BASEC-Nr: 2019-01326

Study protocol

The study potocol is not publicly available, but was made available to all patients prior to study enrollment.

Data collection

All samples and clinical data were collected at the University Hospital Zurich between September 2019 and March 2021.

Outcomes

The TuPro is a prospective observational clinical study. The primary outcome of this study was feasibility of single-cell multi-omics data generation within a clinically meaningful time frame of 4 weeks from sample to clinical report.