nature research

Corresponding author(s): Terry J. Fry, M.D.

☐ 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.

As stated in the methods, all sequentially enrolled patients with ALL were included in the analysis through a specific cutoff date to ensure adequate follow up

2. Data exclusions

Describe any data exclusions.

No data was excluded from the analysis

3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts at replication of the pre-clinical experiments were successful

4. Randomization

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

In the murine experiments, mice were injected with leukemia and randomly distributed (without bias from the luciferase imaging of leukemia burden) to treatment groups

5. Blinding

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

Mouse imaging was performed by an operator who was blinded to treatment group.

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.	Statistics were performed using GraphPad Prism. Details of the sequencing analysis are included in the Methods.

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.	There are no restrictions on availability of material described.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Antibodies used for flow cytometry are listed in Methods. Clinical samples were analyzed in CLIA laboratories at the NIH and were validated in this laboratory. Details on the site density measurement assay is included in Methods.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	The parental Nalm6 cell line was provided by ATCC
b. Describe the method of cell line authentication used.	The Nalm6 cell line formally authenticated by HLA typing and verified to express the relevant antigens (CD19 and CD22) prior to each experiment.
c. Report whether the cell lines were tested for mycoplasma contamination.	All cell lines were tested for mycoplasma contamination and confirmed to be negative
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 mis-identified cell lines were used

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.	Male and female Non-Obese Diabetic, SCID, gamma KO (NSG) mice were use for xenograft studies.

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.	Required information is included in supplemental table.

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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.	Murine bone marrow (see Methods); human blood, bone marrow, cerebrospinal fluid. Processing of human samples per CLIA certified lab.
6. Identify the instrument used for data collection.	Becton Dickinson (BD) Fortessa for preclinical samples, BD Canto for Clinical samples.
7. Describe the software used to collect and analyze the flow cytometry data.	FlowJo used for analysis of preclinical samples, analysis of human samples per CLIA certified lab.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	N/A
9. Describe the gating strategy used.	See supplemental data

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

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