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AUTHOR'S VIEW

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CEA TCB: A novel head-to-tail 2:1 T cell bispecific antibody for treatment of CEA-positive solid tumors

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ABSTRACT
Carcinoembryonic antigen T cell bispecific antibody (CEA TCB) is a bispecific antibody used to recognize CEA and CD3e via a novel molecular format (2:1) that induces T cell-mediated killing of CEA over-expressing tumors while sparing primary cells with low CEA expression. CEA TCB treatment inhibits tumor growth and generates a highly inflamed tumor microenvironment.

Abbreviations: TCB, T cell bispecific; CEA, carcino embryonic antigen; BiTE, bispecific T cell engager

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T cell bispecific antibodies (TCBs) are molecules that recruit and engage T cells through simultaneous binding to the CD3e subunit of the T cell receptor complex (TCR) and a tumor surface antigen (TA), which results in T cell crosslinking. As consequence of TCR engagement, polyclonal T cells undergo potent activation that results in tumor cell killing along with cytokine release and T cell proliferation (e.g., expansion at sites of activation).[1] With the recent approval of blinatumomab and the promise of cancer immunotherapy, TCBs have re-attracted broad interest, and various T cell bispecific antibody formats are in preclinical and clinical development. There are two major classes of TCBs: (1) Fc-free TCBs with a short circulatory half-life and (2) IgG-based TCBs with an extended half-life. The prototype Fc-free TCB is blinatumomab (Blincyto), the CD19 BiTE (Bispecific T cell Engager), which was approved for the treatment of refractory $CD19^+$ ALL in 2014 and consists of two scFvs targeting CD19 and CD3e fused via a flexible linker.[2,3] While the BiTE molecular format is very potent in terms of killing, its clinical application is hampered by its short half-life and requirement for continuous infusion (Fig. 1A). Novel generation IgG-based T cell bispecifics use a human IgG backbone, different technologies to enable formation of the correct asymmetric bispecific antibody and Fc portions with reduced Fc gamma receptor (FcγR) binding, but retained binding to the neonatal Fc receptor FcRn to enable IgG-like pharmacokinetics (Fig. 1B and C).[4-6]

Carcinoembryonic antigen (CEA, CEACAM5, CD66e) is a glycophosphatidylinositol-anchored, 180 kDa glycoprotein broadly known as a common tumor biomarker that is over-expressed in various solid tumor including colorectal, pancreatic, gastric, non-small cell lung, breast, and other cancers. In normal tissues, low level of polarized CEA expression is found on the apical surface of glandular epithelia in the gastrointestinal tract where it is not accessible to therapeutic antibodies due to tight junctions. A short-lived BiTE molecule against CEA, MEDI-565/AMG 211, is currently in Phase 1 clinical trials.[7]

We have recently described CEA TCB (RG7802, RO6958688), a CEA-specific asymmetric 2:1 T cell bispecific antibody with a molecular format that incorporates bivalent binding to CEA and monovalent binding to CD3e. Additional key features of the 2:1 TCB format are the "head-to-tail" fusion (via a flexible linker) of one of the CEA-binding Fabs to the N-terminus of the CD3e binding one, plus an engineered, heterodimeric Fc region with completely abolished binding to FcγRs and C1q (Fig. 1C).[6] Manufacturing of this molecule with two different light chains and two heavy chains is facilitated by CrossMAb and knob-into-hole engineered Fc technologies. Bivalent binding to CEA tumor antigen confers avidity and allows better differentiation between cells with high and low CEA expression. CEA TCB bears monovalent low affinity binding to CD3e to avoid non-specific T cell activation in the peripheral blood, e.g., in absence of simultaneous binding to CEA-expressing tumor cells. Furthermore, CEA TCB is characterized by a geometric arrangement of CEA and CD3e binding Fabs in one arm of the antibody that allows formation of tight immunological synapses between T cells and tumor cells, resulting in efficient killing. CEA TCB targets a membrane-proximal epitope of CEA and does not bind to shed CEA. It is completely devoid of any Fc-mediated effector functions by introduction of P329G LALA mutations (which abrogate binding to FcγRs), to exclude the risk of FcγR-mediated activation. CEA TCB mediates killing of CEA^+ tumor cells paralleled by T cell activation, cytokine release, and T cell proliferation (both $CD8^+$ and $CD4^+$ T cell subsets). The killing of tumor cells is CEA-specific and does not occur in absence of CEA expression. CEA TCB

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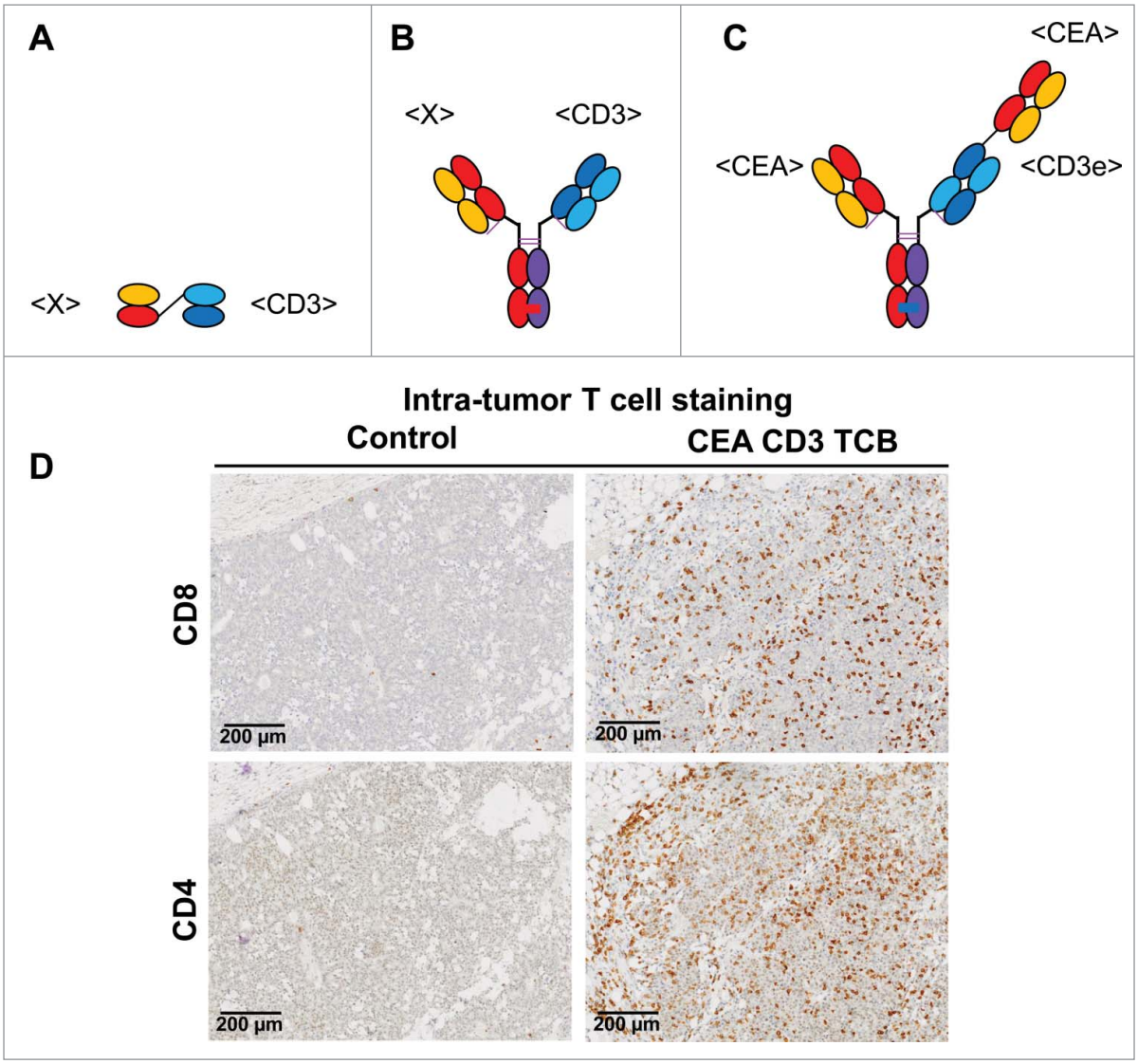

Figure 1. Schematic representation of selected clinical stage T cell bispecific antibodies: (A) tandem scFv (BiTE) format, (B) asymmetric 1:1 heterodimeric IgG-based TCB, (C) asymmetric 2:1 heterodimeric IgG-based CEA CD3 TCB; (D) Infiltration of $CD3^+$ T cells into poorly infiltrated MKN45 tumors in PBMC-engrafted NOG mice treated with vehicle, CEA CD3 TCB and an untargeted control TCB, modified from [6] with permission from Clinical Cancer Research.

activity strongly correlates with CEA expression, with higher potency observed in highly CEA-expressing tumor cells and a threshold of approximately 10,000 CEA-binding sites/cell, which allows distinguishing between high- and low-CEA-expressing tumor and primary epithelial cells, respectively. The CEA threshold required for CEA TCB activity, along with the apical expression of CEA in primary, non-transformed cells mentioned earlier, results in highly tumor-specific activity of CEA TCB. In PBMC engrafted xenograft models, CEA TCB mediates tumor growth inhibition and shows superior tumor targeting as compared to classical asymmetric 1:1 IgG TCBs. *In vitro* and *in vivo* efficacy experiments, along with histology, confocal and intravital imaging studies, demonstrated that CEA TCB mediates efficient T cell-dependent tumor cell lysis by inducing stable crosslinking of multiple T-cells to individual tumor cells.[8] Most notably, CEA TCB demonstrated efficacy in non-inflamed and poorly T-cell-infiltrated tumors and the ability to increase T-cell infiltration in tumors, thus converting the non-inflamed, PD-L1-negative tumors into highly inflamed and PD-L1-positive tumors, resulting in the generation of a more inflamed tumor microenvironment (Fig. 1D). Current studies in tumor-bearing fully immunocompetent mouse models focus on the assessment of secondary adaptive immune response and long-term T cell memory formation along with expansion of (neo-) antigen-specific T cells within tumors, as described *in vitro* for HLA-A2-WT1 specific BiTE.[9]

CEA TCB entered phase I clinical trials in 2014 for the treatment of CEA-expressing solid tumors as one of the first IgG-based TCBs for the treatment of solid tumors. Due to lack of a suitable toxicology species and surrogate molecules the entry-into-human starting dose was based on the MABEL approach.[10] CEA TCB is currently being tested as a single agent (NCT02324257) and in combination with the PD-L1 checkpoint inhibitor atezolizumab (Tecentriq) (NCT02650713). The clinical trial data, expected to be released in the near future, will reveal how the potency of CEA TCB observed in preclinical models translates into patients.

Disclosure of potential conflicts of interest

All authors are employees of Hoffmann La Roche.

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