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T cell engaging bispecific antibody (T-BsAb): From technology to therapeutics

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ABSTRACT

Harnessing the power of the human immune system to treat cancer is the essence of immunotherapy. Monoclonal antibodies engage the innate immune system to destroy targeted cells. For the last 30 years, antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity have been the main mechanisms of anti-tumor action of unconjugated antibody drugs. Efforts to exploit the potentials of other immune cells, in particular T cells, culminated in the recent approval of two T cell engaging bispecific antibody (T-BsAb) drugs, thereby stimulating new efforts to accelerate similar platforms through preclinical and clinical trials. In this review, we have compiled the worldwide effort in exploring T cell engaging bispecific antibodies. Our special emphasis is on the lessons learned, with the hope to derive insights in this fast evolving field with tremendous clinical potential.

1. Introduction

Cancer remains one of the leading causes of death, with the accompanying social and economic burden worldwide. While surgery is effective for locoregional control, chemotherapy and radiation have been mostly ineffective for metastatic cancers, even when pushed to dose and intensity limits, which alone can be harmful because of their inability to discriminate cancer cells from normal bystanders. To minimize toxicity, much efforts have been devoted to identify therapeutic agents that can selectively inhibit the growth of or eradicate cancer cells, while leaving normal cells unscathed - a concept dubbed the “magic bullet” by Paul Ehrlich more than 100 years ago. Before the advent of pathway-specific small molecule inhibitors, antibody-based drugs had been the centerpiece of these efforts and they will likely remain a major player in the coming decades in cancer therapy.

Antibodies are extraordinary molecules vetted through millions of years of evolution. Each antibody molecule has two identical antigen binding sites at the N-terminal variable region that are responsible for the exquisite antigen binding specificity and the binding affinity of these molecules, and a constant fragment crystallizable (Fc) region at the C-terminus that triggers multiple effector mechanisms (Vidarsson, Dekkers, & Rispens, 2014). Depending on the specific antigen/antibody pair, binding alone can physically block the antigen (receptor) or initiate/inhibit signaling through the antigen (receptor) leading to apoptosis of target cells. For the majority of cancer therapeutic IgG antibodies, they execute their immune functions through recruitment of natural killer cells or myeloid cells/macrophages via the Fc region. Furthermore, the Fc region can initiate the classical complement cascade to deposit membrane attack complex on the surface membrane of target cells. These Fc-dependent tumor lysis mechanisms have been extensively studied and exploited in human medicine.

Soon after the discovery of the hybridoma technique by Kohler and Milstein (1975) to immortalize B-cells, the first monoclonal antibody muromonab-CD3 (OKT3) specific for human CD3 was developed and approved in 1985 for treating organ transplant rejection. It took the next decade before the first cancer therapeutic antibody rituximab was approved in 1997 to treat CD20(+) non-Hodgkin lymphoma. Since then, at least 27 therapeutic antibodies for a broad spectrum of human cancers have been approved. The success of these antibody therapeutics firmly established cancer immunotherapy as the fourth modality (after surgery, chemotherapy and radiation) whereby existing defense mechanisms of the human immune system can be mobilized to specifically kill cancer cells. However, naturally occurring IgG antibodies do not have the functionality to directly engage the most efficient “killer” in the immune system, namely, the cytotoxic T lymphocyte (CTL). In order to do that, antibodies have to be engineered to include a second specificity, hence bispecific antibodies (BsAb).

Abbreviations: CRS, cytokine release syndrome; CTL, cytotoxic T lymphocyte; EC_{50}, half maximal effective concentration; Fc, fragment crystallizable; ICI, immune checkpoint inhibitor; pMHC, peptide-major histocompatibility complex; scFv, single chain variable fragment; TandAb, tandem diabody; T-BsAb, T cell engaging bispecific antibody; TCR, T cell receptor; TDCC, T cell dependent cellular cytotoxicity; TIL, tumor infiltrating lymphocyte

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The concept of bispecific antibodies dates back to the 1960s, when Alfred Nisonoff envisioned the potential of replacing one of the two identical antigen binding arms with a different antigen binding specificity (Nisonoff & Rivers, 1961; Nisonoff, Wissler, & Lipman, 1960). This concept was developed further in the 1980s to include a second specificity against T cell determinants. CTLs, like all T cells, express variable T-cell receptors (TCRs) associated with invariable CD3 subunits. Binding of TCR by cognate peptide-major histocompatibility complex (pMHC) initiates the signaling through the CD3 complex, which in turn relays the signal internally to activate T cells. By binding to the CD3 complex, CD3-binding monoclonal antibody can bypass the pMHC restriction, thereby activating polyclonal CTLs. When such CD3 binding specificity was engineered into antibodies that bind to tumor specific antigens, CTL response can be redirected to cancer cells (Perez, Hoffman, Shaw, Bluestone, & Segal, 1985; Staerz, Kanagawa, & Bevan, 1985). This strategy gave rise to a completely new class of therapeutic antibodies for cancer immunotherapy. Although it was later found that this class of antibodies could also activate through CD3 on non-T cells, for the purpose of this review, we refer to them as T cell engaging bispecific antibody, or T-BsAb for short.

Over the past three decades a myriad of T-BsAbs have been developed (discussed below). Although the molecular details differ considerably, they are all grounded on the basic design of combining tumor antigen binding specificity and T cell binding specificity into one molecule, with or without an Fc region. To date, only two T-BsAbs, catumaxomab and blinatumomab, have been approved for clinical use in humans, as compared to the other 25 IgG based antibody drugs. The lag is largely attributed to the difficulties in protein engineering during the manufacture of these antibodies and the uncertain clinical toxicities with these novel constructs. Nevertheless, over the past 30 years, multiple molecular designs have been invented, some of which have entered clinical stages of development and many more are in preclinical testing. In this review, we have compiled all the molecular designs that have been developed so far and discussed different aspects of T-BsAbs, including molecular details of their mechanisms of action, factors that may determine their potency, as well as different challenges lying ahead. We hope to provide a timely summary of all the lessons learned that may provide insights to help T-BsAb development in the coming decades.

2. T-BsAbs developed to date

A few recent comprehensive reviews (Brinkmann & Kontermann, 2017; Kontermann & Brinkmann, 2015; Spiess, Zhai, & Carter, 2015) have summarized the various bispecific antibody designs currently under development or approved. To be consistent, this review will follow the same nomenclature they adopted whenever possible. Multiple technologies have been developed to generate human IgG-like molecules; in this review we refer to them as hIgG. Fig. 1 summarizes the major formats discussed in this review.

2.1. T-BsAbs in clinical development

Table 1 summarizes all T-BsAbs that have reached clinical stages so far. Out of these 23 antibodies, blinatumomab was approved for treatment of refractory/relapse Ph(-) B-ALL and catumaxomab was approved for malignant ascites derived from EpCAM(+) carcinomas. The rest are mostly ongoing or completed phase I clinical trials, except two trifunctional antibodies, FBTA05 and ertumaxomab, which have entered phase II trials for intravenous infusion. However, both studies have since been terminated.

Besides T-BsAbs against antigens expressed by hematopoietic cells, namely, B cells (CD19, CD20, BCMA) and myeloid cells (CD33, CD123 and CLEC12A), it is encouraging to note that T-BsAbs against antigens expressed by solid tumors (CEA, EpCAM, HER2, PSMA, p-Cadherin, pMHC, GPC3, GPA33) are also being tested. Results from these trials will inform future strategies to optimize T-BsAbs. CD19, CD20, EpCAM, CD33 and HER2 are clinically proven targets, as they are also targets of approved IgG drugs; whereas other targets like p-Cadherin, pMHC and GPC3 are important novel targets that have not been drugged with FDA-approved or EMA-approved antibodies.

The most common format is tandem single chain variable fragment (scFv) based on blinatumomab. However, newer formats like tandem diabody (TandAb), DART and DART-Fc, hIgG, Fab-scFv-Fc, TriFab-Fc, scFv-Fc-scFv, BEAT and TCR-αCD3 are also being investigated. The m/rIgG trifunctional format was used by the first T-BsAb approved. However, apart from immunogenicity, it was severely limited by toxicity when delivered systematically (Sebastian et al., 2007). This is likely due to their wildtype Fc with full effector functions; and as a result it has not been widely adopted. All the molecular designs incorporate monovalent CD3 binding except for TandAb and scFv-Fc-scFv, which, at least structurally, could mediate bivalent CD3 binding. The prevalence of monovalent anti-CD3 design probably stemmed from the observation that bivalent anti-CD3 antibodies could result in activation induced T cell death (AICD) (Kuhn & Weiner, 2016) and the concerns that it might cause target independent T cell activation. However, AICD in T-BsAb will likely be platform-specific, since for at least 3 formats using bivalent anti-CD3 design, T cells seemed to be fully functional in vitro and in animal models (discussed below). Therefore, the clinical outcomes of these bivalent formats (two are currently in trial) would be informative in the future design of the optimal T-BsAb.

2.2. Preclinical T-BsAbs

The concept of T-BsAb was explored initially in 1985 in murine system using anti-mouse CD3 antibody; but within a few months the first T-BsAb using anti-human CD3 was developed (Perez et al., 1985; Staerz et al., 1985). The following decades saw an “explosion” of bispecific antibody development (Riethmüller, 2012). T-BsAbs engineered for human use were dominated initially by chemical conjugation of either full-length IgG or F(ab’), or by hybrid hybridoma technology. Since then, a plethora of T-BsAb formats have been described (Table 2). These include most of the formats used by non-T bispecific antibodies (Brinkmann & Kontermann, 2017; Kontermann & Brinkmann, 2015; Spiess et al., 2015). The most frequently used format is tandem scFv (BiTE), partly because it avoids issues of cognate chain pairing in multichain constructs, and partly because of its clinical success epitomized by blinatumomab. With the advent of full-length bispecific Ig formats that overcome these pairing issues (Fig. 1), T-BsAbs with more native conformations can now be more easily manufactured while achieving more desirable PK-profiles than BiTEs (discussed below) and are becoming more widely adopted.

In addition to the large number of formats, more than 44 antigens have been targeted, with varying degrees of success in preclinical models. The majority of these antigens are oncogenic proteins, except for a few targets in infectious diseases which are not the focus of this review. The most commonly targeted antigens are EGFR, CD19, CD20, CD33, CEA, EpCAM and HER2, all of which have been targeted by more than one format. Peptide-MHC is an interesting class of antigens that has emerged in recent years. Traditional targets for therapeutic antibodies are expressed on cell surface, while most oncoproteins are expressed intracellularly and inaccessible to conventional antibodies. However, peptide fragments of some of these proteins generated via protein turnover can be presented by MHC on the cell surface, which greatly expand the repertoire of “druggable” targets. Immunocore Limited has pioneered the affinity maturation of TCR fused to anti-CD3 scFv. Moreover, TCR-like therapeutic antibodies that target pMHC in a similar fashion as TCR are also emerging in the past few years and are currently actively pursued (Dao et al., 2015).

Most T-BsAbs developed so far utilize anti-CD3 moiety for T cell recruitment. Excluding those T-BsAbs that did not disclose their anti-CD3 sequences, most of the T-BsAbs developed to date used clones