#1850

A novel anti-CD137 antibody recognizing the membrane-proximal CD137 domain elicits potent anti-tumor T cell activity in a bispecific antibody format

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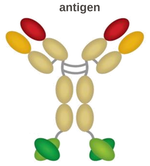
INTRODUCTION

- Although CD137 (4-1BB) is a potent coreceptor augmenting T cell receptor-mediated activation and proliferation, clinical development for therapeutic use has not been successful, specifically due to hepatotoxicity
- Conditional T cell activation in tumor microenvironment is a key for eliciting potent immune response with no risk of peripheral toxicity
- 4-1BB antibody 1A10 with no agonistic activity was selected and designed for Grabody T, a bispecific antibody format, to induce tumor associated antigen (TAA) specific immune responses

SUMMARY

Grabody T Platform

Tumor-specific/associated antigen



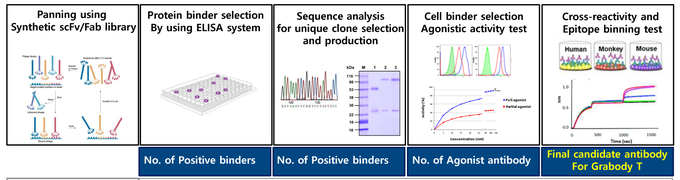
Grabody™ T

4-1BB Binder

- 1A10 binds to CRD4, the membrane proximal domain, of 4-1BB, distinct from binding sites of other 4-1BB antibodies
- 2+2 bispecific format with 1A10 scFv linked to the C-terminal ends of TAA-specific antibody showed the highest antigen binding and 4-1BB activation
- Grabody T-containing assets induce TAA-specific 4-1BB activation across various TAA targets, while Urelumab shows TAA-independent 4-1BB activation
- Grabody T does not activate PBMC leading to cytokine secretion, implicating non-specific CRS-related risks are greatly reduced
- In monkey toxicity study, no significant increase in liver damage-related enzymes or inflammatory cytokines was observed

RESULT

1A10 clone was selected through phage library screening and biological Evaluation

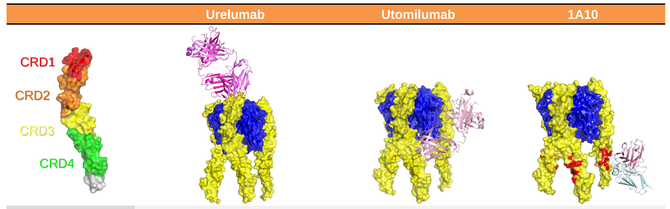


Clone	Biological activity							
	ELISA (EC50, nM)		Affinity to human 4-1BB by Octet (KD, nM)	Epitope binning			Cell based assay[1] (EC50, nM)	PBMC activation Assay[2] (EC50, nM)
	Human 4-1BB	Rhesus 4-1BB		Urelumab competition	Utomilumab Competition	4-1BBL Competition		
TAA1-1A10	**0.07**	**0.11**	**0.26**	No	Yes	No	**0.02**	**0.036**
TAA1-1E7	0.07	No Binding	0.79	ND	ND	ND	ND	0.040
TAA1-1A12	0.25	0.09	1.01	No	Yes	No	0.04	0.041

[1] Cell based assay: 4-1BB reporter bioassay (Promega Kit); [2] PMBC activation assay: Measurement of 4-1BB induced IFN-gamma secretion; ND, Not Determined

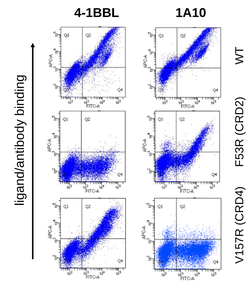
RESULT

1A10 clone binds to the CRD4, the membrane proximal domain of 4-1BB, and does not overlap with the 4-1BBL binding site

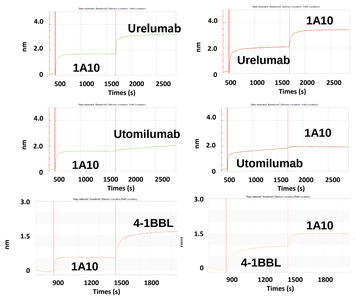


	CRD1	CRD3	CRD4
Epitope domain			
4-1BBL competition	No	Yes	No
4-1BB activation without antibody engagement	Yes	No	No

Flow cytometry binding analysis of 4-1BBL and 1A10 against corresponding epitope mutants of 4-1BB



Epitope binning study of 1A10 against Urelumab, Utomilumab, and 4-1BBL

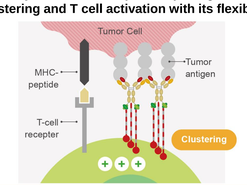


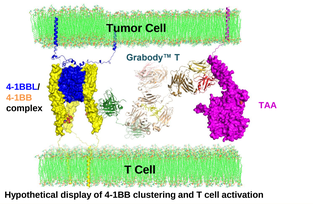
Site-directed 2+2 format showed distinct target binding and 4-1BB activation profiles

Bispecific Format (TAA2x1A10)		Antigen Binding[1]	4-1BB Activity[2]
2 + 2 *Site of scFv Conjugation: Light chain C-term.*	Anti-TAA2 / Anti-4-1BB in scFv	1.27 nM	CAMA-1: 0.150 nM SK-BR3: 0.109 nM
2 + 2 *Site of scFv Conjugation: Light chain N-term.*	Anti-4-1BB in scFv / Anti-TAA2	0.801 nM	CAMA-1: 0.176 nM SK-BR3: 0.134 nM
2 + 2 *Site of scFv Conjugation: Heavy chain N-term.*	Anti-4-1BB in scFv / Anti-TAA2	1.05 nM	CAMA-1: ambiguous SK-BR3: ambiguous
2 + 2 *Site of scFv Conjugation: Heavy chain C-term.*	Anti-TAA2 / Grabody T BsAb (Anti-4-1BB in scFv)	**0.664 nM**	**CAMA-1: 0.101 nM SK-BR3: 0.092 nM**

[1] Dual antigen captured ELISA (EC50); [2] 4-1BB activation assay (EC50) in 2 breast cancer cell lines using Promega kit

Grabody T induces an optimal spacing for 4-1BB clustering and T cell activation with its flexible linker

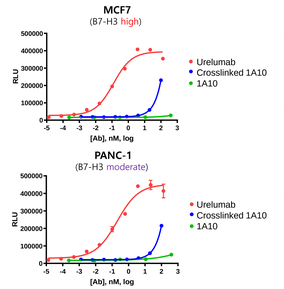




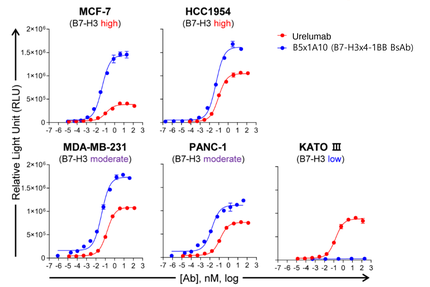
Hypothetical display of 4-1BB clustering and T cell activation

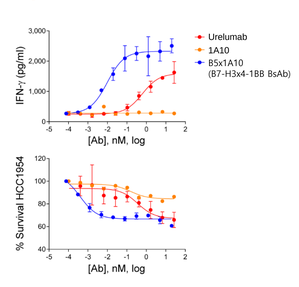
RESULT

1A10 clone induces no or weak 4-1BB activation with Fc-mediated crosslinking, while triggering potent 4-1BB activation in the presence of cancer cell lines in a TAA expression dependent manner



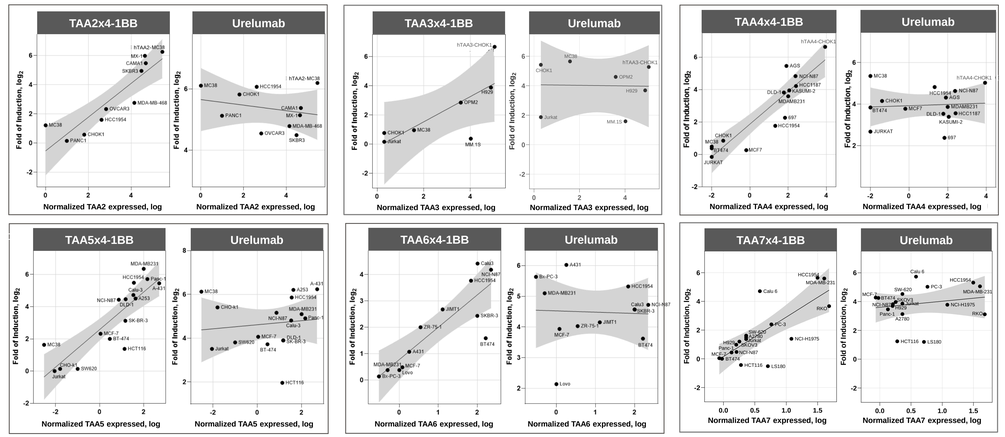
4-1BB activation assay (Promega)





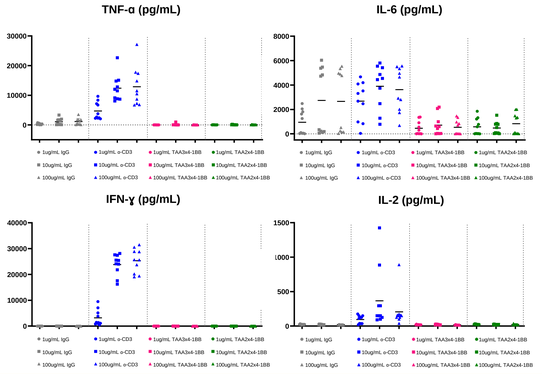
PBMC based assay (IFN-γ secretion, cytotoxicity WST-8)

Grabody T-containing assets show linear correlation between TAA expression and 4-1BB activation

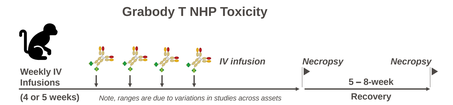


4-1BB activation assay (Promega); TAA expression was determined by FACS analysis

Grabody T-containing assets do not induce cytokine release from PBMCs in the absence of tumor cells



Grabody T assets do not render 4-1BB related liver toxicity



Grabody T Asset	TAA-related Tox	Liver Tox
TAA3x4-1BB	–	–
TAA8x4-1BB	–	–
TAA7x4-1BB	++	–

- ✓ Grabody T showed no transient or permanent liver toxicity in GLP toxicity studies
- ✓ Toxicities for each asset were associated with the specific TAA
- ✓ TAA-mediated toxicities occurred within the target expressing region, with limited systemic involvement
- ✓ Tox readouts are expected based on the specific TAA
- ✓ Tox readouts are not overlapping among Grabody T assets

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