Novel therapeutic and diagnostic mAbs against KIR3DL2, a unique tumor antigen expressed on T Cell Lymphomas

Authors

A.Marie-Cardine[2], N. Viaud[1], A. Dujardin[1], R. Joly[1], N. Granier[1], L. Gauthier[1], Bonnafous[1], M. Bléry[1], C. Paturel[1], A. Bensussan[2], M. Bagot[2] and H. Sicard[1]

[1]Innate Pharma, 117 Av de Luminy – 13009 Marseilles, France
[2]INSERM U976, Hôpital Saint Louis – 75475 Paris, France

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Abstract

KIR3DL2 belongs to the killer immunoglobulin (Ig)-like receptors (KIRs) family and is composed of 3 extracellular Ig-like domains. KIR3DL2 is naturally expressed on some NK cells and minor subpopulations of CD8+ and CD4+ T cells. Physiologically, KIRD3L2 is an inhibitory receptor for human leukocyte antigen (HLA) class I molecules regulating NK cell activation. Remarkably, KIR3DL2 is also aberrantly expressed on several subtypes of T lymphomas/leukemias, such as Sézary Syndrome, transformed Mycosis Fungoides and HTLV1+ Adult T Cell Leukemia, making it a unique therapeutic target in cancer.

We have generated a series of anti-KIR3DL2 monoclonal antibodies (mAbs) binding selectively to KIR3DL2, spanning epitopes on all 3 Ig domains. Their efficacy was evaluated *in vitro* and *in vivo* against KIR3DL2-expressing tumors and Sézary cell lines as disease model. An autologous assay was set up to evaluate the killing of primary Sézary cells by patients' NK effectors in the presence of those mAbs. Potent antibody-dependent cell cytotoxicity (ADCC) was found the main mode of action involved in their anti-tumor activity.

The most promising candidates were humanized as IgG1 mAbs and the final lead molecule was selected for further development, based on several predefined criteria. In parallel, anti-KIR3DL2 mAbs were also developed as unique and sensitive tools for the detection by immunohistochemistry of KIR3DL2 on tumor biopsies. Owing to the promising efficacy profile of our anti-KIR3DL2 mAb candidate and to the restricted expression pattern of the target on some T leukemia/lymphoma cells, a mAb-based therapy targeting KIR3DL2 stands as a unique strategy in several orphan diseases with high unmet medical need.

KIR3DL2 is a specific tumor antigen expressed on advanced CTCL subtypes

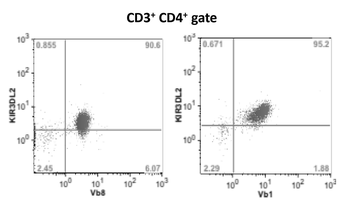
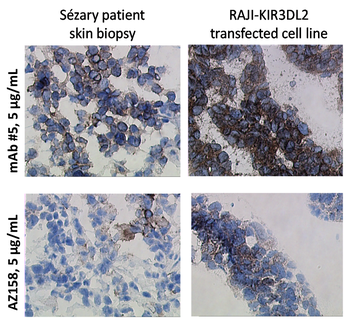


Fig. 1: Clonal Vbeta+ CD4+ blood Sézary cells co-express KIR3DL2, as shown by flow cytometry analysis with mAb clone #4.

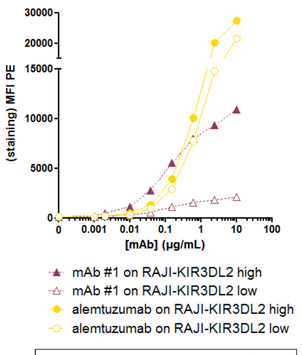
Fig. 2: mAb clone #5 allows more sensitive and selective IHC detection of KIR3DL2 on frozen patient biopsies than historical anti-KIR3DL2 mAb AZ158

Historical studies with mAb AZ158:
Musette, P et al, (2003). Blood
Nikolova, M et al, (2002). Leuk Lymphoma
Wechsler, J et al, (2003). J Pathol



Anti-KIR3DL2 mAbs kill KIR3DL2+ cell lines through allo-ADCC, even at low antigen density

Fig. 3: Comparative binding of alemtuzumab and anti-KIR3DL2 mAb clone #1 on RAJI-KIR3DL2high and RAJI-KIR3DL2low cell line sub clones.

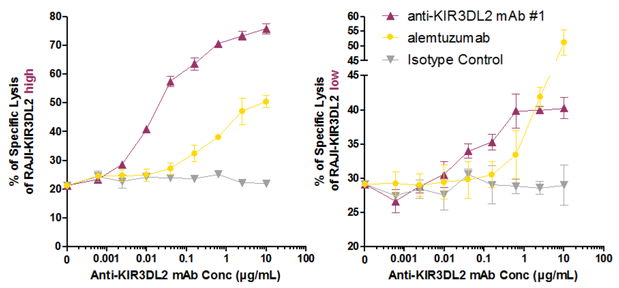


KIR3DL2 Ag densities (sites/cell):
RAJI-KIR3DL2 high = ~28,000
RAJI-KIR3DL2 low = ~6,000

KIR3DL2-transfected RAJI cell lines were sub cloned to generate target cells with various levels of KIR3DL2 antigen (Ag) expression.

Anti-CD52 alemtuzumab is used as positive control in these allo-ADCC experiments with NK cells purified from healthy donors (effector/target ratio = 10/1).

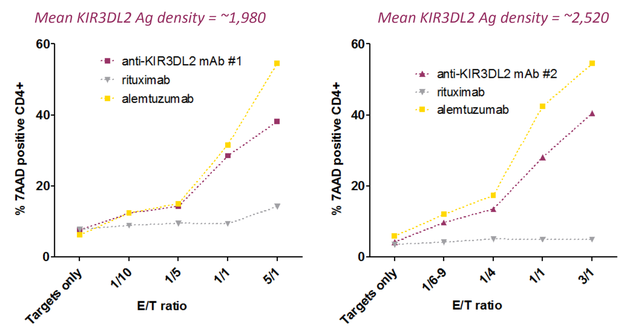
Fig 4: The most potent anti-KIR3DL2 mAb #1 kills KIR3DL2-transfected RAJI sub clones through ADCC, including at low tumor Ag density.



Anti-KIR3DL2 mAbs mediate efficient killing of primary Sézary cells with autologous NK

Fig. 5: Despite low KIR3DL2 Ag density, anti-KIR3DL2 mAb clones #1 and #2 induce potent killing of primary Sézary leukemic cells with autologous NK cells, nearly as efficiently as alemtuzumab, which targets highly and widely expressed CD52.

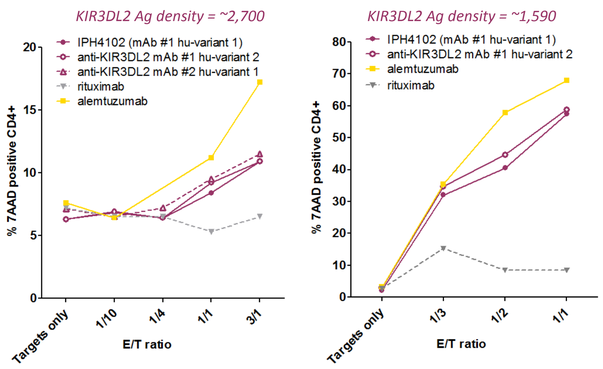
Mean data from n = 4 different patients in each figure.



KIR3DL2 Ag density on primary Sézary cell ranges from 1,000 to 4,000 sites/cell, analyzed with QiFiKit (Dako) on blood samples from n = 15 different Sézary patients.

Fig. 7: Humanized variants of mAb clones #1 and #2 exert similar ADCC efficacy against primary Sézary cells with autologous NK cells.

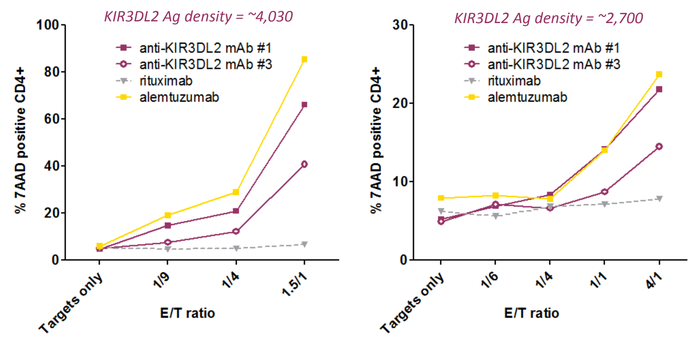
n = 1 patient by figure, which also illustrates inter-individual heterogeneity.



Anti-KIR3DL2 mAbs were evaluated in an autologous ADCC assay using NK cells and primary leukemic cells sorted (by negative selection) from Sézary patient blood samples. Increasing effector-to-target ratios (E/T) were performed according to the numbers of recovered cells. 7AAD incorporation was used as surrogate marker of cell death.

All mAbs were incubated with the cells at 10 µg/mL, including positive control anti-CD52 alemtuzumab and negative control anti-CD20 rituximab, for 4 to 5 hours.

Fig. 6: Anti-KIR3DL2 mAb clone #3 is less efficient in the autologous setting than mAb clone #1 (as in the mouse xenograft model, see also Fig. 8 below) (*n = 1 patient by figure*)



This autologous *ex vivo* killing assay demonstrates that a mAb-based targeted therapy using KIR3DL2 as tumor antigen is feasible in advanced Cutaneous T-Cell Lymphoma patients:

- **NK cells from Sézary patients are functional and able to mediate ADCC**
- **Primary leukemic Sézary cells are sensitive to ADCC induced by anti-KIR3DL2 mAbs**

Also, the efficacy results from various anti-KIR3DL2 mAbs tested in this *ex vivo* assay are consistent with those from other experimental settings and confirm that anti-KIR3DL2 mAb clones #1 and #2 are more efficient than mAb clone #3 (see xenograft models, Fig. 8)

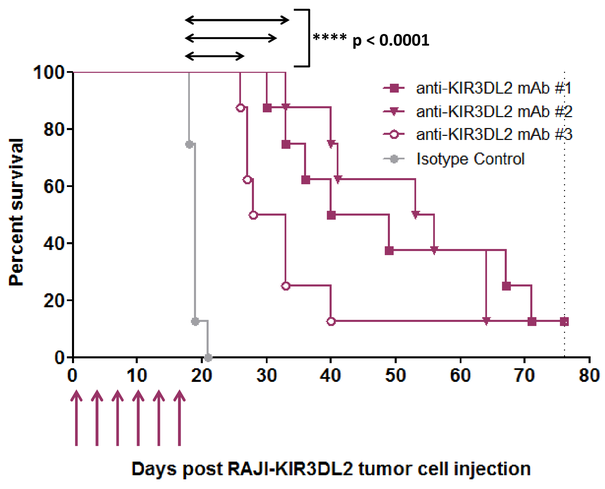
Anti-KIR3DL2 mAbs improve mouse survival in KIR3DL2+ xenograft models

N = 8 SCID mice per group were engrafted IV with 5 M RAJI-KIR3DL2 high cells on Day 0.

From Day 1, mice received twice a week for 3 weeks IP injections of 300 µg pre-selected mAb clones #1, #2 and #3 or irrelevant hu-IgG1 isotype control (IC).

Fig. 8: Mouse survival was monitored for up to 76 days after tumor cell graft and the following median survivals were calculated for each experimental group:
- IC: 19 days
- mAb #1: 44.5 days
- mAb #2: 54.5 days
- mAb #3: 30.5 days

Statistical analyses of increased survival were performed as compared to IC in a Log-rank (Mantel Cox) test.



Conclusions

IPH4102 is the lead humanized anti-KIR3DL2 mAb selected for development

- > 6,000 hybridomas were screened post immunization of mice with KIR3DL2
- Extensive selection was performed, based on non clinical efficacy profile in different models, as presented here
- Additional predefined criteria were included (affinity, functional properties, bound epitopes, lack of unwanted tissue cross-reactivity…) to select 3 mAbs for humanization (#1, 2 & 3)
- Most humanized variants of anti-KIR3DL2 favorite mAb clones #1 and #2 retained full affinity and efficacy profile
- Final selection was made based on best industrial feasibility (pre-CMC attributes) and the highest proportion of Human sequences
- IPH4102 is a humanized variant of anti-KIR3DL2 mAb clone #1, with potent efficacy against KIR3DL2+ tumors
- IPH4102 gathers all pre-defined criteria for further regulatory development
- Biomarker mAb tools were generated in parallel for flow cytometry and IHC detection of KIR3DL2

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