Bispecific claudin-6 x CD3 antibodies in a 2+1 format demonstrate selectivity and activity on human ovarian cancer cells

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Abstract #1860

Introduction

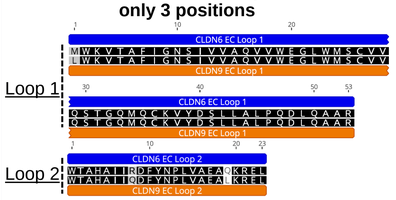
- Claudin-6 (CLDN6) is a tetraspan membrane protein involved in the formation of tight junctions. IHC and bulk RNAseq data show CLDN6 is differentially expressed in ovarian cancers compared to normal tissue.
- There is a large unmet need for targeted therapies to treat ovarian cancer and other solid tumors, and the differential expression of CLDN6 in cancerous tissue makes it a promising target for CD3 bispecific antibody therapeutics.
- A complicating factor is that many members of the claudin family have high sequence identity, with CLDN9 having the most similarity to CLDN6. CLDN6 and CLDN9 extracellular (EC) loops differ at only 3 out of 76 residues. CLDN9 is highly expressed in some normal tissue, which makes selectivity for CLDN6 over CLDN9 a critical component of any CLDN6 targeting antibody-based therapeutic.
- To overcome these challenges, we engineered a highly selective anti-CLDN6 antibody and formatted it into in our XmAb® 2+1 bispecific antibody format. These selective CLDN6 x CD3 bispecifics were tested for in vitro and in vivo activity in multiple models.

RNA-seq reveals CLDN6 as a selective ovarian cancer target, but close homolog CLDN9 is expressed on normal tissues

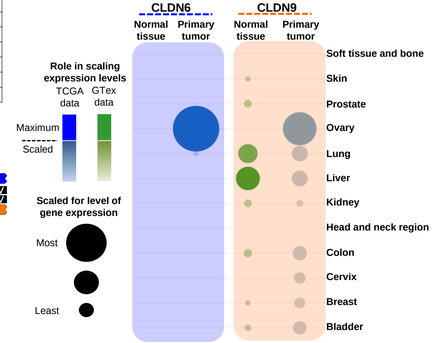
CLDNs most similar to CLDN6

Other CLDNs	Pairwise Identity Shared with CLDN6 (%)	EC Loop Pairwise Identity Shared with CLDN6 (%)
CLDN9	71.8	96.0
CLDN4	59.1	81.6
CLDN3	57.1	78.4
CLDN5	52.7	61.3
CLDN8	44.9	68.4
CLDN17	44.4	51.3

CLDN6 and CLDN9 EC loops differ at only 3 positions

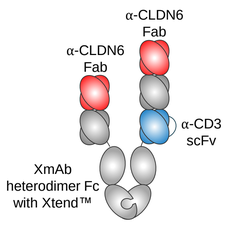


Requirement of selectivity for CLDN6 over CLDN9 revealed by gene expression data

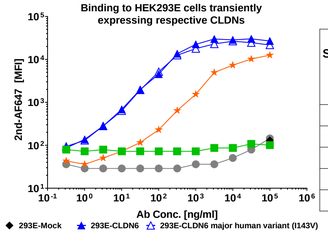


A partially-selective α-CLDN6 mAb was humanized and placed into our XmAb® 2+1 bispecific format

XmAb® 2+1 bispecific



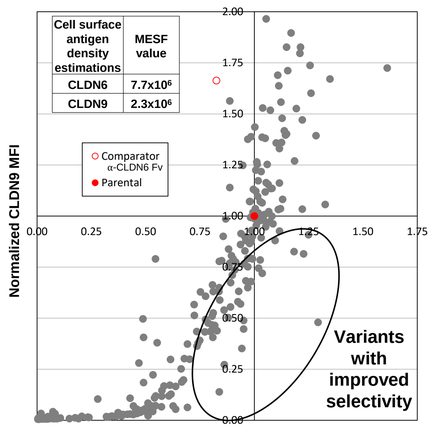
Parental 2+1 bispecific has moderate selectivity



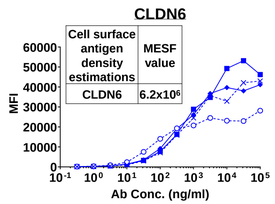
Surface antigen density estimation	MESF value
CLDN6	8.7x10⁶
CLDN6_I143V	7.6x10⁶
CLDN3	3.7x10⁶
CLDN4	4.5x10⁶
CLDN9	1.8x10⁶

Fv engineering further improves CLDN6 selectivity over CLDN9

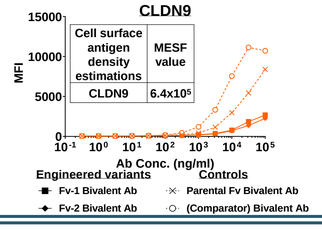
Single concentration screen of ~300 variants, MFI values normalized to the parental Fv



Engineered variants maintain parental levels of CLDN6 binding

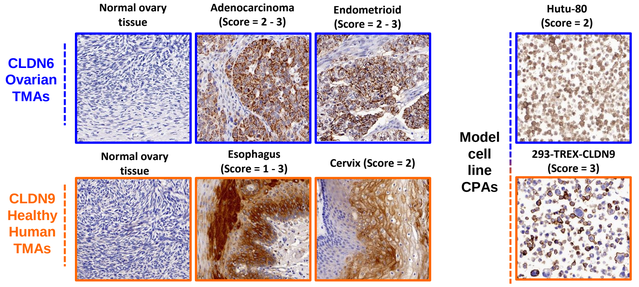


Engineered variants have significantly reduced CLDN9 binding



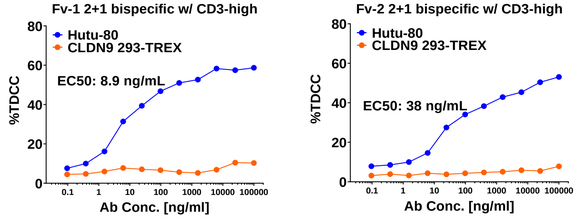
Selective CLDN6 x CD3 bispecifics induce TDCC of CLDN6⁺ cancer cell lines and spare cells with normal tissue levels of CLDN9

IHC confirms RNA-seq data: no CLDN6 expression in healthy tissue, but strong expression in ovarian cancer tissue, and CLDN9 activity must be avoided due to high normal tissue expression.



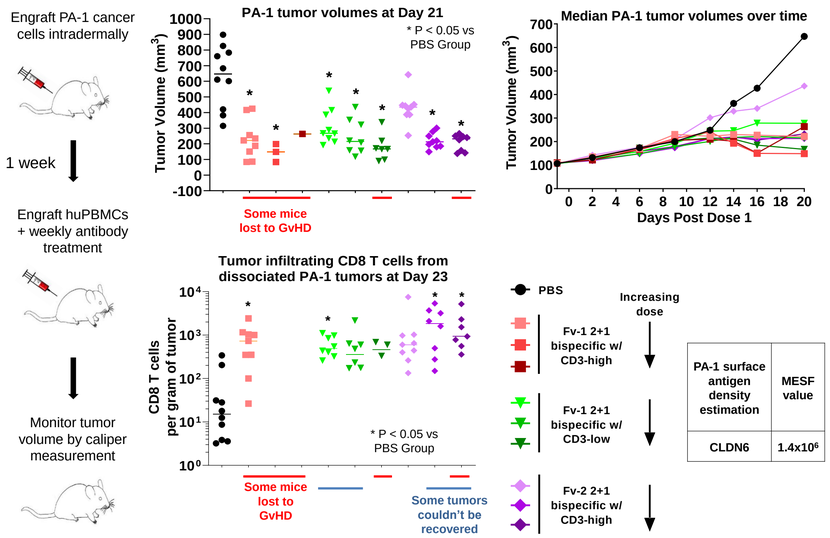
Surface antigen density estimation	MESF value
Hutu-80 (CLDN6)	2.2x10⁵
293-TREX-CLDN9	3.3x10⁵

TDCC with model cell lines verifies selectivity for CLDN6 over CLDN9



- **Hutu-80 was chosen as a representative CLDN6⁺ cell line to match ovarian tumor expression**
- **293-TREX CLDN9⁺ cell line used to match normal tissue CLDN9 expression levels**

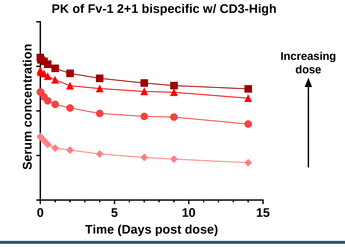
CLDN6 x CD3 lead candidates decrease established tumor size



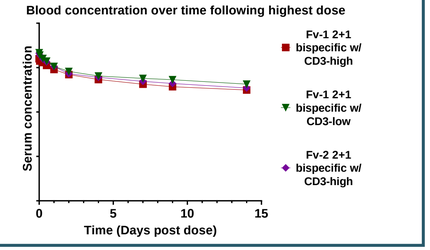
PA-1 surface antigen density estimation	MESF value
CLDN6	1.4x10⁶

CLDN6 x CD3 bispecifics are well-tolerated in non-human primates and have in vivo half-lives of ~2 weeks

PK dose response with most potent bispecific



PK of all bispecifics at highest dose



Summary

The engineered XmAb® 2+1 CLDN6 x CD3 bispecific antibodies are:

- Highly selective for CLDN6 over all other CLDNs.
- Effective in recruiting T cells to kill cancer cells with CLDN6 levels similar to those observed in ovarian cancer patient populations.
- Effective at reducing tumor growth in in vivo xenograft mouse models of ovarian cancer.
- Well-tolerated in non-human primates with favorable PK.

These results support further evaluation of these bispecifics for treatment of ovarian cancer.

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