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Application of Bispecific T Cells Engagers in Hematological Malignancy

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Abstract:

Over the past decade, it has played a significant role in immunology and is commonly used to combat malignant blood tumors. The product that has been invented and is the most successful one is blinatumomab. BiTEs are dual-specificity T-cell engagers composed of two linked antigen-binding variable fragments, and these fragments do not contain the constant domains of their parent antibodies. This makes it a project with great development potential. Indeed, BiTEs still face many challenges, including short half-life, tumor microenvironment (TME), cytokine release syndrome (CRS), and neurotoxicity. For these side effects resulting from the treatments, BiTEs are still in a stage of research and overcoming. Modern medicine is urgently seeking effective and safe methods for treating cancer. Since the advent of BiTEs, a large number of applications have been made to develop the increasing maturity of blinatumomab, which has also led to the wider application of BiTEs. A large number of clinical trials have confirmed that BiTEs have a high rate of targeted therapy for malignant hematological tumors.

Keywords: BiTEs; Hematological Malignancy; Application.

1. Introduction

Hematological malignancies comprise a diverse spectrum of severe disorders primarily originating from dysfunctional hematopoietic stem cells (HSCs). These pathological alterations interfere with normal differentiation pathways, resulting in arrested maturation, suppressed apoptosis, and uncontrolled proliferation. Epidemiological studies indicate that such malignancies represent roughly 6–10% of all cancer cases, accounting for nearly 6% of cancer-related fatalities.Consequently, investigations into disease mechanisms, novel drug discovery, and treatment optimization remain essential to mitigate the burden and improve survival.

In 2022, the World Health Organization (WHO) classified the hematolymphoid tumors mainly into leukemia, lymphoma, myelodysplastic syndromes (MDS), and multiple myeloma (MM). Current therapeutic modalities include chemotherapy, radiation, molecularly targeted agents, hematopoietic stem cell transplantation (HSCT), and immunotherapy. While certain subtypes—such as acute promyelocytic leukemia (APL), chronic myeloid leukemia (CML), and

chronic lymphocytic leukemia (CLL)—exhibit favorable remission rates, the majority still suffer from suboptimal long-term survival and frequent relapse. Additionally, treatment-related toxicities present significant clinical challenges, underscoring the need for more refined approaches [1-3].

Bispecific T-cell engagers (BiTEs) have emerged as a transformative class of immunotherapeutic agents, especially within oncology. Their development and implementation have progressed most rapidly in the context of hematologic malignancies. BiTEs are specially designed proteins that can bind to immune effector cells and are suitable for large-scale standardized production. Their relatively simple single-chain polypeptide structure facilitates efficient production using existing commercial platforms, which not only benefits immunological research but also contributes to clinical translational application [1,2].

Current research is still exploring the potential of BiTE technology in developing more effective and broader applicable anti-cancer therapies. So far, its most mature application field is the treatment of hematological malignancies and other non-solid tumors. Although some preclinical and clinical research evidence indicates its potential application value in solid tumor treatment, the therapeutic effect in this area is still relatively limited. Clearly, BiTE technology is a rapidly developing field with great therapeutic potential. This review will focus on the clinical application of BiTE in hematological malignancies, as its efficacy in this area has been strongly demonstrated.

2. Bispecific T Cell

BiTEs, a major subclass of immune cell engagers (ICEs), have gained increasing prominence in cancer immunotherapy due to their ability to counteract immune evasion and redirect immune cell activity against tumors. Advances in immunology and molecular biology have been instrumental in the development of these molecules. A landmark achievement in this field is blinatumomab, which has demonstrated considerable clinical efficacy in the treatment of ALL. Developed by Amgen, blinatumomab received simultaneous approval from the U.S. FDA and the European EMA in December 2014. As a bispecific antibody targeting CD19 and CD3, it is specifically indicated for B-cell ALL and has catalyzed the development of numerous related agents. Presently, over 100 clinical trials are investigating BiTEs for both hematologic and solid malignancies [1].

The year 2022 marked the approval of three additional BiTEs in both the U.S. and Europe: tebentafusp (targeting CD3 and gp100, for metastatic uveal melanoma), mosunetuzumab (targeting CD3 and CD20, for follicular lymphoma), and teclistamab (targeting CD3 and BCMA, for multiple myeloma). The „Antibodies to Watch“ series in 2023 further identified three late-stage BiTE candidates—odronextamab (anti-CD20 × anti-CD3), linvoseltamab (anti-BCMA × anti-CD3), and talquetamab (anti-GPRC5D × anti-CD3)—that were anticipated to gain regulatory approval that year [1]. According to the 2024 update, talquetamab has since received full FDA approval and conditional EMA authorization, while odronextamab and linvoseltamab remain under review.

Mechanistically, BiTEs are constructed from two single-chain variable fragments (scFvs): one binds to a tumor-associated antigen (TAA) on cancer cells, and the other engages the CD3 subunit of the T-cell receptor (TCR). This dual binding facilitates the formation of an immunological synapse between T cells and tumor cells, triggering T-cell activation and the release of perforin and granzymes, which mediate rapid tumor cell killing. By targeting the CD3ϵ subunit, BiTEs activate T cells in a TCR-independent manner, bypassing MHC restriction and enabling broad redirection of polyclonal T cells against tumors. This mode of action may also promote epitope spreading by engaging T cells with diverse specificities. Furthermore, BiTE-induced activation drives the expansion of CD8+ T cells, particularly cytotoxic effector memory subsets, enhancing antitumor immunity. Importantly, because BiTE-mediated killing does not require TCR recognition of peptide–MHC complexes, it circumvents a common immune escape mechanism wherein tumors downregulate MHC-I expression [3, 4].

Furthermore, BiTEs have been demonstrated to induce T cell-mediated cytotoxicity both in vivo and in vitro at extremely low concentrations and low effector-to-target cell ratios (E:T). Single-chain bispecific antibody fragments have also been shown to exhibit up to 100,000-fold higher antitumor cytotoxicity in vitro compared to conventional monoclonal antibodies (mAbs) [1]. However, it is important to recognize that in vitro findings may not accurately reflect the in vivo behavior of therapeutic agents. Retrospective studies comparing antibody efficacy in these two settings have revealed notable differences in binding properties. Many in vitro efficacy assays fail to fully capture steady-state target interactions and are therefore limited in their ability to comprehensively assess ligand–target kinetics. However, there are still significant challenges remain, which will be discussed in the following parts.

3. Appplication in ALL

Currently, bispecific T cells engagers is mainly used for the treatment of non-solid tumors. Among them, its devel-

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opment and therapeutic effects in malignant hematological tumors are the most remarkable. The most groundbreaking and successful development of bispecific T cells engagers is Blinatumomab. Blinatumomab is a CD19×CD3-directed bispecific T-cell engager that has become an essential component of acute B lymphoblastic leukemia (BCP-ALL) treatment. It is utilized in cases of relapsed/refractory disease, minimal residual disease (MRD), and as a first-line treatment. Blinatumomab is particularly effective in MRD-positive BCP-ALL, inducing a high rate of deep molecular response and demonstrating very good overall tolerability. The protein measures around 500 amino acids in length, roughly one-third the size of a traditional antibody with a molecular weight around 55 kDa [5]. The smaller size of this non-glycosylated fusion protein provides the advantage of improved penetration ability into tumors. It consists of two scFv arranged in a tandem configuration. The first scFv specifically targets the CD19 surface antigen, which is expressed on nearly all B cells, with the exception of hematopoietic stem cells and plasma cells that do not express this antigenic marker. The second scFv binds to the fifth subunit of the CD3 invariant antigen, a core component of the T-cell receptor (TCR) complex. Each scFv fragment includes an extended linker region to facilitate antigen binding. These fragments are connected by a short, flexible linker consisting of five amino acids. The linker sequence ensures a high degree of rotational flexibility, which making both scFv domains to bind simultaneously to their cell surface targets, respectively.

Immunotherapy has greatly transformed the treatment landscape of B-cell acute lymphoblastic leukemia (B-ALL), enabling more precise elimination of leukemia cells and offering a more favorable safety profile compared to traditional cytotoxic chemotherapy. Among the various immunotherapeutic modalities, blinatumomab is widely recognized as one of the most influential and successful agents in modern treatment paradigms. As a BiTE, it redirects CD3-positive cytotoxic T cells to engage and destroy CD19-expressing B cells, thereby harnessing the patient's own immune system to target CD19-positive leukemic blasts. Initially approved by the US FDA in late 2014, its indications have expanded, and it is now indicated for patients from one month of age with relapsed/refractory (R/R) or newly diagnosed CD19-positive. These advancements over the past decade have established blinatumomab as a foundational treatment of treatment for the majority of B-ALL patients [3].This paper aims to synthesize the key clinical trials that supported these regulatory decisions, with particular emphasis on pediatric applications.

The utility of blinatumomab has been further validated in the frontline setting for newly diagnosed B-ALL. Building on evidence of its efficacy in adults with measurable residual disease (MRD), a major randomized trial (ECOG-ACRIN E1910) assigned patients aged 30-70 years with BCR.ABL-negative disease who achieved MRD-negativity following induction to receive consolidation chemotherapy with or without several cycles of blinatumomab, followed by maintenance. After a median follow-up period exceeding three years, the incorporation of blinatumomab resulted in statistically significant improvements in both overall survival (OS) and relapse-free survival (RFS) compared to chemotherapy alone. These compelling findings prompted a label extension from the FDA to include blinatumomab in the consolidation therapy for eligible patients with newly diagnosed disease [6,7].

A multinational phase 2 investigation evaluated the integration of one cycle of blinatumomab into the established Interfant-06 protocol for infants with KMT2A-rearranged (KMT2Ar) B-ALL. The study enrolled infants under one year of age with newly diagnosed disease who had a favorable response to induction therapy. Following a single blinatumomab infusion, the vast majority of patients achieved either MRD-negative status or very low MRD levels. Per protocol, high and medium-risk patients with elevated MRD prior to a specific multi-agent chemotherapy block were candidates for hematopoietic stem cell transplantation (HSCT) in first complete remission (CR1). Most high-risk patients proceeded to transplant, while only a few medium-risk patients did. The two-year survival outcomes were notably higher than those observed in historical control groups treated with the Interfant-06 regimen alone. The relapse rate was relatively low, with all recurrences involving the central nervous system (CNS) and retaining CD19 expression without evidence of lineage switch [6-8].

4. Current Challenges that Facing

4.1 Short Half Life

First-generation bispecific T-cell engagers are relatively small in size, typically around 55 kDa, which renders them prone to rapid renal clearance and results in a short plasma half-life of approximately 2.5 hours. This limitation not only restricts the duration of their therapeutic effect but also poses practical challenges in clinical administration, often requiring continuous infusion to maintain effective drug concentrations. To overcome this issue, more recent design strategies have integrated additional components, such as Fc regions, full-length antibody constructs, or albumin-binding domains, with the aim of prolonging the circulating half-life and enhancing therapeutic efficacy.

4.2 Tumor Microenvironment (TME)

One of the main obstacles in the application of bispecific T-cell engagers (BiTEs) in glioblastoma is the highly immunosuppressive microenvironment of the tumor. This environment is characterized by the presence of regulatory T cells (Tregs; $CD4^+CD25^+FOXP3^+$), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs). These cell types work together to promote T-cell dysfunction and apoptosis, while weakening the activity of innate natural killer (NK) cells, which play a crucial role in tumor recognition and clearance [1,2]. Moreover, glioblastoma cells continuously secrete the HLA-G molecule, further inhibiting the function of NK cells.

The stromal cells in this microenvironment also produce potent immunosuppressive cytokines, including transforming growth factor-β (TGF-β) and interleukin-10 (IL-10). Additionally, tumor cells mainly rely on anaerobic glycolysis - a metabolic process known as the Warburg effect - which leads to lactate accumulation, thereby weakening the function of cytotoxic T lymphocytes (CTL) and impairing their migratory ability [1].Furthermore, tumor cells exacerbate T cell dysfunction by expressing hypoxia-inducible factor-1 alpha (HIF-1α), which promotes angiogenesis and tumor growth while reducing CTL infiltration into the tumor site through the down-regulation of CD62L, a key homing receptor. So the TME is a significant challenge which needs to overcome.

4.3 Cytokine Release Syndrome (CRS)

CRS characterized by a systemic inflammatory response mainly because of the high tumor burden and rapid T cell proliferation post-activation which leads to more T cell engagement. Furthermore, during the therapy many patients show a syndrome which is overactivation of T cells majorly generate to excessive cytokine like CL-6, IFN-v, TNF-α etc, release. Most patients with CRS exhibit characteristic symptoms such as fever, hypotension, organ dysfunction, confusion and seizures, and aphasia, respectively. (e.g., approximately 15% of patients treated with blinatumomab) [9].

4.4 Neurotoxicity

Neurological complications often present with signs of encephalopathy, such as dizziness, tremors, and speech difficulties. Without appropriate intervention, these symptoms may progress to more advanced stages of encephalopathy, which could result in impaired consciousness. Seizures might also arise, either in conjunction with or separately from encephalopathic events. Early recognition of neurotoxicity is essential; therefore, educating both patients and their families is strongly advised. Routine writing exercises can serve as a practical tool for detecting early encephalopathic changes. Management strategies include administering dexamethasone and temporarily halting treatment infusions. For severe or persistent neurotoxic events, reducing the dosage upon reinitiation of therapy—for example, from 28 μg/day to 9 μg/day—may be warranted. In most cases, neurotoxicity is reversible with timely intervention and does not lead to permanent deficits. It is also noteworthy that high-grade neurotoxic events occur less frequently with this treatment than with other immunotherapies, such as CAR-T cell therapy. Headaches are commonly reported but are typically not indicative of neurotoxicity; they often respond well to analgesics like paracetamol or metamizole [1,9].

5. Conclusion

BiTEs represent a highly specific and potent anti-tumor therapeutic strategy, capable of overcoming the restrictions encountered by conventional immunotherapeutic solutions in penetrating immune-privileged sites, for example, the hematological malignancy. However, there are still some challenges, including short half-life, the existence of a strong immunosuppressive tumor microenvironment, cytokine release syndrome and neurotoxicity in a large number of samples, all of which may impair the function of T cells and hinder the development of BiTE. In the future, combination strategies will likely be necessary, potentially involving immune checkpoint inhibitors (ICI) or cytokines, which have the potential to reinvigorate antitumor immune responses. Although both of them still not mature at all. However, it is certain that BiTEs have made significant contributions and are poised to play a crucial role in the future development of immunology, along with their great potential. Just like other treatment methods, such as CAR-T, BiTEs are expected to develop in a more effective direction.

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