Review

Current Advances in Bispecific T Cell–Engaging Therapies

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Review

Current Advances in Bispecific T Cell–Engaging Therapies

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Abstract: The emergence of bispecific antibodies has transformed cancer immunotherapy, highlighting increased clinical efficacy, especially in hematological malignancies. These innovative molecules uniquely target two distinct tumor antigens or separate epitopes simultaneously, demonstrating potent antitumor activity across various cancers. Despite their promise, challenges like rapid drug clearance, off-target effects, and cytokine release syndrome hinder their widespread therapeutic application. Recent engineering advancements in bispecific antibody systems aim to overcome these challenges, broadening therapeutic coverage. This review offers insights into the latest clinical and preclinical progress in bispecific immunotherapy, outlining key challenges faced by the technique, and exploring emerging strategies to address these obstacles.

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Introduction

Before the introduction of bispecific antibodies (BsAbs), traditional cancer immunotherapy relied on monoclonal antibody (MoAb) molecules targeting a single tumor antigen **[1]**. However, the complex nature of some cancers, with their ability to switch signaling pathways and evade immune responses, posed challenges for this approach **[2]**. A prime example is the interaction between Programmed Cell Death Protein 1 (PD-1) and Programmed Cell Death Ligand 1 (PD-L1), where tumor cells exploit this interaction to attenuate the immune response **[3]**. This manipulation involves inducing apoptosis in antigen-specific T cells and inhibiting the apoptosis of regulatory T cells, affecting the efficacy of single antibody-targeted immunotherapy **[3]**. The arrival of bispecific antibodies marks a significant shift in addressing these challenges, offering a promising avenue for more effective cancer treatment.

BsAbs are a promising type of therapy that can target two different tumor antigens simultaneously **[4]**. These antibodies typically consist of two single-chain variable fragment (scFv) antigen-binding parts linked by a flexible amino acid linker, offering a more refined approach against cancer cells **[5]**. In the case of the PD-1/PD-L1 axis, bispecific antibodies can be designed to bind both PD-1 and a tumor-specific antigen at the same time. This disrupts immune evasion mechanisms and strengthens the immune response **[6]**. Over 100 bispecific antibodies have been evaluated across various cancer types, with many receiving marketing approvals **(Table 1) [7, 8]**. A significant achievement occurred in 2022 when the FDA approved a Bispecific T cell Engager (BiTE) product targeting CD3/BCMA for treating relapsed or refractory multiple myeloma **[9]**. Subsequently, talquetamab and elranatamab, both CD3 T-cell engagers, received FDA approval in 2023 for multiple myeloma treatment **(Table 1) [10, 11]**. These approvals mark substantial progress in treating adult patients with relapsed or refractory multiple myeloma.

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Table 1. Summary of BsAbs approved for market worldwide for clinical use, as of 2014.

Drug (Company)	Trade name	Target antigen	Approved Countries	Year Approved	Approved indications
Blinatumomab (Amgen)	Blincyto	CD3/CD19	FDA	2014	adults and children with B-cell precursor acute lymphoblastic leukemia (ALL) in first or second complete remission with minimal residual disease (MRD) greater than or equal to 0.1%.
Emacizumab-kxwh (Genentech)	Hemlibra	FIXa/ FX	FDA	2017	the treatment is recommended for adult and pediatric patients, including newborns, with hemophilia A. This includes individuals with congenital factor VIII deficiency, whether or not they have developed factor VIII (FVIII) inhibitors
Amivantamab-vmjw(Janssen Biotech)	Rybrevant	EGFR/c-Met	FDA/EMA	2021	adult patients with locally advanced or metastatic non-small cell lung cancer who have EGFR exon 20 insertion mutations

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					and have previously received platinum-based chemotherapy
Tebentafusp-tebn (Immunocore)	Kimmtrak*	CD3/ gp100	FDA	2022	for the treatment of adult patients with unresectable or metastatic uveal melanoma who are HLA-A*02:01-positive.
Faricimab-svoa (Roche)	Vabysmo	VEGF-A/Ang-2	FDA	2022	To treat neovascular (wet) age-related macular degenerated and diabetic macular edema
Mosunetuzumab-axgb (Genentech)	Lunsumio	CD3/CD20	EMA/FDA	2022	Patients with advanced non-small cell lung cancer (NSCLC), harboring EGFR exon 20 insertion mutations, facing disease progression after platinum-based chemotherapy,
Cadonilimab (Akeso)	Kaitanni	PD-1/CTLA-4	CFDA	2022	For patients with relapsed or metastatic cervical cancer (r/mCC) who have experienced disease progression following platinum-based chemotherapy

 doi:10.20944/preprints202401.1861.v1

Teclistamab-cqyv (Janssen Biotech)	Tecvavli	CD3/BCMA	EMA/FDA	2022	adult patients with relapsed or refractory multiple myeloma who have received at least four prior lines of therapy, including a proteasome inhibitor, an immunomodulatory agent, and an anti-CD38 monoclonal antibody
Epcoritamab-bysp (Genmab)	Epkinly	CD3/CD20	FDA/EMA	2023	adults with relapsed or refractory diffuse large B-cell lymphoma (DLBCL), including cases arising from indolent lymphoma and high-grade B-cell lymphoma after two or more lines of systemic therapy
Glofitamab-gxbm (Genentech)	Columvi	CD3/CD20	FDA	2023	For adult with relapsed or refractory diffuse large B-cell lymphoma (DLBCL, NOS) or large B-cell lymphoma (LBCL) arising from follicular lymphoma, after two or more lines of systemic therapy.

 doi:10.20944/preprints202401.1861.v1

Talquetamab-tgvs (Janssen Biotech)	Talvey	GPRC5D/ CD3	EMA/FDA	2023	adults with relapsed or refractory multiple myeloma who have undergone at least four prior lines of therapy, including a proteasome inhibitor, an immunomodulatory agent, and an anti-CD38 monoclonal antibody.
Elranatamab (Pfizer)	Elrexfio	BCMA/CD3	FDA/EMA	2023	for adults with relapsed or refractory multiple myeloma who have received at least four prior lines of therapy, including a proteasome inhibitor, an immunomodulatory agent, and an anti-CD38 monoclonal antibody.
Odronextamab*	Regeneron	CD20/CD3	FDA	FDA decision is on March 31, 2024.	adult patients with relapsed/refractory (R/R) follicular lymphoma (FL) or R/R diffuse large B-cell lymphoma (DLBCL) who have progressed after at least two prior systemic therapies

*__Kimmtrak__ is technically a bispecific molecule, not a bispecific antibody. Like some of the other bispecific antibodies used to treat some cancers, Kimmtrak has one arm using an antibody fragment to bring killer T cells

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to the tumor. Kimmtrak's other arm is an analogous structure found on T cells, the T cell receptor, instead of an antibody fragment to target a tumor antigen.

While most bispecific antibodies focus on cancer treatment, some are directed at chronic inflammatory, autoimmune, neurodegenerative diseases, and infections. Examples include emacizumab and faricimab, both developed for hemophilia A and retinal vascular disease treatment, respectively **[12, 13]**. These diverse applications highlight the expanding role of bispecific antibodies in transformative therapeutic interventions. While bispecific antibodies have shown success in cancer treatment, they still face challenges like a short in vivo half-life, on-target off-tumor effects, cytokine release syndrome, and issues in manufacturing [**14-16]**. These challenges hinder their broad application. However, recent advancements have led to innovative approaches addressing these challenges, paving the way for improved clinical practices. In this review, we shed light on the evolving field of bispecific antibodies, providing insights into their present status in clinical development. Additionally, we delve into the challenges associated with bispecific antibodies and explore recent modifications aimed at enhancing their therapeutic efficacy.

Bispecific T Cell Engager

The concept of bispecific antibodies (BsAbs) has evolved significantly since their initial description by Nisonoff in 1960, resulting in the development of several hundred formats categorized into six diverse mechanisms of action: (1) bridging cells, (2) receptor inhibition, (3) receptor activation, (4) co-factor mimetic, (5) piggybacking I, and (6) piggybacking II **[8, 17]**. These diverse BsAb formats have been engineered to target various components such as tumor signaling pathways, immune checkpoint inhibitors (ICIs), inflammatory cytokines, and more **[18-20]**. Among these formats, the bridging cell or Bispecific T-cell Engager stands out as the most common BsAb employed for the treatment of both liquid and solid tumors **[21]**. A crucial aspect of BiTEs is their ability to redirect naïve T cells to target tumor cells, leading to T-cell activation, clonal expansion, and subsequent tumor cytotoxicity **[21, 22]**. First-generation BiTE constructs were typically designed with two monoclonal antibody (mAb) moieties tandemly fused, with one moiety targeting a specific tumor antigen and the other binding to CD3 antigen on T-cell surfaces. This design ensures that T cells engaged by BiTE molecules become activated and effectively eliminate malignant cells **[23]**. More than six decades, seven BiTEs are approved for cancer treatment (**see Table 1**), and several more are undergoing clinical testing **[24]**. Despite their efficacy, the use of bispecific T-cell engagers has faced challenges associated with 'on-target, off-tumor' toxicities **[25, 26]**. BiTE therapy primarily involves identifying suitable tumor-associated antigens (TAAs) on target cells that differ from those on normal cells, aiming to prevent on-target/off-tumor toxicity **[25].** However, the identification of antigenic targets exclusive to tumor cells presents challenges, as many target antigens are expressed on both normal and tumor cells **[27]**. Even minimal antigen expression on normal cells can result in adverse on-target off-tumor toxicities leading to cytokine release syndrome (CRS). CRS, characterized by an excessive immune response leading to the release of proinflammatory cytokines, can potentially result in organ failure and, in severe cases, death **[28]**. Currently, the primary clinical interventions to manage CRS in TCE therapies involve dose reduction or the administration of anti-interleukin antibodies and corticosteroids **[28]**. While these interventions have proven effective in certain scenarios, they do not provide a complete prevention of CRS. Accordingly, increasing reports have highlighted the occurrences of off-target, on-target toxicity associated with bispecific antibody molecules, especially BiTE therapeutics **[23, 29, 30]**.

To overcome the significant challenge of on-target, off-tumor adverse effects including CRS, and enhanced the therapeutic index of BsAbs particularly in the context of solid malignancies, researchers have been exploring several modification strategies. One such strategy focuses on employing avidity-mediated specificity or the 2 + 1 architecture **[31, 32]**. In this novel approach, a bivalent antibody with low affinity for the tumor antigen is combined with a monovalent anti-CD3 molecule **[32]**. This unique design enables the BiTE to selectively bind to tumor cells that overexpress the target tumor-

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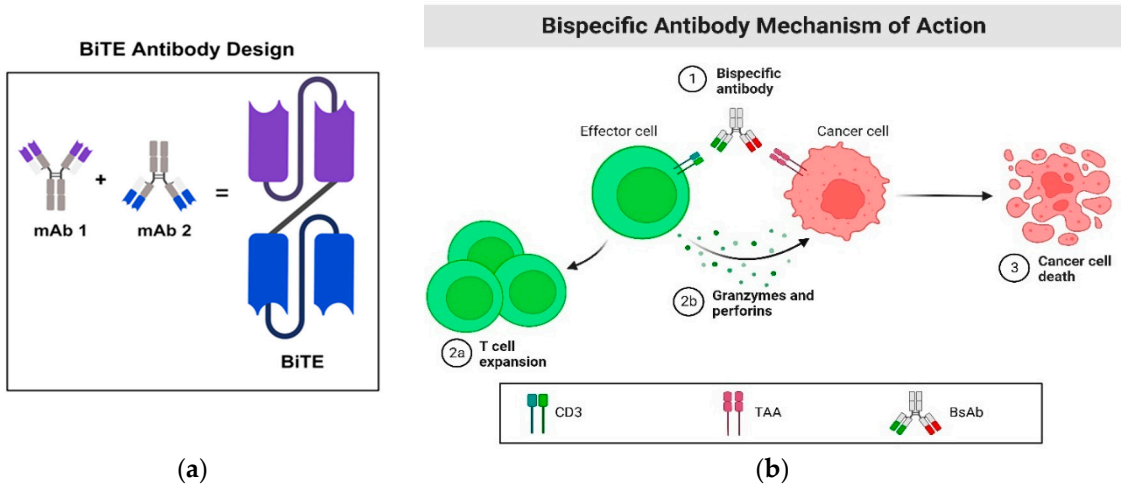
associated antigen (TAA), facilitating the specific killing of tumor cells while sparing normal cells expressing the target antigen at lower densities.

A study conducted by Bacac et al. exemplifies this approach, utilizing a bivalent anti-CEA scFv domain linked with a monovalent anti-CD3 domain for the treatment of solid tumors expressing carcinoembryonic antigen (CEA) **[33]**. CEA, also known as carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), is associated with glycosylphosphatidylinositol and is overexpressed in various cancers, playing a role in adhesion and invasion **[34]**. The resulting CEA T cell bispecific (TCB) demonstrated sustained antitumor activity in a preclinical model, exhibiting a notable increase in T-cell longevity **[33]**. Moreover, the CEA+CD3 TCB transformed PD-L1-negative tumors into PD-L1-positive, creating a highly inflamed tumor microenvironment. This promising development has advanced to phase 1 clinical investigation (NCT02324257), showcasing pronounced efficacy and manageable safety profiles **[33]**.

In line with these advancements, another group used an anti-HER2/CD3 T cell-dependent bispecific (TDB) antibody to redirect T cells to eliminate HER2-overexpressing cells, demonstrating potent antitumor activity **[31]**. This suggests that avidity-mediated selection holds promise for treating solid tumors, as its potentially addresses one of the major challenges associated with TCE therapies, offering a more targeted and controlled immune response. However, since high expression levels of the TAA are crucial for avidity specificity and bispecific antibody-mediated tumor lysis, this strategy is applicable primarily to cancer cells expressing very high levels of the target antigen. The challenge arises when dealing with solid tumors expressing variable densities of the target antigen. To address this challenge and enhance the versatility of the approach, future studies are needed to develop a dual bispecific antibody with a 2+1+1 architecture, where one target incorporates avidity-mediated specificity and the other features high-affinity binding. This approach would offer a comprehensive solution to rapidly target and eliminate solid tumors expressing differential levels of the target antigen.

Generally, there is currently no FDA-approved BiTE molecule for treating solid malignancies. However, catumaxomab, the first bispecific T-cell engager approved by the EMA in 2009 to treat malignant ascites of epithelial cancers, was later withdrawn from the market due to severe adverse events, including CRS and dose-dependent liver toxicity **[35]**. Ongoing research and development aim to address these challenges and further enhance the clinical applicability of BiTEs, emphasizing their significance in advancing cancer immunotherapy.



(**a**) (**b**)

Figure 1. BiTE and its mechanism of action. a. BiTE antibody construct comprises two single-chain variable fragments of monoclonal antibodies linked together through a flexible linker. b. One arm of the BiTE molecule is designed to bind to CD3, an antigen located on the surface of T cells. Simultaneously, the other arm is engineered to bind to a tumor-associated antigen (TAA). Upon successful binding of both arms to their specific targets, a synapse is formed between the T cell and the cancer cell. Subsequently, the T cells undergo expansion and release perforin, creating a pore in

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the cancer cell's membrane. This pore allows toxic molecules called granzymes to flow through, ultimately inducing the death of the cancer cell.

While BiTEs encounter challenges in battling solid tumors, a promising alternative, immune-mobilizing monoclonal T-cell receptors against cancer (ImmTACs), has emerged **[36]**. Like BiTEs, ImmTACs facilitate the interaction between cancer cells and T cells by simultaneously engaging their proteins. However, ImmTACs take a different approach by employing a T-cell receptor instead of an antibody fragment to recognize proteins on cancer cells [36]. This unique strategy allows ImmTACs to bind to intracellular proteins processed and presented externally, expanding their target range beyond cell surface proteins. This characteristic makes ImmTACs more effective in addressing solid tumors, where many cancer-specific proteins are primarily expressed inside the cell. Tebentafusp (Kimmtrak), an ImmTAC therapeutic, has already gained approval for treating uveal melanoma **[37]**. Considering the risks associated with BiTEs in solid tumors, especially CRS, ImmTACs emerge as a promising class of therapeutics, offering cancer-fighting immune cells a distinct advantage.

Blinatumomab, the First FDA Approved BiTE Construct

Blinatumomab stands out as a significant success in BiTE therapy, marking the first FDA-approved BiTE molecule to treat B-cell acute lymphoblastic leukemia (ALL) **[38]**. This therapy combines anti-CD19 and anti-CD3 single-chain variable fragment (scFv) domains, demonstrating notable clinical efficacy. Many patients experienced complete tumor regression, contributing to improved overall survival rates **[39]**. In a study with 54 relapsed or refractory (R/R) patients, 91% (49/54) achieved a complete response with blinatumomab treatment, highlighting its clinical effectiveness in challenging R/R settings **[40]**. These outcomes emphasize Blinatumomab's therapeutic potential and its crucial role in advancing treatment options for B-cell ALL patients. Importantly, Blinatumomab's activity is independent of major histocompatibility complex activation, ensuring rapid activation of T cells and the destruction of tumor cells **[41]**.

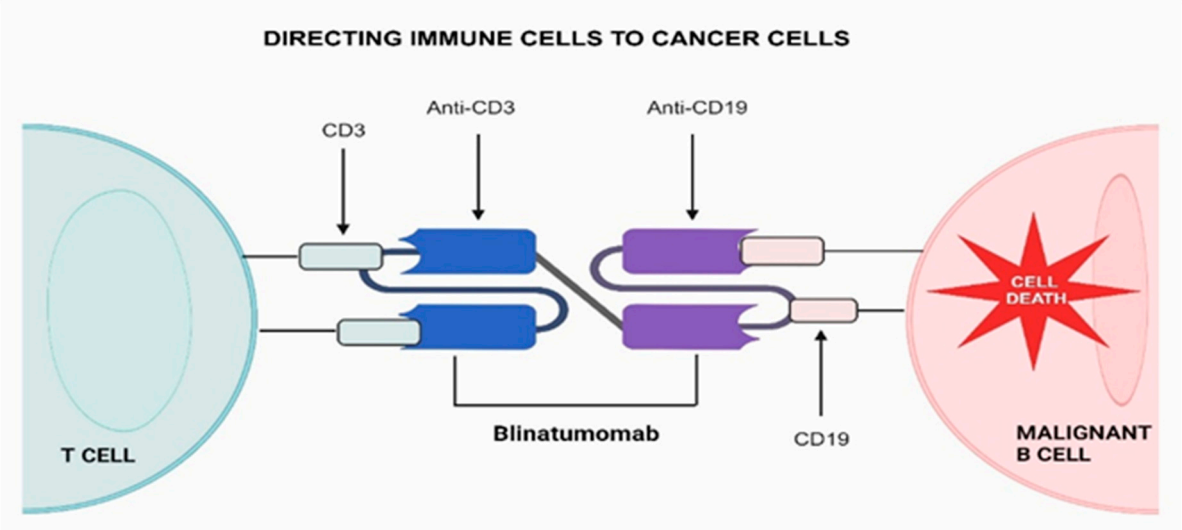

Figure 2. The mechanism of action for blinatumomab, the first-in-class bispecific T cell engager (BiTE), involves one arm binding to CD3 and the other to CD19. This interaction activates unstimulated T cells, initiating their attack on CD19+ cells.

Although Blinatumomab has demonstrated significant success, crucial challenges persist. Factors such as rapid drug clearance, on-target/off-tumor adverse effects, cytokine release syndrome, and activation of peripheral immune cells may potentially limit therapeutic efficacy in both hematological malignancies [42]. Recent reports indicate instances of relapse among patients following Blinatumomab treatment, with the phenomenon associated not only with the loss of CD19 but also CD58, as proposed by Jabbour et al. **[43]**. Previous research has explored mechanisms

 doi:10.20944/preprints202401.1861.v1

contributing to CD19 escape, including CD19 mutations, CD19-mutant allele-specific expression, low CD19 RNA expression, and mutations in CD19 signaling member CD81 **[44]**. However, limited attention has been given to CD58 loss and its mechanism in the context of Blinatumomab treatment.

A recent study by Yizhen et al. has identified a crucial intrinsic factor, PAX5 mutation, significantly downregulating CD58. This downregulation has been linked to reduction in Blinatumomab activity, particularly observed in patients with acute lymphoblastic leukemia (ALL) **[45]**. Further research is needed to address the PAX5 mutation in ALL models under Blinatumomab treatment, providing a more comprehensive understanding of the role of PAX5 in CD58 loss. Moreover, additional studies have suggested regulatory T cells (Tregs) as potential regulators in the resistance process against Blinatumomab, indicating that multiple factors may contribute to resistance and a reduced response rate to this therapeutic approach **[44]**. These findings indicate the complexity of the mechanisms behind resistance to Blinatumomab, emphasizing the necessity for ongoing research to unravel these intricacies and ultimately pave the way for more effective and personalized treatment strategies.

Furthermore, the phenomenon of lineage switch represents a significant challenge associated with blinatumomab treatment, wherein refractory B lymphoblastic leukemia (B-ALL) can undergo a transition to acute myeloid leukemia (AML) **[46-48]**. This shift in lineage was initially documented by Stass and colleagues following standard chemotherapy for acute leukemia **[49]**. The occurrence of lineage switching has been observed not only in blinatumomab therapy but also in other immunotherapies, including CD19-specific chimeric antigen receptor (CAR) T cells **[50]**. It is particularly noteworthy that this switch occurs when CD19 B-cells acquire a distinct phenotype after the loss of CD19 **[51-53]**. While other several theories have been proposed to explain the mechanisms leading to lineage switch **[54, 55]**, the prevailing view suggests that the selective pressure resulting from CD19-directed therapy plays a crucial role in this phenotypic transition **[56-58]**. Studies on lineage switching highlight various rearrangements of the gene encoding histone–lysine N-methyl-transferase 2A (KMT2A, also known as mixed-lineage leukemia, MLL) as a key regulator of this switch **[59-61]**. The development of this immunophenotype is recognized as a critical factor contributing to relapses and resistance to several antibody-targeted therapies.

In the case of blinatumomab, five chromosomal rearrangements linked to lineage switch have been identified: KMT2A-AFF1 **[62, 63]**, KMT2A/AFF4 **[58]**, BCR-ABL1 **[64]**, hyperdiploidy **[65]**, and KMT2A/EPS15 **[66]**. The t(4;11) (q21;q23) rearrangement with the KMT2A/AFF1 fusion protein is particularly common, especially in infants with ALL **[67-69]**. Lineage conversion has been observed in pediatric patients with ALL, impacting blinatumomab treatment monitoring. A switch from CD19-positive B-precursor ALL to CD19-negative AML has been documented following blinatumomab therapy **[47]**. Efforts to overcome this challenge include incorporating blinatumomab into the Interfant-06 backbone regimen. In an analysis of 30 infants with acute leukemia treated with standard chemotherapy and post-induction blinatumomab, no lineage switches were observed **[citation needed]**. Similarly, promising outcomes have been reported in infants with KMT2A-rearranged ALL, where the addition of blinatumomab to the Interfant-06 chemotherapy trial significantly improved the 2-year overall survival compared to the Interfant-06 alone **[70]**. It is essential to note that the follow-up time in these studies was relatively short, and longer-term monitoring is required to comprehensively evaluate the safety and efficacy of this combined therapy. Furthermore, blinatumomab has shown promise as an effective salvage therapy following anti-CD19-CAR-T failure, surpassing chemotherapy options. In R/R B-ALL patients, blinatumomab showed an improved complete remission rate, even in those expressing low CD19 levels **[71]**. However, inconsistent findings warrant further comparable studies to validate its potency as a rescue or pretreatment therapy, as some reports suggest prior blinatumomab treatment can maintain anti-CD19-CAR-T efficacy **[72].**

Immune Checkpoint Bispecific Antibodies

In cancer immunotherapy, the use of immune checkpoint inhibitors (ICIs) has been a major breakthrough, particularly when used as monotherapies **[73, 74]**. These inhibitors tap into the

 doi:10.20944/preprints202401.1861.v1

potential of natural T cells that infiltrate tumors. Cancer cells often exploit immune checkpoints to avoid immune responses, and ICIs counteract this by blocking specific checkpoints **[75, 76]**. Approvals of drugs like ipilimumab, pembrolizumab, and nivolumab signify significant strides in ICI development **[73]**. However, the effectiveness of single antibody targets against immune checkpoints and their ligands has shown limited impact, especially in treating "cold tumors" – tumors that hinder immune responses by preventing the infiltration of immune cells into the tumor **[77, 78]**. Consequently, only a minimal fraction of the patient population has experienced significant benefits from ICI monotherapies.

Recent advancements in bispecific antibodies have addressed this limitation by focusing on the dual targeting of immune checkpoints, encompassing both receptors and ligands **[79]**. Notably, programmed death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) checkpoint inhibitors have gained attention for their ability to restore T cells exhausted due to tumor-induced suppression **[79]**. PD-L1 and PD-L2, widely expressed ligands across various cancer types, have been a focus of study. PD-L2, known to bind PD-1 more strongly than PD-L1, presents an opportunity for more impactful outcomes when targeted **[80, 81]**. In contrast to monospecific PD-1 and PD-L1 antibodies, bispecific antibodies targeting both PD-1 and PD-L1 have demonstrated powerful antitumor responses. LY3434172, a bispecific antibody co-targeting PD-1 and PD-L1, exhibited significant in vivo antitumor potency even at lower doses in preclinical studies, suggesting a synergistic effect and a distinctive pathway interaction in modulating immune responses **[82]**.

Approximately 60% of cancers express both PD-L1 and PD-L2, while around 30% express either PD-L1 or PD-L2, expanding the binding effect and reducing off-target toxicities of bispecific antibody constructs **[83]**. Ongoing studies are exploring dual-specific antibodies to co-target stromal cells, T regulatory cells (Tregs), and myofibroblasts in the tumor microenvironment, facilitating the influx of T cells into poorly infiltrated tumors **[84]**. Emerging strategies aim to target specific surface proteins, including PD-L1/PD-L2, CD25/CTLA-4, PD-L1/ICOS, PD-1/CD47, and PD-L1/T cell immunoreceptor with Ig and ITIM domains (TIGIT) (**Table 1**) **[85, 86]**. For instance, dual-specific monoclonal antibodies designed to bind PD-L1 and PD-L2 have demonstrated enhanced immune-driven anti-tumor activity **[87]**. In the context of treating HER2-positive solid tumors, a bispecific combination of PD1 and HER2 exhibited high effectiveness in killing HER2-positive tumor cells through antibody-dependent cellular cytotoxicity **[88]**.

Undoubtedly, bispecific antibodies tailored against PD-L1 and PD-L2 play a pivotal role in facilitating the migration of host immune responses to tumor cells, thereby enhancing antitumor responses. The targeting of PD-L1 in dual antibody regimens has demonstrated effectiveness in various settings of human tumors, as evidenced by the numerous ongoing clinical trials exploring PD1/PDL1 combination regimens **[89]**.

Table 2. Studies investigating the efficacy of PD1/PDL1 combination regimens in patients with advanced solid tumors (Clinical trials are registered at clinicaltrials.gov).

Target	Name	Condition	Status	Phase	NCT ID
PD-L1 and TGF-β	SHR-1701	Advanced solid tumors	Unknown	Phase I	NCT03710265
CTLA-4×PD-L1	KN064	Advanced Solid Tumors	Completed	Phase 1	NCT03733951
PD-1 and CTLA-4	MEDI5752	Advanced solid tumors	Recruiting	Phase I	NCT03530397

 doi:10.20944/preprints202401.1861.v1

	MGD019	Advanced solid tumors	Active, not recruiting	Phase 1	NCT03761017
	AK104	Hepatocellular carcinoma	Recruiting	Phase I/II	NCT04444167
	COMPASSION-03	Advanced solid tumors	Active, not recruiting	Phase I/II	NCT03852251
LAG-3 × PD-L1	ABL501	Advanced solid tumors	Recruiting	Phase I	NCT05101109
	FS118	Advanced solid tumors	Active, not recruiting	Phase I/II	NCT03440437
	AK104	NSCLC	Active, not recruiting	Phase I/II	NCT04646330
LAG-3 × PD-1	MGD013	Advanced liver cancer	Terminated	Phase I/II	NCT04212221
	RG6139	Advanced solid tumors	Recruiting	Phase I/II	NCT04140500
	Not Given	Advanced solid tumors	Recruiting	Phase I	NCT05577182
TIM-3 × PD-L1	LY3415244	Advanced solid tumors	Terminated	Phase I	NCT03752177
	ABL501	Advanced solid tumors	Recruiting	Phase I	NCT05101109
TIGIT×PD-L1	HLX301	Advanced solid tumors	Recruiting	Phase I/II	NCT05102214

 doi:10.20944/preprints202401.1861.v1

TIGIT×PD-1	ARTEMIDE-01	Advanced NSCLC	Recruiting	Phase I/II	NCT04995523
	LB1410	Advanced Solid Tumor	Recruiting	Phase I	NCT05357651
TIM-3 × PD-1	AZD7789	Lymphoma	Recruiting	Phase I/II	NCT04931654
	RG7769	Advanced Solid Cancer	Recruiting	Phase I	NCT03708328
	Lomvastomig	Advanced Solid Cancer	Active, not recruiting	Phase II	NCT04785820
	Tobemstomig	Non-small Cell Lung Cancer	Recruiting	Phase II	NCT05775289
4-1BB×PD-L1	ABL503	Advanced Solid Cancer	Recruiting	Phase I	NCT04762641
	PRS-344	Advanced Solid Cancer	Recruiting	Phase I/II	NCT05159388
	GEN1046	Advanced Solid Cancer	Recruiting	Phase I/II	NCT03917381
CD27×PD-L1	CDX-527	Advanced Solid Cancer	Completed	Phase I	NCT04440943
PD-L1 and CD137	MCLA-145	Advanced Solid Cancer	Recruiting	Phase I	NCT03922204
	AP203	Advanced Solid Cancer	Not yet recruiting	Phase I/II	NCT05473156
	FS222	Advanced Solid Cancer	Recruiting	Phase I	NCT04740424

 doi:10.20944/preprints202401.1861.v1

PD-L1 and VEGF	PM8002	Advanced Solid Cancer	Recruiting	Phase II	NCT05879055
	HB0025	Advanced Solid Cancer	Recruiting	Phase I	NCT04678908
	IMM2510	Advanced Solid Cancer	Recruiting	Phase I	NCT05972460
PD-1/ VEGF	AK112	NSCLC	Recruiting	Phase II	NCT04736823

Future Directions

Looking ahead, the success of Bispecific Antibodies (BsAbs) in effectively treating hematological malignancies is evident with FDA approvals. However, it's noteworthy that there is currently no FDA-approved BiTE molecule for addressing solid malignancies. Ongoing initiatives are exploring innovative approaches, such as incorporating masks linked through protease-cleavable linkers into first-generation TCEs, including Conditional Bispecific Redirected Activation, Probody TCB, and precision-activated TCEs**.** These attempts aim to enhance the therapeutic efficacy of bispecific T-cell engagers in treating solid tumors.

In addressing complications like cytokine release syndrome (CRS) associated with BsAbs therapy, future research is focused on optimizing design to trigger immunological responses exclusively towards tumors. Unlike previous designs involving a single BsAb agent, emerging strategies adopt a unique approach by employing two Bispecific Antibodies (BsAbs) components. Each component features a split anti-CD3 paratope and a binding moiety for a tumor antigen. These advancements signify a promising direction in the evolution of bispecific T-cell engagers for more effective and targeted treatments of solid tumors.

Conclusions

The field of bispecific antibodies (BsAbs), particularly exemplified by bispecific T cell–engaging therapies, has witnessed remarkable strides in cancer immunotherapy, and appears superior to conventional chemotherapy, in at least hematological malignancy settings. The clinical success of over 100 evaluated bsAbs, with seven BiTE approved for market use, highlights their remarkable achievements. However, challenges such as rapid drug clearance, off-target effects, and cytokine release syndrome persist, limiting their widespread application. Despite, innovative modifications, including avidity-mediated specificity, paratope masking and two BsAbs system hold promise in addressing on-target/off-tumor adverse effects. Moreover, immune checkpoint bispecific antibodies, co-targeting receptors and ligands like PD-1 and PD-L1, present a paradigm shift in cancer immunotherapy, offering enhanced antitumor responses. The evolving landscape of bispecific immunotherapeutic holds great potential in advancing personalized and effective cancer treatments, emphasizing the need for ongoing research and development to overcome existing challenges and broaden therapeutic applications.

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