mAbs initiated the unprecedented breakthroughs in cancer immunotherapy and are rapidly evolving with multiple therapeutic platforms. One next-generation strategy engineers multivalent proteins that ligate single-chain variable fragments targeting cellular effectors, tumor-associated antigens, and cytokines. These developing therapeutics target and regulate cellular effector bioactivity and significantly improve clinical outcomes. Clin Cancer Res. 22(14): 3419–21. ©2016 AACR.

See related article by Vallera et al., p. 3440

In this issue of Clinical Cancer Research, Vallera and colleagues (1) report the generation of a trispecific killer engager (TriKE) that incorporates single-chain variable fragments (scFV) against CD16 and CD33. This engineered protein crosslinks natural killer (NK) and myeloid tumor cells while also stably incorporating IL15 to induce the expansion, activation, and survival of NK cells. The preclinical therapeutic potential of this drug was documented using immunodeficient mice bearing an acute myelogenous leukemia cell line (HL-60), resulting in the improved survival of NK cells and prolonged survival of the tumor-bearing hosts. In these studies, the therapeutic activity of engineered CD16 × CD33 scFVs with or without IL15 was compared. Both therapeutics demonstrated improved control of tumor burden relative to no-drug control mice, while long-term activity was significantly improved with the engineered therapeutic incorporating IL15 relative to the drug without IL15.

Tumor-specific mAbs provide effective cancer immunotherapy for patients with a variety of malignancies (2). The first therapeutic antibody, rituximab was approved by the FDA in 1997. These first-generation mAbs have achieved significant success in the treatment of neoplasia and since rituximab’s approval, numerous mAbs have been approved. Next-generation monoclonal variants have been engineered and are undergoing clinical development with encouraging results, suggesting increased potency and an improved safety profile, resulting in an expanding focus on engineered mAb development (3). Indeed, two such engineered antibodies have received regulatory approval and more than 30 are in clinical development. These engineered proteins include bispecific Abs that simultane-ously target tumor cell antigens and re-target NK or T cells to the tumor cells. NK cells have traditionally been considered innate immune cells, and initial therapeutic strategies focused on NK cells utilizing IL2 administration to expand their num-

bers, an approach that received regulatory approval associated with both a significant clinical response (around 15%) and toxicity (4). Thus, therapeutic strategies to target NK cells to tumors without systemic expansion are expected to reduce toxicity with the retention of therapeutic activity.

Mechanistically, mAbs utilize a variety of strategies to mediate antitumor activity (2). One such mechanism, antibody-dependent cellular cytotoxicity (ADCC), involves an interaction between the antibody Fc region and Fc receptors on immune cells, including NK cells, macrophages, and neutrophils, resulting in the lysis of infectious agents and infected and malignant cells. Despite their noteworthy clinical achievements, therapeutic mAbs are still not approved for the majority of human cancers, primarily due to the lack of suitable “tumor cell–specific” antigens, as well as challenges with mAb pharmacology (5). A second confounder to the routine clinical use of mAbs is that standard-of-care interventions, chemotherapy, and radiation can be cytotoxic to NK cells, hampering their ability to mediate ADCC.

In recent years, bispecific mAbs and engineered molecules have been used to redirect NK cells to tumor cells (6) with a mechanism of action dependent on ADCC. The CD16-specific scFVs have advantages over monospecific mAbs, as they result in stronger NK cell ADCC and improved pharmacodynamic characteristics (7). A second approach to improving their immunotherapeutic activity has been the combination of bispecific mAbs with cytokines, such as IL2 or IL15 (Fig. 1), that have the potential to improve the efficacy of mAb therapy through their ability to activate and expand NK cells in vivo, to upregulate NK cell-activating receptors, and override inhibitory interactions mediated by self-MHC class I molecules that can suppress NK cell ADCC. The current focus is on IL15, which has a critical role in NK cell developmental homeostasis, proliferation, survival, and activation (8) in the absence of the toxicities associated with systemic IL2 administration. One additional advantage of IL15 over IL2 therapy is that it does not engage Treg suppressor (9).

In the article by Vallera and colleagues (1), it is reported that the trispecific CD16 × IL15 × CD33 recombinant molecule increased ADCC toxicity in vitro and in vivo as compared with the bispecific antibody without IL15. Furthermore, this trispecific molecule was demonstrated to expand and sustain NK cells viability in vivo using a xenograft model. This preclinical model of therapeutic activity
tumor targets, resulting in accelerated tumor cell killing. Immune lytic synapse forms between the NK effector cells and proximity with tumor cells is critical. As part of this interaction, an fully understood, although their ability to bring NK cells into close proximity, cytokine secretion of cytotoxic mediators (left). These engineered proteins are generated by a linker between an scFV targeting a specific tumor-associated antigen (CD33) and a CD16 scFV that ligation NK cells. These are combined with a second linker to a cytokine (IL15; right).

Figure 1. Trispecific engineered proteins can enhance an immunotoxic synapse by a variable scFV to a tumor-associated antigen (CD33) linked to a targeting and activating scFV (CD16) for NK cells combined with a second linker to IL15. The addition of IL15 can prolong NK cell survival, increase proliferation and activation, resulting in NK-cell secretion of cytotoxic mediators (left). These engineered proteins are generated by a linker between an scFV targeting a specific tumor-associated antigen (CD33) and a CD16 scFV that ligation NK cells. These are combined with a second linker to a cytokine (IL15; right).

Supports the potential utility of TriKEs to increase the selectivity of NK cell therapy not only for myeloid malignancies, but indirectly also for solid tumors if appropriate tumor antigens are targeted. The mechanism by which these recombinant molecules act is not fully understood, although their ability to bring NK cells into close proximity with tumor cells is critical. As part of this interaction, an immune lytic synapse forms between the NK effector cells and tumor targets, resulting in accelerated tumor cell killing.

Similar to bispecific antibodies, immunocytokines are mAb/ scFV and cytokine fusion proteins. These engineered proteins can use full mAbs or scFVs linked to cytokines (10). Following tumor cell binding, immunocytokines and their associated proinflammatory cytokines can mediate multiple bioactivities. For example, IL2, IL12, and TNF can stimulate leukocytic infiltration into a tumor mass, which may result in therapeutic activity (11). As such, bispecific and trispecific mAbs can be directed against tumor cell antigens, nontumor-associated targets found in the tumor extraacellular matrix, such as endothelial cells, effector cells such as NK cells and T cells, and may include a variety of cytokines to manipulate cellular effector or target effector cells to tumor cells or all of the above.

The success of engineered molecules critically relies on the identification of functional tumor-associated antigens and their ligation by scFVs to effector cells and immunoregulatory cytokines. Challenges to the success of this therapy strategy include impediments similar to chemotherapy, whereby the downregulation of the targeted tumor antigens allows the tumor to become refractory to intervention. Another challenge to the success of engineered multivalent proteins is cellular targeting by the cytokine as opposed to targeting by scFVs to tumor cells. The scFVs are expected to mediate tumor targeting; however, in at least one study, the cytokine moiety was shown to govern biodistribution, resulting in the unexpected loss of tumor targeting (12). Similar challenges may be associated with abnormal cellular biodistribution following ligation, based on capillary arrest in nontumor organs, such as the lungs, liver, or spleen. Depending on the characteristics of the engineered fusion product, as well as the linked cytokine, the mononuclear phagocyte system may regulate biodistribution of an engineered molecule bound to a target cell(s). Therefore, careful evaluation of the biodistribution and pharmacokinetics of engineered molecules is critical to assess the influence of cytokine affinity, receptor distribution, size, and avidity on the effectiveness of engineered protein/effector cell accumulation. Despite these challenges, based on the results from the studies by Vallera and colleagues, the future is bright for trispecific engineered therapeutics.

Disclosure of Potential Conflicts of Interest
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Genetically Engineered Multivalent Proteins for Targeted Immunotherapy

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