

A Novel Preclinical and Translational CD3 ϵ Humanized Model to Study the Efficacy of T-Cell Engagers

A commentary

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Humanized mouse models to evaluate human CD3-mediated therapeutics

Following the clinical success of immune checkpoint antibodies targeting PD-1, PD-L1 or CTLA-4, T-cell engagers (TCEs) have emerged as a new class of immunotherapies for the treatment of cancer. As a result, numerous TCEs are currently in clinical and preclinical development.¹ The advancement of therapeutic TCE candidates into clinical trials requires the evaluation of their translatability and therapeutic efficacy in in vivo models: that is why robust and translatable preclinical models are essential for the development and evaluation of new therapeutics.²

CD3 is a complex of transmembrane proteins that non-covalently associate with the T-cell receptor (TCR) to trigger a cascade of signaling pathways, which ultimately leads to T-cell activation, proliferation, cytokine production and effector functions.^{1,3,4} CD3 consists of four chains (CD3 δ , CD3 ϵ , CD3 γ and CD3 ζ), and three subunits, two heterodimers (CD3 $\gamma\epsilon$ and CD3 $\delta\epsilon$) and one homodimer (CD3 $\zeta\zeta$). Previous experiments have shown that animals knocked out for CD3 ϵ exhibit an early arrest in T-cell development, suggesting that this chain is absolutely required for the formation and signaling pathway of the TCR-CD3 complex.^{5,6}

Given its key role in T-cell activation, a number of models expressing the human CD3 ϵ have already been developed to assess the efficacy of human CD3-specific therapeutics. One of the main challenges in designing such models, though, consists in maintaining the complex interactions between CD3 ϵ with CD3 δ and CD3 γ : if altered, the formation and function of the TCR-CD3 complex is compromised. Along these lines, modifying the amino acid sequence or the tertiary structure of CD3 ϵ , by expressing a fully humanized CD3 ϵ for example, abolishes T-cell activation.⁷ Similarly, experiments conducted on independent lines of transgenic mice containing high copy numbers of hCD3 ϵ demonstrate that these animals display a severe immunodeficiency due to a complete loss of T lymphocytes and natural killer cells, suggesting that physiological CD3 ϵ expression level is critical to maintain normal immune responses.⁸

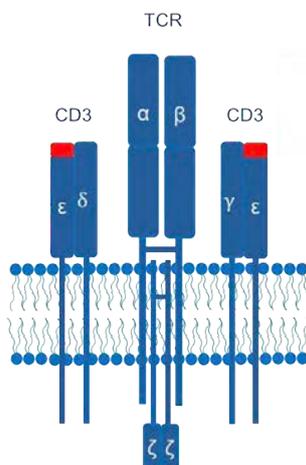


Figure 1 | Structure of the TCR-CD3 complex. In red, the N-terminal epitope of the CD3 ϵ chains humanized in our hCD3 ϵ model.

These limitations and our previous know-how in developing humanized mouse models prompted us to develop a novel mouse strain ([hCD3 \$\epsilon\$](#)). More specifically, this model expresses the human N-terminal epitope of the CD3 ϵ chains and the murine extracellular CD3 ϵ domains. In addition, it possesses murine transmembrane and intracellular domains, thereby maintaining salt bridges interaction as well as interaction with the CD3 ζ subunits, and preserving the signaling cascade within murine cells. Notably, the CD3 ϵ N-terminal human epitope is recognized by the great majority of the T-cell engagers currently available on the market (Figure 1).

Our analyses show that the humanization of the N-terminal epitope of CD3 ϵ does not alter the immune cells distribution in hCD3 ϵ mice (comparable frequency of T, B and NK cells, monocytes, dendritic cells). Moreover, we found that the TCR complex in these mice is functional, as the activation of T-cells with anti-human CD3 induces T-cell proliferation and cytokine production. Moreover, the cooperation between T and B cells seems to be functional. Finally, we observed that TCEs targeting both human CD3 and a tumoral antigen induce a dose-dependent anti-tumor effect in vivo (Figure 2).⁹

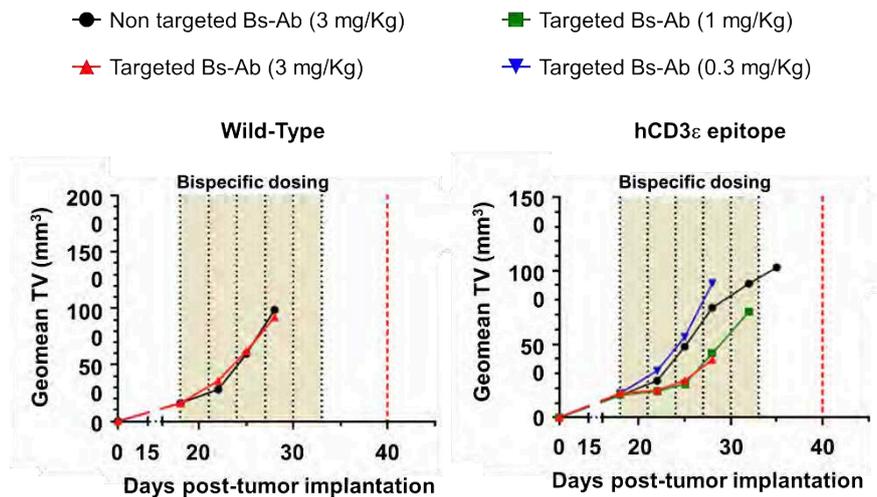


Figure 2 | T cell engagers efficacy assessment. Tumor growth curves in wild-type (left panel) and hCD3 ϵ mice (right panel).

As shown above, this model is physiological and can be used to assess anti-human CD3 antibodies targeting the N-terminal epitope of the CD3 ϵ subunit. In order to evaluate new and more innovative therapeutics for cancer immunotherapies, a more versatile humanized CD3 model is currently under development.

The New and the Old TCEs in Cancer Immunotherapy

The first TCEs to be developed were bispecific antibodies or BiTEs[®] (for bispecific T-cell engagers), i.e., antibodies with affinity for two different epitopes.¹ More specifically, BiTEs[®] are engineered to recognize and physically link one T cell to one tumor cell; this is made possible thanks to their structure, which consists of two single-chain variable fragments (scFv) from two different antibodies, one targeting a constant-component of T-cells, such as CD3, and the other one binding to a tumor-associated antigen (TAA) (Figure 3).¹⁰

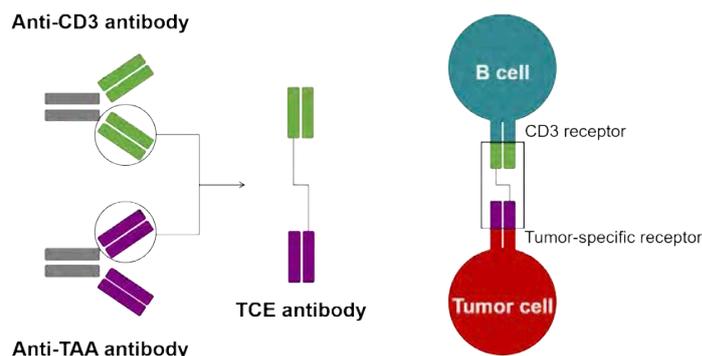


Figure 3 | Structure and basic mechanism of action of TCEs. Modified from Sigmund *et al* BLCTT 2020.

In physiological conditions, the main mediator of T-cell activation is the TCR, a protein complex that recognizes the antigen carried by the major histocompatibility complex (MHC) on the surface of an antigen-presenting cell (APC). Cancer cells, however, have evolved several mechanisms to lower the innate immune barriers and escape immune surveillance. One of those consist in downregulating cell surface display of MHC which, in turn, leads to the downmodulation of T-cell activation and, therefore, to evasion of antitumor immunity.¹¹ Since BiTEs[®] bypass the physiological TCR-MHC interaction to trigger T-cell activation, they possess important benefits as immunotherapies: they can initiate polyclonal T-cell responses, and they are not affected by MHC downregulation by cancer cells.¹²

These characteristics, combined with their cancer-targeting properties, give BiTEs[®] exceptional clinical potential for a variety of tumors, including hematological malignancies and solid cancers.^{13,14} Currently, 57 oncology-related bispecific antibodies are in clinical trials, and 38 of them are BiTEs[®]; interestingly, of those 38, 36 engage T-cells via CD3.^{1,3} To date, two BiTEs[®] have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for use in patients: catumaxomab (Removab, Fresenius Biotech) and blinatumomab (Blincyto, Amgen). The first is an anti-EpCAM/anti-CD3 antibody for the intraperitoneal treatment of malignant ascites in adults with EpCAM-positive carcinomas; approved in 2009, catumaxomab was withdrawn in 2017 at the request of the marketing authorization holder.¹⁵⁻¹⁸ The second was approved in 2014 by the FDA, and in 2015 by the EMA; it targets CD19 and CD3, and it is used to treat B-cell acute lymphoblastic leukemia in patients who still have detectable traces of cancer after chemotherapy.¹⁹⁻²¹

The growing success of BiTEs[®] has driven the development of a new generation of TCEs, with increased efficiency and reduced systemic activation of T-cells, such as the one recently developed by Panchal and colleagues engineered to become an active antibody only when it reaches tumor microenvironment, or the trispecific antibody developed by Wu et al. targeting a cancer cell, a receptor that activates T-cells, and a T cell protein that promotes long-lasting T cell activity against the cancer cell.^{22,23}

In summary, TCEs can transform cancer therapies, offering patients valid alternatives to existing treatments. Nevertheless, many TCE therapeutics have failed to enter clinical trials due to safety and efficacy issues. More robust and translatable animal models are therefore needed to continuously improve the design and effectiveness of these molecules for meaningful therapeutic benefits to patients.

References:

1. Suurs FV, Lub-de Hooge MN, de Vries EGE, de Groot DJA. A review of bispecific antibodies and antibody constructs in oncology and clinical challenges. *Pharmacol Ther.* 2019;201:103-119. doi:10.1016/j.pharmthera.2019.04.006
2. Voynov V, Adam PJ, Nixon AE, Scheer JM. Discovery Strategies to Maximize the Clinical Potential of T-Cell Engaging Antibodies for the Treatment of Solid Tumors. *Antibodies (Basel).* 2020;9(4). doi:10.3390/antib9040065
3. Zhang H, Lim H-S, Knapp B, et al. The contribution of major histocompatibility complex contacts to the affinity and kinetics of T cell receptor binding. *Sci Rep.* 2016;6:35326. doi:10.1038/srep35326
4. Dong D, Zheng L, Lin J, et al. Structural basis of assembly of the human T cell receptor–CD3 complex. *Nature.* 2019;573(7775):546-552. doi:10.1038/s41586-019-1537-0
5. Ueda O, Wada NA, Kinoshita Y, et al. Entire CD3 ϵ , δ , and γ humanized mouse to evaluate human CD3-mediated therapeutics. *Sci Rep.* 2017;7:45839. doi:10.1038/srep45839
6. DeJarnette JB, Sommers CL, Huang K, et al. Specific requirement for CD3epsilon in T cell development. *Proc Natl Acad Sci U S A.* 1998;95(25):14909-14914. doi:10.1073/pnas.95.25.14909
7. Martínez-Martín N, Risueño RM, Morreale A, et al. Cooperativity between T cell receptor complexes revealed by conformational mutants of CD3epsilon. *Sci Signal.* 2009;2(83):ra43. doi:10.1126/scisignal.2000402
8. Wang B, Biron C, She J, et al. A block in both early T lymphocyte and natural killer cell development in transgenic mice with high-copy numbers of the human CD3E gene. *Proc Natl Acad Sci U S A.* 1994;91(20):9402-9406. doi:10.1073/pnas.91.20.9402
9. Martin, Gaëlle, Sônego, Fabiane, Beringer, Audrey, et al. 14 Novel CD3 epsilon humanized N-terminal epitope model for assessment of efficacy of T-cell engagers. In: ; 2020. https://jitc.bmj.com/content/8/Suppl_3/A7.3.info
10. Huehls AM, Coupet TA, Sentman CL. Bispecific T-cell engagers for cancer immunotherapy. *Immunol Cell Biol.* 2015;93(3):290-296. doi:10.1038/icb.2014.93
11. Cornel AM, Mimpfen IL, Nierkens S. MHC Class I Downregulation in Cancer: Underlying Mechanisms and Potential Targets for Cancer Immunotherapy. *Cancers (Basel).* 2020;12(7). doi:10.3390/cancers12071760
12. Ellerman D. Bispecific T-cell engagers: Towards understanding variables influencing the in vitro potency and tumor selectivity and their modulation to enhance their efficacy and safety. *Methods.* 2019;154:102-117. doi:10.1016/j.ymeth.2018.10.026
13. Velasquez MP, Bonifant CL, Gottschalk S. Redirecting T cells to hematological malignancies with bispecific antibodies. *Blood.* 2018;131(1):30-38. doi:10.1182/blood-2017-06-741058
14. Yu S, Li A, Liu Q, et al. Recent advances of bispecific antibodies in solid tumors. *J Hematol Oncol.* 2017;10(1):155. doi:10.1186/s13045-017-0522-z
15. Fresenius Biotech Receives Approval for Removab® by European Commission - First Drug Worldwide for Treatment of Malignant Ascites. Accessed February 2, 2021. <https://www.fresenius.com/1930>
16. Linke R, Klein A, Seimetz D. Catumaxomab: clinical development and future directions. *MAbs.* 2010;2(2):129-136. doi:10.4161/mabs.2.2.11221
17. Krishnamurthy A, Jimeno A. Bispecific antibodies for cancer therapy: A review. *Pharmacology & Therapeutics.* 2018;185:122-134. doi:10.1016/j.pharmthera.2017.12.002
18. Heiss MM, Murawa P, Koralewski P, et al. The trifunctional antibody catumaxomab for the treatment of malignant ascites due to epithelial cancer: Results of a prospective randomized phase II/III trial. *Int J Cancer.* 2010;127(9):2209-2221. doi:10.1002/ijc.25423
19. Sigmund A, Sahasrabudhe K, Bhatnagar B. Evaluating Blinatumomab for the Treatment of Relapsed/Refractory ALL: Design, Development, and Place in Therapy. *BLCTT.* 2020;Volume 10:7-20. doi:10.2147/BLCTT.S223894
20. Pulte ED, Vallejo J, Przepiorka D, et al. FDA Supplemental Approval: Blinatumomab for Treatment of Relapsed and Refractory Precursor B Cell Acute Lymphoblastic Leukemia. *The Oncol.* 2018;23(11):1366-1371. doi:10.1634/theoncologist.2018-0179
21. Topp MS, Gökbuğet N, Stein AS, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. *The Lancet Oncology.* 2015;16(1):57-66. doi:10.1016/S1470-2045(14)71170-2
22. Panchal A, Seto P, Wall R, et al. COBRATM: a highly potent conditionally active T cell engager engineered for the treatment of solid tumors. *MAbs.* 2020;12(1):1792130. doi:10.1080/19420862.2020.1792130
23. Wu L, Seung E, Xu L, et al. Trispecific antibodies enhance the therapeutic efficacy of tumor-directed T cells through T cell receptor co-stimulation. *Nat Cancer.* 2020;1(1):86-98. doi:10.1038/s43018-019-0004-z