KIR3DL2 MUTATION MAY DEFINE A HIGH RATE OF RESPONSE OF AITL TO TIPIFARNIB

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INTRODUCTION

Killer-cell immunoglobulin-like receptors (KIRs) are transmembrane glycoproteins expressed in NK/T cells that play key regulatory functions, including the control of chemokine/cytokine release and angiogenesis. KIRs interact with HLA and transduce activatory (KIR-DS) or inhibitory (KIR-DL) signals through their cytoplasmic domains.

Tipifarnib downregulates CXCL12 and tipifarnib treatment as monotherapy translates to objective responses in patients with tumors that express CXCL12, including angioimmunoblastic T-cell Lymphoma (AITL) (Witzig, EHA 2019). However, differences in outcomes with tipifarnib treatment were observed among AITL patients that could not be completely explained by levels of tumor CXCL12 expression. The present study was undertaken to further elucidate the molecular mechanisms of sensitivity and resistance to tipifarnib treatment in AITL patients.

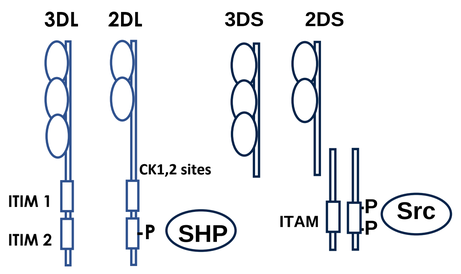
	HISTOL	RESP	CXCL12/CXCR4
6	AITL	PR	7.42
28	AITL	CR	2.4
34	AITL	PD/NE	1.5
36	NOS	SD	1.3
17	NOS	SD	0.85
32	AITL	PD/NE	0.8
37	NOS	SD	0.6
1	AITL	CR	0.48
30	AITL	PR	0.4
7	AITL	PD/NE	0.43
18	NOS	SD	0.40
12	NOS	CR	0.37
2	AITL	CR	0.36
3	AITL	PR	0.35
16	NOS	SD	0.30
31	AITL	PR	0.28
5	AITL	SD	0.28
8	AITL	PD/NE	0.19
23	NOS	PD/NE	0.18
40	NOS	PD/NE	0.17
19	NOS	PD/NE	0.16
4	AITL	PR	0.15
26	ALCL	PD/NE	0.14
41	NOS	SD	0.1
10	AITL	PD/NE	0.12
11	AITL	PD/NE	0.12
20	NOS	PD/NE	0.10
25	NOS	PD/NE	0.08
33	AITL	SD	0.1
21	NOS	PD/NE	0.05
29	AITL	CR	0.04
13	NOS	PD/NE	0.03
9	AITL	PD/NE	0.03
24	NOS	PD/NE	0.03

Tumor CXCL12 expression identifies sensitivity to tipifarnib in T cell lymphoma

Pre-treatment biopsy samples of patients enrolled in trial KO-TIP-002 (NCT02464228) were analyzed using RNA Seq. CR, complete response; PR, partial response; SD, stable disease, PD, progressive disease. AITL patients with KIR3DL2 336/386 wt, mutant or unknown sequences are included.

KIR-DL MUTATIONS IN AITL

- KIR-DS receptors signal intracellularly through ITAM motifs and SRC whereas KIR-DL receptors signal through ITIM motifs and SHPs. SHPs dephosphorylate SRC phosphorylation targets.
- Next Generation Sequencing (NGS) was conducted in pre-treatment biopsy samples from 17 AITL patients treated with tipifarnib in study KO-TIP-002. Increased KIR-DL gene variation was observed in patients who responded to tipifarnib treatment.
- The most notable tumor KIR-DL mutations[1] found in patients who experienced objective responses with tipifarnib were located in:
  - —the ITIM2 domain of KIR3DL1 and KIR2DL3, and
  - —the vicinity of the CK1,2 phosphorylation sites of KIR2DL3 and KIR3DL2.
- Approximately half of the AITL tumor samples carried KIR3DL2 mutations.



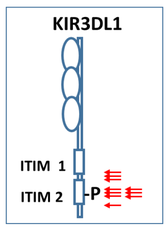
1. The term tumor mutation is employed but germinal/somatic status was not determined. KIR3DL1 I426T, L427M, (T429M not reported) and V440I are reported in COSMIC as mutations. KIR3DL2 Q386E is reported in COSMIC as a mutation but C336R has been flagged as a potential polymorphism.

KIR3DL1 Mutations

Sample no.	6	30	5	33	3	4	28	29	1	2
RESP.	PR	PR	SD	SD	PR	PR	CR	CR	CR	CR
									R409T	R409T
									R413C	R413C
									R413P	R413P
ITIM 2			I426T			I426T		I426T	I426T	I426T
			L427M			L427M		L427M	L427M	L427M
			T429M			T429M		T429M	T429M	T429M
			S438fs							
			V440I					V440I	V440I	V440I

ITIM 1 PKC site PDK site ITIM 2

DPEEVTYAQLDHCVFTQRKITRPSQRPKTPPTDTILYTELPNAKPRSKVVS



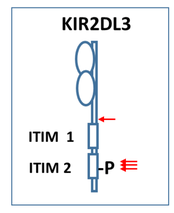
- Extensive mutation in ITIM2, domain including loss of Threonine 429 and a novel Threonine at position 426, was observed in KIR3DL1

KIR2DL3 Mutations

Sample no.	6	30	5	33	3	4	28	29	1	2
RESP.	PR	PR	SD	SD	PR	PR	CR	CR	CR	CR
			E295D			E295D				E295D
ITIM 2			I330T			I330T				
						I331T				
						V332M				

CK2 site ITIM1 PKC site ITIM2

NREDSDEQDPQEVTYAQLNHCVFTQRKITRPSQRPKTPPTDIIVYTELPNAEP



- Mutation of the ITIM2 domain was only observed in 2 AITL patients responding to tipifarnib.
- E295D (rs76449138) located near the CK2 phosphorylation site was observed only in 3 AITL responders. It was also present in 1 unique case of CR in 21 PTCL NOS patients with NGS data (study KO-TIP-002)[1] and 1 unique case of PR in 6 Hodgkin lymphoma patients with NGS data (study LS038B)[2].

1. NCT02464228. 2. NCT00082888

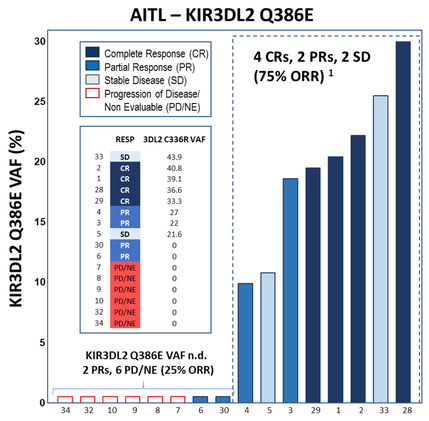
KIR3DL2 Mutations

Sample no.	6	30	5	33	3	4	28	29	1	2
RESP.	PR	PR	SD	SD	PR	PR	CR	CR	CR	CR
						P319S				
						W323S	W323S			
						P324S	P324S			
						S333T	S333T			
					C336R	C336R	C336R	C336R	C336R	C336R	C336R	C336R
					V341I	V341I		V341I	V341I	
					Q386E	Q386E	Q386E	Q386E	Q386E	Q386E	Q386E	Q386E

- A significant association between pre-treatment AITL tumor KIR3DL2 C336R/Q386E and clinical benefit from tipifarnib was observed (8 CR-PR-SD/8 KIR3DL2m vs 3/9 KIR3DL2wt, p=0.009).
- KIR3DL2 Q386E is reported in COSMIC as a tumor mutation but C336R has been flagged as a potential SNP (COSM5765149).

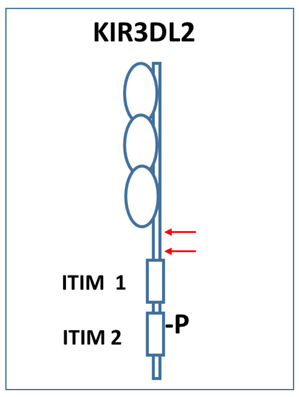
HIGH VAF OF KIR2DL3 C336R/Q386E PREDICTED CR WITH TIPIFARNIB

- KIR2DL3 C336R/Q386E variant allele frequency (VAF) as determined by NGS was predictive of complete responses with tipifarnib treatment (Receiver Operator Curve AUC=0.94, p<0.0001, for KIR3DL2 Q386E VAF>19% and p<0.001 KIR3DL2 C336R VAF>27%)



	KIR3DL2	Wild Type	KIR3DL2 Mutant
N		8	8
Overall Best Response			
Complete Response (CR)		0	4
Partial Response (PR)		2	2
Stable Disease (SD)		0	2
Progressive Disease (PD)		6	-
Not efficacy evaluable (NE)		-	-
Response Rate (CR + PR)		**25%**	**75%**

1. ORR = Objective Response Rate by IWGC. KIR3DL2 mutant patients were 88% Caucasian, 75% male, 62% stage IV, 50% had B symptoms, and 88% had prior transplants. KIR3DL2 wt patients were 75% caucasian, 75% male, 75% stage IV, 50% had B symptoms, 37% had prior transplants



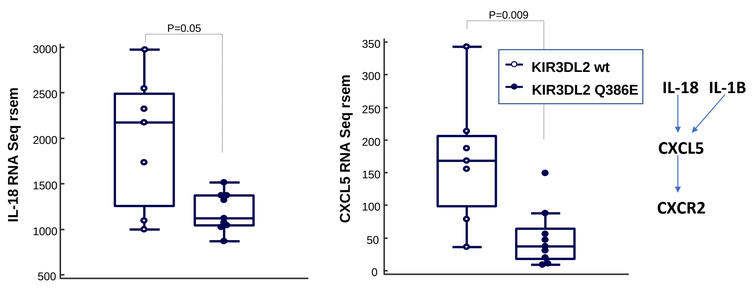
Potential Structure/Function Effects of KIR3DL2 Variants

- C336R may likely interfere with disulphide-bonded dimerization of KIR2DL2 whereas Q386 could have functional effects due to its location near the CK1,2 phosphorylation sites.
- Q386E replaces a neutral amino acid (Q, glutamine) with a charged one (E, glutamic acid). Potential phospho-mimetic effect activating KIR3DL2/SHP signaling

	CK1	CK2
2DL1	NKKNAAVMDQESAGNRTANSEDSDEQDPQEVTYTQLN	
2DL2	NKKNAAVMDQESAGNRTANSEDSDEQDPQEVTYTQLN	
2DL3	NKKNAVVMDQEPAGNRTVNREDSDEQDPQEVTYAQLN	
2DL4	KKKNAAVMDQESAGHRTVNREDSDEQDPQEVTYAQLN	
2DL5	NKKNAAVMDQEPAGDRTANSEDSDEQDPQEVTYAQLN	
3DL1	NKKNAAVMDQEPAGNRTANSEDSDEQDPQEVTYAQLN	
3DL2	NKKNAAVMDQEPAGNRTVNRQDSDEQDPQEVTYAQLN	
3DL3	NKKNAVVMDQEPAGNRTVNREDSDPQDPQEVTYAQLN	

Alignment of human killer cell immunoglobulin-like receptor (KIR) cytoplasmic domains in the vicinity of pos 386. CK1, casein kinase 1; CK2, casein kinase 2

CXCL5 Downregulation in KIR3DL2 Variant AITL Tumors



- Expression of IL-18 and CXCL5 were significantly lower in AITL tumors carrying KIR3DL2 C336R/Q386E.
- AITL may express high levels of CXCL5 that binds and activates CXCR2. CXCL12 but not CXCL5 is downregulated by tipifarnib (not shown)
- SHP-2 previously reported to downregulate CXCL5 [1] suggesting that C336R/Q386E may enhance the anti-inflammatory activity of KIR3DL2/SHP-2

1. Coulombe etal., 2013. Mol Cell Biol. 33: 2275–2284.

KIR3DL2 Variation may Define Poor SOC Prognosis in AITL

- AITL patients with KIR3DL2 gene variants appeared to experience better outcome with tipifarnib than with SOC treatment in their last prior line of therapy.

KIR3DL2 Q386E VAF(%)	Last Prior Therapy			Tipifarnib		
	Treatment (line)	Resp	PFS (mo)	Resp	PFS (mo)	Status
30.4	Nivolumab (3rd)	PR	3.8	CR	5.5	Progression/death
25.5	BEAM/ASCT (2nd)	PD	2.7	SD	3.6	Progression
22.2	DICE (4th)	PD	3.4	CR	4.2+	ASCT/FU
20.4	BEAM/ASCT (2nd)	CR	6.6	CR	3.6+	ASCT/FU
19.5	CHOP-E (1st)	PR	15.4	CR	5.1+	ongoing
18.6	Brentuximab ved. (4th)	SD	10.3	PR	4.4	Progression
10.8	CEOP (1st)	SD	7.7	SD	11.1+	ongoing
9.9	GemDOX (2nd)	PR	36.2	PR	8.4	Progression

BEAM=carmustine, etoposide, cytarabine, and melphalan
ASCT=Autologous Stem Cell Transplant
DICE=Dexamethasone, Ifosfamide, Cisplatin, Etoposide
CHOP-E=Cyclophosphamide, doxorubicin, vincristine, prednisone, and etoposide
CEOP=Cyclophosphamide, epirubicin, vincristine, and prednisone
GemDOX=Gemcitabine,prednisone, oxaliplatin

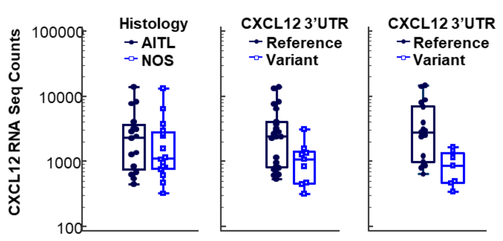
CONCLUSIONS

- **CXCL12/CXCR4 and CXCL5/CXCR2 appear to drive respectively sensitivity and resistance to tipifarnib.**
- **Lymphomas of AITL histology and those (PTCL NOS, DLBCL) carrying CXCL12 reference (wild type) sequences express high levels of CXCL12 and are sensitive to tipifarnib therapy.**
- **AITL expresses also CXCL5; however, a subset of AITL (~50%) carries mutations of KIR-DL genes and express very low levels of this chemokine, suggesting that KIR-DL variants may increase the anti-inflammatory properties of KIR-DL/SHP signaling, including CXCL5 downregulation.**
- **KIR3DL2 C336R/Q386E were the most prevalent KIR-DL mutations in AITL and were associated with high activity of tipifarnib: 50% CR rate, 75% ORR, 100% clinical benefit rate.**
- **High Variant Allele Frequency of KIR3DL2 C336R/Q386E predicted complete response to tipifarnib treatment.**
- **AITL patients carrying KIR3DL2 C336R/Q386E appeared to experience a better outcome with tipifarnib treatment than with prior SOC treatment.**

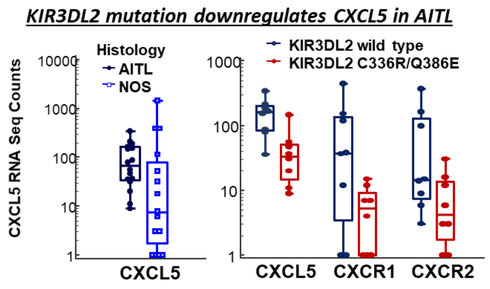
GENETICS OF TIPIFARNIB'S ACTIVITY IN PTCL

Genetics

AITL histology and reference 3'UTR upregulate CXCL12

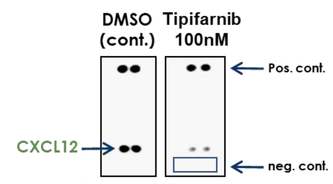


KIR3DL2 mutation downregulates CXCL5 in AITL

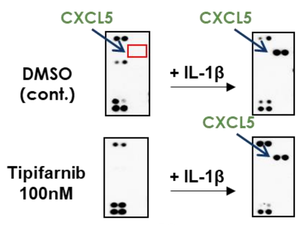


Mechanism of Action

Tipifarnib downregulates CXCL12 secretion



Tipifarnib does not inhibit CXCL5 secretion



Activity

N= 32 AITL/NOS cases with response, NGS and RNA Seq. data [1]

AITL (KIR3DL2wt) + NOS	Ref. 3'UTR
N	17
Overall Best Response	
Complete Response (CR)	1
Partial Response (PR)	3
Stable Disease (SD)	6
Progressive Disease (PD)	-
Not evaluable (NE)	-
Overall Response Rate	**23.5%**
Clinical Benefit Rate	**59%**

	AITL	KIR3DL2 Mutant
N		8
Overall Best Response		
Complete Response (CR)		4
Partial Response (PR)		2
Stable Disease (SD)		2
Progressive Disease (PD)		-
Not evaluable (NE)		-
Overall Response Rate		**75%**
Clinical Benefit Rate		**100%**

1. 34 cases have available RNA Seq data. 1 subject does not have NGS data; 1 subject is ALCL