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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

No statistical methods were used to predetermine sample size. Sample size was based on experimental feasibilty, sample availability, and N necessary to obtain definitive results.

2. Data exclusions

Describe any data exclusions.

None.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All experimental findings were reproduced at least twice, with the exception of the in vivo data in Fig. 3i, j.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Randomization was not part of the design for the phase I/II trials NCT01915498 and NCT02074839. Randomization is not relevant to the current study as the object was to decipher the mechanisms of acquired clinical resistance to targeted therapies within individual patients.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not part of the design for the phase I/II trials NCT01915498 and NCT02074839. Blinding is not relevant to the current study as the object was to decipher the mechanisms of acquired clinical resistance to targeted therapies within individual patients.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	GraphPad Prism v7 was used for general data analyses and statistical tests. Quantasoft v1.7 was used to analyze the droplet digital PCR data. MassHunter vB.08.00 was used to analyze mass spectrometry data. Maestro v11.2, Prime v4.8, and PyMOL v1.8.2.0 were used for structural modeling.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No restrictions.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	The antibodies described in the Methods section are widely used, commercially available antibodies validated by the companies and publications cited on the company websites. Primary antibodies used included: anti-FLAG (Sigma, F1804; clone M2; mouse; 1:1000); anti-GAPDH (Cell Signaling Technology, 5174; clone D16H11; rabbit; 1:1000); anti-HA (Cell Signaling Technology, 2367S; clone 6E2; mouse; 1:1000); anti-IDH2 (Abcam, ab55271; no clone name; mouse; 1:1000); anti-vinculin (Cell Signaling Technology, 4650; no clone name; rabbit; 1:1000).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	293T cells were purchased from ATCC. Ba/F3 cells were purchased from DSMZ.
b. Describe the method of cell line authentication used.	Short tandem repeat (STR) profiling.
c. Report whether the cell lines were tested for mycoplasma contamination.	Cells were routinely tested for mycoplasma.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used in the study.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Mus musculus C57Bl/6 Mx-Cre(+) Idh2 (R140Q) and Mx-Cre(+) Idh2 (R140Q) Flt3 (ITD) mice were previously described (Shih et al, Cancer Discovery 2017). Hematopoietic stem/progenitor cells were harvested from female and male mice at age 8-10 months as previously described (Shih et al, Cancer Discovery 2017).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The patients described were enrolled on the phase I/II studies NCT02074839 or NCT01915498 (Stein el al, Blood 2017). Enrollment was open to all patients with relapsed or refractory acute myeloid leukemia with a mutation in IDH1 (NCT02074839) or IDH2 (NCT01915498) identified locally and confirmed centrally. Patients were required to be 18 years or older at the time of study entry. Both men and women were enrolled on the studies. Patients were required to have a performance status of 2 or better, adequate organ function as defined in the study protocols. Clinical data, blood, and bone marrow samples from patients with acute myeloid leukemia were obtained after receiving written informed consent from patients. Approval was obtained from the Institutional Review Board at each institution participating in these clinical trials. Additional consent was obtained from participants at Memorial Sloan Kettering Cancer Center with analyses performed on the institutional biobanking protocol approved by the Institutional Review Board. Patient biospecimens were anonymized by creating unique identifiers with no associated PHI and keeping the key on a password-protected server. Data collection and research was performed in compliance with all relevant ethical regulations for human research participants.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Blood was collected from mice and red blood cells were lysed. The remaining cells were pelleted and then analyzed for mCherry positivity
6. Identify the instrument used for data collection.	BD LSRFortessa
7. Describe the software used to collect and analyze the flow cytometry data.	Data was collected by BDFACS Diva software and analyzed using FCS Express 6 Flow.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	No sorting was performed.
9. Describe the gating strategy used.	Gating was done on the DAPI– mCherry+ population

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