A Novel Preclinical and Translational CD3ε Humanized Model to Study the Efficacy of T-Cell Engagers

Commentary by Alessia Armezzani, PhD 16/02/2021

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 (CD3δ, CD3ε, CD3γ and CD3ζ), and three subunits, two heterodimers (CD3γε and CD3δε) and one homodimer (CD3ζζ). Previous experiments have shown that animals knocked out for CD3ε 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 CD3ε 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 CD3ε with CD3δ and CD3γ: 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 CD3ε, by expressing a fully humanized CD3ε for example, abolishes T-cell activation.[7] Similarly, experiments conducted on independent lines of transgenic mice containing high copy numbers of hCD3ε demonstrate that these animals display a severe immunodeficiency due to a complete loss of T lymphocytes and natural killer cells, suggesting that physiological CD3ε expression level is critical to maintain normal immune responses.[8]

These limitations and our previous know-how in developing humanized mouse models prompted us to develop a novel mouse strain (hCD3ε). More specifically, this model expresses the human N-terminal epitope of the CD3ε chains and the murine extracellular CD3ε domains. In addition,

it possesses murine transmembrane and intracellular domains, thereby maintaining salt bridges interaction as well as interaction with the CD3ζ subunits, and preserving the signaling cascade within murine cells. Notably, the CD3ε N-terminal human epitope is recognized by the great majority of the T-cell engagers currently available on the market (Figure 1).

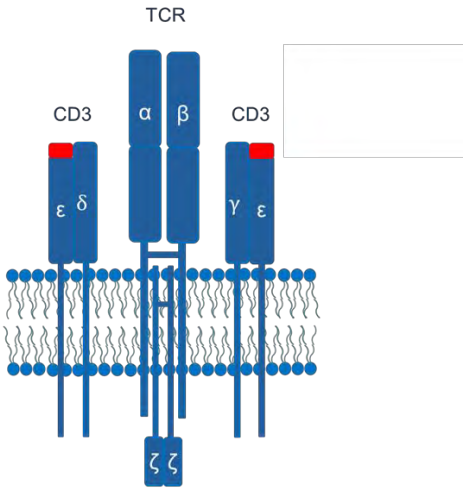

Figure 1 | Structure of the TCR-CD3 complex. In red, the N-terminal epitope of the CD3ε chains humanized in our hCD3ε model.

Our analyses show that the humanization of the N-terminal epitope of CD3ε does not alter the immune cells distribution in hCD3ε 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]

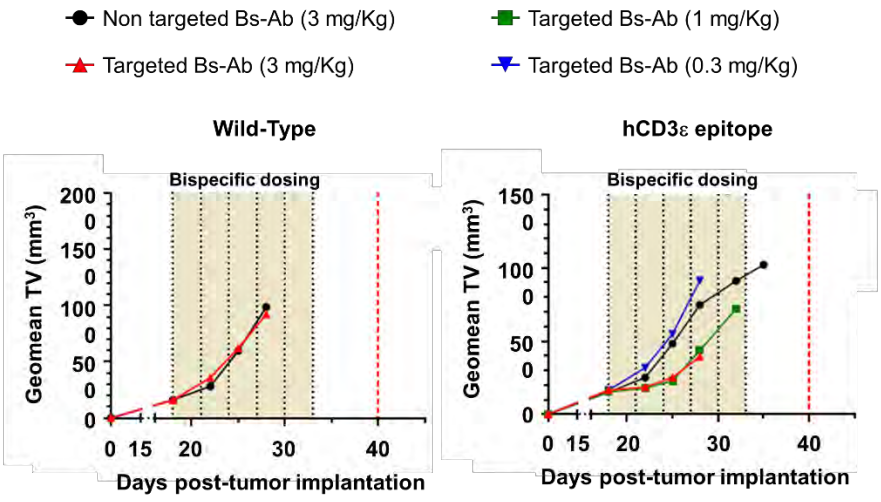

Figure 2 | T cell engagers efficacy assessment. Tumor growth curves in wild-type (left panel) and hCD3ε mice (right panel).

As shown above, this model is physiological and can be used to assess anti-human CD3 antibodies targeting the N-terminal epitope of the CD3ε 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]

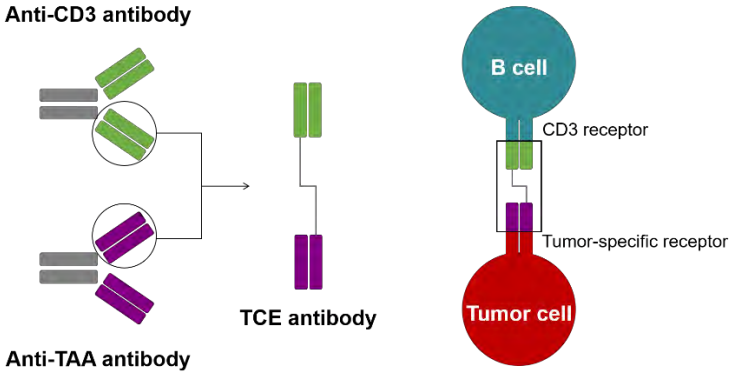

Figure 3 | Structure and basic mechanism of action of TCEs. Modified from Sigmund *et al* BLCTT 2020.

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.

Alessia Armezzani is Scientific Communication Manager at genOway

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