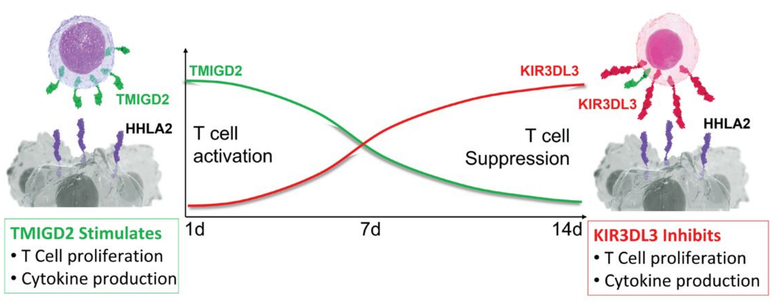
NPX887, a fully human monoclonal antibody targeting HHLA2, blocks KIR3DL3-mediated immunosuppression and initiates antibody-dependent cellular cytotoxicity of HHLA2$^+$ tumors

McKenna Lamb[1], Melissa W. Hayes[1], Rachel O'Connor[1], Emilien Loeuillard[1], Jamie Strand[1], Riale Gilligan[1], Arianna Manlangit[1], Austin Dulak[1], Yasmin Hashambhoy-Ramsay[1], Katalin Kis-Toth[1], Bijan Etemad-Gilbertson[1], Matthew Rausch[1]

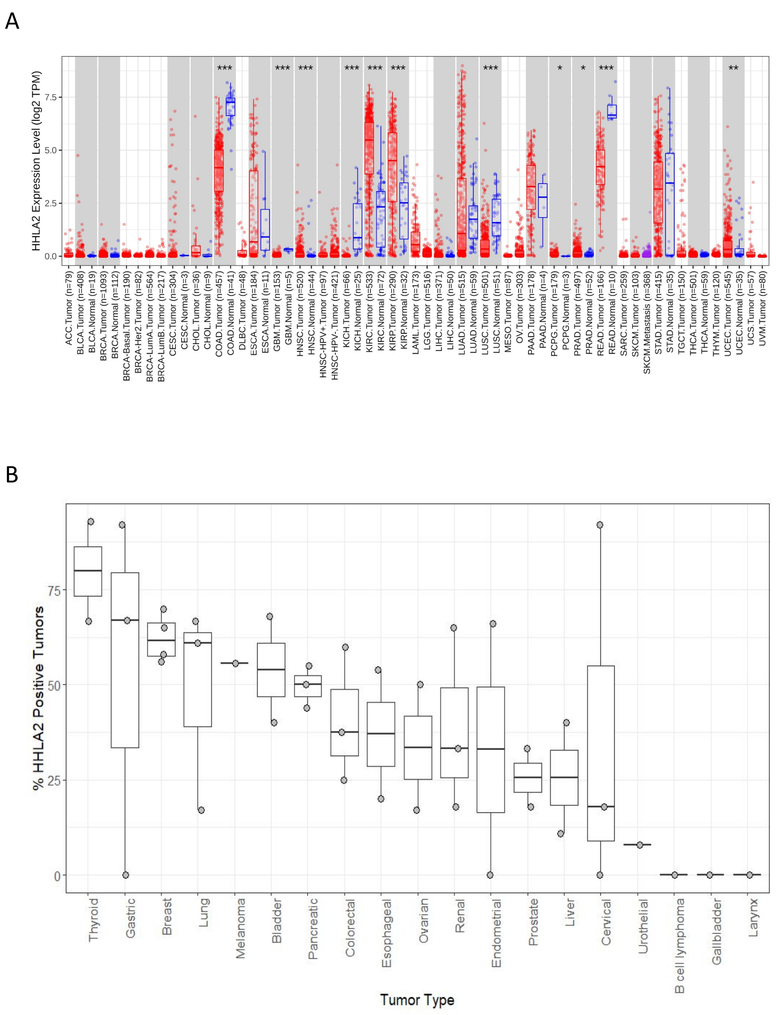
[1]NextPoint Therapeutics, Cambridge, MA

Background

Human endogenous retrovirus H long terminal repeat associating protein 2 (HHLA2) is a member of the B7 family that has both immune suppressive and stimulatory functions on T and NK cells through interaction with either the co-inhibitory receptor killer cell immunoglobulin-like receptor, three immunoglobulin domains and long cytoplasmic tail 3 (KIR3DL3) or the co-stimulatory receptor transmembrane and immunoglobulin domain containing 2 (TMIGD2). HHLA2 binding to KIR3DL3 blunts downstream immune activation while the interaction of HHLA2 with TMIGD2 enhances T and NK cell activity. The HHLA2-KIR3DL3 axis is emerging as an important immune checkpoint in cancer. While HHLA2 expression in normal tissues is limited, it is highly expressed in many cancers where it is often associated with more severe pathology, fewer tumor infiltrating lymphocytes, and poor patient outcome.



HHLA2 Expression is Elevated in Tumors

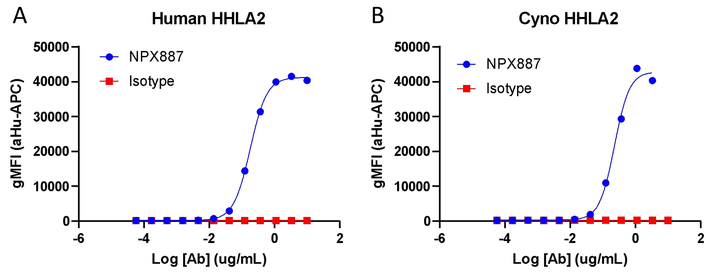


A) HHLA2 gene expression was analyzed using the "Gene_DE" module of TIMER 2.0[1] to explore the differences in HHLA2 expression across tumors of TCGA cohorts and their adjacent normal tissues. Statistical significance is computed by the Wilcoxon test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). B) % HHLA2 positive tumors reflect publicly available IHC data, specifically percent positive samples[2,3] and fraction of samples that showed 2+ or 3+ staining[4].

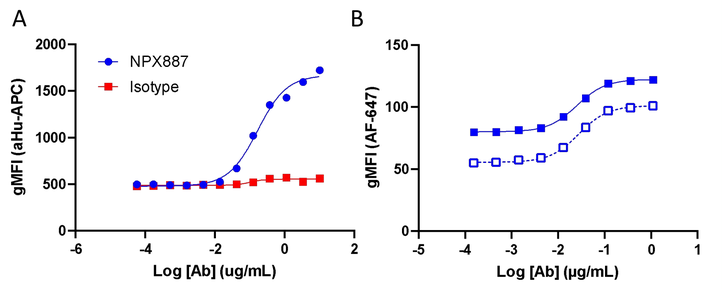
References

1. Li et al, Nucleic Acids Res, 2020 (PMID 32442275).
2. Janakiram et al, Clin Can Res, 2015 (PMID 25549724).
3. Uhlén M et al, Science, 2015 (PMID 25613900).
4. Zhang et al, Front Cell Dev Biol, 2020 (PMID 32509772).
5. Bhatt er al, Cen Immunol Res, 2020 (PMID 33229411).

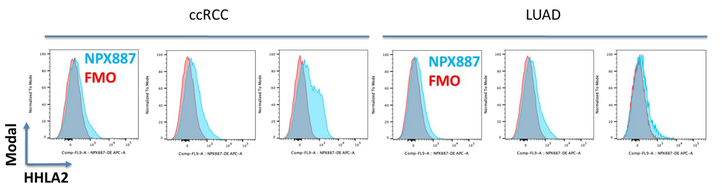
NPX887 Binds to HHLA2$^+$ Cells



A) Human HHLA2-300.19 cells or B) Cynomolgus HHLA2-300.19 cells were treated with NPX887 or human IgG1 isotype control. Flow cytometric analysis of NPX887 binding was detected using an anti-human APC secondary antibody. NPX887 binds to human or cynomolgus HHLA2 in a concentration dependent manner.

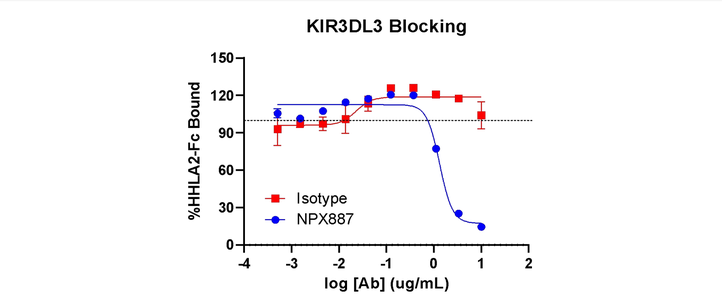


A204 (●), HT-29 (□), and HCC827 cells (■) were treated with NPX887 or human IgG1 isotype control (isotype not shown for HT-29 or HCC-827). Flow cytometric analysis of NPX887 binding was detected using an anti-human IgG APC (A) or using NPX887 conjugated to Alexa Fluor 647 (B). NPX887 binds cell lines endogenously expressing HHLA2.



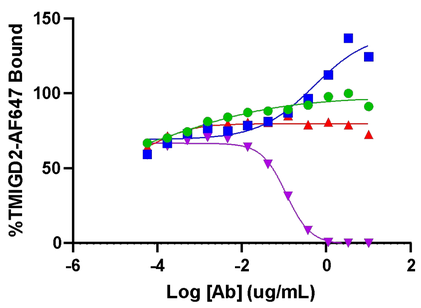
Primary human disassociated tumor cells (CD45 negative, EpCAM$^+$) were treated with 10 µg/mL NPX887 or no antibody (FMO). Flow cytometric analysis of NPX887 binding was detected using NPX887 conjugated to Alexa Fluor 647. NPX887 binds primary human dissociated tumor cells.

NPX887 Blocks The Interaction of HHLA2 With KIR3DL3



Biotinylated HHLA2 (5µg/mL) was pretreated with NPX887 or IgG1 isotype control then added to KIR3DL3-300.19 cells. Bound HHLA2 was detected using APC-streptavidin. Percent inhibition was calculated using APC gMFI. Max/Min inhibition was determined in the absence of NPX887 or isotype, with and without APC-streptavidin.

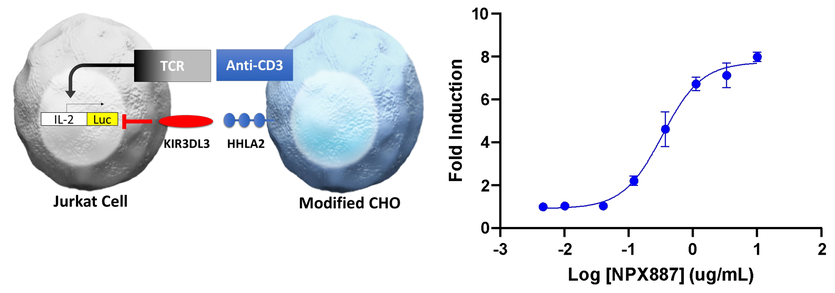
NPX887 Spares HHLA2 Binding With TMIGD2



HHLA2 antibody	Ligand Blocking	
	KIR3DL3	TMIGD2
NPX887	√	-
2C4	√	-
6F10	√	√
Isotype	N/A	N/A

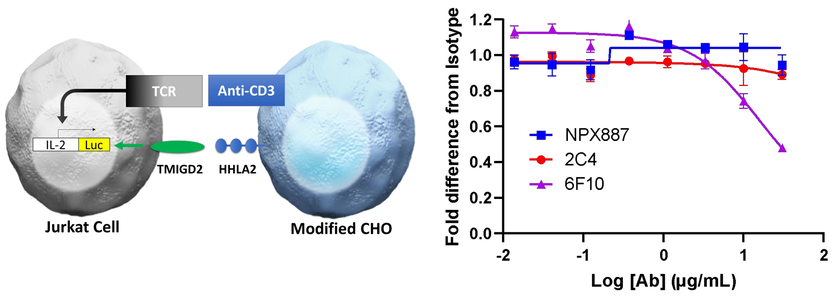
HHLA2-300.19 cells were pretreated with NPX887, IgG1 isotype, 2C4, and 6F10 antibodies, then treated with 5µg/mL recombinant TMIGD2 protein labeled with Alexa Fluor 647. Max/Min binding was set using samples with a saturating dose of isotype (0% inhibition) or a saturating dose of the dual blocking 6F10 antibody (100% inhibition).

NPX887 Blocks KIR3DL3-mediated Supression



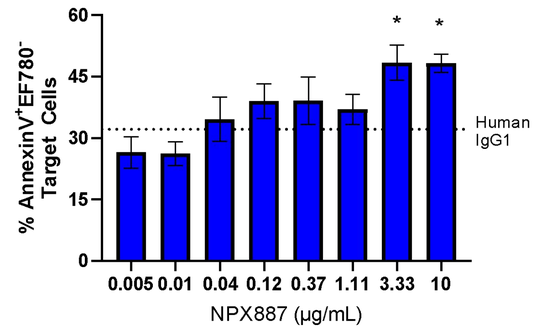
NPX887 treated CHO/TCR/HHLA2 cells were co-cultured with Jurkat/IL-2/KIR3DL3 cells and an anti-CD28 agonist antibody. NPX887 relieves the HHLA2/KIR3DL3 mediated suppression of Jurkat/IL-2/KIR3DL3 activation.

NPX887 Spares TMIGD2-mediated Co-stimulation



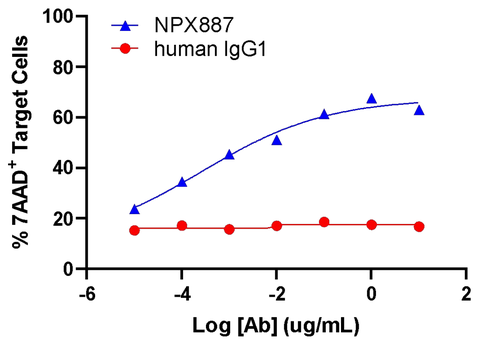
NPX887 or isotype treated CHO/TCR/HHLA2 cells were co-cultured with Jurkat/NFAT/TMIGD2 cells. NPX887 treatment had no effect on HHLA2/TMIGD2-mediated costimulation similar to the HHLA2 specific antibody 2C4. The HHLA2 specific antibody 6F10 which blocks TMIGD2 signaling inhibited Jurkat/NFAT/TMIGD2 co-stimulation in this assay.

NPX887 NK cell-Mediated Killing of HHLA2$^+$ Tumors



NK-92 MI cells were incubated with K-562-HHLA2-GFP cells at an E:T ratio of 1:1000 cells in the presence of NPX887. Early apoptotic cells were identified as CD56$^-$/GFP$^+$/Annexin V$^+$ and negative for a fixable viability stain. Statistical significance was calculated by two-sided Student's T-test. NPX887 enhanced NK-92 MI killing of HHLA2$^+$ K-562 tumor cells. (* $p<0.05$ compared to isotype control)

NPX887 Induces ADCC of HHLA2$^+$ Tumors



Cell Trace Violet labeled HT-29 cells were incubated with NPX887 or human IgG1 before adding PBMC from a healthy human donor at an E:T ratio of 50:1. Cells were stained with 7-AAD to detect frequency of dead HT-29 cells (CTV$^+$/7AAD$^+$). NPX887 treatment of HT-29 tumor cells induces PBMC-mediated killing in a dose dependent manner compared to human IgG1 isotype control with an EC_{50} of 0.27 ng/mL, or 1.8 nM.

Conclusions

The HHLA2 axis represents a novel immune checkpoint that mediates tumor immune evasion by suppressing both NK and T cell activity. Furthermore, HHLA2 is an attractive tumor antigen given its limited expression in normal tissue and enhanced expression in many tumors. NPX887, a fully human, monoclonal antibody targeting HHLA2 represents an attractive approach to treat certain HHLA2$^+$ cancers by both inducing ADCC and by potentiating anti-tumor immune responses.

nextpoint