Supporting Information

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SI Materials and Methods

Shotgun Cloning. Genomic DNA was digested with MseI or NlaIII, followed by ligation of linkers. MseI+ 5′-GTAATACGACTCACTA TAGGGCTCCGCTTAAGGGAC-3′ and MseI– 5′-Phos-TAGTC CCTTAAGCGGAG-C3spacer-3′. NlaIII+ 5′- GTAATACGACT CACTATAGGGCTCCGCTTAAGGGACCATG-3′ and NlaIII– 5′-Phos-GTCCCTTAAGCGGAG-C3spacer-3′. Fragments were digested with EcoRV to remove distal linker. MOL4070LTR junction fragments were amplified with primers to the LTR and linker using 5′-GCTAGCTTGCCAAACCTACAGGTGG-3′ and 5′-GTAATACGACTCACTATAGGGCTCCG-3′. Fragments were further amplified using nested primers 5′-CCAAACCTA CAGGTGGGGTCTTTC-3′ and 5′-AGGGCTCCGCTTAAGG GAC-3′. The PCR products were cloned into Zero Blunt Cloning Kit (Invitrogen), and 96 clones were sequenced per enzyme (192 total per T-ALL). Sequencing was performed by Functional Biosciences.

Retroviral Transduction and Adoptive Transfer. Bone marrow from 6-week-old *Mx1-Cre, Kras*^{G12D} and wild-type littermate mice were retrovirally transduced as described previously (1) with Murine Stem Cell Virus (MSCV) biscitronic retroviral vector expressing Ik6 (*IKZF1* lacking coding exons 3–6) cloned from a patient with BCR-ABL1 acute lymphoblastic leukemia, and GFP graciously provided by Charles Mullighan. Recipient mice were irradiated with 950 cGy and retroorbitally injected with transduced bone marrow cells and 5×10^5 Sca-1 depleted normal helper bone marrow cells. Three weeks after transplantation, recipient mice were injected with poly-inosinic-polycytidilic acid to induce expression of *Kras*^{G12D}.

Western Blot Analysis. Cells were lysed in 1% Nonidet P-40 buffer containing 30 mM NaF, 30 mM β-glycerophosphate, 20 mM Na_4P2O_7, 1 mM Na_3VO_4, and Complete (Roche). Antibodies included anti-Pan-Ras (Ab-3) (Calbiochem), anti-phospho-ERK1/2 (Thr202/Tyr204), anti-β-actin, anti-PTEN, anti-cleaved Notch1 (Val1744), anti-phospho-s6 (Ser2352/Ser236) (Cell Signaling Technology), anti-pAkt (Ser473) (Invitrogen), and anti-Ikaros (H-100) (Santa Cruz Biotechnology). Nuclear extracts were isolated using an NE-PER kit (Pierce), and the Ras-GTP pulldown assay with GST-Raf1 RBD beads (Upstate) was performed according to the manufacturer's instructions.

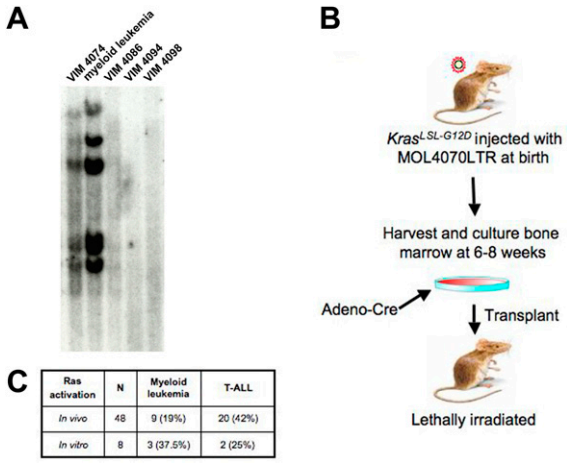
Notch1 Mutation Analysis. Genomic DNA was amplified using 5′-ATAGCATGATGGGGCCACTA-3′ and 5′-GCCTCTGGAA TGTGGGTGAT-3′. Taqman assays were designed to preferentially amplify each of the mutations. Three primers were used for each mutation: (*i*) wild-type specific primer, (*ii*) mutant-specific primer, and (*iii*) primer that amplifies both wild-type and mutant samples (available upon request). Reactions were performed using 1× Taqman Universal PCR Master Mix (ABI), 250 nM Notch PEST domain probe, 900 nM of each primer, and 100 ng of DNA in a final volume of 20 μL. Reactions were run in triplicates on an ABI 7900HT under the following conditions: 90 °C for 10 min, then 40 cycles of 90 °C for 15 s and 60 °C for 1 min.

Quantitative RT-PCR. Taqman assays were used to quantify the transcriptional levels of Notch1 target genes. Reactions were performed with 1× SYBR Green (Applied Biosystems), 500 nM of each primer, and varying concentrations of cDNA. Reactions were run in triplicates on an ABI 7900HT under the following conditions: 95 °C for 10 min and then 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

The following primers were used:

GapDH forward: TGTGTCCGTCGTGGATCTGA
GapDH reverse: CCTGCTTCACCACCTTCTTGA
Hes1 forward: AAAGTCATCAAAGCCTATCATGGAG
Hes1 reverse: GCCGGGAGCTATCTTTCTTAAG
Deltex1 forward: ATCAGTTCCGGCAAGACACAG
Deltex1 reverse: CGATGAGAGGTCGAGCCAC

1. Schubbert S, et al. (2005) Functional analysis of leukemia-associated PTPN11 mutations in primary hematopoietic cells. *Blood* 106:311–317.



C

Ras activation	N	Myeloid leukemia	T-ALL
In vivo	48	9 (19%)	20 (42%)
In vitro	8	3 (37.5%)	2 (25%)

Fig. S1. MOL4070-induced myeloid leukemia. (*A*) In contrast to T lineage acute lymphoblastic leukemia (T-ALL), Southern blot analysis with a probe to the MOL4070 LTR indicates a clonal insertion pattern in both the primary virus-injected mouse (VIM) (lane 1) and in a recipient of this bone marrow that developed myeloid leukemia (lane 2). Southern blot analysis of myeloproliferative disease bone marrows that did not give rise to leukemia did not reveal a dominant clone (lanes 3–5). (*B* and *C*) The frequency of myeloid leukemia vs. T-ALL in $Kras^{G12D}$ mice is increased by delaying $Kras^{G12D}$ expression. (*B*) Experimental protocol for delayed $Kras^{G12D}$ activation ex vivo by infecting bone marrow from MOL4070-injected mice with an *Adenol-Cre* vector followed by adoptive transfer into lethally irradiated mice. (*C*) Incidence of transplantable myeloid malignancies and T-ALL induced by MOL4070 in vivo in *Mx1-Cre,* $Kras^{G12D}$ mice and after ex vivo transduction of $Kras^{G12D}$ bone marrow with *Adeno-Cre*.

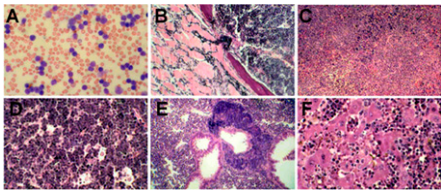


Fig. S2. Pathologic features of T lineage acute lymphoblastic leukemia. (*A*) Cardiac blood smear stained with Wright-Giemsa. (*B–F*) Paraffin-embedded sections stained with hematoxylin and eosin: (*B*) sternum, (*C*) spleen, (*D*) thymus, (*E*) lung, and (*F*) liver.

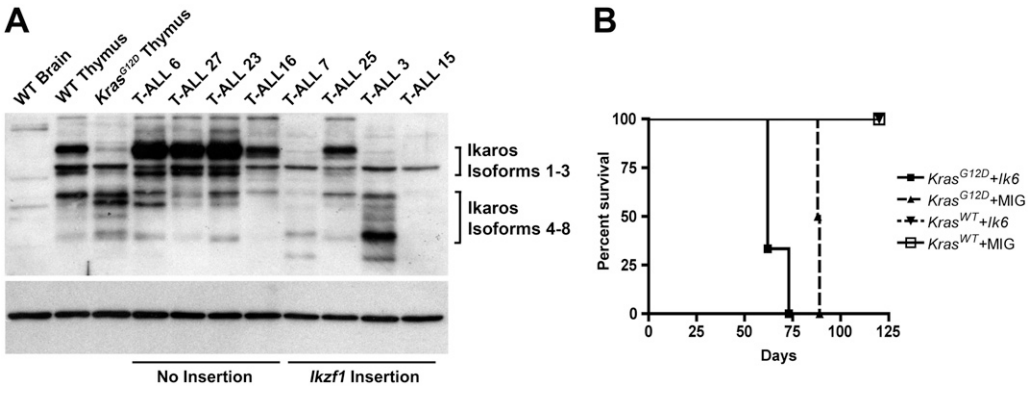
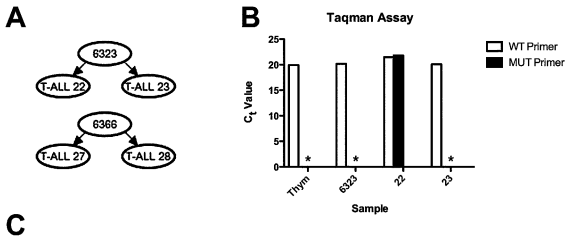


Fig. S3. Ikaros isoform expression is deregulated and *Ik6* cooperates with oncogenic Kras. (*A*) Western blot of Ikaros in nuclear extracts of primary T lineage acute lymphoblastic leukemia (T-ALL) cells with and without *Ikzf1* integrations. (*B*) Survival analysis of mice transplanted with $Kras^{G12D}$ or $Kras^{WT}$ bone marrow retrovirally transduced with either dominant negative Ik6 or vector (MIG). $Kras^{G12D}$+*Ik6* (n = 3) develop T-ALL, whereas $Kras^{G12D}$+MIG (n = 2) develop both myeloid leukemia and T-ALL. $Kras^{WT}$+*Ik6* (n = 3) and $Kras^{WT}$+MIG (n = 3) transplant recipients did not develop leukemia by 120 days.



C

Notch1 Mutation	VIM 6323	T-ALL 22	T-ALL 23
c.7273delGinsCCC	not detected	0.8	not detected
c.7161delGinsCC	not detected	not detected	0.8

Notch1 Mutation	VIM 6366	T-ALL 27	T-ALL 28
c.7408_7409delGC	not detected	0.4	not detected
c.7161delGinsCC	not detected	not detected	0.5

Fig. S4. Molecular analysis infers that *Notch1* mutations are a late event in *Kras*^{G12D} T lineage acute lymphoblastic leukemia (T-ALL). (*A*) Overview of virus-injected mouse (VIM) and T-ALL samples used for quantitative PCR analysis of *Notch1* mutations. (*B*) Representative data to assess *Notch1* mutation copy number in T-ALLs 22 and 23, which were both initiated from VIM #6323 but showed distinct retroviral integrations and different *Notch1* mutations. Asterisks (*) denote a C_t value of >40 (no product detected after 40 rounds of amplification). (*C*) Summary of quantitative PCR to assess for specific insertion/deletion mutations (rows) in distinct samples (columns) reveals that unique mutations are present in individual T-ALL samples derived from the same VIM and are not detected in the parental marrow. Values represent the ratio of the mutant allele relative to the WT *Notch1* allele in each sample.

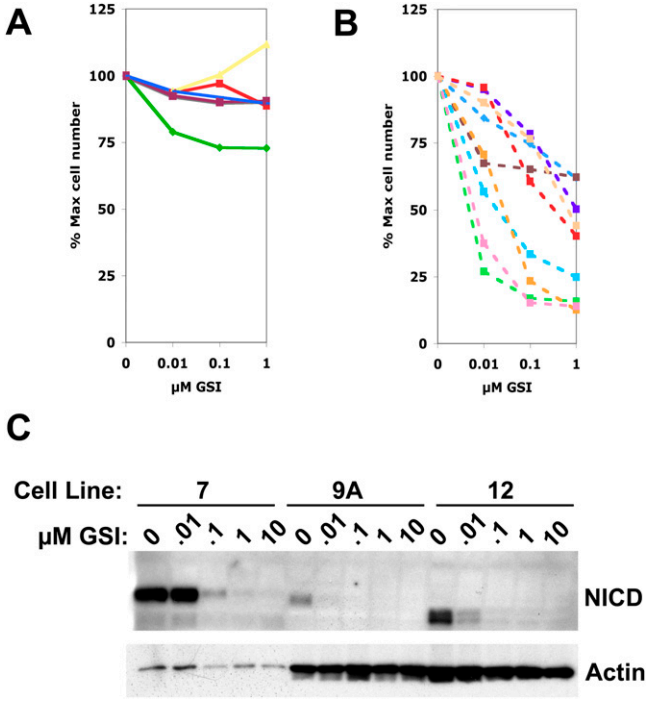


Fig. S5. Sensitivity of individual T lineage acute lymphoblastic leukemia (T-ALL) cell lines to a γ-secretase inhibitor (GSI). (*A* and *B*) T-ALL cell lines (3–5 × 10^5 cells per well) were grown in lymphocyte media containing DMSO (vehicle control) or GSI and counted after 5–7 days. The vehicle control for each line was taken as 100% growth, and the percentage reduction in cell number was calculated for each concentration of GSI. (*A*) Cell lines without detectable Notch1 intracellular domain (NICD) (solid lines; n = 5); (*B*) cell lines with NICD (dashed lines; n = 9). (*C*) Cell lines expressing NICD were cultured with varying concentrations of GSI for 48 h. Western blotting with an antibody that detects cleaved Notch1 demonstrates inhibition by the GSI.

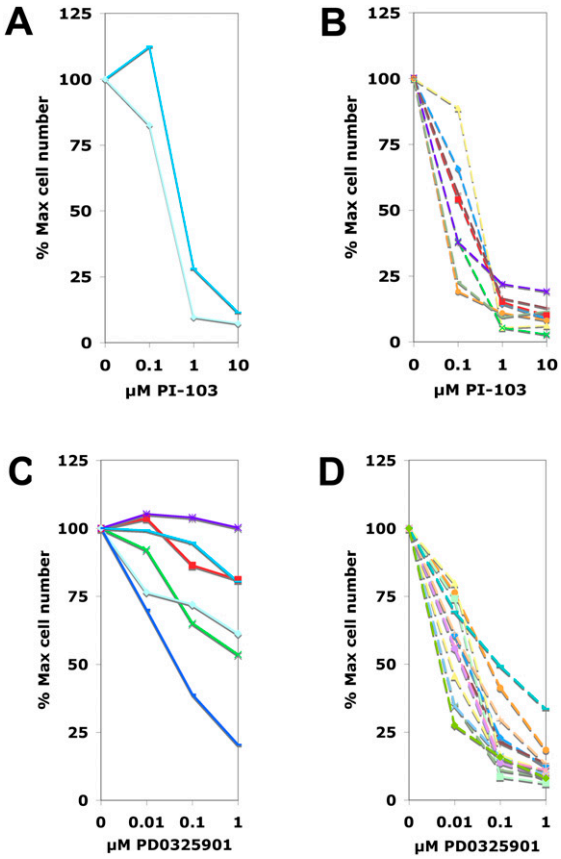


Fig. S6. Sensitivity of individual T lineage acute lymphoblastic leukemia cell lines to targeted agents. Tumor-derived cell lines were grown for 5–7 days over a range of drug concentrations, and growth inhibition was assessed as described in Fig. S4. (*A*) PI-103 $Kras^{WT}$, $n = 2$. (*B*) PI-103 $Kras^{G12D}$, $n = 8$. (*C*) PD0325901 $Kras^{WT}$, $n = 6$. (*D*) PD0325901 $Kras^{G12D}$, $n = 13$.

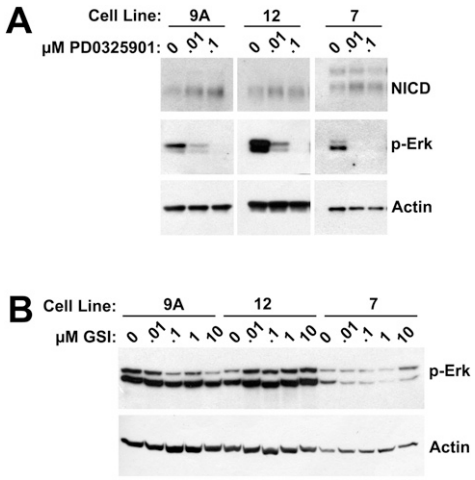


Fig. S7. PD0325901 has no effect on Notch1 intracellular domain (NICD) cleavage, and γ-secretase inhibitor (GSI) does not alter p-ERK levels in T lineage acute lymphoblastic leukemia (T-ALL) cell lines. (*A*) PD0325901 has no effect on NICD cleavage at doses that block Erk activation. (*B*) GSI does not inhibit Erk phosphorylation. T-ALL cell lines were cultured with either inhibitor for 48 h, lysed, and analyzed by Western blot.

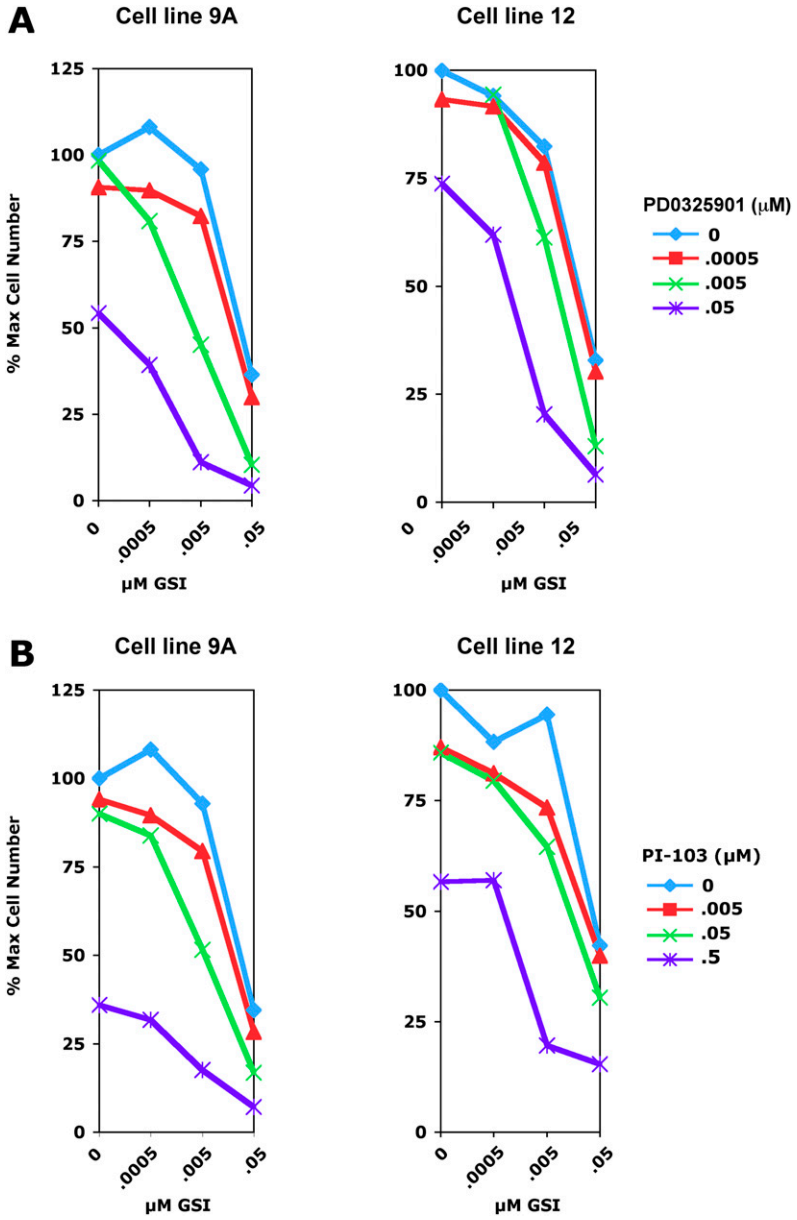


Fig. S8. Sensitivity of additional T lineage acute lymphoblastic leukemia cell lines to combined inhibitor treatment. Two cell lines that were sensitive to Compound E [γ-secretase inhibitor (GSI)], PD0325901, and PI-103 were grown in increasing concentrations of both (*A*) GSI and PD0325901 or (*B*) GSI and PI-103. The *x* axis is increasing doses of the GSI, and each line represents a different dose of (*A*) PD0325901 or (*B*) PI-103. The blue line (GSI alone) shifts to the left (green and purple lines) when combined with PD0325901 or PI-103, thereby reducing the IC_{50} concentration of GSI.

Table S1. Common insertion sites identified in *Kras* ^{WT} and *Kras*^{G12D} T-ALLs

Gene	T-ALL	Genotype	Location	Effect	Direction	Address*	Frequency
Ikzf1	3	*Kras*^{G12D}	Intron 3	Disrupt CDS	Inv	11640820	9
Ikzf1	3	*Kras*^{G12D}	Intron 1	Not disrupt CDS	Inv	11588821	45
Ikzf1	5	*Kras*^{G12D}	Intron 3	Disrupt CDS	Inv	11626720	1
Ikzf1	5	*Kras*^{G12D}	Intron 3	Disrupt CDS	Inv	11612305	1
Ikzf1	7	*Kras*^{G12D}	Intron 3	Disrupt CDS	Same	11614435	10
Ikzf1	15	*Kras*^{G12D}	Intron 3	Disrupt CDS	Same	11615515	2
Ikzf1	15	*Kras*^{G12D}	Intron 3	Disrupt CDS	Inv	11615251	104
Ikzf1	15	*Kras*^{G12D}	Intron 3	Disrupt CDS	Inv	11615248	1
Ikzf1	25	*Kras*^{G12D}	Intron 3	Disrupt CDS	Inv	11622678	16
Ikzf1	26	*Kras*^{G12D}	Intron 3	Disrupt CDS	Inv	11618019	3
Ikzf1	26	*Kras*^{G12D}	Intron 1	Not disrupt CDS	Inv	11591855	5
Ikzf1	29	*Kras*^{G12D}	Intron 3	Disrupt CDS	Inv	11615831	1
Ikzf1	32	*Kras*^{G12D}	Intron 3	Disrupt CDS	Inv	11624649	7
Ikzf1	32	*Kras*^{G12D}	Intron 3	Disrupt CDS	Same	11620497	1
Ikzf1	32	*Kras*^{G12D}	Intron 3	Disrupt CDS	Inv	11614950	2
Ikzf1	32	*Kras*^{G12D}	Intron 3	Disrupt CDS	Same	11610649	2
Ikzf1	42	*Kras*^{G12D}	Intron 1	Not disrupt CDS	Inv	11591205	15
Ikzf1	42	*Kras*^{G12D}	Intron 1	Not disrupt CDS	Inv	11590911	2
Ikzf1	49	*Kras*^{G12D}	Intron 3	Disrupt CDS	Same	11625202	13
Ikzf1	49	*Kras*^{G12D}	Intron 1	Not disrupt CDS	Same	11594250	5
Ikzf1	49	*Kras*^{G12D}	Intron 1	Not disrupt CDS	Inv	11591856	1
Ahi1	C7	WT	Intron 19	Disrupt CDS	Same	20738849	5
Ahi1	6	*Kras*^{G12D}	Intron 15	Disrupt CDS	Inv	20684543	10
Ahi1	28	*Kras*^{G12D}	3′	4.189 kb	Same	20774033	1
Ahi1	42	*Kras*^{G12D}	Intron 21	Disrupt CDS	Same	20759403	5
Rasgrp1	C3	WT	Intron 1	Disrupt CDS	Same	117033870	19
Rasgrp1	C4	WT	5′	64.569 kb	Same	117098887	4
Rasgrp1	C7	WT	5′	58.846 kb	Same	117093164	15
Lmo2	C7	WT	5′	67.398 kb	Inv	103703663	1
Lmo2	C7	WT	5′	35.014 kb	Same	103736047	10
Lmo2	3	*Kras*^{G12D}	5′	67.399 kb	Inv	103703662	5

T-ALL, T lineage acute lymphoblastic leukemia; Inv, inverse orientation; CDS, coding sequence.
*Refers to the reference genome (University of California, San Francisco mouse genome assembly mm8; February 2006).

Table S2. *Notch1* mutations in $Kras^{G12D}$ and $Kras^{WT}$ T-ALLs

T-ALL	Genotype	Mutation
1	$Kras^{G12D}$	c.7161_7162delGinsTTTCT
2	$Kras^{G12D}$	c.7160_7163delCGGCinsGAGGT
3	$Kras^{G12D}$	c.7162_7163insG
5	$Kras^{G12D}$	c.7161_7162delGinsTACAGGAACCACCC; c.7272insAAGAGG
6	$Kras^{G12D}$	c.7160_7161insACCCC
7	$Kras^{G12D}$	c.7271_7272delTCinsGTGAGGG
9	$Kras^{G12D}$	c.7148_7170delCTGCCCAACACACGGCTGGCAAC
12	$Kras^{G12D}$	c.7354_7356delTCTinsGG
14	$Kras^{G12D}$	c.7337_7338insC
15	$Kras^{G12D}$	c.7273_7274delGinsAAAAC
16	$Kras^{G12D}$	c.7158_7159insG
18	$Kras^{G12D}$	c.7300_7301insGAGGGAGC
20	$Kras^{G12D}$	c.7161_7162delGinsCCTTC
22	$Kras^{G12D}$	c.7273_7274delGinsCCC
23	$Kras^{G12D}$	c.7161_7162delGinsCC
24	$Kras^{G12D}$	c.7164_7165insAGTAGTT
25	$Kras^{G12D}$	c.7162_7163insGTAGCATTG
26	$Kras^{G12D}$	c.7297_7298insGTTCCTGG
27	$Kras^{G12D}$	c.7408_7409delGC
28	$Kras^{G12D}$	c.7161_7162delGinsCCTTC
29	$Kras^{G12D}$	c.7328_7335delGATGTACA
32	$Kras^{G12D}$	c.7161_7162delGinsCT
34	$Kras^{G12D}$	c.7161delinsCCCC
35	$Kras^{G12D}$	c.7161_7162insCC
36	$Kras^{G12D}$	Frameshift before PEST domain
37	$Kras^{G12D}$	c.7161_7162insC
40	$Kras^{G12D}$	c.7161_7162insCCCCCCC
42	$Kras^{G12D}$	c.7161_7162delGinsCC; c.7306_7307delAG
43	$Kras^{G12D}$	Frameshift before PEST domain
50	$Kras^{G12D}$	c.7161_7162delGinsTT
C3	WT	7162_3insG
C2	WT	7272_7274delGGinsAGAAAAA
C12	WT	7272_3delGinsGAGGC

T-ALL, T lineage acute lymphoblastic leukemia.