Tumor-specific CD28 costimulatory bispecific antibodies enhance T cell activation in solid tumors

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Abstract #2983

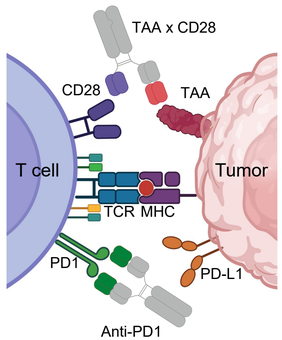
Introduction

- T cells in the tumor microenvironment require TCR/peptide MHC (pMHC; Signal 1) and costimulatory receptor (Signal 2) engagement to achieve optimal activation
- Tumor cells do not typically express CD28 ligands (CD80/86); this lack of costimulation may compromise the activity of CD3 engagers or anti-PD1 therapies in the clinic
- Therefore, we generated bispecific antibodies that conditionally provide CD28 costimulation only in the presence of tumor-associated antigen (TAA) and TCR engagement using Xencor's XmAb® bispecific platform

CD28 x TAA bispecific antibodies may expand the utility of checkpoint blockade and CD3 T cell engagers

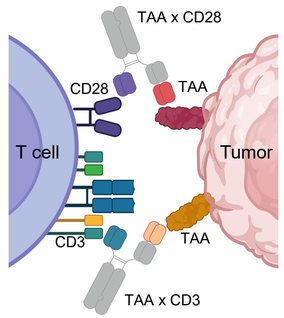
In combination with anti-PD1

Signal 1: Neoantigen recognition



In combination with CD3 T cell engagers

Signal 1: TAA x CD3

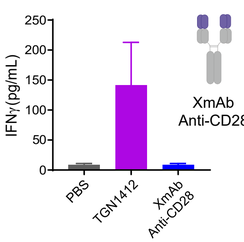


The XmAb heterodimeric Fc platform allows for well-behaved CD28 bispecific antibodies

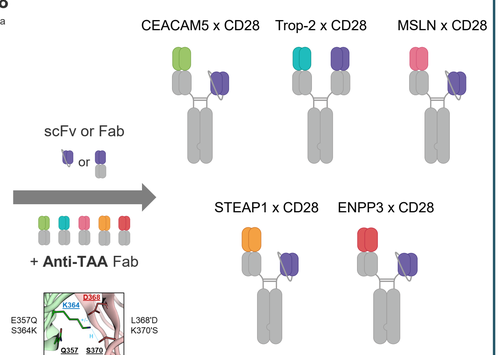
Non-superagonist anti-CD28

No cytokine release in dry coat assay[a]

[a]Stebbings R, et al. *J Immunol.* 2007.



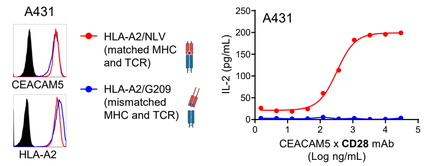
1+1 format (monovalent CD28)



- $Fc\gamma R$ interactions silenced
- Xtend™ (LS) half-life extension
- Highly stable scFv (T_m > 70°C)

CEACAM5 x CD28: Colorectal and other GI tract cancers

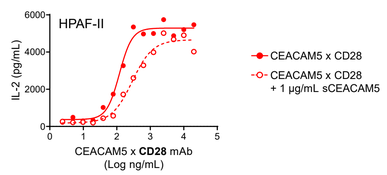
CEACAM5 x CD28 promotes T cell activation by TCR-pMHC recognition



A431-β2M-null cells stably expressing CEACAM5 were engineered to express a CMV-derived (HLA-A2/NLV[a]) or a melanoma-derived (HLA-A2/G209; negative control) pMHC antigenic complex. Cells were cocultured with PBMCs from an HLA-A2+, CMV-seropositive donor, and treated with a dose titration of CEACAM5 x CD28 mAb.

[a]Carreno BM, et al. *J Immunol.* 2012.

CEACAM5 x CD28 tolerates high concentrations of soluble CEACAM5

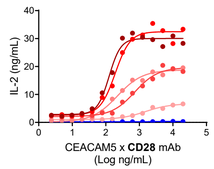


Cocultures of T cells and cancer cells were treated with 1 µg/mL B7H3 x CD3 mAb and a dose titration of CEACAM5 x CD28 mAb, with and without 1 µg/mL sCEACAM5.

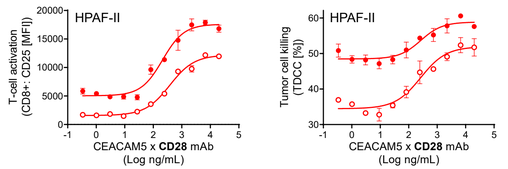
CEACAM5 x CD28 enhances T cell activation in combination with a CD3 T cell engager

CEACAM5 x CD28 requires CEACAM5 expression levels representative of tumors for activity

Cell line	CEACAM5 density
MKN45	High
HPAF-II	High
LS1034	Medium
LS174T	Medium
Lovo	Medium
HT29	Low



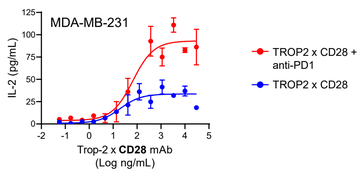
CEACAM5 x CD28 synergizes with high and low concentrations of T cell engager



Cocultures of T cell and cancer cells were treated with 1 µg/mL (solid circles) or 0.1 µg/mL (open circles) B7H3 x CD3 mAb, and a dose titration of CEACAM5 x CD28 mAb.

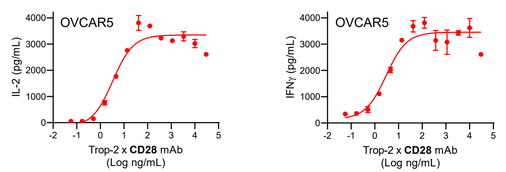
Trop-2 x CD28: Broad applicability across solid tumors

Trop-2 x CD28 enhances alloreactivity alone and in combination with anti-PD1



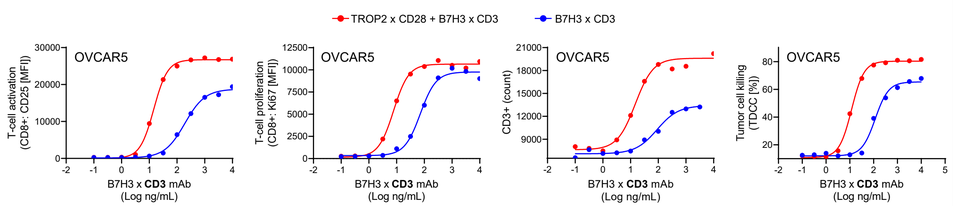
Cocultures of T cells and MDA-MB-231 were treated with a dose titration of Trop-2 x CD28 mAb, with and without 10 µg/mL anti-PD1.

Trop-2 x CD28 enhances T cell activation in combination with a CD3 T cell engager



Cocultures of T cells and OVCAR5 cells were treated with 1 µg/mL B7H3 x CD3 mAb and a dose titration of Trop-2 x CD28 mAb.

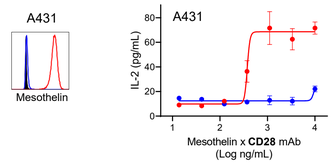
Trop-2 x CD28 improves T cell expansion and cytotoxicity of a CD3 T cell engager



Cocultures of T cells and OVCAR5 cells were treated with a dose titration of B7H3 x CD3 mAb, with and without 1 µg/mL Trop-2 x CD28 mAb.

Mesothelin x CD28: Ovarian cancer, mesothelioma

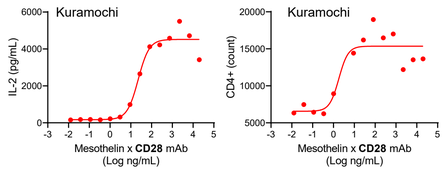
Mesothelin x CD28 promotes T cell activation by TCR-pMHC recognition



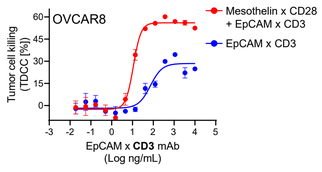
A431-β2M-null cells stably expressing a CMV-derived antigenic complex (HLA-A2/NLV[a]) were cocultured with T cells from an HLA-A2+, CMV-seropositive donor, and a dose titration of mesothelin x CD28 mAb.

[a]Carreno BM, et al. *J Immunol.* 2012.

Mesothelin x CD28 enhances T cell activation in combination with a CD3 T cell engager



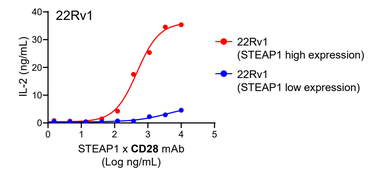
Cocultures of T cells and Kuramochi cells were treated with 1 µg/mL EpCAM x CD3 mAb and a dose titration of mesothelin x CD28 mAb.



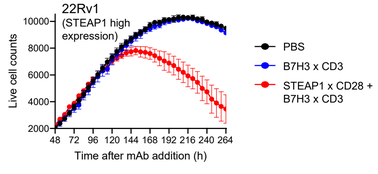
Coculture of T cells and OVCAR8 cells were treated with a dose titration of EpCAM x CD3 mAb, with and without 1 µg/ml mesothelin x CD28.

STEAP1 x CD28: Prostate cancer

STEAP1 x CD28 enhances T cell activation in combination with a CD3 T cell engager



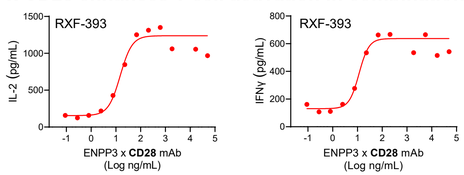
Cocultures of T cells and cancer cells were treated with 1 µg/mL B7H3 x CD3 mAb and a dose titration of STEAP1 x CD28 mAb.



Cocultures of T cells and cancer cells were treated with 0.5 µg/mL B7H3 x CD3 mAb, with or without 1 µg/mL STEAP1 x CD28 mAb, or PBS.

ENPP3 x CD28: Renal cell carcinoma

ENPP3 x CD28 enhances T cell activation in combination with a CD3 T cell engager



Cocultures of T cells and cancer cells were treated with 1 µg/mL ENPP3 x CD3 mAb and a dose titration of ENPP3 x CD28 mAb.

Summary

- The XmAb bispecific antibody platform uses a modular, non-superagonist anti-CD28 domain to rapidly produce TAA x CD28 bispecific antibodies that conditionally provide T cell costimulation dependent upon TCR engagement and TAA expression
- TAA x CD28 bispecific antibodies can be used as a single agent, or in combination with anti-PD1 or TAA x CD3 T cell engagers
- TAA x CD28 bispecific antibodies show compelling preclinical activity and warrant further exploration for clinical development

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