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KIR3DL2 contributes to delineate the Acute-type and is a therapeutic target in Adult T-cell leukemia/lymphoma

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INTRODUCTION

Adult T-cell leukemia (ATL) is a lymphoid neoplasm of CD4+ T lymphocytes caused by the human T-cell leukemia virus type I (HTLV-1), which is classified into 4 clinical subtypes (ie, smoldering, chronic, acute, and lymphoma).

Natural killer receptors (NKR) were previously identified on T-cell lymphomas[1].

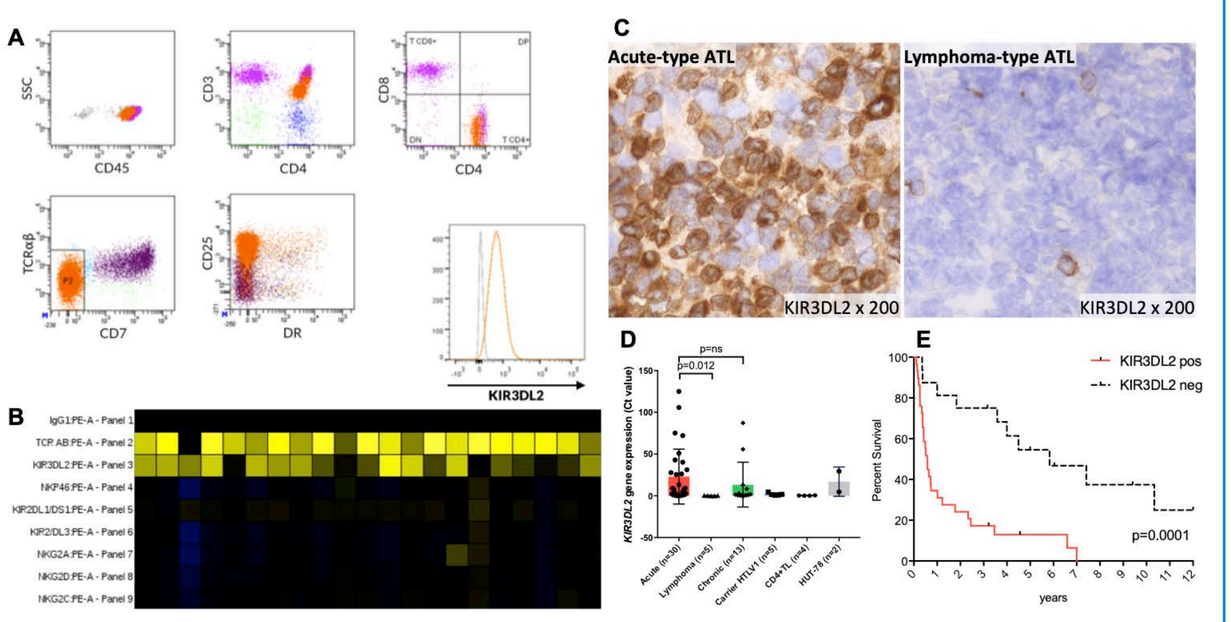
OBJECTIVES

Based on these new findings, we made the hypothesis that NKR could :

- **be expressed on ATL cells and help to discriminate the different ATL forms,**
- **participate to the pathophysiology of ATL,**
- **serve as new therapeutic targets in this disease with a dismal prognosis.**

RESULTS

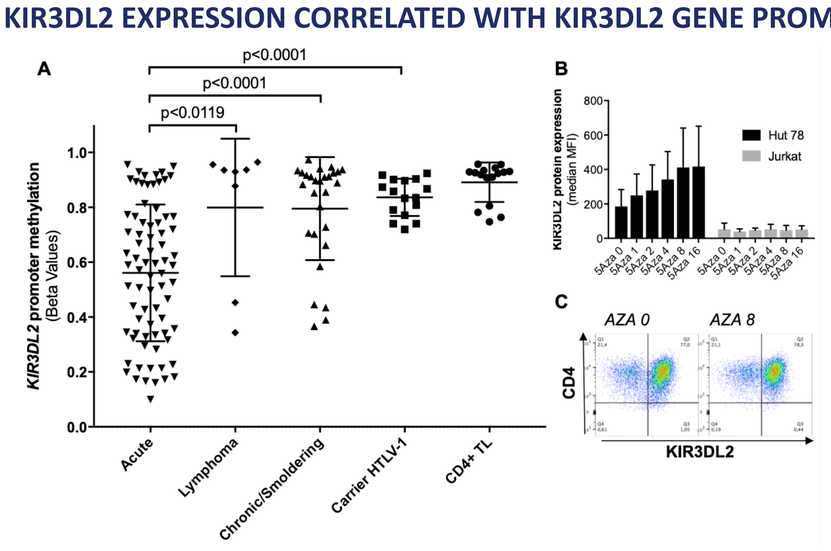
KIR3DL2 PROTEIN EXPRESSION IS ASSOCIATED WITH ACUTE-TYPE ATL



ATL cells were identified by the low expression of CD3, CD4 and activation markers (CD25 and/or HLA-DR) and the absence of CD7 (**Fig. 1A**). KIR3DL2 was the only NKR that was expressed by CD4+ CD7- CD25+ ATL tumor cells (**Fig. 1B**). In 11/21 ATL patients, abnormal lymphocytes harbored heterogeneous KIR3DL2 expression by IHC (**Fig. 1C**). KIR3DL2 expression in acute ATL is confirmed by mRNA analysis (**Fig. 1D**). KIR3DL2 expression is associated with poorer survival in ATL (**Fig. 1E**).

In almost all acute ATL patients, abnormal lymphocytes harbored KIR3DL2 positivity (n=28/30, 93%). In contrast, lymphoma and chronic/smoldering cases were often negative for KIR3DL2 (n=2/8 and n=2/12 KIR3DL2+ respectively, p=0.001).

KIR3DL2 EXPRESSION CORRELATED WITH KIR3DL2 GENE PROMOTER HYPOMETHYLATION



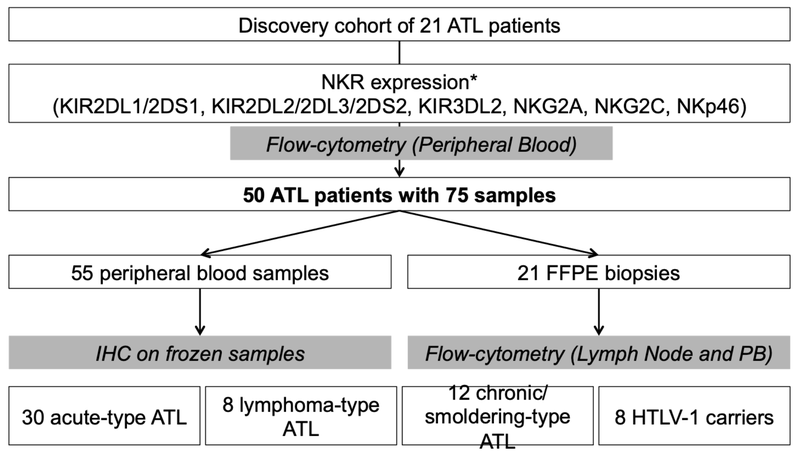
The *KIR3DL2* promoter was significantly hypomethylated in acute ATL compared to lymphoma, chronic/smoldering types and HTLV-1 AC (p=0.0119, p<0.0001, and p<0.0001, respectively; unpaired t-test) that was consistent with KIR3DL2 expression (**Fig. 2A**).

Upon 5Aza incubation, KIR3DL2 protein expression was efficiently induced with a dose-dependent effect on the cell surface of Hut 78 but not on Jurkat cells (**Fig. 2B**). Moreover, KIR3DL2 expression was not increased on primary PBMC from healthy donors and from 4 KIR3DL2+ ATL patients upon 5Aza treatment *ex-vivo* (**Fig. 2C**).

REFERENCES

1. Battistella M, et al. KIR3DL2 expression in cutaneous T-cell lymphomas: expanding the spectrum for KIR3DL2 targeting. Blood. 2017;130(26):2900–2902.
2. Bagot M, et al. IPH4102, a first-in-class anti-KIR3DL2 monoclonal antibody in patients with refractory cutaneous T cell lymphoma: An international multicentre phase 1 trial. Lancet Oncology, *in press*.

METHODS



***NKR assessment**

Multiparameter flow cytometry was performed with 8-color mixes with the anti -KIR2DL1/2DS1-PE (11BP6 Miltenyi), -KIR2DL3/2DL2/2DS2-PE (GL183 Beckmann), -NKG2A-PE (Z199 Beckmann), -NKG2C-PE (134591 R&D), -KIR3DL2-PE (13E4) and -NKp46-PE (9E2; Innate Pharma).

Methylation

Array-based analysis of genomic DNA methylation patterns of *KIR3DL2* promoter was assessed. Cell lines, PBMC from healthy donors and ATL patients, were treated with 5-aza-2-deoxycytidine (5-Aza) and analyzed for KIR3DL2 expression by flow cytometry after 72 hours of incubation.

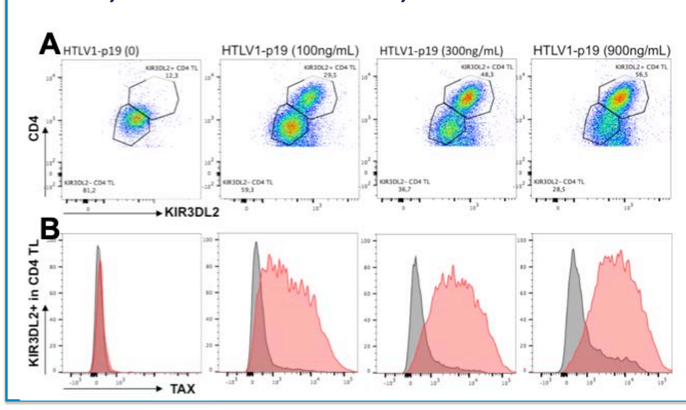
HTLV-1 infection *in-vitro*

To explore the role of HTLV-1 on KIR3DL2 expression, KIR3DL2 and *TAX* mRNA expressions were assessed by prime-flow RNA assay on primary ATL cells and on activated CD4+ T cells that were infected with HTLV-1 *in-vitro*.

***Ex-vivo* autologous antibody dependent cell cytotoxicity (ADCC)**

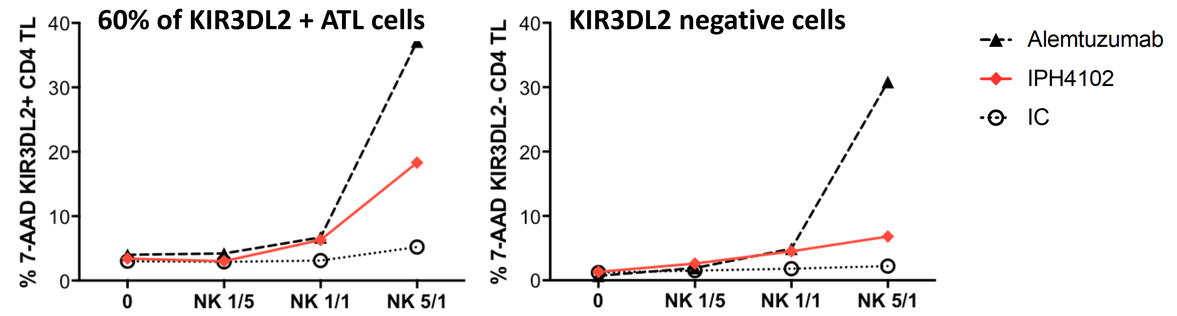
ADCC was performed on sorted primary ATL cells with IPH4102, a monoclonal anti-KIR3DL2 antibody that has shown robust clinical activity in Phase I in patients with relapsed relapsed/refractory advanced CTCL (NCT02593045)[2].

HTLV-1, BUT NOT TAX ALONE, IS ABLE TO INDUCE KIR3DL2 EXPRESSION ON CD4+ T-CELLS



Purified HTLV-1 virions induced KIR3DL2 expression by CD4+ T-cells that was dependent on the quantity of HTLV-1 (p19 equivalent, n=3; **Fig. 3A**). *TAX* mRNA was mostly expressed in KIR3DL2 positive CD4+ cells, while KIR3DL2 negative CD4+ cells were also negative for *TAX* mRNA (**Fig. 3B**)

IPH4102 EFFICIENTLY ELIMINATES KIR3DL2+ PRIMARY ATL TUMOR CELLS BY AUTOLOGOUS NK CELLS *EX VIVO*



Antitumor activity of IPH4102 against primary ATL cells was observed in all KIR3DL2 positive patient samples tested (n=3) and increased with the E/T ratio (**Fig. 4**) (IC: isotype-matched control mAb). Moreover, IPH4102 did not mediate killing of KIR3DL2 negative ATL patient samples (n=5).

CONCLUSIONS AND PERSPECTIVES

1. **KIR3DL2 expression is mainly associated with acute-type ATL.**
2. **Induction of KIR3DL2 gene transcription may be triggered by HTLV-1 infection followed by transcription maintenance due to DNA hypomethylation of the gene promoter.**
3. **The benefit of targeting KIR3DL2 by IPH4102 should be further investigated in ATL patients.**