

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



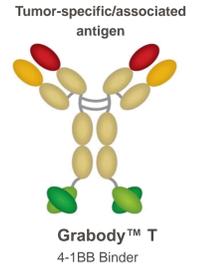
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INTRODUCTION

- 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

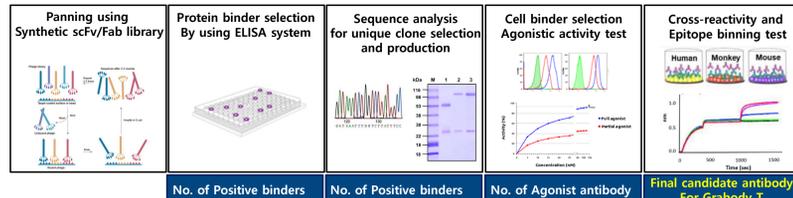
Grabody T Platform



- 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



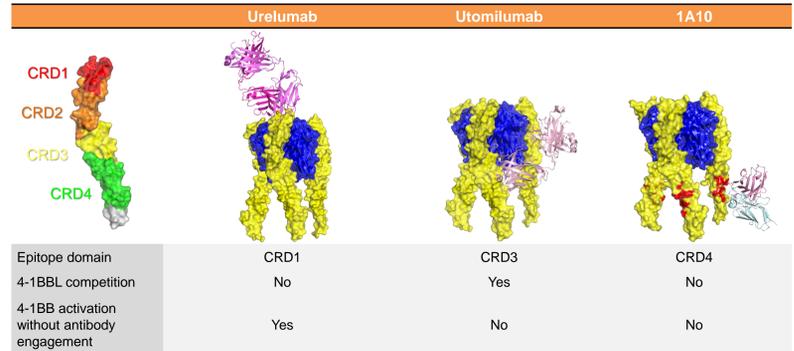
Library	No. of Positive binders	No. of Positive binders	No. of Agonist antibody	Final candidate antibody For Grabody T
Human scFv library Lambda	> 300 clones	5 clones	3 clones	2 clones TAA1, TAA2 1A10, 1A12, 1E7
Human scFv library Lambda and Kappa				
Human Fab library Kappa				

Clone	Biological activity			Epitope binning			Cell based assay ¹ (EC50, nM)	PBMC activation Assay ² (EC50, nM)
	ELISA (EC50, nM)	Affinity to human 4-1BB by Octet (KD, nM)		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

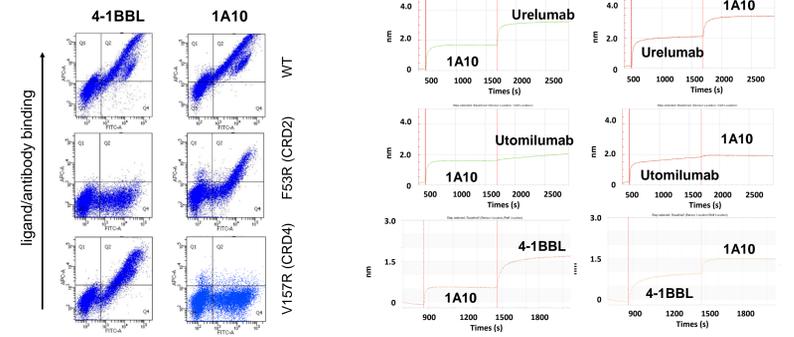
¹ Cell based assay: 4-1BB reporter bioassay (Promega Kit); ² PBMC activation assay: Measurement of 4-1BB induced IFN-gamma secretion; ND, Not Determined

RESULT

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



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

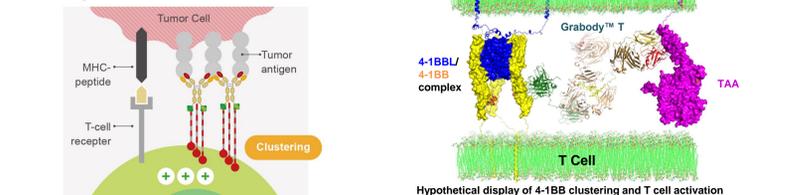


Site-directed 2+2 format showed distinct target binding and 4-1BB activation profiles

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

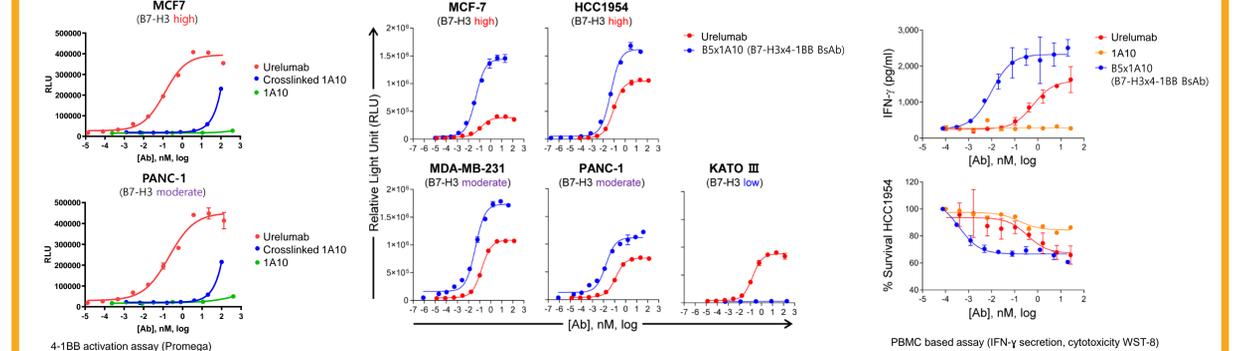
¹ Dual antigen captured ELISA (EC50); ² 4-1BB activation assay (EC50) in 2 breast cancer cell lines using Promega kit

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

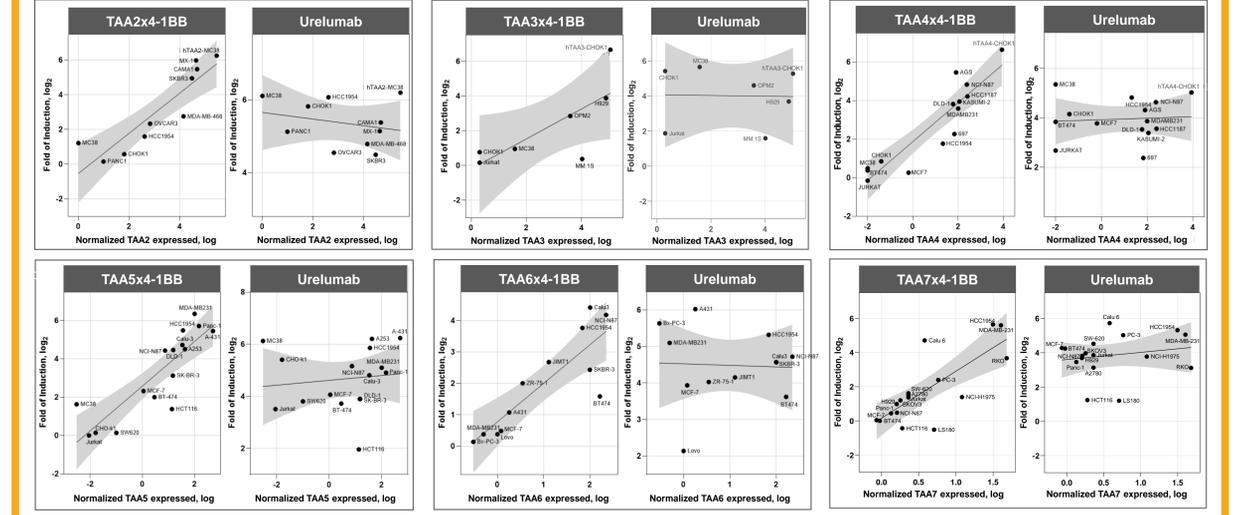


RESULT

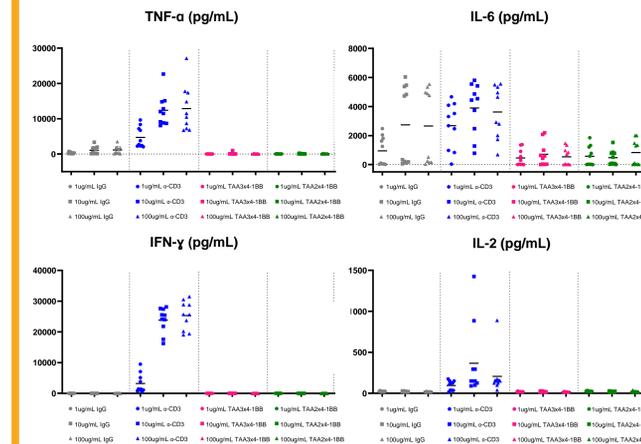
1A10 clone induces no or weak 4-1BB activation with Fc-mediated crosslinking, while triggering potent 4-1BB activation in the presence of cancer cell lines in a TAA expression dependent manner



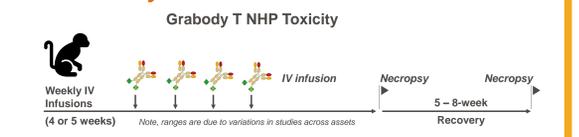
Grabody T-containing assets show linear correlation between TAA expression and 4-1BB activation



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