# Tumor-targeted CD28 bispecific POWERbody™ for safe and synergistic T cell-mediated immunotherapy

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## BACKGROUND AND SIGNIFICANCE

Targeting CD28 by TGN1412 agonist antibody for systemic T cell activation resulted in a near-fatal clinical disaster due to severe cytokine storm and multiorgan failure. However, tumor specific CD28 T-cell engager (TCEs) in TAA×CD28 bispecific format, similar to the clinically validated TAA×CD3 bispecific TCEs, offers an attractive solution to mitigate the serious safety concerns associated with systemic CD28 activation while delivering potent T-cell costimulatory signal. Our goal is to combine tumor specific bispecific TCEs engaging both CD3 and CD28, the two key and synergistic pathways in T cell activation, for safe, potent, and durable T cell-mediated synthetic immunotherapies.

Following our success in utilizing the CD3 TCE POWERbody™ platform for tumor targeting with tight safety control, we further applied our suite of three-body technologies to develop tumor specific CD28 bispecific TCE POWERbody programs (Fig 1). Our NEObody™ enabled us to identify the species cross-reactive anti-CD28 antibodies targeting a unique and conserved epitope of CD28 in combination with the precision masking of antigen binding sites using SAFEbody® technology for conditional activation in tumor microenvironment (TME).

Our data show that the tumor specific TAA×CD28 bispecific TCE POWERbody has achieved the following targeted product profiles: 1) anti-CD28 NEObodies targeting unique epitopes of CD28 with species cross-reactivity against human, monkey and mouse CD28 for high fidelity translational studies; 2) the anti-CD28 are NOT superagonist, but activate T cells only in the presence of the primary TCR signal; 3) Tumor specific bispecific B7H3×CD28 and HER2×CD28 induce potent T-cell co-stimulation; 4) these TAA×CD28 bispecific antibodies show synergistic T cell-mediated tumor cell killing in combination with TAA×CD3 TCEs and/or with checkpoint inhibitor anti-PD-1 *in vitro*; 5) these bispecific antibody B7H3×CD28 can synergize with masked HER2×CD3 ADG138 Powerbody to mediate strong antitumor efficacy; 6) masked tumor specific CD28 bispecific antibodies are further developed using SAFEbody technologies to increase tumor specificity by conditional activation in TME.

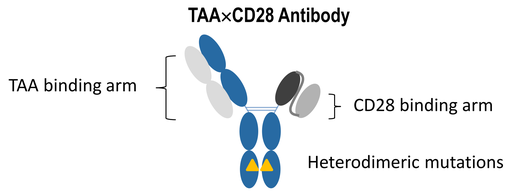


**Fig 1.** Tumor-targeted CD28 bispecific antibody. The structure of the bispecific antibody TAA×CD28 is shown here.

## RESULTS

### Anti-CD28 antibodies with varying binding affinities and mouse cross-reactivity

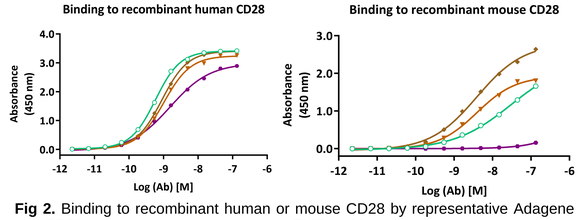


**Fig 2.** Binding to recombinant human or mouse CD28 by representative Adagene anti-CD28 antibodies, as determined by ELISA. Multiple antibodies exhibit cross-reactivity to both human and mouse CD28.

## RESULTS

### Anti-CD28 antibodies co-activate human T cells in the presence of anti-CD3

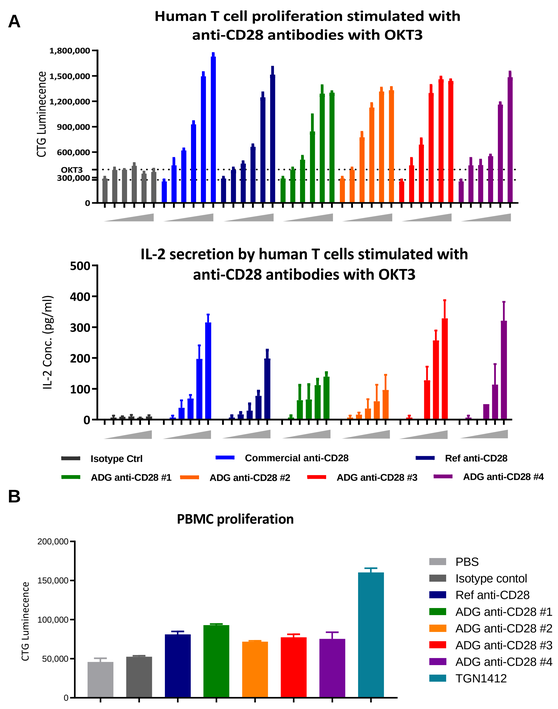


**Fig 3. (A)** In vitro stimulation of human T cells by anti-CD28 antibodies in the presence of anti-CD3 (OKT3). T cell proliferation (upper) and IL-2 secretion (lower) were examined as readouts of T cell activation. The results indicate that all Adagene (ADG) anti-CD28 antibodies can co-activate human T cells in the presence of anti-CD3, similar to a commercial anti-CD28 (clone CD28.2), or a reference anti-CD28. **(B)** The superagonist activity of anti-CD28 antibodies were examined with a dry-coat PBMC assay. All test antibodies were dry-coated onto the wells of 96-well plate and incubated with human PBMCs. The PBMC proliferation was determined by a CTG assay. The results indicate that Adagene anti-CD28 antibodies behaved similarly as a reference antibody without superagonist activity, whereas TGN1412, a superagonistic anti-CD28, significantly stimulated PBMC proliferation in this assay.

### TAA×CD28 and TAA×CD3 cooperate to activate NFκB T cell signaling

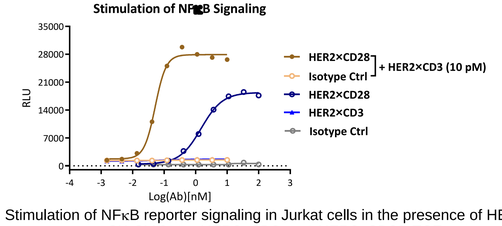


**Fig 4**. Stimulation of NFκB reporter signaling in Jurkat cells in the presence of HER2-positive cancer cells SK-OV-3, by HER2×CD3 and HER2×CD28 TCEs alone or in combination. Non-competing HER2 were used in the HER2×CD3 and HER2×CD28 bispecific antibodies.

### Combinations of TAA×CD3 and TAA×CD28 TCEs enhance T cell mediated killing of target cells

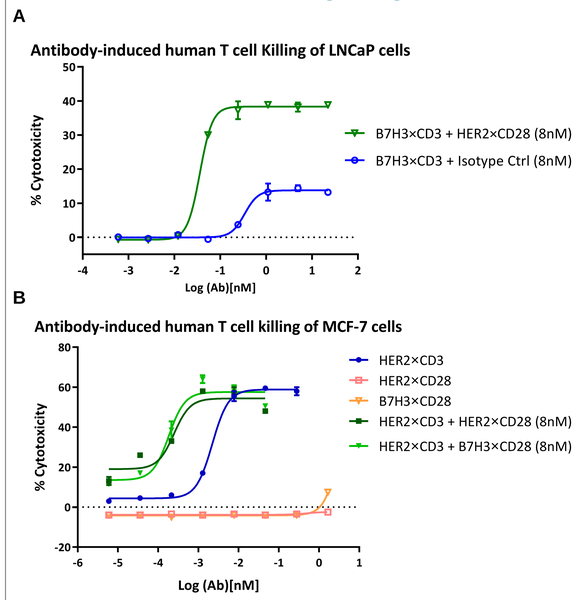


**Fig 5. (A)** Stimulation of T cell mediated killing of LNCaP cells by B7H3xCD3, HER2xCD28, or the combination. The results demonstrate that HER2xCD28 can greatly enhance the cytotoxicity of B7H3xCD3 TCE. **(B)** In vitro human T cell mediated killing of HER2-expressing MCF-7 cells induced by HER2xCD3, HER2xCD28 or B7H3xCD28, and the combinations. The results demonstrate that both HER2xCD28 and B7H3xCD28 can significantly enhance the cytotoxicity of HER2xCD3 TCE. In A and B, non-competing HER2, or B7H3 were used in the CD3 or CD28 TCEs.

### Combination of TAA×CD3 TCE with TAA×CD28 demonstrates enhanced *in vivo* antitumor effect

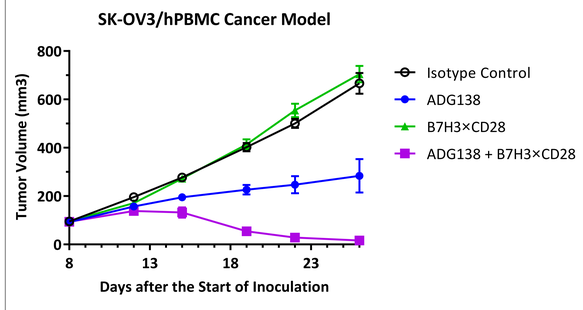


**Fig 6.** The in vivo anti-tumor efficacy of the double masked HER2×CD3 (ADG138), B7H3×CD28 TCE, or the combination, was assessed in the SK-OV3 ovarian cancer xenograft tumor model in NSG mice engrafted with human PBMC. Antibodies were *i.p.* administered twice a week.

### TAA×CD28 with anti-PD-1/PD-L1 enhances T cell activation

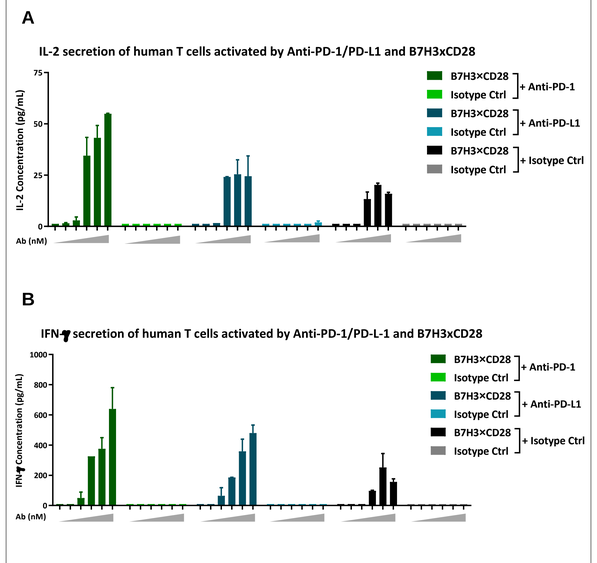


**Fig 7.** T cell activation by B7H3×CD28, anti-PD-1, anti-PD-L1, or the combinations, by one-way MLR assay. MDA-MB-231 cancer cells were mixed with isolated human T cells in culture condition in the presence of different test antibodies alone or in combinations. Anti-PD-1 (pembrolizumab) or anti-PD-L1 (atezolizumab) was tested at fixed concentration. IL-2 (A) and IFN-γ (B) levels were measured in the supernatants following the co-culture. The results indicate that combination of B7H3xCD28 and anti-PD-1 or anti-PD-L1 can cooperate to enhance T cell activation.

## SUMMARY

- Through Adagene's NEObody platform, anti-CD28 antibodies with varying affinities were developed that target unique and conserved epitopes of CD28 with broad species cross-reactivity against primate and mouse CD28.
- ADG anti-CD28 lead antibodies can co-activate T cells in the presence of priming signals such as anti-CD3, but did not show superagonist activity.
- Tumor associated antigen (TAA)×CD28 bispecific antibodies were developed, such as B7H3×CD28 and HER2×CD28, to deliver potent T-cell costimulatory signal in tumor microenvironment with high expression of these TAAs.
- When combined with TAA×CD3 TCEs in vitro, these TAA×CD28 bispecific antibodies exhibited much enhanced T cell signaling activation and T cell-mediated tumor cell killing.
- Tumor targeted CD28 bispecific antibody can also cooperate with checkpoint inhibitors anti-PD-1/PD-L1 to mediate enhanced T cell activation in vitro.
- In mouse SK-OV3/hPBMC xenograft tumor model, tumor-targeted CD28 bispecific antibody B7H3×CD28 can synergize with masked HER2×CD3 Powerbody ADG138 to mediate strong antitumor efficacy.
- CD28-masked tumor-targeted bispecific antibodies are further developed using SAFEbody technologies to increase tumor specificity by conditional activation in the TME to reduce on-target off-tumor toxicities and prevent systemic cytokine release syndrome.

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