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AK130, a first-in-class Fc-mutant anti-TIGIT antibody fused with TGF-βRII protein, elicits potent anti-tumor efficacy in pre-clinical studies

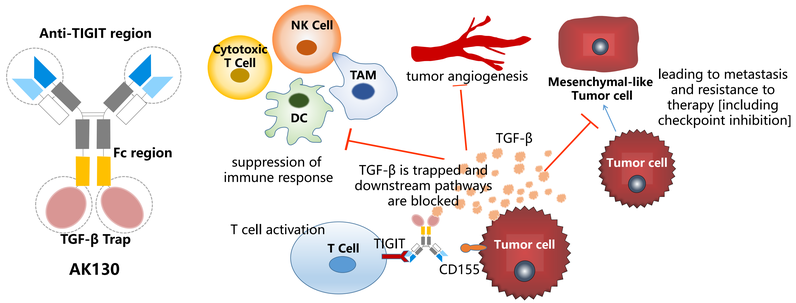
Jing Min, Tingting Zhong, Zhaoliang Huang, Xinghua Pang, Chunshan Jin, Na Chen, Dennis Xia, Peng Zhang, Max Wang, Michelle Xia, Baiyong Li.

Akesobio

Introduction

TIGIT (T-cell immunoglobulin and ITIM domain), which is expressed on T and NK cells, can interact with its ligands (i.e., CD155 and CD122), leading to inhibitory signaling in T cells and promoting exhaustion of lymphocytes. Although TIGIT is considered as a promising immune checkpoint molecule, the single-agent efficacy of anti-TIGIT therapy is limited. TGF-β (Transforming growth factor-beta), served as an immune regulator in the tumor microenvironment (TME), is elevated in various tumor types and contributes to the resistance to checkpoint inhibitors. Consequently, a novel anti-TIGIT antibody fused with TGF-βRII protein (AK130) was designed to inhibit TIGIT-mediated immunosuppression while decreasing the TGF-β levels in the TME. Mutations were introduced in the Fc region of the antibody with IgG4 backbone, in order to avoid antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) to minimize lymphocyte loss.

Figure 1. Schematic diagram of the structure and mechanism of action of AK130.

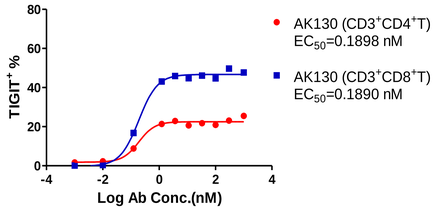


Methods

Binding activity of AK130 with TIGIT or TGF-β were assessed by Fortebio and flow cytometry. The bioactivity of AK130 to block the TIGIT or TGF-β signaling pathways was determined in luciferase reporter cell assays. Effector functions of AK130 were measured in ADCC and CDC assays. The anti-tumor activity of AK130 was investigated in BALB/c-hTIGIT transgenic mice implanted with H22 (a mouse hepatocarcinoma cell line) cells. Mice were treated with isotype control antibody, anti-HEL&TGF-βRII that could bind to TGF-β but not TIGIT or AK130 (4 mg/kg; 36 mg/kg) via intraperitoneal injection. The tumor volume was measured.

Results

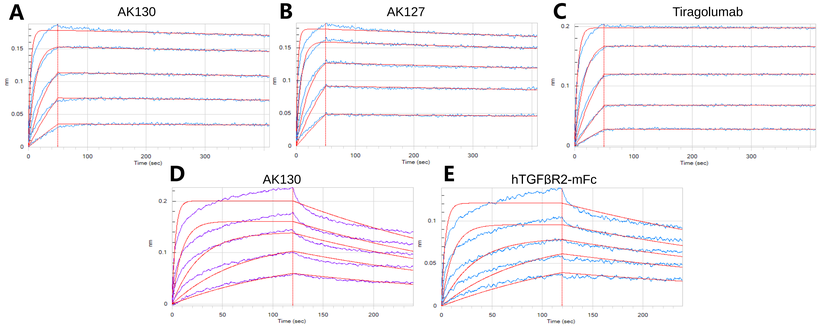
Figure 2. Binding activity of AK130 to TIGIT expressed on human T cells.



Note: both CD3+CD4+ T and CD3+CD8+ T cells were found to endogenously express TIGIT on cell surface.

Results

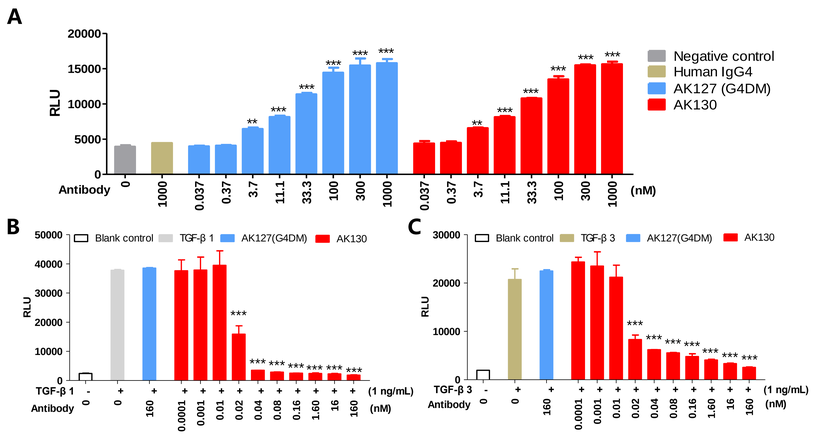
Figure 3. Binding activity of AK130 to TIGIT or TGF-β detected by Fortebio.



Target	Figure	Antibody	KD (M)	kon (1/ms)	kdis (1/s)	Rmax (nm)
TIGIT	A	AK130	9.07E-11	1.34E+06	1.22E-04	0.15-0.23
	B	AK127	9.54E-11	1.87E+06	1.78E-04	0.15-0.24
	C	Tiragulomab	1.77E-12	9.47E+05	1.68E-06	0.17-0.26
TGF-β	D	AK130	2.47E-09	1.52E+06	3.76E-03	0.16-0.20
	E	hTGFβR2-mFc	1.91E-09	1.31E+06	2.49E-03	0.09-0.14

Note: AK127 is an IgG1 mAb targeting TIGIT developed by AkesoBio. (A-C) human TIGIT-mFc-Biotin or (D, E) hTGFβ1FL-NHis-biotin were immobilized onto SA sensor, gradient concentrations of antibodies or protein (2.47, 7.41, 22.23, 66.69, 200 nM) were then flowed over the chip surface.

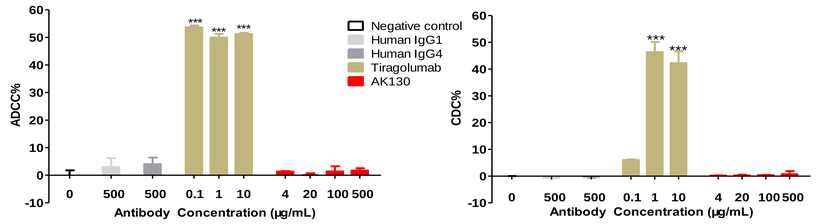
Figure 4. Bioactivity of AK130 to block the TIGIT/TGF-β-mediated signaling pathway was determined in reporter gene assay.



Note: AK127(G4DM) is the parental antibody for AK130. (A) When CHO-K1-aAPC-PDL1-PVR cells and Jurkat-TIGIT-NFAT-Luc cells were co-cultured , the TIGIT/PVR interaction inhibits NFAT-mediated luminescence, blocking the interation of TIGIT with PVR leads to enhancement of luminescence; (B-C) When 293T-hTGFβRII-Luc cells cultured with TGF-β1 (B) or TGF-β3 (C), the TGF-β/TGFβRII interaction activates SMAD-mediated luminescence, while blocking the interaction TGF-β with TGFβRII leads to reduction of luminescence.
*Data was shown as mean±SEM (N=2), the statistical differences among groups were assessed using one-way ANOVA. (A) *P<0.05, **P<0.01, ***P<0.001 vs. human IgG4; (B-C) *P<0.05, **P<0.01, ***P<0.001 vs. AK127 (G4DM).*

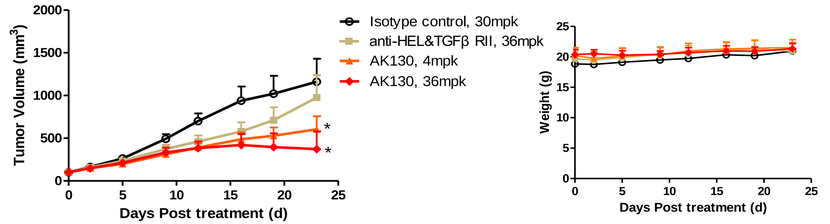
Results

Figure 5. ADCC and CDC activity of AK130 against CHO-K1-TIGIT Cells.



*Note: Data was shown as mean±SEM (N=2), the statistical differences among groups were assessed using one-way ANOVA. *P<0.05, **P<0.01, ***P<0.001 vs. Isotype control (human IgG1 or human IgG4).*

Figure 6. *In vivo* activity of AK130 in BALB/c-hTIHIT transgenic mice H22 tumor model.



*Note: anti-HEL&TGFβRII is a fusion protein with a similar structure to AK130, which can bind to HEL (Hen Egg Lysozyme) and TGF-β. "mpk" is short for "mg/kg" . H22 cells were subcutaneously inoculated in the right hind limb of BALB/c-hTIGIT transgenic mice. The day of animal grouping was defined as day 0 of treatment and mice were treated with Isotype control, anti-HEL&TGFβ or AK130 via intraperitoneal injection on day 0, 3, 7, 10, 14 and 17. Tumor volume data was presented as mean±SEM and body weight data was presented as mean±SEM (N=8). The statistical differences among groups were assessed using two-way ANOVA. *P<0.05 vs isotype control*

Conclusion

AK130 could specifically bind to human TIGIT and TGF-β with high affinity. In reporter assays, AK130 efficiently blocked the interaction between TIGIT and CD155, as well as TGF-β1/TGF-β3 and TGFβRII. As expected, AK130 did not show ADCC or CDC activity when compared with tiragolumab. AK130 also demonstrated strong anti-tumor efficacy in mice.

AK130, a humanized Fc-mutant anti-TIGIT antibody fused with TGFβ-RII protein, shows great anti-tumor efficacy in a mouse tumor model and does not have ADCC and CDC effects, supporting its clinical development for the treatment of human cancers.

Reference

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