In vitro validation of γ-secretase inhibitors alone or in combination with other anti-cancer drugs for the treatment of T-cell acute lymphoblastic leukemia

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Supplementary appendix

Telomerase assays

Telomerase activity in cells treated for 2, 6 or 12 days with 1 μM Compound E was compared to that in DMSO-treated cells with the polymerse chain reaction (PCR)-based telomeric repeat amplification protocol (TRAP) from the TeloTAGGG Telomerase PCR ELISA PLUS kit (Roche). TRAP products were quantified by ELISA and 20 μl of the product were separated on a 12% non-denaturing polyacrylamide gel and visualized with SYBR safe (Invitrogen).

Real-time quantitative PCR

Trizol Reagent (Invitrogen, Carlsbad, CA, USA) was used for total RNA extraction from cells treated for 12 days with 1 μM Compound E or with DMSO. Extracted RNA was treated with DNase and used for cDNA synthesis with SuperScript RTII reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Real-time PCR was performed on an ABI Prism 7000 Sequence detection system (Applied Biosystems, Foster City, CA) using SYBR Green for detection and TERTF (5'-GCAGAGCTCCTCCCTGAATG) and TERTR (5'-GCGTGGTGGCACAGAAG) primers for TERT and ActinF (5'-CACCCTGAAGTACCCCA TCG) and actinR (5'-TGCCAGATTTTCTCCATGTCG) for beta-actin transcript amplification. Relative expression levels were calculated with the delta-delta Ct method, beta-actin was used as the reference gene for normalization.

Telomere length analysis

DNA was isolated from cells treated for 14 days with 1 μM Compound E or DMSO using the Wizard genomic DNA purification kit (Promega). DNA was digested with RsaI and *HinfI*, separated by field inversion gel electrophoresis, blotted onto nylon membranes and hybridized to a P^{32} radiolabeled 5- mer synthetic oligonucleotide telomeric probe $(CCCTAA)_5$. Telomere restriction fragment length (TRF) was determined as described previously.[41]

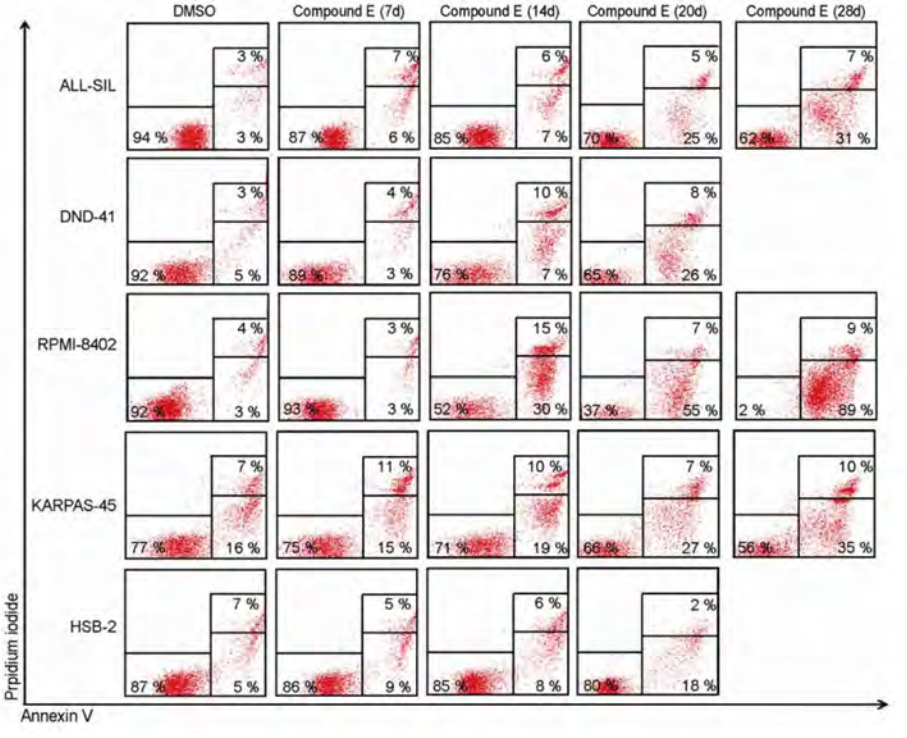

Figure S1. Long-term gamma-secretase inhibition treatment is required for induction of apoptosis. FACS analysis of annexin V/propidium iodide stained cells that were treated for 7, 14, 20 or 28 days with DMSO or with 1 µM Compound E.

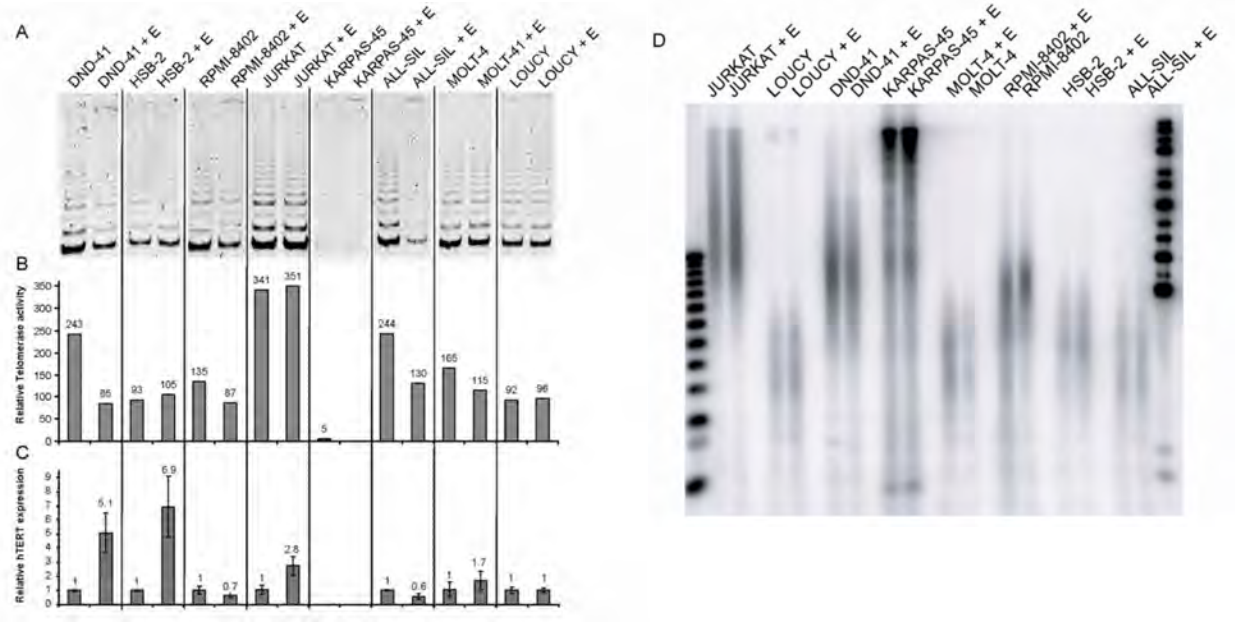

Figure S2. Gamma-secretase inhibition treatment can affect telomerase activity (A, B) Telomerase activity in cells treated for 12 days with 1 µM Compound E or DMSO was compared in a TRAP assay. TRAP products were analyzed by gel electrophoresis (A) and quantified by ELISA (B). (C) Real-time PCR comparison of TERT expression levels in cells treated for 12 days with 1 µM Compound E or DMSO. KARPAS-45 data were unreliable due to low TERT expression levels and have been left out. (D) Comparison of telomere length between cells treated for 14 days with 1 µM Compound E or DMSO.

Table S1. *NOTCH1* heterodimerization domain (HD) and PEST domain mutation detection in T-ALL cell lines and samples from patients.

	NOTCH1 PEST domain mutations	*NOTCH1 HD mutations*	*CDKN2A*	*Proliferation/ survival defects*	*Transcription factor defects*	*Other*
ALL-SIL	7372 C>G => L2458V 7420-7421 ins (GG) => 2475 AHP*STOP	4781 T>C => L1594P	deleted[a]	NUP214-ABL1	TLX1 MYB	
DND-41	7330 del G ins (TGT) => 2444 CCSHWAPAAWRCTIFCPRRAP PCPRRCHPRWSHP*STOP	4781 T>C => L1594P 4829 A>T => D1610V	wt[a]		TLX3	
HSB-2	-	-	NA	LCK overexpression		
JURKAT	-	-	deleted[a]		TAL1 overexpression	PTEN null
KARPAS-45	-	5129 C>T => L1710P	wt[a]		MLL-AFX and AFXMLL fusion	
MOLT-4	7544 del (CT) => 2515 RVP*STOP	4802 T>C => L1601P	deleted[a]	RAS mutation	MYB	PTEN null
RPMI-8402	-	4754 ins (CCGTGGAGCTGATG CCGCCGGAGC) => 1584 ins PVELMPPE	deleted[a]		SIL-TAL1 LMO1 MYB	
LOUCY	-	-	deleted[a]			
Patient 1	-	4736 T>G =>V1579G	+/+[b]	NUP214-ABL1	TLX1	
Patient 2	-	4781 T>C =>L1594P	-/-[b]	NUP214-ABL1	TLX1	
Patient 3	-	4735 del (GTG) => 1579 del V	-/+[b]	NUP214-ABL1	TLX1	
Patient 4	7563 G>A => W2521STOP	-	-/+[b]	NUP214-ABL1	TLX3	
Patient 5	-	5027 ins (CCGCTTTCC) => I1676T 1676-1677 ins (AFL)	+/+[b]	NUP214-ABL1	NA	
Patient 6	7478 C>A => S2493STOP	-	-/+[b]	EML1-ABL1	TLX1	

Table legend: [a]determined by array comparative genomic hybridization ; [b]determined by fluorescence in situ hybridization ; NA: not analyzed
Note: The HD-domain mutation we find in ALL-SIL is the same a that described by Weng et al., although Weng et al. reported a different position for this mutation. We found a HD mutation in our KARPAS-45 cell line while Weng et al. described a PEST mutation in their KARPAS-45 cell line.