Leukemic cell xenograft in zebrafish embryo for investigating drug efficacy

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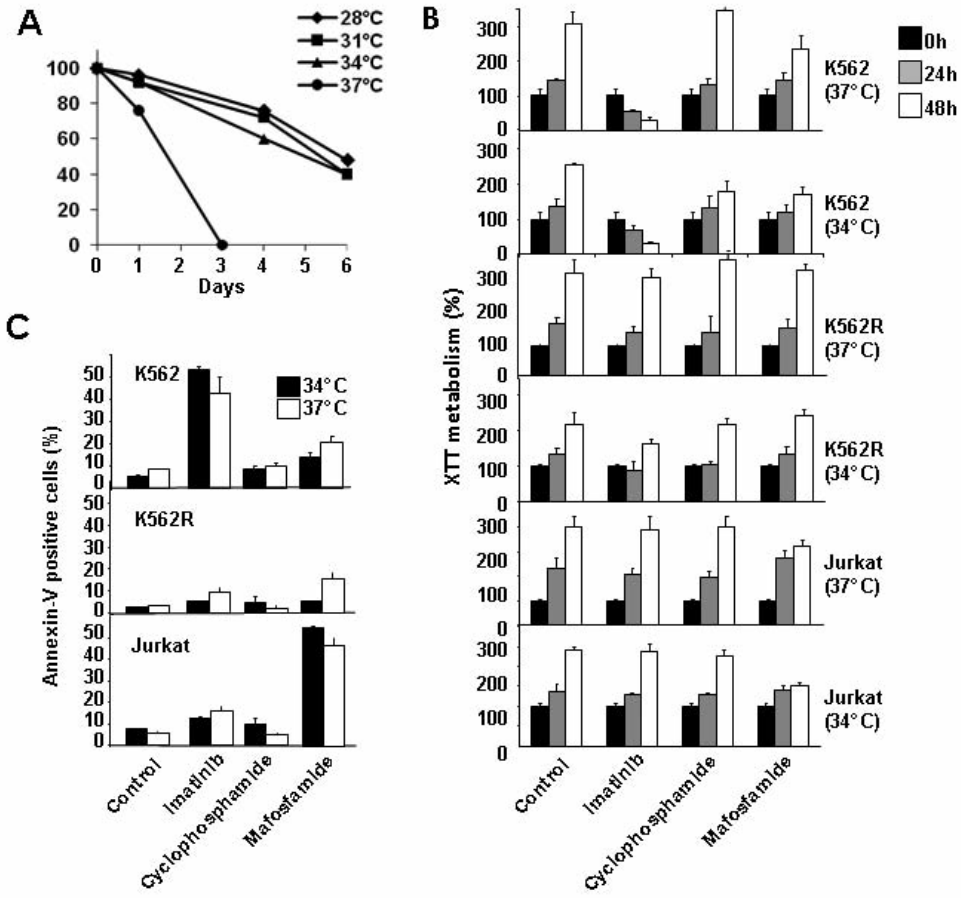
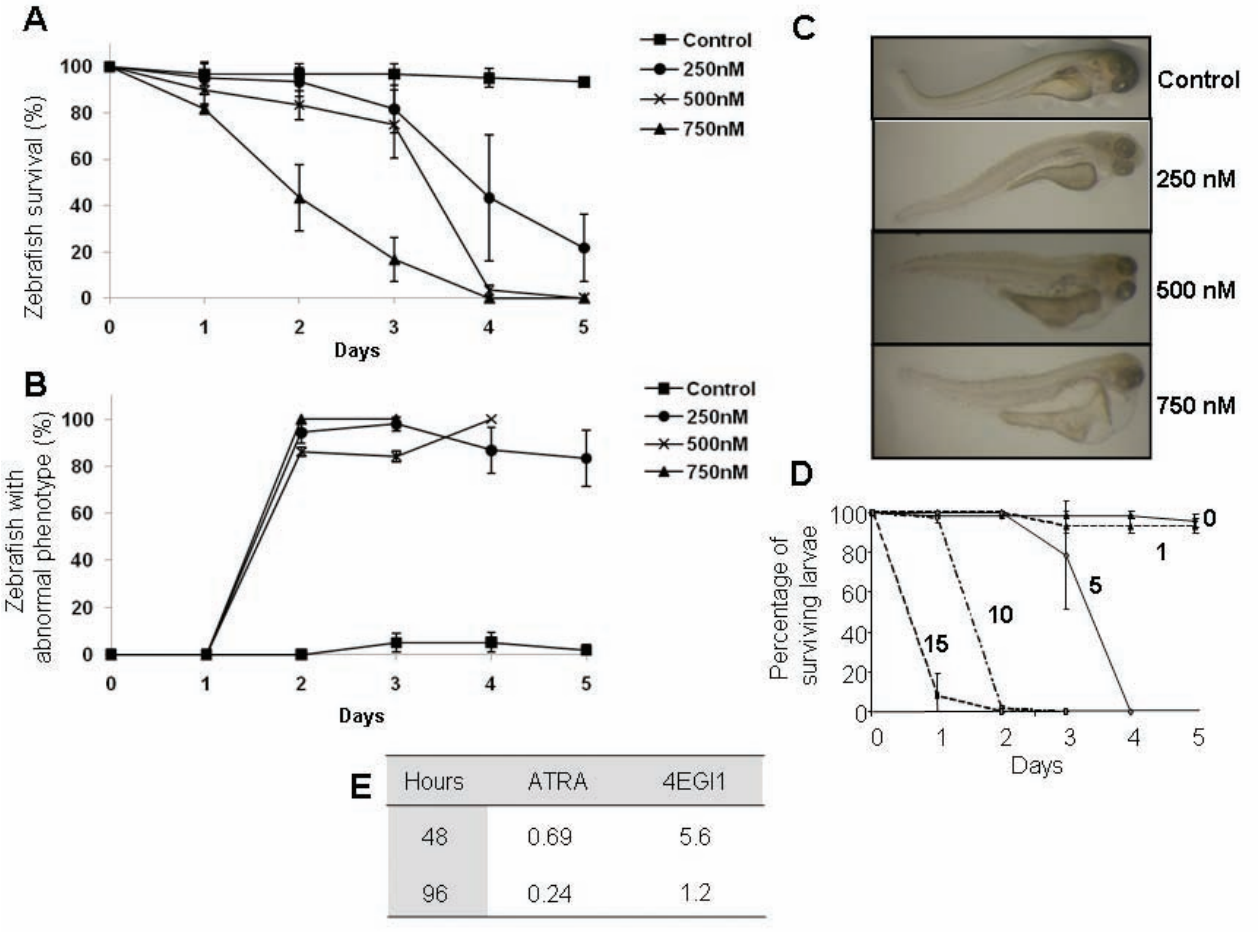


Online Supplementary Figure S1. Definition of experimental conditions. (A) Water temperature and fish survival. Zebrafish embryos are usually maintained at 28°C. Their development was followed for six days at four different temperatures. The embryos developed normally from fertilization through 6 dpf at temperatures up to 34°C. All the embryos died before 4 dpf at 37°C. (B) Temperature and cell proliferation. K562, K562R and Jurkat human leukemic cell lines are usually grown at 37°C. Their proliferation was explored using an XTT assay. 15×10^3 cells/100 mL in a 96-well plate were incubated with the indicated drug (3 μM imatinib; 10 μg/mL cyclophosphamide, or 10 μg/mL mafosfamide) for 24 (gray) or 48 (white) h before adding 50 μL of XTT reagent (sodium 30-[1- (phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate) and measuring the absorbance at 490 nm of the formazan dye produced by cells that actively metabolize the tetrazolium salt Mean +/- SD of quadruplicates in one representative of 3 independent experiments. (C) Temperature and cell death by apoptosis. K562, K562R and Jurkat cells were treated with the indicated drug (3 μM imatinib; 10 μg/mL cyclophosphamide, or 10 μg/mL mafosfamide) for 48 h at either 37°C (black) or 34°C (white) before assessing apoptosis by flow cytometry using a combination of Annexin V and propidium iodide (Results expressed as in B).

Online Supplementary Figure S2. K562 cells were labeled with CM-Dil and injected into 2-days old zebrafish larvae that were incubated for 1 h at 28°C, then at 34°C and examined at 1 day post-injection by video-microscopy. Leukemic cells can be easily observed in the circulation and part of them are fixed into tissues. SEE MOVIE AND LISTEN MP4.

Online Supplementary Figure S3. Jurkat cells were labeled with CM-Dil and injected into 2-days old zebrafish larvae that were incubated for 1 h at 28°C, then at 34°C and examined at 1 day post-injection by video-microscopy. Leukemic cells can be easily observed in the circulation whereas part of these cells are fixed into tissues. SEE MOVIE AND LISTEN MP4.

Online Supplementary Figure S4. All-*trans* retinoic acid (ATRA) and 4EGI-1 teratogenic effects. Zebrafish embryos were treated with indicated doses of ATRA added every day or 4EGI-1 added at day 0 to the aquarium water at 34°C. (A) Survival of zebrafish embryos after ATRA repeated treatment (Mean ± SEM of at least 25 larvae per point). (B) Percentage of fish with an aberrant phenotype under exposure to various concentrations of ATRA as in A. (Mean ± SEM of at least 25 injected larvae per point). (C) Contrast microscopy examination of embryos at day 4 of treatment with indicated doses of ATRA. (D) Survival of zebrafish embryos after addition of 4EGI1 at indicated concentration (μM) at day 0 in the fish water (Mean ± SEM of at least 25 larvae per point). (E) LD50 values (μM) measured at indicated times (hours) after daily addition of ATRA or a unique addition of 4EGI1 to fish water (25 larvae per dose).



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Hours	ATRA	4EGI1
48	0.69	5.6
96	0.24	1.2