Novel therapeutic and diagnostic mAbs against KIR3DL2

a unique tumor antigen overexpressed on T-Cell Lymphomas

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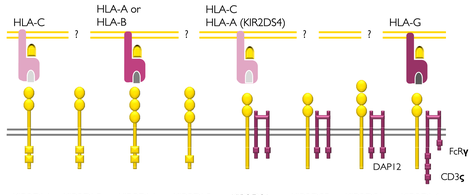
Abstract

KIR3DL2 belongs to the killer immunoglobulin (Ig)-like receptors (KIRs) family and bears 3 extracellular Ig-like domains. KIR3DL2 is naturally expressed on some NK cells and minor subpopulations of CD8+ and CD4+ T cells. Physiologically, KIR3DL2 is an inhibitory receptor for human leukocyte antigen (HLA) class I regulating NK cell activation. Remarkably, KIR3DL2 is also aberrantly overexpressed on subtypes of T lymphomas/leukemias, such as Sezary Syndrome, transformed Mycosis Fungoides and HTLV-1+ Adult T Cell Leukemia (Obama, Brit. J. Hematol. 2007), making it a unique therapeutic target in cancer.

We have generated a series of anti-KIR3DL2 monoclonal antibodies (mAbs) binding selectively to KIR3DL2. Their efficacy was evaluated *in vitro* and *in vivo* against KIR3DL2-expressing tumors and Sezary cell lines as disease model. Various modes of action, such as complement-dependent cytotoxicity (CDC) and antibody-dependent cell cytotoxicity (ADCC) were found involved in their anti-tumor activity. In parallel, other anti-KIR3DL2 mAbs were also developed as sensitive tools for the detection by immunohistochemistry (IHC) of KIR3DL2 on tumor biopsies.

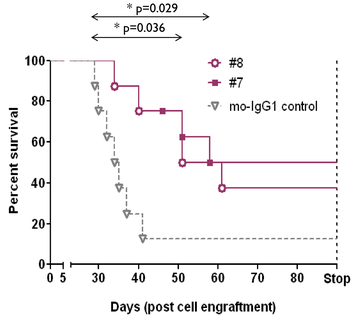
Owing to the highly restricted expression pattern of the target on some T leukemia/lymphoma cells and to the promising efficacy profile of our anti-KIR3DL2 mAb candidates, an antibody-based therapy targeting KIR3DL2 stands as a potentially unequalled strategy in several orphan diseases with critical unmet medical need.

The KIR molecules and their ligands



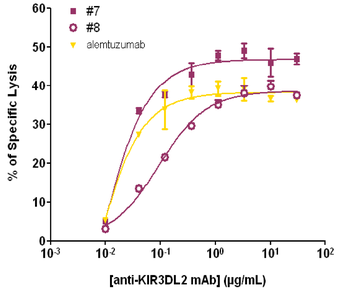
(Adapted from Thielens *et al.*, Curr. Op. Immunol. 2012)

Efficacy *in vivo* in mouse xenograft models



Murine mAbs #7 and #8 (300 µg/injection, twice/week) were tested in NOD-SCID mice engrafted with 5.10^6 B221-KIR3DL2 cells IV (n = 8 mice per group). Control mice were treated with same doses of isotype control IgG1. Mice survival was followed for 90 days post-tumor engraftment. Differences in median survival were analyzed statistically with Log-Rank (Mantel Cox) test.

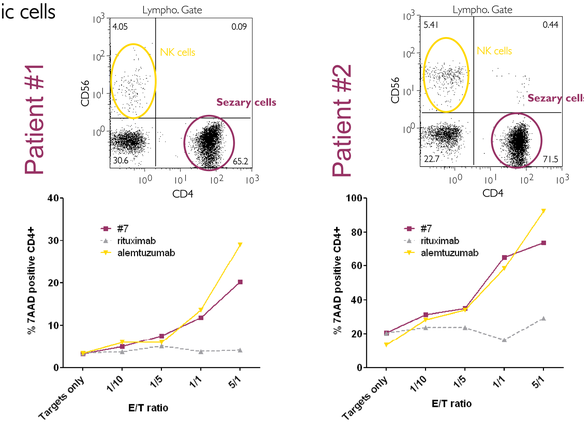
Allo-ADCC *in vitro* against HUT78 Sezary cell line



Dose-ranging ADCC activity of mAbs #7 and #8 against HUT78, with allo-NK cells purified from healthy volunteers' blood, at effector/target (E/T) ratio = 10/1. The activity of anti-CD52 alemtuzumab is evaluated as positive control.

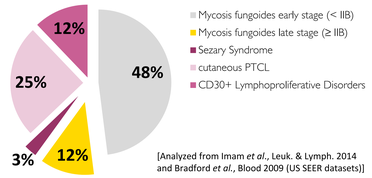
Autologous *ex vivo* NK killing of primary Sezary leukemic cells

Sezary patient PBMC were prepared immediately after blood sampling and analyzed by flow cytometry: representative images of double staining for CD56 and CD4 cells are displayed on the upper panels.
NK ($CD56^+CD3^-$) and leukemic ($CD4^+$) cells were sorted and used in an autologous cytotoxic assay: anti-KIR3DL2 mAb #7 (10 µg/mL) was incubated for 4 to 6 h with primary Sezary cells and sorted autologous NK cells at increasing E/T ratios. Anti-CD52 alemtuzumab and anti-CD20 rituximab were used respectively as positive and negative controls. Dying cells were monitored through 7AAD incorporation (lower panels).



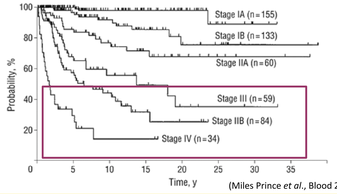
Epidemiology & Clinical Data

CTCL Incidence: ~7,000 new cases/year EU+US



[Analyzed from Imam *et al.*, Leuk. & Lymph. 2014 and Bradford *et al.*, Blood 2009 (US SEER datasets)]

Survival in MF & SS according to clinical stage at diagnosis



(Miles Prince *et al.*, Blood 2009)

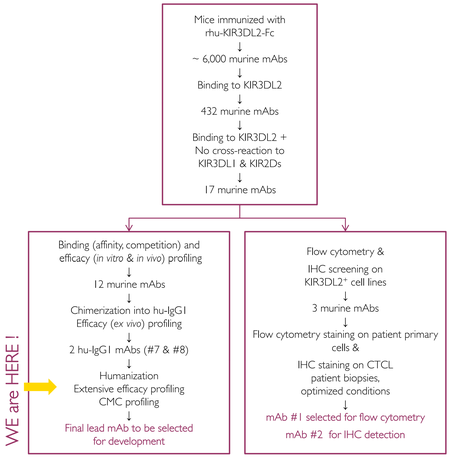
Key numbers in HTLV-1^+ ATL

- Adult T-Cell Leukemia (ATL): clonal malignant expansion of CD4+ induced by the HTLV-1 retrovirus
- ~20 million HTLV-1 infected people around the world, found in specific endemic areas. Highest prevalence in Japan, Africa, the Caribbean Islands, Central & South America:

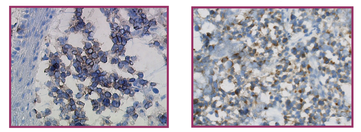
(Utsch Gonçalves *et al.*, Clin. Microbiol. Rev. 2010)

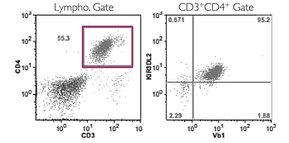
- 2 to 5% HTLV-1 carriers will develop ATL (O'Connor, Lymphoma 2008), inducing for example 1,000 new cases per year in Japan
- Four-year survival for the most aggressive subtypes of ATL is less than10%

mAb selection process



Biomarker and diagnostic tools





IHC images (upper panels) of biopsies from (a) Sezary Syndrome and (b) transformed Mycosis Fungoides patients stained with mAb #2. Flow cytometry detection with mAb #1 (lower panels) of KIR3DL2 biomarker on the $CD4^+$ leukemic clone defined with Vβ expression in a Sezary patient blood sample illustrates its remarkable homogeneity of expression.

Perspectives

We have generated a series of novel unique anti-KIR3DL2 mAbs. Based on *in vitro*, *ex vivo* and *in vivo* results, we have humanized 2 mAbs (#7 & #8) for further development. The final anti-KIR3DL2 mAb lead candidate will be chosen by mid-2013 and IND-enabling regulatory studies will be initiated. Two mAbs have also been selected for tumor biomarker identification and diagnostic purposes allowing specific detection of KIR3DL2 by IHC on tumor biopsies (#2) or by flow cytometry on circulating leukemic cells (#1).

We have successfully completed most steps in the selection of our mAb candidate that will be developed for the treatment of KIR3DL2 positive cancers: these include several T-cell leukemia/lymphoma subtypes that have so far been subject to very few dedicated therapeutic developments.