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Bispecific antibody platforms for cancer immunotherapy

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Abstract

Over the past decades advances in bioengineering and expanded insight in tumor immunology have resulted in the emergence of novel bispecific antibody (bsAb) constructs that are capable of redirecting immune effector cells to the tumor microenvironment. (Pre-) clinical studies of various bsAb constructs have shown impressive results in terms of immune effector cell retargeting, target dependent activation

Abbreviations: V_H, variable heavy chain domain; V_L, variable light chain domain; mAb, monoclonal antibody; bsAb, bispecific antibody; scFv, single chain variable fragment; Triomab, trifunctional hybrid antibody; TaFv, tandem single chain variable fragment; BiTE, bispecific T-cell engager; bsDb, bispecific diabody; scDb, single chain diabody; DART, dual affinity retargeting molecule; VHH, variable domain of heavy chain-only Ab.

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and the induction of anti-tumor responses. This review summarizes recent advances in the field of bsAb-therapy and limitations that were encountered. Furthermore, we will discuss potential future developments that can be expected to take the bsAb approach successfully forward.

Keywords: Bi-specific antibodies; Dual specific retargeting; Immune effector cells; Anti-cancer therapy

1. Introduction

Several clinically available therapeutic monoclonal antibodies (mAbs) can induce immune-mediated tumor cell killing through mechanisms that include complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). Following binding of the mAb to its tumor target, interactions of the Fc-portion with Fcγ-receptors (FcγR) expressed by effector cells (*e.g.* natural killer (NK) cells, macrophages and γδ T-cells) may result in CDC and ADCC and subsequent antitumor cytotoxicity and/or phagocytosis. In clinical series, ADCC has been demonstrated to significantly enhance the efficacy of various mAbs, including rituximab (anti-CD20), trastuzumab (anti-human-epidermal-growth-factor receptor 2 (Her2)) and cetuximab (anti-epidermal-growth-factor receptor (EGFR)) [1]. Although data are inconsistent, clinical responses may be influenced by FcγR polymorphisms [2]. Granting their therapeutic efficacy can in part be attributed to beneficial secondary immune effects, it is clear that mAbs still do not exploit the full potential of the immune system as effects are *e.g.* hampered by circulating immunoglobulins (Ig) competing for FcγR binding spots on immune effector cells, and inadequate tumor-target penetration due to their relatively large size (∼150 kDa) [3]. Furthermore, binding to inhibitory FcγR on immune cells may result in internalization of the mAb-tumor target-FcγR complex reducing its therapeutic efficacy [4].

Bispecific Abs (bsAbs), capable of binding two targets simultaneously, lack several of the above described limitations and can potentially induce a more powerful anti-tumor immune response. The first bsAbs were engineered by either chemical crosslinking or exchange of different heavy chains as a result of fusion of two hybridoma cell lines (*i.e.* hybrid hybridomas or quadromas). Despite some clinical effects, none of these bsAbs made it to advanced stage clinical trials, as a low production yield owing to random association of heavy/light chains and immunogenicity caused by human anti-mouse/rat antibodies (HAMA/HARA) severely hampered clinical applicability [5]. It was not until 1995 that bsAb research was sparked again by the introduction of fused mouse-rat hybrid antibodies and tandem single-chain variable fragments (TaFv) [6,7].

Here, we will review the main bsAb formats that are currently being developed for tumor retargeting of immune cells and discuss thus far obtained (pre-)clinical results and encountered limitations. Furthermore, we will elaborate on potential ways to take the bsAb approach forward.

2. Currently available bispecific antibody platforms

2.1. Trifunctional hybrid antibodies (Triomab)

Introduced in 1995, this platform offered a solution to the random association of heavy/light chains observed in classic quadroma technology. By combining the halves of two distinct antibodies, a tumor-specific mouse IgG2a and a CD3-specific rat IgG2b, a full-size functional mAb was engineered and termed Triomab (Trion pharma Inc.) (Fig. 1b). Due to species-preferential heavy/light chain pairing random association was greatly reduced. Interestingly, the hybrid mouse/rat Fc-portion was able to activate FcγR$^+$ accessory cells [6,8,9].

Preclinical studies with a Triomab targeting epithelial cell adhesion molecule (EpCAM), expressed by the majority of epithelial cancers, and CD3 expressed by T-cells, demonstrated redirection and activation of T- and accessory cells (*e.g.* NK cells, dendritic cells (DC) and macrophages). T-cell activation was complemented by the induction of T-cell mediated tumor lysis, cytotoxic cytokine release, and ADCC in the picomolar range [8,9]. The additive value of a functional Fc-portion was underscored by enhanced tumor protection in mice treated with a Triomab compared to a similar bsAb consisting of two chemically cross-linked fragment antigen binding (Fab) regions ($F(ab)_2$) (Fig. 1c). *In vivo* assessment of the Triomab injected intraperitoneally (i.p.) in a syngeneic C57BL/6 and BALB/c mouse model using (human) EpCAM expressing B16-melanoma and A20-lymphoma cells, respectively, demonstrated a significantly improved tumor cell elimination compared with the simultaneous administration of both parental antibodies. In the Triomab treated group a 100% survival rate, complete tumor eradication and protection against tumor rechallenge was reported. Selective depletion of both CD4$^+$ and CD8$^+$ T-cells resulted in a marked loss of tumor protection and survival. Of note, only the animals injected with human EpCAM expressing tumors and Triomab treatment developed strong anti-EpCAM specific humoral immune responses [10], with additional evidence suggestive of epitope spreading. These data resulted in the development and clinical evaluation of a number of Triomabs, including catumaxomab, ertumaxomab and FBTA05 which will be discussed below.

2.1.1. Catumaxomab

Catumaxomab, an anti-EpCAM-anti-CD3 Triomab, was the first Triomab studied in patients. A phase-I trial of a single intravenous (i.v.) dose of catumaxomab in patients with non-small cell lung cancer (NSCLC), established a maximum