CXCL12 AND CXCR3 MAY IDENTIFY COMPLETE RESPONSE IN ACUTE MYELOID LEUKEMIA PATIENTS TREATED WITH TIPIFARNIB

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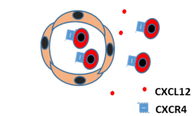
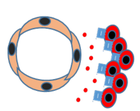
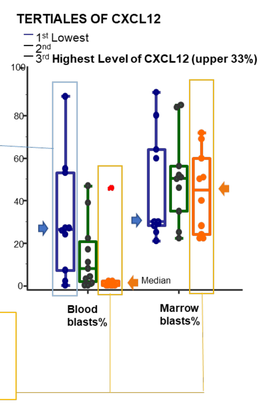
HIGH CXCL12 AND BONE MARROW HOMING ARE MARKERS OF TIPIFARNIB'S ACTIVITY



Patients with low bone marrow (BM) CXCL12 had high peripheral blood blast counts



Patients with high BM CXCL12 had very low peripheral blood blast counts, despite having a high number of BM blast counts



Bone Marrow Homing is a Surrogate of Tipifarnib Response (Trial CTEP-20)

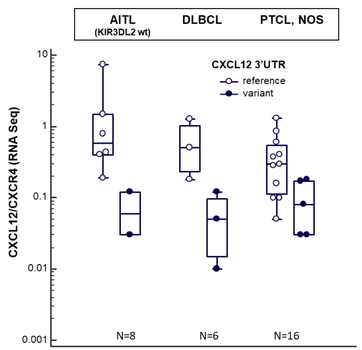
Blood blasts	Marrow blasts	Resp	CXCL12/CXCR4
0	72	CR	1.33
0	35	CR	0.30
0	22	CR	0.28
8	80	PD	0.21
0	24	PD	0.12
0	28	CR	0.10
0	21	CR	0.09
11	15	SD	0.09
55	64	PD	0.06
13	32	SD	0.06
39	56	HI	0.06
33	78	CR	0.06
2	28	SD	0.04
3	22	HI	0.04
55	84	PD	0.04
22	85	PD	0.04
89	80	PD	0.03
46	60	SD	0.03
7	77	CR	0.03
1	50	HI	0.03
14	46	PD	0.03
0	21	PD	0.03
12		PD	0.02
33	25	PD	0.02
26	60	SD	0.02
39	29	PD	0.02
53	30	SD	0.02

CXCL12/CXCR4

CR, complete response; HI, hematological improvement; SD, stable disease; PD, progressive disease . CTEP20 (NCT00027872) . Global gene expression data was generated using the Affymetrix U133A gene chip

HIGH CXCL12 IN PATIENTS WITH REFERENCE 3'UTR/INTRON CXCL12

- The presence of a CXCL12 3'UTR gene variant (rs1801157) has previously been shown to be associated with decreased CXCL12 levels (Soriano 2002) and to decrease bone marrow homing of blasts and increase extramedullar disease in AML (Dommange 2006).
- We observed that the presence of the rs2839695 A>G 3'UTR/intron CXCL12 gene sequences translated to lower levels of CXCL12 gene expression in T-cell lymphoma (AITL, NOS).
- Mutation of CXCL12 3'UTR/intron sequences translated to lower CXCL12 expression in B-lymphoma (DLBCL). Most CXCL12 variants in DLBCL were due to mutation.



AITL, angioimmunoblastic T cell lymphoma; DLBCL, diffuse large B cell lymphoma, PTCL NOS, peripheral T cell lymphoma not otherwise specified. Patients with RNA Seq data available only. KIR3DL2 wt status in AITL was determined by NGS.

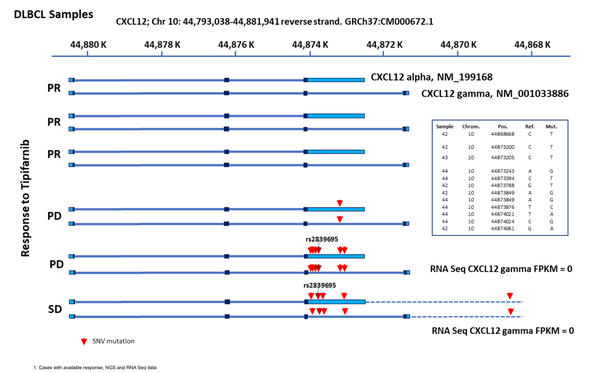
REFERENCE 3'UTR/INTRON CXCL12 LYMPHOMA RESPONDERS

CXCL12 expression and response to tipifarnib in lymphoma pts with reference 3'UTR CXCL12 sequences [1]

	HISTOL	RESP	CXCL12/CXCR4	3UTR
6	AITL	PR	7.42	reference
34	AITL	PD/NE	1.5	reference
35	DLBCL	PR	1.28	reference
36	NOS	SD	1.3	reference
17	NOS	SD	0.85	reference
32	AITL	PD/NE	0.8	reference
37	NOS	SD	0.6	reference
38	DLBCL	PR	0.50	reference
30	AITL	PR	0.4	reference
7	AITL	PD/NE	0.43	reference
18	NOS	SD	0.40	reference
18	NOS	CR	0.37	reference
16	NOS	SD	0.30	reference
33	NOS	PR	0.28	reference
8	AITL	PD/NE	0.19	reference
39	DLBCL	PR	0.18	reference
23	NOS	PD/NE	0.18	variant
40	NOS	PD/NE	0.17	variant
19	NOS	PD	0.16	reference
26	AITL	PD/NE	0.14	variant
41	NOS	SD	0.1	reference
10	AITL	PD/NE	0.12	variant
42	DLBCL	SD	0.12	variant
11	AITL	PD/NE	0.12	reference
20	NOS	PD/NE	0.10	reference
25	NOS	PD/NE	0.08	variant
21	NOS	PD	0.05	reference
43	DLBCL	PD/NE	0.05	variant
13	NOS	PD/NE	0.03	variant
9	AITL	PD/NE	0.03	variant
24	NOS	PD/NE	0.03	variant
44	DLBCL	PD/NE	0.01	variant

1. Cases with available response, NGS and RNA Seq data. DLBCL biopsy samples were from tipifarnib study LS0388 (NCT00028388); AITL and PTCL NOS biopsy samples were from tipifarnib study KO-TIP-002 (NCT02466228). All AITL cases listed were KIR3DL2 336/386 wt as determined by tumor NGS .

DLBCL Samples

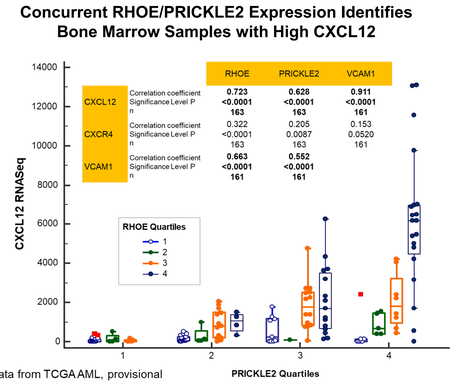


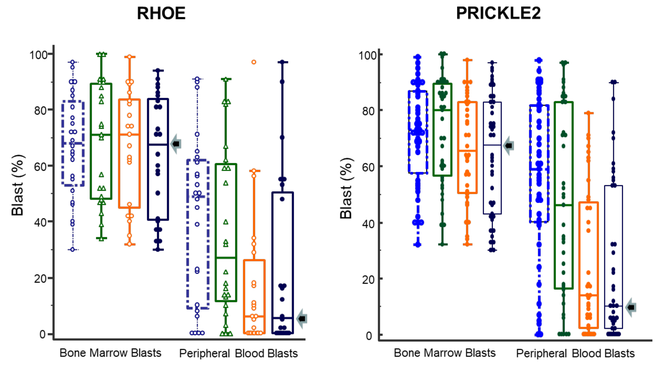
1. Cases with available response, NGS and RNA Seq data

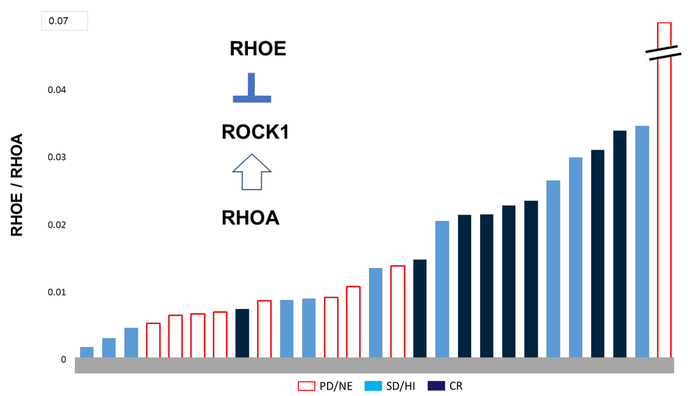
SCREENING FOR FARNESYLATED TARGETS OF TIPIFARNIB IN AML

- Pre-treatment high tumor CXCL12 expression identifies potential responders to tipifarnib therapy but the relationship between farnesylation and CXCL12 is poorly understood.
- Hypothesis: Because CXCL12 expression and bone marrow homing are associated with the activity of tipifarnib in AML, expression of the farnesylated target(s) of tipifarnib could be expected to correlate with the expression of CXCL12 in AML. Expression of a farnesylated target should also be associated with bone marrow homing.
- Method: Determine the strength of the relationship between the expression of genes encoding farnesylated proteins and that of CXCL12 using the Spearman's rank order test. Database: TCGA AML, Provisional, N=173, RNA Seq V2 RSEM.
- Genes screened were identified using PRENbase (Parameters: KNOWN FT required, GGT1-GGT2 excluded, Eukaryotic Mammalia, Minimum cluster=3): RAB28, RASD1, RASD2, NAP1L1, GNGT1, PTP4A2, RILP, PRICKLE1, PRICKLE2, LMO6, INPP5A, INPP5E, Gbp5, Gbp1, PLA2G4C, PTGIR, CLN3, TIMAP, MYPT3, RHOQ, TC10, RHOU, RHOD, RHOE, HRAS, RAP2A, RAP2C, ERAS, DNAJA4, Lmnb2, Lmnb3, CENPF, CENPE, STK11, GRK1, PHKB, RHEB, PTPRB, PDE6C, PDE6A/C.
- Results: Sperman's rho ~500 (highly significant) observed for RHOE (RND3, 0.723), PRICKLE2 (0.628)

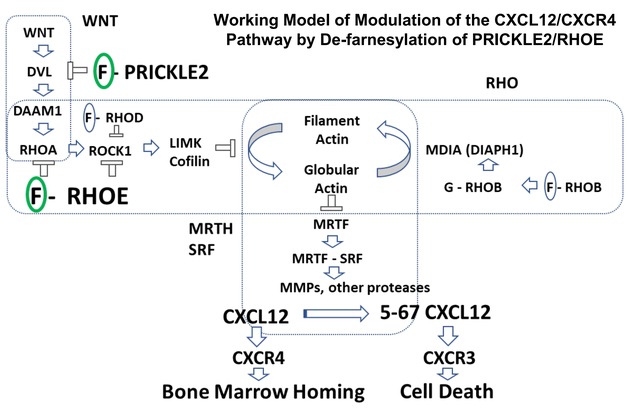
HIGH PRICKLE2/RHOE EXPRESSION ASSOCIATED BONE BLAST MARROW HOMING





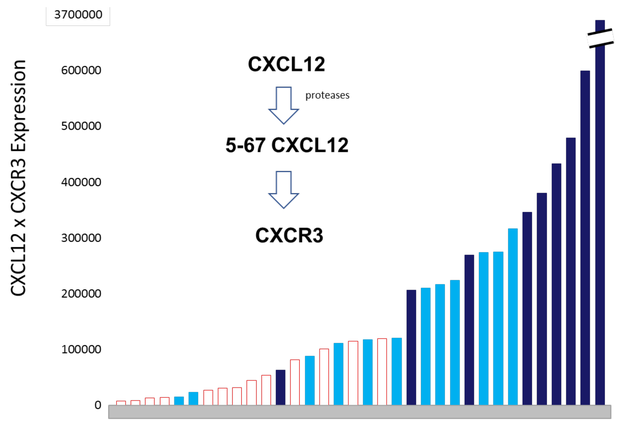


WORKING MODEL



In this model, de-farnesylation of PRICKLE2/RHOE triggers a dowstream switch from activation of CXCR4 by full length CXCL12 to activation of CXCR3 and induction of cell death by a 5-67 CXCL12 fragment

CXCR3 AND CXCL12 EXPRESSION IDENTIFY COMPLETE RESPONSES TO TIPIFARNIB IN ELDERLY UNFIT AML (CETP-20)



CONCLUSIONS

- In AML, bone marrow expression of farnesylated RHOE and PRICKLE2 strongly correlated with CXCL12 expression and homing of AML blasts to the bone marrow.
- CXCL12, RHOE and CXCL12 appear to be of stromal origin based on its strong correlation with VCAM1.
- CXCL12 expression in lymphoma is determined in part by 3UTR/intron polymorphisms.
- High bone marrow RHOE, CXCL12, CXCR3 and homing of AML blasts to the bone marrow are observed in AML patients who respond to tipifarnib therapy .
- These data provide a working model by which farnesylation regulates the activity of the CXCL12 pathway.

References: Soriano 2002, J Infect Dis 186:922-31. Dommange 2006, FASEB J 20:1913-5