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TCR/CD3 activation and co-stimulation combined in one T cell retargeting system improve anti-tumor immunity

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We have recently described a novel modular targeting platform for T cell recruitment that not only efficiently replaces but also is superior to conventional T cell-engaging bispecific antibodies as it allows for the flexible targeting of several antigens and the delivery of co-stimulatory ligands to malignant lesions, thereby enhancing the antitumor potential of redirected T cells.

Bispecific antibodies (bsAbs) are a powerful tool to activate polyclonal T cells in an antigen-specific manner, resulting in the lysis of antigen-expressing target cells. A large number of in vitro and in vivo studies from our group as well as from others have demonstrated that the redirection of T cells to tumor-associated antigens (TAAs) with bsAbs leads to efficient antitumor responses.[1–3] As a front-runner, the CD19-CD3-targeting bsAb blinatumomab has been shown to be safe and to exert profound anti-cancer activity in preliminary clinical trials.[4] However, as single agents bsAbs are intrinsically limited in that they target only one specific TAA, which will eventually lead to the development of antigen-loss tumor escape variants. Moreover, in our experience the development of new bsAbs for T cell engagement requires a time-consuming and cost-intensive series of optimization steps and might not necessarily leads to a product that is fully satisfying in terms of in vitro and in vivo efficiency.[5] Indeed, the experience accumulated with the design of pre-existing bsAbs, with regard to optimal single-chain fragment variable (scFv) arrangements, linker length and so forth is often not applicable to the optimization of new molecules.

To overcome these limitations, we developed a modular T-cell retargeting system which splits a conventional bsAb into 2 components (**Fig. 1**).[6] First, an effector module (EM) that is basically a bsAb with one scFv specific for the CD3 complex on the surface of T cells and one scFv targeting a motif of 10 amino acids from the human nuclear protein La (also known as Sjögren syndrome antigen B).[7] Second, an independent target module (TM), which provides the antigen specificity to the modular system. The TM essentially consists of a TAA-binding moiety, e.g., a TAA-specific scFv, fused to the La peptide recognized by one of the arms of the EM. The interaction between the T cell-recruiting EM and the target cell-binding TM leads to the establishment of an immune synapse between T lymphocytes and target cells, stimulating T cell effector functions. As a proof-of-concept, we demonstrated that the in vitro and in vivo efficiency of this modular system is comparable to that of a conventional CD33-targeting bsAb designed for the treatment of acute myeloid leukemia (AML).[6] Based on these initial findings, we were able to expand our modular system to new TAAs expressed by AML blasts, leukemias of B-cell origin and solid tumors including prostate carcinoma within a few weeks (Bachmann et al., unpublished observations). So far, we have used scFvs as target modules in our studies. However, essentially any antigen-binding moiety including recombinant variable T cell receptor (TCR) domains and other peptides can be functionalized as TMs, thus expanding the range of TAAs that can be potentially targeted with this approach.

Beside its inherent flexibility for new target antigens, our modular system opens the door to deliver additional payloads to tumor cells via the TM. In this way, we fused tumor necrosis factor (ligand)

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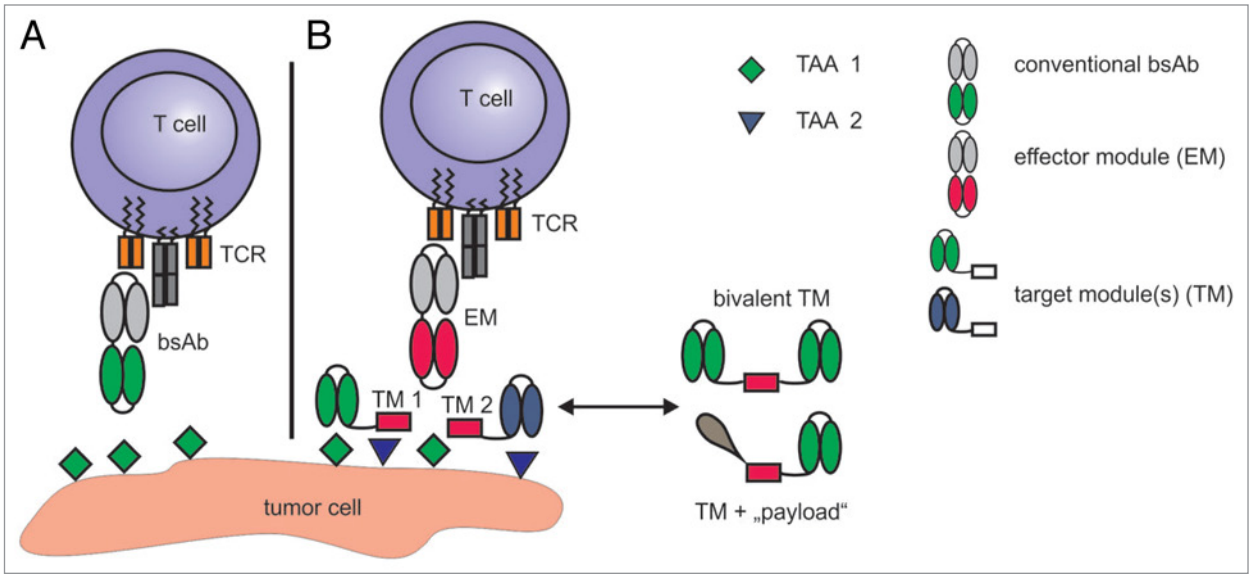

Figure 1. T-cell recruitment by conventional bispecific antibodies and a novel modular system. (**A**) T cell engaging bispecific antibodies (bsAbs) are fusion proteins comprising 2 single-chain variable fragments (scFvs). One of such scFvs is directed against an activating receptor of T cells, most commonly the CD3 molecule, while the other is specific for a tumor-associated surface antigen. (**B**) In our modular T-cell recruitment system, the 2 binding arms of a conventional bsAb are split into 2 separate units. The first unit is an effector module (EM) in the bsAb format, with one scFv specific for CD3 and one for a 10-amino acid motif from the human nuclear protein La (also known as Sjögren's syndrome antigen). The second unit is a target module (TM) that consists of an antigen-binding domain, e.g., a scFv, coupled to the La epitope recognized by the EM. In this setting, the cross-linkage of T lymphocytes and their targets is mediated by the interaction between the T cell-recruiting EM and the target cell-binding TM. TMs directed against different target antigens can be easily exchange to avoid the emergence of antigen-loss tumor escaped variants. Moreover, TMs can be functionalized with additional payloads, e.g., T cell-activating ligands, and can be rendered bivalent, to increase the avidity of binding, as well as bi-or multispecific, to recruit T cells against several antigens simultaneously.

superfamily member 9 (TNFSF9), a co-stimulatory molecule better known as 4–1BBL or CD137L, to the CD33-specific TM.[6] Unpublished data from our lab demonstrate that the lysis of target cells carrying no co-stimulatory ligands on their surface is efficiently induced by conventional bsAbs. However, in the absence of co-stimulation, redirected T cells release only modest amounts of mitogenic cytokines like interleukin-2 (IL-2) and pro-inflammatory factors such as interferon-γ (IFN-γ) and tumor necrosis factor (TNF). Consequently, upon cross-linkage by bsAbs, T cells do not initiate a proliferative response, or do so only to marginal extents (Bachmann et al., unpublished observations). The same occurs when T cells are cross-linked to their target cells by our modular system. However, the use of a TM fused to CD137L results in the delivery of a co-stimulatory signal to T cells via CD137, boosting cytokine secretion and initiating a proliferative responses among T cells, as only in this setting absolute T-cell numbers increase over a period of 6 d.[6] These results are in line with observations made by other investigators, who delivered co-stimulatory signals to T cells via a second bsAb independent of the primary CD3-crosslinking bsAb.[8] The improvement of bsAb-induced T cell responses obtained by the addition of co-stimulatory signals mirrors observations that were made years ago in the field of chimeric antigens receptor (CAR) research (reviewed in ref. 9). In clinical trials testing T lymphocytes that had been modified to express first-generation CARs, these T cells could only be detected in vivo for very short periods, and no objective tumor response was observed. In contrast, in ongoing trials, the use of T cells expressing second-generation CARs, which harbor a co-stimulatory CD28 or CD137 domain in addition to the activating CD3ζ signaling chain, resulted in profound antitumor responses, and CAR-expressing T cells could be detected in the circulation of patients for several months up to now.[10] Thus, the additional costimulation signals enhance in vivo survival of engineered T cells, which seems to be one critical factor for the outcome of this therapeutic approach. In the setting of bsAb-based therapy, redirected effector T cells can be constantly replenished from the circulation and the long-term fate of redirected T cells might be less important for maintaining anti-tumor response. However, in clinical situations in which the number of effector T cells that can be recruited by bsAbs is limited, for instance in the case of solid tumors, the delivery of a potent co-stimulatory signal by bsAbs might be critical for the success of therapy. We have demonstrated that the additional CD137-dependent co-stimulatory signal provided by the CD137L-TM fusion protein significantly increases the lysis of AML blasts with low antigen density at low effector to target cell ratios.[6] Thus, the incorporation of additional T cell co-stimulatory signals in bsAb constructs should be considered as a feasible strategy to improve the clinical outcome of bsAb-based immunotherapy.

Disclosure of Potential Conflicts of Interest

Michael Bachmann, Slava Stamova, and Gerhard Ehninger have filed provisional patent application related to the antibody directed to CD33.

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