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 anti-tumor responses. 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. 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.

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). 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). 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). 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)
superfamily member 9 (TNFSF9), a co-stimulatory molecule better known as 4–1BBL or CD137L, to the CD33-specific TM. 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 the CD137 domain in addition to the activating CD3ζ signaling chain, resulting in profound antitumor responses, and CAR-expressing T cells could be detected in the circulation of patients for several months up to now. 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. 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.
References
1. Arndt C, von Bonin M, Cartellieri M, Feldmann A, Koristka S, Michalk I, Stamova S, Bornhäuser M, Schmitz M, Ehninger G, et al. Redirection of T cells with a first fully humanized bispecific CD33-CD3 antibody efficiently eliminates AML blasts without harming hematopoietic stem cells. Leukemia 2013; 27:964-7; PMID:23325142; http://dx.doi.org/10.1038/leu.2013.18
2. Feldmann A, Arndt C, Töpfer K, Stamova S, Krone F, Cartellieri M, Koristka S, Michalk I, Lindemann D, Schmitz M, et al. Novel humanized and highly efficient bispecific antibodies mediate killing of prostate stem cell antigen-expressing tumor cells by CD8+ and CD4+ T cells. J Immunol 2012; 189:3249-59; PMID:22875801; http://dx.doi.org/10.4049/jimmunol.1200341
3. Stamova S, Cartellieri M, Feldmann A, Bippes CC, Bartrich H, Wehner R, Schmitz M, von Bonin M, Bornhäuser M, Ehninger G, et al. Simultaneous engagement of the activatory receptors NKG2D and CD3 for retargeting of effector cells to CD33-positive malignant cells. Leukemia 2011; 25:1053-6; PMID:21458350; http://dx.doi.org/10.1038/leu.2011.42
4. Bargou R, Leo E, Zugmaier G, Klinger M, Goebeler M, Knop S, Noppeney R, Viardot A, Hess G, Schuler M, et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. Science 2008; 321:974-7; PMID:18703743; http://dx.doi.org/10.1126/science.1158545
5. Stamova S, Cartellieri M, Feldmann A, Arndt C, Koristka S, Bartrich H, Bippes CC, Wehner R, Schmitz M, von Bonin M, et al. Unexpected recombinations in single chain bispecific anti-CD3-anti-CD3 antibodies can be avoided by a novel linker module. Mol Immunol 2011; 49:474-82; PMID:22014687; http://dx.doi.org/10.1016/j.molimm.2011.09.019
6. Arndt C, Feldmann A, von Bonin M, Cartellieri M, Ewen EM, Koristka S, Michalk I, Stamova S, Berndt N, Goehr A, et al. Costimulation improves the killing capability of T cells redirected to tumor cells expressing low levels of CD33: description of a novel modular targeting system. Leukemia 2013; 10; PMID:23958923; http://dx.doi.org/10.1038/leu.2013.243
7. Koristka S, Cartellieri M, Arndt C, Bippes CC, Feldmann A, Michalk I, Wiefel K, Stamova S, Schmitz M, Ehninger G, et al. Retargeting of regulatory T cells to surface-inducible autoimmune La/SS-B. J Autoimmun 2013; 42:105-16; PMID:23522111; http://dx.doi.org/10.1016/j.jaut.2013.01.002
8. Hornig N, Kermor V, Frey K, Dieboldt P, Kontermann RE, Müller D. Combination of a bispecific antibody and costimulatory antibody-ligand fusion proteins for targeted cancer immunotherapy. J Immunother 2012; 35:418-29; PMID:22576347; http://dx.doi.org/10.1097/CJI.0b013e3382594387
9. Cartellieri M, Bachmann M, Feldmann A, Bippes C, Stamova S, Wehner R, Temme A, Schmitz M. Chimeric antigen receptor-engineered T cells for immunotherapy of cancer. J Biomed Biotechnol 2010; 2010:956304; PMID:20467460; http://dx.doi.org/10.1155/2010/956304
10. Davila ML, Brentjens R, Wang X, Riviere I, Sadelain M. How do CARs work?: Early insights from recent clinical studies targeting CD19. OncolImmunology 2012; 1:1577-83; PMID:23264903; http://dx.doi.org/10.4161/onci.22524