To determine the optimal route and schedule for R788 administration, we investigated R406 activity in mouse plasma after administration of 40 mg/kg R788 by intraperitoneal injection (I.P.) or oral gavage. Samples were drawn before, at 30/45 and 180 minutes after R788 administration. R406 activity in the plasma samples was evaluated using BAF3 B-cells that express a constitutively active TEL-Syk protein. Reduction of PLCγ2, AKT and ERK phosphorylation was used as a readout for inhibition of Syk activity. Mouse plasma samples were diluted 3 times in complete RPMI. Serial dilutions of R406 in the same medium were used as a positive control. Both routes of R788 administration resulted in efficient but short-lived Syk inhibition. Syk was completely inhibited at 30 and 45 min after R788 administration, but its activity returned to basal levels within 3 hours (left panel). The sample collected 45 min after I.P. administration inhibited Syk-induced PLCγ2 and ERK phosphorylation to approximately the same extent as 2.5 µM R406 (right panel). Considering that the plasma samples were diluted 3 times, this experiment suggested that the plasma concentration of R406 exceeded 7.5 µM after administration of 40 mg/kg R788. This concentration is higher than the maximum plasma concentration (3.0–3.5 µM) achieved in the clinical trials of rheumatoid arthritis, ITP and NHL. However, the plasma half-life of R406 in humans is considerably longer and reaches approximately 15 hours.

REFERENCES

Figure S2. Representative examples of flow-cytometry analysis of mice with adoptively transferred TCL1-002, TCL1-551, and TCL1-870 leukemia treated with R788 or vehicle control
Peripheral blood samples from animals with adoptively-transferred TCL1-002 and TCL1-551 leukemia were collected on the last day of treatment (day 21 and day 24, respectively). Samples from animals with TCL1-870 leukemia were collected on day 74. The percentage of malignant (CD5⁺/B220⁺) and normal (CD5⁻/B220⁺) B cells is indicated.
Figure S3. Immunophenotype of TCL1 leukemias treated with R788 in vivo