A transgenic mouse model of Down syndrome acute lymphoblastic leukemia identifies targetable vulnerabilities

Authors

Jacob J. Junco,[1] Max Rochette,[1] Michelle Alozie,[1] Raushan Rashid,[1] Maci Terrell,[1] Barry Zorman,[1] Pavel Sumazin,[1] Lauren Rowland,[2] Gino Dettorre,[2] Reid T. Powell,[3,4] Clifford C. Stephan,[3,4] Peter J. Davies,[3,4] Margarita Martinez-Moczygemba,[3,4] Jun J. Yang[2] and Karen R. Rabin[1]

[1]Texas Children's Cancer Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX; [2]Department of Pharmacy and Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN; [3]Institute of Biosciences and Technology, Texas A&M University, Houston, TX and [4]Department of Translational Medical Sciences, School of Medicine, Texas A&M University, Houston, TX, USA

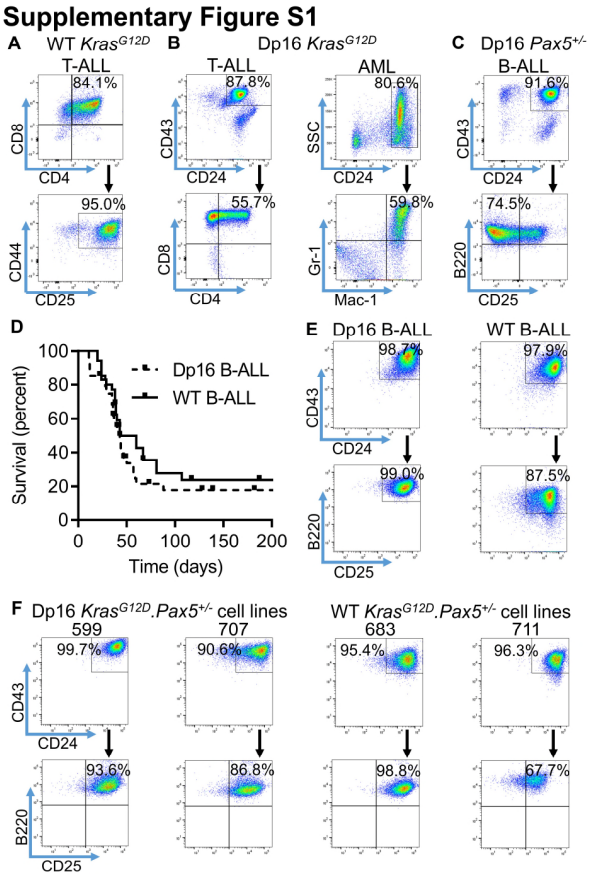
Correspondence:
K.R. RABIN - krrabin@texaschildrens.org
J.J. JUNCO - junco@bcm.edu

https://doi.org/10.3324/haematol.2023.284761

Received: November 27, 2023.
Accepted: July 16, 2024.
Early view: July 25, 2024.

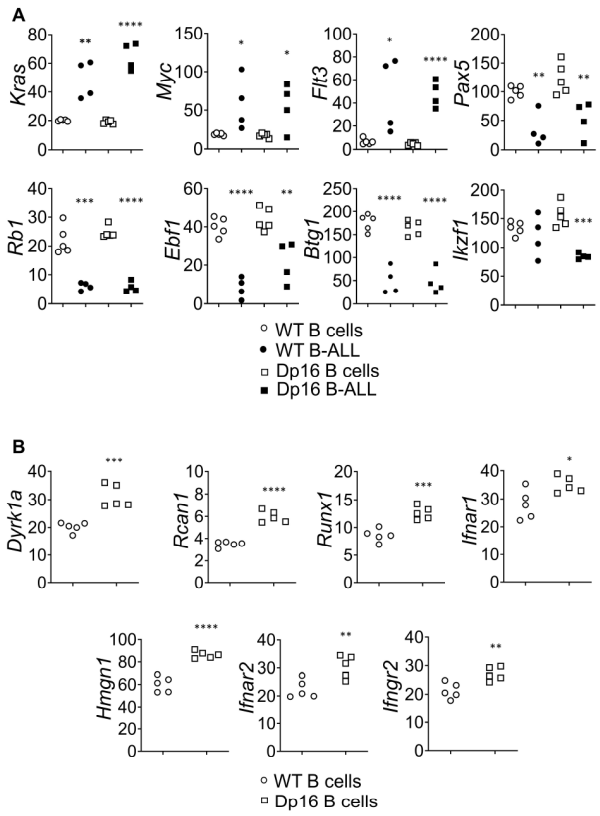
A transgenic mouse model of Down syndrome acute lymphoblastic leukemia identifies targetable vulnerabilities

Supplementary Figures



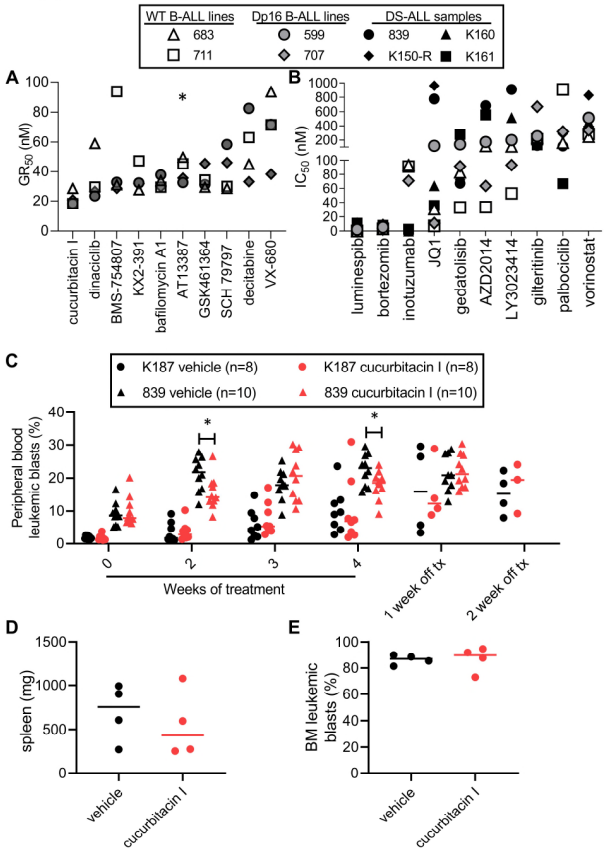
Supplementary Figure S1. Dp16 and WT *Kras^{G12D}* mice develop leukemias of different lineages, and NSG recipients of Dp16 and WT *Kras^{G12D}.Pax5$^{+/-}$* B-ALL cells develop B-ALL with similar latency and the same immunophenotype as the primary sample and generate novel B-ALL cell lines. (A) WT *Kras^{G12D}* mice (n=2) developed disease with an immunophenotype consistent with T-ALL. **(B)** Dp16 *Kras^{G12D}* mice developed disease with an immunophenotype consistent with T-ALL (n=2) or acute myeloid leukemia (n=4). **(C)** Dp16 *Pax5$^{+/-}$* mice (n=4) developed disease with an immunophenotype consistent with B-ALL. For **(A-C)**, representative dot plots from spleen cells are shown. **(D)** NSG recipients of Dp16 or WT B-ALL primary samples both had a median latency to disease of 43 days (p=0.25). **(E)** Spleen cells from leukemic NSG recipients demonstrated a high burden of disease with the same B-ALL immunophenotype (typically B220$^+$CD43$^+$CD24$^+$CD25$^+$) as the primary mouse sample (representative Dp16 and WT samples shown). **(F)** The two Dp16 (599 and 707) and two WT (683 and 711) *Kras^{G12D}.Pax5$^{+/-}$* B-ALL lines used in drug screening experiments displayed a B220$^+$CD43$^+$CD24$^+$CD25$^+$ immunophenotype, similar to the primary mouse leukemia samples. Three additional Dp16 *Kras^{G12D}.Pax5$^{+/-}$* B-ALL lines displayed a similar immunophenotype (data not shown).

Supplementary Figure S2



Supplementary Figure S2. Dp16 and WT *Kras^{G12D}.Pax5$^{+/-}$* B-ALL, and Dp16 and WT non-leukemic bone marrow B cells, demonstrate gene expression changes similar to human cells. (A) Expression of genes in signaling pathways frequently activated in B-ALL, including *Kras*, *Myc,* and *Flt3,* were significantly upregulated in both the Dp16 and WT *Kras^{G12D}.Pax5$^{+/-}$* B-ALL models compared to control B cells. The expression of tumor suppressor genes frequently mutated in DS-ALL and non-DS ALL, including *Pax5*, *Ikzf1*, *Rb1*, *Ebf1*, and *Btg1*, were significantly downregulated in the Dp16 *Kras^{G12D}.Pax5$^{+/-}$* B-ALL model compared to control Dp16 B cells. *Pax5*, *Rb1*, *Ebf1*, and *Btg1* were also significantly downregulated in WT *Kras^{G12D}.Pax5$^{+/-}$* B-ALL compared to control WT B cells (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 for significance relative to B cell control for each genotype, Student's t-test). **(B)** A number of genes shown to be upregulated in DS lymphoblastoid cells were significantly upregulated in B220+ BM B cells from Dp16 compared to WT healthy non-leukemic mice (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 for significance relative to WT BM B cells, Student's t-test). Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values are displayed.

Supplementary Figure S3



Supplementary Figure S3. Dp16 and WT B-ALL lines and DS-ALL patient samples are sensitive to novel targeted agents, and cucurbitacin I displays limited efficacy *in vivo*. (A) Two Dp16 and two WT *Kras*^{G12D}*.Pax5*$^{+/-}$ B-ALL cell lines were tested in a high-throughput screen using the Broad Institute Informer Set. Cells were incubated with 10 nM, 100 nM, or 1 µM of drug for 72 hours before viable cells were quantified using a flow cytometry-based assay. Of 365 unique small molecules tested, 44 reduced the growth of all four tested B-ALL cell lines. For clarity, the individual GR_{50} values (in nM) for the 10 most effective agents inhibiting the growth of all four cell lines are shown (*$p<0.05$ for the average GR_{50} in Dp16 vs WT). **(B)** Cell lines were incubated with a targeted panel of 35 drugs for 72 hours prior to CellTiter-Glo viability assay. DS-ALL patient samples were incubated in the same panel for 96 hours prior to imaging assays. The 10 compounds displayed were effective at nanomolar-range concentrations in most tested samples. Samples which were insensitive to any particular drug are not displayed. IC_{50} values in nM are indicated. The legend for both **(A)** and **(B)** is indicated above. **(C)** NSG mice xenografted with DS-ALL patient samples K187 (circles) or 839 (triangles) were treated with vehicle (black) or 1 mg/kg cucurbitacin I (red) via i.p. injection 5 days per week for 4 weeks. Cucurbitacin I significantly reduced PB disease burden at weeks 2 and 4 of the treatment phase in mice xenografted with DS-ALL 839, but had no effect in mice with DS-ALL K187 (*$p<0.05$, Student's t-test, investigators were blinded to PB sample identity). Cucurbitacin I had no effect on **(D)** spleen weight or **(E)** BM leukemic disease burden after 4 weeks of treatment in K187 mice (n=4).