Mouse	Gender	Age at	Spleen	WBC	%Jag1+	GSI
		death (mo)	(g)	(K/uL)		responsive
3842	М	5	1.14	11.3	34.5	ND
13441	F	11.5	1.21	11.5	51.5	ND
13497	М	8	1.15	25.8	92.8	ND
13843	М	5	1.03	45.2	46.8	ND
2894	М	7	2.29	109.5	19.3	Yes
2972	F	11	1.02	94.4	19.3	No
3149	F	8	1.55	35.5	SF	Yes
3430	М	8	1.61	182.3	29.9	Yes
3673	М	5	0.97	224	SF	No
13346	F	8	1.7	17	36.1	No
13355	М	9	0.93	18.8	90.7	Yes
13499	М	6	1.23	69	SF	Yes
13659	M	7.5	0.75	29.8	15.5	Yes

Supplemental Table 1: Characteristics of *Ctsg-PML-RARA* mice and murine APL samples. ND, not determined due to failure of colony formation; SF, sample failure.





A) JAG1 expression in the CALGB set of 93 de novo AML samples, showing the same probeset as in Figure 1A (probe set 209099). B) qRT-PCR measurement of *JAG1* expression in 11 APL, 12 other AML (4 samples each of M0, M1, M2, and M4 FAB subtypes) and 4 flow-sorted normal promyelocyte samples. Data are normalized to GAPDH expression. C) Summary of the data in B. Each data point represents one patient sample or one normal sample.



Figure S2. Expression of genes encoding Notch signaling components in APL (closed bars) and normal promyelocytes (open bars). (A). Gamma secretase components; B). Additional enzymes involved in Notch cleavage; C). Transcriptional cofactors of cleaved Notch; D). Notch modifying enzymes; E). E3 ubiquitin ligases involved in Notch signaling. Data are the mean +/- standard deviation of the probeset with the highest average signal intensity for 22 APL and 5 normal promyelocyte samples present in the Washington University *de novo* AML set. Genes present in the APL dysregulome are marked with an asterisk.



Enrichment plot: GSI-Notch

Α



Figure S3: Expression of Notch signatures in APL

A) GSEA plot and heat map of 22 APL samples compared to 5 normal promyelocyte (Pro) samples demonstrates significant enrichment in APL of a previously published set of genes downregulated in T-ALL by gamma secretase inhibitor treatment³⁰. Normalized enrichment score 1.8927. Heatmaps of a (B)previously described set of Notch transcriptional targets in T-ALL³¹ and (C) a previously published set of transcriptional targets of Notch whose expression is inhibited by both gamma secretase inhibitor treatment and DNMAML expression³².





Figure S4: JAG1 protein is found in an intracellular compartment in PR-9 cells. Flow cytometry plots showing staining of Zn²⁺-induced PR-9 cells with an isotype control antibody, JAG1 antibody by surface staining or JAG1 antibody by intracellular staining following fixation and permeabilization.

Α **GSI-Notch**

02481624	
	SLC44A1
	GIMAP6
	PPP4R2
	HK2
	KIAA0922
	IKBP4
	MLN
	PUS7
	RNU3IP2
	JTV1
	BOP1 /// LOC653119
	AKAP1
	SHQ1
	NOLC1
	JTV1
	GTPBP4 SLC29A1
	DDAN
	C60RF66
	DTX1
	C10RF33
	LYAR
	DKC1
	POLR1C
	CCDC86
	HSPC111
	SLC19A1
	TRMT1 DHV33
	LOC348180
	DPH2
	SH01
	DNAJC11
	GPATC4
	NAT10
	POLR3H
	WDR12
	PSMB5
	DDIE
	NOL1
	PDSS1
	TRMT1
	USP36
	HSPC111
	WDR74
	MRPL4
	CTPS
	IFRDZ
	CNL3
	BXDC1
	KTAA0664
	MRPL24
	GPATC4
	MKI67IP
	MYC
	EVL
	BYSL
	ETF1
	PWPZH
	POLR3E
	NOLE
	D21S2056F
	APRT
	NOB1
	PUS1
	PPRC1
	ZCD1
	MATR3
	ISG20L1

Notch-GSI-DNMAML

Β



Figure S5: Expression of Notch signatures in induced PR-9 cells.

Heat maps showing expression of A) the GSI-Notch³⁰ and B) Notch-GSI-DNMAML³² signatures in PR-9 cells 0, 2, 4, 8, 16 or 24 hours after Zn²⁺ induction of *PML-RARA* expression.



Figure S6: Expression of JAG1 and Notch target genes in PR-9 cells Microarray gene expression data for JAG1 and three Notch target genes (*PHB, TASP1* and *GSPT1*) in PR-9 cells after Zn^{2+} induction of *PML-RARA* expression.



Figure S7: JAG1 protein is found in an intracellular compartment in murine APL. Flow cytometry plots showing staining of three independent murine APL samples with an isotype control antibody, JAG1 antibody by surface staining or JAG1 antibody by intracellular staining following fixation and permeabilization. Counterstaining with the myeloid marker Gr-1 is provided as a control.

PR		wт			
781 782 784 785	NT1	EE E			
but Dut Dut Dut Dut 1			SampleNa	ame	
			AHSA1	AHSA1	AHA1, activator of heat shock 90kDa protein ATPase homolog 1 (yeast)
			KARS	KARS	lysyl-tRNA synthetase
			HIST1H2BN	HIST1H2BN	histone cluster 1, H2bn
			MYC	MYC	v-myc myelocytomatosis viral oncogene homolog (avian)
			DDX56	DDX56	DEAD (Asp-Glu-Ala-Asp) box polypeptide 56
			WDR46	WDR46	WD repeat domain 46
			TMEM59	TMEM59	transmembrane protein 59
			UMPS	UMPS	uridine monophosphate synthetase (orotate phosphoribosyl transferase and orotidine-5'-decarboxylase)
			UTP14A	UTP14A	UTP14, U3 small nucleolar ribonucleoprotein, homolog A (yeast)
			CSDA	CSDA	cold shock domain protein A
			ITPR2	ITPR2	inositol 1,4,5-triphosphate receptor, type 2
			FYC01	FYCO1	FYVE and coiled-coil domain containing 1
			SNX5	SNX5	sorting nexin 5
			SF3B3	SF3B3	splicing factor 3b, subunit 3, 130kDa
			HES1	HES1	hairy and enhancer of split 1, (Drosophila)
			WDR12	WDR12	WD repeat domain 12
			PWP1	PWP1	PWP1 homolog (S. cerevisiae)
			SRPRB	SRPRB	signal recognition particle receptor, B subunit
			TIMM13	TIMM13	translocase of inner mitochondrial membrane 13 homolog (yeast)
			NOL5A	NOL5A	nucleolar protein 5A (56kDa with KKE/D repeat)
			PMM2	PMM2	phosphomannomutase 2
			GM2A	GM2A	GM2 ganglioside activator
			CDKN1B	CDKN1B	cyclin-dependent kinase inhibitor 1B (p27, Kip1)
			PPAT	PPAT	phosphoribosyl pyrophosphate amidotransferase
			POLR2I	POLR2I	polymerase (RNA) II (DNA directed) polypeptide I, 14.5kDa
			NAT10	NAT10	N-acetyltransferase 10
			DDX28	DDX28	DEAD (Asp-Glu-Ala-Asp) box polypeptide 28
			TASP1	TASP1	taspase, threonine aspartase, 1
			GSPT1	GSPT1	GI to S phase transition 1
			MRPL4	MRPL4	mitochondrial ribosomal protein L4
			SERBP1	SERBP1	SERPINE1 mRNA binding protein 1
			TRUB2	TRUB2	TruB pseudouridine (psi) synthase homolog 2 (E. coli)
			IFRD2	IFRD2	interferon-related developmental regulator 2
			SLC25A19	SLC25A19	solute carrier family 25 (mitochondrial deoxynucleotide carrier), member 19
			KPNB1	KPNB1	karyopherin (importin) beta l
			NDRG1	NDRG1	N-myc downstream regulated gene 1
			PHB	PHB	prohibitin
			MRPL24	MRPL24	mitocnondriai ribosomai protein L24
			MTMR4	MTMR4	myotupularin related protein 4
			COX7C	COX/C	cytochrome c oxidase subunit Viic
			JARIDZ	JARIDZ	jumonji, AT rich interactive domain 2
			SH01	SH01	SHO1 homolog (S. cerevisiae)

Figure S8: Expression of Notch signature in Ctsg-PML-RARA KLS cells

Heat map of 6 KLS samples sorted from preleukemic *Ctsg-PML-RARA* (PR) mice compared to 4 wildtype C57BL/6 KLS (WT) samples, demonstrating significant enrichment of the previously described Notch-Targets³¹ signature in *Ctsg-PML-RARA* KLS cells.







С

Figure S9: Inhibition of Notch signaling does not change colony formation by wildtype marrow cells.

A). Relative colony formation for 3 wildtype C57BL/6 marrow samples plated in Methocult (3534) after a two day treatment with DMSO, 2 μ M compound E, or 25 μ M compound IX. Data shown are mean +/- standard deviation. Data are normalized to the DMSO treated control colony counts. B). Relative colony formation for 3 wildtype C57BL/6 marrow samples transduced with either MSCV- DNMAML-GFP or GFP control, sorted for GFP+ cells and plated in methylcellulose media. Data shown are mean +/- standard deviation and are normalized to the GFP control. In both A and B, cells from each animal were plated in triplicate for all treatment conditions. C) Summarized data for 3 wildtype 129SvJ/B6 F1 marrow samples treated for 48 hours with 1 μ M ATRA, 2 μ M compound E, 25 μ M compound IX or DMSO control, and then plated in methylcellulose media without drugs in triplicate. Data shown are mean +/- standard deviation and are normalized to the average colony formation for the DMSO control of each animal. NS=Not Significant.



Figure S10: Response of Murine APL Cells to Inhibition of Notch Signaling. Colony formation data for 5 murine APL samples treated for 48 hours with 1 μ M ATRA, 2 μ M compound E, 25 μ M compound IX, or DMSO vehicle control, and then plated in methylcellulose media supplemented with IL-3, IL-6 and SCF in triplicate. Data bars are the mean numbers of colonies per 10,000 cells plated. Error bars represent standard deviations. In all graphs, one asterisk (*) indicates p<0.05, two asterisks (**) indicates p<0.001. NS=Not Significant.



С

		% Blasts/Promyelocytes					
UPN	Age	Sex	FAB	PB	BM	Mutations	
884751*	54	F	M3	70	86	<i>FLT3</i> D835	
757199	50	F	M3	0	90	MT-ND1, CALR, OR11H12, PHIP, FAM155A, MLLT4, SEPX1	
478908	50	М	M3	10	74	FLT3-ITD, CACNA2D3, XIRP1, ASTE1, DCT, RBKS	
179016	42	Μ	М3	2	88	TTN, CALR, DCHS2, LRRC4, NMUR2, RBM16, CTNNA3, DYRK4, EXD1, PCDH11X, TCL1A, TJAP1	

Figure S11. Variable responses of patient-derived APL samples to gamma-secretase inhibition. A) Graph depicting proliferation of 4 patient-derived APL samples relative to a DMSO control after 5 days of treatment with compound IX at indicated concentrations, or with 1 μ M ATRA. Two patient samples responded in a dose-dependent manner (884751 and 757199). Cells were expanded using a stromal co-culture system, as described previously (Klco JM, et. al., *Blood, in press*). Error bars represent s.e.m. * indicates p < 0.05 using unpaired two-sided t test, n=3-4, technical replicates. B) Histogram of CD11b cell surface expression assessed by FACS analysis of cells from UPN 884751 from same experiment described in (a). The highest concentration of compound IX (100 μ M) minimally altered CD11b expression compared to 1 μ M ATRA, suggesting a minimal effect on differentiation. This result was representative of the other doses and samples. Recurrently mutated genes in bold. *All patient specimens underwent whole genome/exome sequencing except for 884751, which underwent standard clinical FLT3 and NPM1 testing. FAB, French-American-British classification; PB, peripheral blood; BM, bone marrow.