HCAb Based Bispecific Immune Cell Engager (HBICE) Platform Enables Fast And Convenient Generation of the Next Generation Bispecific Antibodies with High Potency, Promising Safety Profiles and Excellent Developability

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Abstract

Immunotherapies based on immune cell engagers have been inspiring yet challenging due to limited efficacy and significant safety concerns. It has been well established that 4-1BB signaling provides co-stimulatory signals for various types of immune cells. However, clinical application of conventional anti-4-1BB agonistic antibodies has been limited, due to severe toxicity and/or low response rate. The next generation bispecific antibodies derived by targeting both immune cells and tumor associated antigens (TAA) confer advantages over the conventional mono-specific molecules.

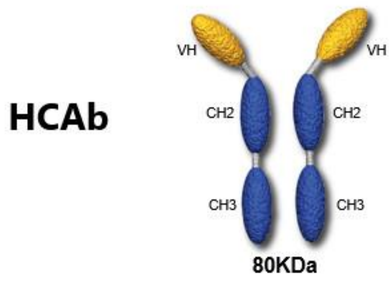
Bispecific antibodies selectively activate immune cells in the tumor microenvironment, thereby preventing non-specific activation of peripheral immune cells. Furthermore, they directly link immune cells to tumor cells resulting in efficient lysis of tumor cells. However, generation of bispecific antibodies can be often difficult due to mis-match paring of heavy and light chains. Our approach is to take advantage of the fully human heavy chain only antibody (HCAb), which is about half the size of a typical IgG and consists of a single heavy chain. Recently, we developed the HCAb based immune cell engager (HBICE™) platform, utilizing our unique H2L2 and HCAb Harbour Mice®. The antibodies carry an inert Fc backbone and are comprised of only two chains, minimizing chain associated issues that many bispecific antibody formats have. The HBICE platform provides a fast and convenient way to generate bispecific antibodies.

Here we demonstrate the efficiency of the HBICE platform in the generation of TAA x 4-1BB bispecific antibodies (BsAbs). These antibodies display high efficacy and specificity. The lead candidate specifically activates the NF-kB pathway and co-stimulates T cells in a TAA-dependent manner. *In vivo* studies further validate a robust anti-tumor activity which also relies on TAA-mediated crosslinking. Therefore, the TAA x 4-1BB HBICE™ not only displays high efficacy in the T cell co-stimulation and tumor growth inhibition, but also demonstrates greatly improved safety profile due to its strict dependency on TAA-mediated crosslinking. The lead candidate also shows typical human IgG pharmacokinetics profile.

In summary, our TAA x 4-1BB HBICE™ exemplifies the power of HBICE platform for development of next generation therapeutic antibodies. The platform enables the facile development of products with attributes not achievable by conventional antibody platforms or a simple combination of two monoclonal antibodies.

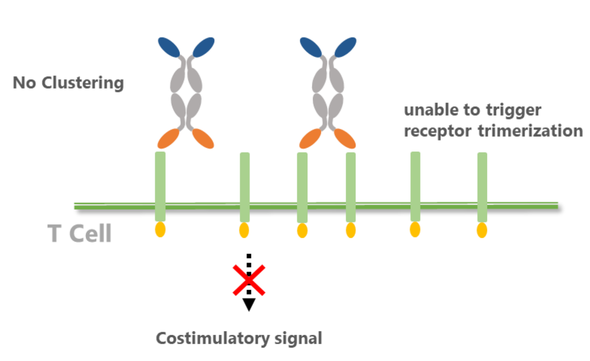
HBICE™ Platform

A **Heavy chain only antibodies (HCAb)**



No need for additional engineering or humanization.
Minimization of the problem of light chain mispairing and heterodimerization.

B **Costimulatory signaling can not be amplified in absence of TAA-mediated cross-linking**



Costimulatory signaling cascade by TAA-mediated cross-linking to avoid systemic T cell activation

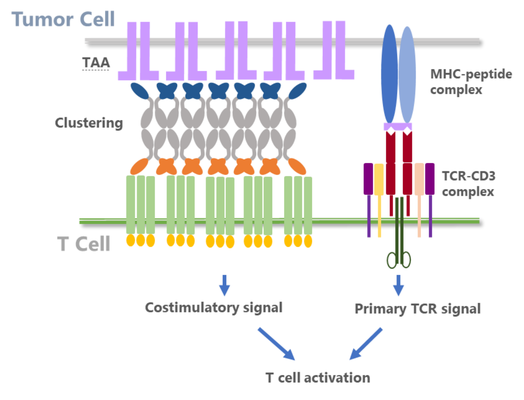
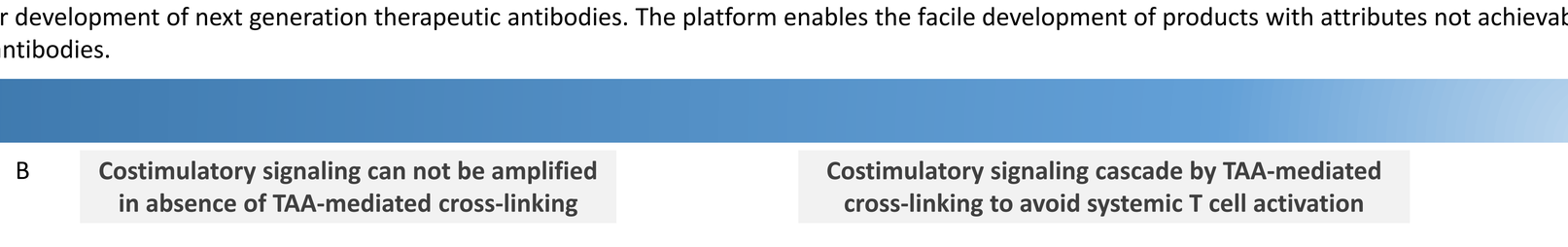


Figure 1. Introduction of the HBICE™ platform. A. HCAb antibodies serve as versatile and modular binding moieties for the platform. B. Exemplification of TAA x 4-1BB HBICE™ developed with the HBICE™ platform. In this specific example, the generated HBICE™ targets both TAA on tumor cells and co-stimulatory molecules on immune cells. Redirection of immune cells in the tumor microenvironment (TME) by HBICE™ results in selective activation of immune cells in the TME, thereby preventing non-specific activation of peripheral immune cells.

TAA-dependent Activity of a Representative TAA x 4-1BB HBICE™

Figure 2. Activation of the immune cells with a representative TAA x 4-1BB HBICE™ relies on clustering of co-stimulatory molecules on immune cells via TAA-mediated crosslinking. (A) The TAA x 4-1BB HBICE™ cannot co-stimulate T cells in the absence of TAA. (B) The TAA x 4-1BB HBICE™ activates T cells in the presence of TAA.



Formats *vs*. Functions

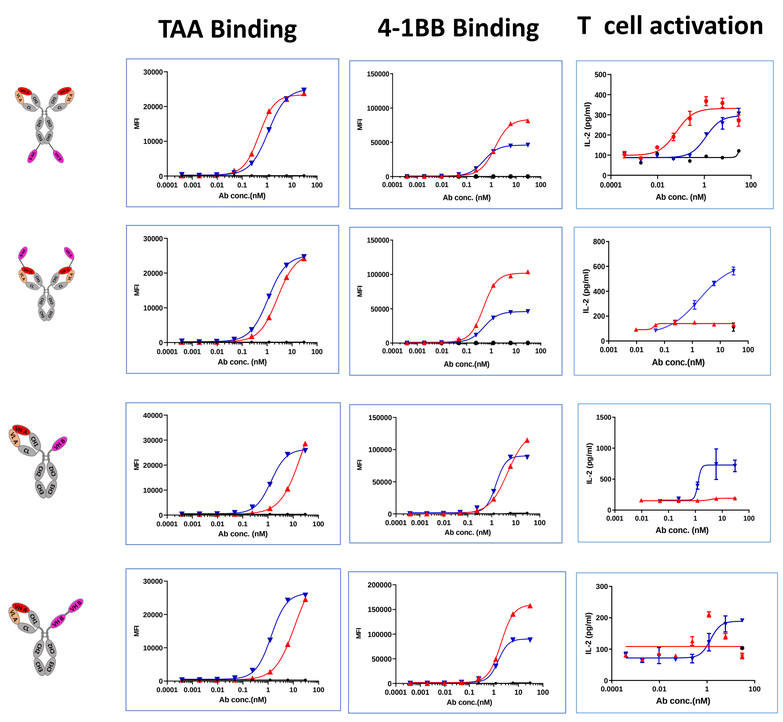


Figure 3. TAA x 4-1BB HBICE™ of different formats. Although the TAA and 4-1BB binding affinities of the antibodies are similar, the T cell activation activities are significantly different.

In vivo studies

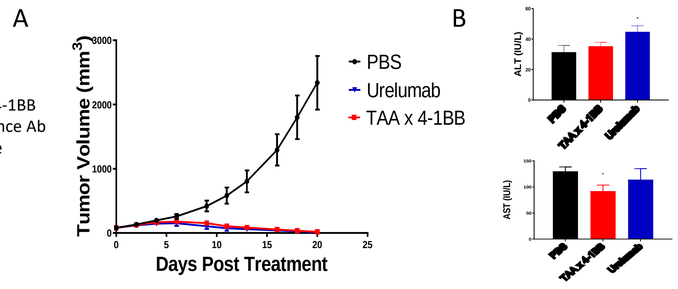


Figure 4. *In vivo* study of a TAA x 4-1BB HBICE™ with the CT26-TAA/4-1BB knock-in syngeneic mouse model. The mice (n=6) were treated on day 0, 4, 7, 11, 14, and 18. Tumor growth and body weight was recorded twice weekly. (A)Tumor growth. (B) Serum ALT and AST levels at the end of the study.

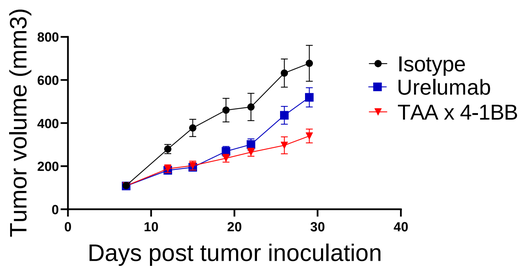


Figure 6. *In vivo study* with the NSG human PBMC CDX mouse model. The mice (n=6) were treated on day 7, 12, 15, 19, 22, and 26. Tumor growth and body weight was recorded twice a week.

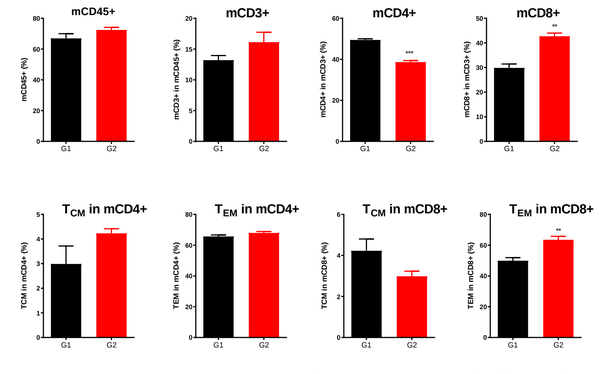


Figure 5. TILs analyzed by FACS. Total tumor infiltrating leukocytes were analyzed by flow cytometry 24 hours after 2 doses. TAA x 4-1BB HBICE™ significantly increased the ratios of CD8+ T cells and effector memory CD8+ T cells.

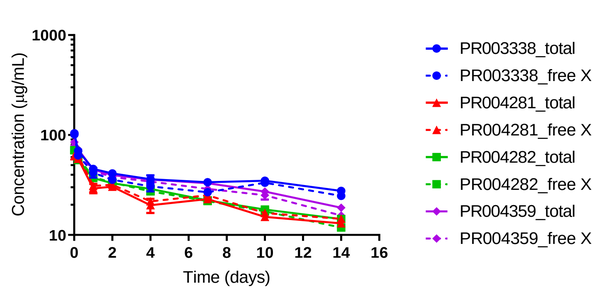


Figure 7. PK profile of several TAA x 4-1BB HBICE™. The antibodies show typical human IgG pharmacokinetics profile.

Summary

- ✓ **The TAA x 4-1BB HBICE™ exemplifies the power of HBICE platform for development of next generation therapeutic bispecific antibodies.**
- ✓ **The representative TAA x 4-1BB HBICE™ not only displays high efficacy in the T cell co-stimulation and tumor growth inhibition, but also demonstrates greatly improved safety profile due to its strict dependency on TAA-mediated crosslinking.**
- ✓ **The lead candidate shows typical human IgG pharmacokinetics profile.**
- ✓ **The platform enables the facile development of products with attributes not achievable by conventional antibody platforms or a simple combination of two monoclonal antibodies.**

References

1. Janssens R, Dekker S, Hendriks RW, Panayotou G, van Remoortere A, San JK, et al.Generation of heavy-chain-only antibodies in mice. Proc Natl Acad Sci U S A. 2006;103(41):15130–5.
2. Laventie B-J, Rademaker HJ, Saleh M, de Boer E, Janssens R, Bourcier T, et al. Heavy chain-only antibodies and tetravalent bispecific antibody neutralizing Staphylococcus aureus leukotoxins. Proc Natl Acad Sci U S A. 2011;108(39):16404–9.

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