Review

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Therapeutic antibodies for precise cancer immunotherapy: current and future perspectives

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Abstract: Antibodies, as one of the most important components of host adaptive immune system, play an important role in defense of infectious disease, immune surveillance, and autoimmune disease. Due to the development of recombinant antibody technology, antibody therapeutics become the largest and rapidly expanding drug to provide major health benefits to patients, especially for the treatment of cancer patients. Many antibody-based therapeutic strategies have been developed including monoclonal antibodies, antibody-drug conjugates, bispecific and trispecific antibodies and pro-antibodies with promising results from both clinical and pre-clinical trials. However, the response rate and side-effect still vary between patients with undefined mechanisms. Here, we summarized the current and future perspectives of antibody-based cancer immunotherapeutic strategies for designing next-generation drugs.

Keywords: antibody; cancer; engineering; immunotherapy.

Introduction

Since 1990s, over 100 antibody-based therapeutics have been approved for the treatment of serious human disease including infectious disease, cancer, and immune-related disorders [1–3]. However, the application of antibody treatment has also been extensively applied to other human disease including hematology, neurology, and metabolic disease. Recent approvals or emergency authorizations of anti-SARS-CoV-2 monoclonal antibodies (mAbs) (casirivimab plus imdevimab, bamlanivimab, bamlanivimab plus etesevimab) highlighted the importance of neutralization antibodies in treatment of acute respiratory virus infection [4, 5]. The research on monoclonal antibodies for cancer treatment started in 1970s, when the methods of producing antibodies using hybridomas were established [6, 7]. MAbs against melanoma showed antitumor efficacy in nude mice first followed by clinical trials of mAb treatment on lymphoma patients [8, 9]. However, the high immunogenicity of murine derived mAbs limited the further application of these therapeutic strategies. With the rapid development of yeast display, recombinant antibody and humanized antibody technologies, more and more antibody-based therapy has been studied for the treatment of cancer [10]. Since 1995, increasing numbers (1–8 per year) of antibody treatment has been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA).

Antibodies as therapeutic agent for cancer treatment has several advantages. First, antibodies are relatively stable with extended *in vivo* half-life mediated by neonatal Fc receptor (FcRn) [11]. With such properties, antibodies can be given intravenously and penetrate to the targeted tissue with durable concentration. Second, antibodies can not only block the growth factor signal transduction in tumor cells, but also activate innate immune response to kill tumor cells through antibody-dependent cellular cytotoxicity (ADCC) [12]. Third, compared with traditional therapy, the specificity of antibody treatment is much higher. Recognition of antigens through variable region of antibody can elicit strong tumor specific immune response while minimizing toxicity and adverse events. Based on these properties, many advanced antibody-based therapeutic strategies have been developed including antibody-drug conjugate (ADC) which conjugate potent cytotoxic agents to specific sites on antibodies, bispecific antibody (BsAb) which can simultaneously bind two targets and antibody fusion protein which can target the tumor microenvironment to deliver cytokines.

Even though promising results have been observed in pre-clinical and clinal trials of antibody-based immunotherapies. There are still several major hurdles to overcome

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to benefit more patients suffering from different kind of cancers. For example, the "on-target off-tumor" distractions and the undefined mechanism of drug resistance [13, 14]. In addition, the innovation in antibody engineering, target screening and therapeutic regimen still have plenty of room for further improvement [15].

Here, we review the design of mAbs, ADCs, BsAb and antibody fusion proteins for cancer immunotherapy. We also discussed the mechanism of action, side-effect, clinical uses, combination treatment strategies and future perspectives in antibody-based therapeutics.

Therapeutic monoclonal antibodies

Therapeutic monoclonal antibodies targeting tumor associated antigens

Tumor associated antigens (TAAs) is a category of tumor antigens which overexpressed or accumulated in cancer cells compared with normal cells [16, 17]. Candidate targets are protein or glycoproteins which highly expressed on the surface of tumor cells. The growth factor receptors are one of the most common TAAs identified in a variety of cancer patients including epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) [18–21]. Antibodies targeting EGFR have generated antitumor responses in colorectal cancer patients and four EGFR monoclonal antibody drugs (cetuximab, panitumumab, nimotuzumab, and necitumumab) are already approved by the FDA [22–24]. Similarly, trastuzumab (Herceptin) was first approved in 1998 for the treatment of HER2 positive breast cancer patients followed by other antibodies like pertuzumab and the most recent approval of margetuximab in 2020 [25, 26]. The mechanisms involved in anti-EGFR/HER2 mediated antitumor effect includes blocking the tyrosine kinases signaling induced malignant cell proliferation and differentiation, inhibiting hetero-dimerization and internalization of receptors. Host immune system also play an important role in anti-EGFR/Her2 mediated antitumor effect. ADCC, complement-dependent cytotoxicity (CDC) and antibody-dependent cellular phagocytosis (ADCP) are the most frequently reported mechanisms of innate immune system mediated tumor killing [27–29]. In a preclinical model, the antitumor effects of rituximab were completely abolished in complement cascade component C1q knockout mice [30]. In addition, Park et al. found that the antitumor effect of anti-HER2/neu antibody depends on both innate and adaptive immunity [31]. The antitumor effect of anti-neu was completely abolished in *Rag* knockout mice and dramatically reduced in wild-type (WT) mice after CD8 T cell depletion, indicating that host cytotoxic T cells also contribute to these monoclonal antibodies mediated tumor regression. Consistently, anti-CD8 depletion also abolished cetuximab mediated antitumor effect [32]. All these independent studies demonstrated the efficiency of mAbs in regulating host immune system to kill malignant cells.

Besides mAbs targeting growth factor receptors, mAbs targeting other TAAs has also been extensively studied. One of the most ideal targets is CD20, which expressed on B cell lymphoma. Rituximab was the first FDA approved anti-CD20 mAb, followed by ofatumumab and obinutuzumab. Rituximab alone or the combination with chemotherapy is still one of the first-line therapies of CD20 positive B-cell non-Hodgkin lymphomas [33–35]. Compared with targeting growth factor receptors which usually expressed on solid tumor, mAbs targeting CD20 has unique advantages. For example, the expression of CD20 is more specific than other TAAs, only on the surface of B cells in the late pre-B cell through mature memory B cell stages [36, 37]. Early pro-B cells and plasma cells are negative for CD20. Even though using anti-CD20 to treat lymphoma may deplete B cells as well, but antibody producing cells are preserved, thus minimizing the side effect [38, 39]. In addition, B cells can be constantly reconstituted by the bone marrow, which also prevent the severe toxicity. The mechanism of anti-CD20 mediated tumor clearance has been extensively studied by Ravetch et al. They demonstrated that Fc γ receptor (FcγR) expressed by $CD11c^+$ antigen-presenting cells is required to generate anti-tumor T cell responses upon ADCC-mediated tumor clearance. The engagement of FcγR on both macrophage and dendritic cell (DC) can generate synergistic antitumor effect [40]. Recently, more and more specific targets for solid tumors have been identified for antibody therapeutics. For example, claudin18.2 (CLDN18.2), one of the tight junction proteins, is specifically expressed on differentiated epithelial cells of the gastric mucosa. However, abnormal overexpression has also been found in many cancer types, especially in digestive system malignancies [41]. Zolbetuximab (IMAB362), an anti-CLDN monoclonal antibody drug has been evaluated in a phase 3 clinical trial in combination with chemotherapy [42]. Besides proteins, other biomacromolecules can also be ideal target of mAbs. Mucin 1 (MUC1) is a glycoprotein that has been demonstrated to be involved in the metastasis and invasion of multiple tumor types [43]. Anti-MUC1 antibodies have also been extensively studied in multiple preclinical and clinical trials [44].

Even though the therapeutic benefits have been observed in TAA-targeting mAb treatment. Patients still responded to these treatments partially with relapse.

Mechanisms involved in mAb treatment resistance includes: (1) mutation of the antibody target; (2) induction of alternative growth signaling pathways, studies have shown that c-MET promotes resistance to EGFR-targeting therapies; (3) adaptive immune editing; (4) the negative regulation of host immune response. Combination treatment with chemotherapy, targeted therapy (tyrosine kinase inhibitors) and immune checkpoint blockades (ICBs) might be required to overcome these resistance [45, 46].

Therapeutic monoclonal antibodies targeting immune checkpoints

Immune checkpoints are regulators of the immune systems. These pathways play an important role in T cell development and the maintenance of self-tolerance [47]. However, cancer cells also take advantages of these unique pathways to shut down host antitumor immune responses [48]. Therapeutic strategies that can interrupt these signal pathways in the tumor microenvironment may restore the antitumor immune response by "take the brakes off" effect [49, 50]. Since 1990s, many immune checkpoints have been identified including programmed cell death protein 1 (PD-1), programmed cell death 1 ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin mucin receptor 3 (TIM-3), lymphocyte-activation gene 3 (LAG-3). And the most successful applications of immune checkpoint blockade for cancer treatment are antibodies targeting PD-L1 and CTLA-4 [51].

PD-1 expression is driven by T-cell receptor (TCR) signaling and mainly expressed on activated T cells [52, 53]. One of the PD-1 ligands, PD-L1, was expressed by myeloid cells including macrophages and dendritic cells. Some cancer cells also intrinsically or extrinsically express PD-L1, and the latter usually induced by interferons (IFNs) [54]. Upon the binding of PD-L1 with PD-1, phosphatase like phosphatase and tensin homolog (PTEN) will inhibit the phosphoinositide 3-kinase (PI3K)-AKT signaling to restrain T cell activation and proliferation [55, 56]. This pathway has been demonstrated as a major mechanism of T cell exhaustion in multiple preclinical models of cancer and chronic infection disease. Many monoclonal antibodies targeting PD-L1/PD-1 has been studied in the clinical trials with six FDA approved drugs, including atezolizumab, avelumab and durvalumab for PD-L1 and pembrolizumab, nivolumab and cemiplimab for PD-1 [57, 58]. Even though these immunotherapies have observed promising antitumor effect in some patients [59, 60]. The overall response rate of anti-PD-1/L1 monotherapy is still below 25%. Several mechanisms have been reported to affect the efficacy of anti-PD-1/L1 treatment [61–64]. First, the T cell infiltration level. Since the aim of blocking PD-L1/PD-1 interaction is to rescue T cells from exhaustion, the level of T cell infiltration, especially CD8 T cell, are critical for the therapeutic effect of anti-PD-1/L1 [65]. Second, lack of tumor antigens. The genetic mutations are key drivers in the oncogenic process, which may lead to tumor immunogenicity and provide an opportunity for cancer immunotherapy. The tumor immunogenicity is closely related to the ability of T cells to recognize tumor cells and thus affect the efficacy of PD-L1/PD-1 blockade. Studies have showed that mutation burden and the number of neoantigens is closely related to the outcome of PD-L1/PD-1 blockade [66, 67], which has been confirmed in tumors with DNA mismatch repair (dMMR) deficiency and microsatellite instability (MSI) high [68]. In addition, studies have also showed that dMMR deficiency tumor cells can induce more cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) activation on dendritic cells to enhance the antitumor effect of PD-L1/PD-1 blockade [69]. Third, the level of stimulatory cytokines. Some preclinical studies have shown that the level interleukin-2 (IL-2) can determine the efficacy of PD-L1/PD-1 blockade [70]. Fourth, host gut microbiome can also influence the efficacy of ICB treatment [71, 72]. Finally, the induction of other immune checkpoints expression. The terminal exhausted phenotype of T cells can be maintained by multiple inhibitory pathways. High expression of CTLA-4, TIM-3 and LAG-3 on T cells will contribute to acquired resistance to PD-L1/PD-1 blockade [73, 74]. Besides tumor tissue, PD-L1 can also restrain T cell function in the tumor draining lymph nodes (TDLNs) [75]. Thus, PD-L1/PD-1 blockade could work on both priming and effector stage of the cancer-immune cycle. Moreover, studies also found that dendritic cells but not tumor cells expressed PD-L1 contribute to the PD-L1 blockade induced antitumor immunity [76, 77]. Mechanistically, interruption of PD-1/PD-L1 interaction will reconstitute B7-CD28 dependent costimulatory signaling for proper T cell function [78, 79].

CTLA-4 is another well-identified immune checkpoint [80]. Different from PD-L1/PD-1, CTLA-4 expression is upregulated on regulatory T cells (Tregs) within 24–48 h after activation. Mechanistically, CTLA-4 can first bind to B7 molecules with higher affinity than CD28 and then remove these ligands by transendocytosis, which results in impaired costimulation of T cells via CD28 [81–83]. Anti-CTLA-4 mAbs have been extensively studied in both preclinical and clinical models. Currently, ipilimumab is the first and only anti-CTLA-4 mAb approved by FDA for the treatment of melanoma, renal cell carcinoma and colorectal cancer, which has a human IgG1 isotype [84]. Two possible mechanisms of anti-CTLA-4 mediated T cell activation includes

enhancing the B7-CD28 signaling on effector T cells and the Treg depletion [85]. However, the Treg depletion mechanism is still controversial [86]. In mouse models, anti-CTLA-4 mediated Treg depletion has been observed, but similar observations have not been reported in clinical applications [86, 87]. The difference in the isotypes of anti-CTLA-4 mAbs can contribute to distinct efficacy of ADCC or CDC mediated Treg depletion. Further studies are required to define the detailed mechanism. Finally, studies have shown that combination treatment of anti-PD-L1 and anti-CTLA-4 can generate synergistic antitumor effect by coordinately enhancing the B7-CD28 signaling [88, 89]. Even though anti-PD-L1 and anti-CTLA-4 have observed promising antitumor effect. The efficacy of monoclonal antibodies against other immune checkpoints remains to be validated in clinical trials, including anti-TIM-3, anti-immunoreceptor with Ig and ITIM domains (TIGIT) and anti-LAG-3.

In addition to mAbs against co-inhibitory molecules, agonist mAbs that can activate co-stimulatory signaling has also been evaluated for cancer treatment. CD28, inducible T-cell costimulator (ICOS), CD137 (4-1BB) and CD40 are representative targets that have been broadly investigated [90–92]. In 2006, anti-CD28 monoclonal antibody TGN1412 was reported to induced strong cytokine storm in health volunteers [93], and was withdrawn from development. In 1997, Melero et al. have demonstrated that monoclonal antibodies against 4-1BB can eradicate established tumors in preclinical models [94]. However, clinical development of anti-CD137 is facing the same toxicity issue with anti-CD28. Clinical trial on 4-1BB agonist antibody, urelumab, had to be terminated because of treatment-related severe adverse effects in the form of liver inflammation [95]. Thus, severe side effect remains the major hurdle for systemic treatment of agonist antibody against costimulatory molecules.

Recent years, investigators started to develop antibodies to target “innate immune checkpoints”. The most representative molecule is CD47. CD47 is ubiquitously expressed in human cells and binds to signal regulatory protein α (SIRPα) expressed on macrophages to deliver a “don’t eat me” signaling to avoid macrophage mediated phagocytosis [96]. Many cancer cells can escape from this innate immune surveillance by overexpressing CD47 [97, 98]. Anti-CD47 blocking antibody can induce more macrophage-dependent phagocytosis and T-cell dependent antitumor immune responses [99]. However, Liu et al. also reported that the antitumor effect of anti-CD47 depends on dendritic cells and cGAS-STING dependent DNA sensing [100]. Thus, CD47 is a unique immune checkpoint which can bridge innate and adaptive immune system for effective tumor control. Even though CD47 is a promising target for antibody therapeutics, it still has the toxicity issues. Since CD47 is ubiquitously expressed in human, systemic delivery of anti-CD47 may induce severe adverse effect, as represented by red blood cell (RBC) depletion [101]. Alternative strategies have been investigated to improve the efficacy while reduce the toxicity. For example, Weiskopf et al. have engineered SIRPα variants with much higher affinity than wild-type SIRPα to enhance antibody mediated ADCC effect for cancer immunotherapy [102]. Bispecific antibodies targeting TAA and CD47 have also been engineered and studied [103, 104]. Clinical development of antibody therapeutics targeting CD47 have been extensively performed, but no drugs have been approved by the FDA yet. The safety and efficacy of magrolimab, a first-in-class anti-CD47 antibody, have not been established since FDA puts clinical hold on trials concerning the safety. Similar phagocytosis checkpoint has also been identified including Stanniocalcin 1, but the efficacy of antibody therapeutics against these target remains to be determined in clinical trials [105]. In summary, antibodies against innate and adaptive immune checkpoint have showed impressive potency (Table 1). Delivery strategies that can overcome systemic toxicity are required to improve the efficacy and broaden the application.

Antibody-drug conjugates for cancer therapy

Besides monoclonal antibodies, ADCs are the most common format for antibody-based cancer immunotherapeutic drugs [106]. To date, ten ADCs have been approved by the FDA. In addition, more than 80 ADCs are being developed in clinical as monotherapy or combination therapy for the treatment of different cancer types [107]. ADCs are complex molecules which conjugate a potent cytotoxic agent to specific sites on antibody via a chemical linker. The cytotoxic agent also named as “payload”, which includes anti-mitotic tubulin disruptors, DNA alkylating agents, RNA polymerase II and toll-like receptor agonists [108, 109]. Mechanistically, ADCs depend on the specificity of antibodies to target tumor cells first, followed by ADC internalization, and subsequent payload release to achieve the cytotoxicity. Even though most FDA approved ADCs are for the treatment of hematological malignancies, more ADCs are being developed to treat solid tumor (Table 2). For example, trastuzumab emtansine (T-DM1) and sacituzumab govitecan are well-defined ADCs for breast cancer, which can significantly prolong progression-free and overall survival in metastatic triple-negative breast cancer patients [110]. The major barrier

Table 1: US FDA-approved monoclonal antibody for cancer treatment.

Target	mAb name	1st approval year	Format	Application
CD20	Rituximab	1997	Chimeric IgG1	Non-Hodgkin lymphoma
	Ofatumumab	2009	Human IgG1	Chronic lymphocytic leukemia
	Obinutuzumab	2013	Humanized IgG1	Chronic lymphocytic leukemia
HER2	Trastuzumab	1998	Humanized IgG1	Breast cancer
	Pertuzumab	2012	Humanized IgG1	Breast cancer
EGFR	Cetuximab	2004	Chimeric IgG1	Colorectal cancer
	Necitumumab	2015	Human IgG1	NSCLC
	Panitumumab	2006	Human IgG2	Colorectal cancer
PD-1	Cemiplimab	2018	Human mAb	Cutaneous squamous cell carcinoma
	Nivolumab	2014	Human IgG4	Melanoma, NSCLC
	Pembrolizumab	2014	Human IgG4	Melanoma
CTLA-4	Ipilimumab	2011	Human IgG1	Metastatic melanoma
PD-L1	Atezolizumab	2016	Humanized IgG1	Bladder cancer
	Avelumab	2017	Human IgG1	Merkel cell carcinoma
	Durvalumab	2017	Human IgG1	Bladder cancer
SLAMF7	Elotuzumab	2014	Humanized IgG1	Multiple myeloma
CD38	Daratumumab	2015	Human IgG1	Multiple myeloma
	Isatuximab	2021	Chimeric IgG1	Multiple myeloma
CD52	Alemtuzumab	2001	Humanized IgG1	Chronic myeloid leukemia
VEGF-A	Bevacizumab	2004	Humanized IgG1	Colorectal cancer
VEGFR2	Ramucirumab	2014	Human IgG1	Gastric cancer
CD19	Tafasitamab	2020	Humanized IgG1	Diffuse large B-cell lymphoma
LAG-3	Relatlimab	2022	Human IgG4	Melanoma

EGFR, epidermal growth factor receptor; HER2, epidermal growth factor receptor 2; PD-L1, programmed cell death 1 ligand 1; FDA, Food and Drug Administration.

Table 2: US FDA-approved antibody-drug conjugates for cancer treatment.

Name	Target	Application	FDA approval year
Loncastuximab tesirine-lpyl	CD19	Large B-cell lymphoma	2021
Belantamab mafodotin-blmf	BCMA	Relapsed or refractory multiple myeloma	2020
Sacituzumab govitecan	Trop-2	Metastatic triple-negative breast cancer	2020
Trastuzumab deruxtecan	HER2	Unresectable or metastatic Her2-positive breast cancer	2019
Enfortumab vedotin	Nectin-4	Advanced or metastatic urothelial cancer	2019
Polatuzumab vedotin	CD79b	Diffuse large B-cell lymphoma	2019
Moxetumomab pasudotox	CD22	Relapsed or refractory hairy cell leukemia	2018
Inotuzumab ozogamicin	CD22	Relapsed or refractory B-cell precursor ALL	2017
Trastuzumab emtansine	HER2	HER2-positive metastatic breast cancer	2013
Brentuximab vedotin	CD30	Relapsed or refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma	2011
Gemtuzumab ozogamicin	CD33	Acute myeloid leukemia	2000/2017

BCMA, B-cell maturation antigen; HER2, epidermal growth factor receptor 2; FDA, Food and Drug Administration.

for ADCs is still the 'on-target off-tumor' side effect, since ADCs can induce stronger cytotoxicity compared with mAbs. Future engineering directions for ADCs includes target selection, linker chemistry, novel payload development, conjugation method optimization and enhancing internalization. Recently, proteolysis-targeting chimera (PROTAC) has been applied as a payload for ADC and termed as degrader-antibody conjugates (DAC) [111]. Several preclinical studies have demonstrated the potential efficacy of this next-generation drug [112]. More data from clinical trials are required for better characterization of these novel ADCs.

Bispecific antibodies for cancer immunotherapy

CD3-based bispecific antibodies for cancer immunotherapy

Since 1988, Jeffrey Bluestone group first reported that *in vivo* administration of anti-CD3 mAb can prevent malignant progressor tumor growth [113]. Antibody therapeutics targeting CD3 have been studied and developed for more than 30 years. To date, anti-CD3 based bispecific antibody is still the major topic among all the antibody therapeutics [114, 115]. In physiological conditions, the TCR/CD3 complex is cross-linked upon recognition of MHC-peptide

complex, thus leading to specific activation of T cells [116]. However, anti-CD3ε mAb can directly cross-link CD3 complex to non-specifically activate T cells [117]. Due to this unique function, anti-CD3 has been broadly investigated and applied to prevent allograft rejection, to treat diabetes and for cancer immunotherapy [118]. Since systemic delivery of anti-CD3 can non-specifically activate all the T cells, the treatment related side-effect remains the major concern. In order to target tumor cells to activate T cells, bispecific T cell engager (BiTE) concept was introduced as early as in 1980s [119]. To use antibody to 'cross-link' the CD3 signaling, one additional binding site is required besides CD3. According to this unique feature, BiTE was designed as two single-chain variable fragment (ScFv) linked with a flexible linker which can simultaneously bind to CD3 and TAA. This structure has several advantages over traditional mAbs. First, it can bridge T cells with TAA expressing cell for effective killing. Second, it can target TAA expressing cells to cross-link TCR signaling, thus may reduce the systemic toxicity. To date, different formats of BiTE have been developed such as Fab_2, diabody and dual-affinity re-targeting (DART) [120]. But only the CD19xCD3 BiTE, blinatumomab, has been approved by the FDA to treat adults and children with B-cell precursor acute lymphoblastic leukemia (ALL) who are in remission but still have minimal residual disease (MRD) [121]. Similar with mAbs, the major concern of BiTE treatment is still the 'on-target off-tumor' side effect. Since TAAs also expressed in some normal tissue, BiTE induced T cell activation may cause cytokine storm and tissue damage [122]. In addition, the short half-life of BiTE also limits its application in solid tumors. Even for blinatumomab treatment, patients need daily intravenous infusion of BiTE for 4 weeks as a treatment cycle. The penetration of BiTE against solid tumors is still problematic. To extend the *in vivo* half-life of BiTE, Fc region was engineered with BiTE and termed as bispecific antibody (BsAb). Several alternative

Table 3: Summary of clinical bispecific antibodies for cancer treatment.

Target 1	Target 2	Name	Application	Status
CD3	CD19	Blinatumomab	ALL	FDA approved
		A-319	ALL	Phase I (NCT04056975)
		AMG562	Lymphoma	Phase I (NCT03571828)
	CD20	Mosunetuzumab	NHL	Phase I (NCT05169658)
		Odronextamab	NHL	Phase II (NCT03888105)
		Plamotamab	Diffuse large-cell B-cell lymphoma	Phase II (NCT05328102)
	CD38	ISB 1342	Multiple myeloma	Phase I/II (NCT03309111)
	BCMA	AMG 420	Multiple myeloma	Phase I (NCT03836053), completed
		AMG 701	Multiple myeloma	Phase I (NCT03287908)
	CD123	APVO436	AML	Phase I (NCT03647800)
		XmAb14045	ALL	Phase I (NCT02730312)
	CEA	RO6958688	Solid tumor	Phase I (NCT02650713)
	PSMA	Acapatamab	Prostate cancer	Phase I (NCT03792841)
		BAY2010112	Prostate cancer	Phase I (NCT01723475), completed
	HER2	M802	Solid tumor	Phase I (NCT04501770)
		Runimotamab	Solid tumor	Phase I (NCT03448042)
	MUC16	REGN4018	Ovarian cancer	Phase I/II (NCT 03564340)
	GPC3	ERY974	Solid tumor	Phase I (NCT02748837), completed
	EGFR vIII	AMG 596	Glioblastoma or malignant Glioma	Phase I (NCT03296696), completed
	CLDN18.2	AMG 910	Gastric and Gastroesophageal junction adenocarcinoma	Phase I (NCT04260191), completed
CD47	PD-1	HX009	Relapsed/Refractory lymphoma	Phase I (NCT05189093)
	PD-L1	IBI322	Advanced malignant tumor	Phase I (NCT04338659)
	CD19	TG-1801	B-cell lymphoma	Phase I (NCT03804996)
PD-1	ICOS	XmAb®23,104	Advanced solid tumor	Phase I (NCT03752398)
	CTLA-4	MEDI5752	Advanced RCC	Phase I (NCT04522323)
	PD-L1	LY3434172	Advanced cancer	Phase I (NCT03936959), completed
	TIM3	RO7121661	Advanced and/or metastatic solid tumor	Phase I (NCT03708328)
PD-L1	LAG-3	FS118	Advanced malignancies	Phase I (NCT03440437)
	CTLA-4	KN046	Thymic carcinoma	Phase II (NCT04469725)
EGFR	c-MET	Amivantamab	NSCLC	Phase I (NCT04077463)
HER2	HER3	MCLA-128	Breast cancer	Phase II (NCT03321981)

BCMA, B-cell maturation antigen; EGFR, epidermal growth factor receptor; HER2, epidermal growth factor receptor 2; PD-L1, programmed cell death 1 ligand 1; NSCLC, non-small cell lung cancer.

formats of BsAb have also been investigated including ScFv-knobs-into-holes (KIH), dual variable domain (DVD)-IgG and CrossMab [123]. Among them, catumaxomab is a rat-mouse hybrid monoclonal antibody which simultaneously binds to CD3 and EpCAM. It was first approved by EMA to treat malignant ascites in 2009 but withdrawn 8 years later due to commercial reasons [124]. In early 2022, the US FDA approved tebentafusp-tebn, a bispecific gp100 peptide-HLA-directed CD3 T cell engager, for HLA-A*02:01-positive adult patients with unresectable or metastatic uveal melanoma [125]. This is a unique and next-generation design of bispecific antibody. Unlike traditional bispecific antibodies, tebentafusp-tebn binds to the gp100 peptide-HLA complex instead of TAAs. Thus, it can increase the specificity of tumor-targeting while reducing the side effect. This therapeutic strategy could benefit selective patients with well-identified mutant peptide and specific HLA alleles. Similar constructs are designed to target the common oncogenes such as p53 and KRAS with TCR mimic antibodies [126]. Based on the successful application of blinatumomab in leukemia, more and more bispecific antibodies have been developed to treat hemopoietic malignance. Most recently, the EMA has approved CD20xCD3 bispecific antibody (mosunetuzumab) for patients with relapsed or refractory disease of follicular lymphoma and BCMAxCD3 bispecific antibody (teclistamab) for patients with relapsed and refractory multiple myeloma [127, 128]. Compared with hemopoietic malignance, solid tumors usually form an inhibitory tumor microenvironment that limits the efficacy of bispecific antibodies [129]. T cell infiltration level, antibody penetration and the expression of immune checkpoint molecules are major barriers for BsAb treatment against solid tumors. Resistance to BiTE treatment in T cell—cold solid tumors can be overcome by combination treatment [130]. Many targets on solid tumors have been extensively evaluated as candidates for BsAb, including EGFR, carcinoembryonic antigen (CEA), prostate-specific membrane antigen (PSMA) and HER2 (Table 3). But the efficacy and safety have not been established yet [131, 132].

Since the treatment related severe adverse effects is one of the major barriers for BsAb. Several studies have investigated the potential mechanism. Li et al. showed that anti-HER2/CD3–induced cytokine release is not required for T cell cytotoxic activity. Anti-TNFα can prevent monocytes derived IL-1 and IL-6 to induce toxicity while keep the antitumor effect from T cells [133]. Cytokine release syndrome (CRS) was only observed at 24 h post the first dose of treatment in CD20xCD3 treatment [134, 135]. These reports indicate that the treatment-related side effect can be overcome by combination treatment. Besides toxicity, strategies to improve the efficacy have also been investigated. Belmontes et al. have shown that resistant to CLDN18.2 specific BiTE treatment can be overcome by the combination with either anti-PD-L1, anti-CTLA-4 or anti-4-1BB [130]. Bispecific antibody targeting the immune checkpoint PD-L1 and CD3 generate much better antitumor effect than traditional TAA-targeting BiTE by enhancing B7-CD28 mediated costimulatory signaling from dendritic cells [136]. These studies indicate that target the immune checkpoint may generate better T-cell activation than targeting TAAs on the cancer cells by enhancing the crosstalk between immune cells. In addition, several studies have also reported that TAA-targeting BiTE treatment can induce activation-induced cell death (AICD) on T cells, thus prevent the establishment of memory T cell response [137]. Concurrent delivery of costimulatory signaling by anti-CD28 can overcome this limitation by potentiating T cell activation [138, 139].

TAA and immune checkpoint targeting bispecific antibodies for cancer immunotherapy

Apart from CD3-based bispecific antibodies, other dual functional bispecific antibodies have also been developed. The first category is dual receptor blockade antibody. cMet/EGFR and HER2/HER3 bispecific antibodies are representative drugs under clinical investigation [140, 141]. These antibodies are designed to simultaneously block multiple growth factor signaling to limit the adaptive resistance of mAb treatment. In 2021, amivantamab-vmjw, the cMet/EGFR bispecific antibody was approved by FDA for patients with metastatic non-small cell lung cancer (NSCLC). The second category is dual immune checkpoint blockade antibody. Several drugs are being evaluated in phase I to III clinical trials including PD-1xCTLA-4, PD-L1xLAG3, PD-L1xCD47. The purpose of these drugs is to target tumor tissue first and then simultaneously block multiple immune checkpoints [104, 142, 143]. In addition, bispecific antibodies are also designed to deliver agonist or antagonist in the tumor tissue. Anti-4-1BB is a powerful but toxic drug. Like CD3 and some TNF receptor superfamily members, trimerization is required for 4-1BB signaling. According to this unique feature, 4-1BBxHer2 and 4-1BBxPD-L1 have been designed [144–146]. Finally, cytokine receptors are also ideal targets for bispecific antibodies. M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-β have showed effective antitumor effect in multiple mouse tumor models [147]. Despite failing in NSCLC patients, bintrafusp alfa (M7824) will continue to be evaluated in several additional clinical trials for different cancer types.

Antibody-cytokine fusion proteins for cancer immunotherapy

Cytokines are soluble proteins that mediate cell-to-cell communication, especially between immune cells. Studies showed that several cytokines can limit cancer cell growth by both direct anti-proliferative or pro-apoptotic activity, and indirectly activating host adaptive immune responses [148]. IFNα, TNFα and IL-2 have been extensively studied and applied for cancer treatment [59, 149, 150]. Since antibodies can provide unique tumor-targeting property by binding either TAAs or the immune checkpoints. People also engineered cytokines to these antibodies to increase the efficacy and reduce the toxicity [151–154]. Yang et al. reported that type-I interferon fused to anti-EGFR (cetuximab) can overcome therapeutic resistance to cetuximab by increasing DC function and CD8 T cell immune responses [155]. Similar result was also observed in CD20xIFNα treatment study [156, 157]. IL-2 is a potent cytokine for T cell activation and proliferation [158]. System delivery of recombinant IL-2 have been approved by FDA for patients with metastatic renal cell carcinoma and metastatic melanoma in 1990s. However, high dose IL-2 treatment is toxic and may also expand Tregs which express high levels of IL-2 receptor alpha [59, 159]. Several antibody-based IL-2 delivery strategies have been designed to overcome these limitations [160, 161]. In addition, Sun et al. designed an EGFR targeting super mutant IL-2 and demonstrated that tumor-targeting delivery of CD8-T cell-biased IL-2 generate better antitumor effect than WT IL-2 [162]. In another study, Qiao et al. demonstrated that EGFR-targeting delivery of interlukin-10 prevents dendritic cell mediated $CD8^+$ tumor-infiltrating lymphocyte apoptosis through regulating IFN-γ production [163, 164]. In summary, antibodies can be a powerful platform to deliver cytokines in the tumor microenvironment. More clinical validations are required for establishing the efficacy and safety profile for cancer patients.

Strategies to overcome immune-related adverse events in antibody therapeutics

The fundamental rationale of antibody-based drugs is targeting tumor tissue to deliver therapeutic agents and activate immune responses. To date, very few cancer-specific antigens have been identified except some shared/public neoantigens and oncogenic viral antigens [165]. Most antibodies are still targeting the tumor-associated antigens which are also expressed in normal tissues. Systemic delivered antibody therapeutics may also penetrate to these normal tissue, induce inflammation and tissue damage [166]. To overcome this “on-target off-tumor” side effect, several strategies have been developed. The most common design is antibody prodrugs [167]. The principle of these prodrugs is using a protease substrate peptide to link a ‘mask’ to the antibody. Thus, the antibodies will be masked in the peripheral circulation to avoid off-tumor binding. When the antibodies penetrate to the tumor tissue, tumor associated protease will ‘cut’ the substrate linker, remove the mask, and release the drug [168, 169]. This two-step targeting approach could dramatically improve the tumor-targeting efficacy and reduce the toxicity. Representative constructs using this strategy is the dual variable domain Ig (DVD-Ig). Pai et al. demonstrated that anti-CTLA4 DVD-Ig markedly reduced multiorgan immune toxicity by preserving tissue-resident Tregs. In this study, the anti-CTLA4 antibody was shielded by an outer tumor-targeting anti-prostate stem cell antigen (PSCA) domain. Activated membrane type-serine protease 1 (MT-SP1), which was highly expressed in the tumor microenvironment can cleave this shield to expose the inner CTLA4-binding site [170]. Other advanced antibody prodrugs in development are the Probody™ Therapeutics [171–173]. This platform usually needs two steps screening. First, the identification of appropriate mask by screening the peptide libraries. Second, the identification of a suitable protease substrate [174, 175]. Many antibody therapeutics are developed and under clinical trials, including CX-072 (pacmilimab) for anti-PD-L1 [176], CX-904 for EGFRxCD3 and BMS-986249 for anti-CTLA4. The efficacy of peptide masking and protease digestion need to be future investigated for broader application. The immunogenicity of the peptide mask also needs to be identified for the safety.

Summary and future prospect

To date, more than 100 antibody-based therapeutic drugs have been approved by the FDA and most of them are for cancer treatment [3]. Due to the advanced technology in recombinant and humanized antibody engineering, more and more candidates are being screened preclinically and clinically. However, detailed mechanism studies on efficacy and toxicity are required for the translation (Figure 1 and Table 4). Several drugs have observed good efficacy and limited toxicity in mouse models, but immune-related adverse events (irAE) were observed in human despite the efficacy [177, 178]. This means that there are still some undiscovered differences in the immune system between

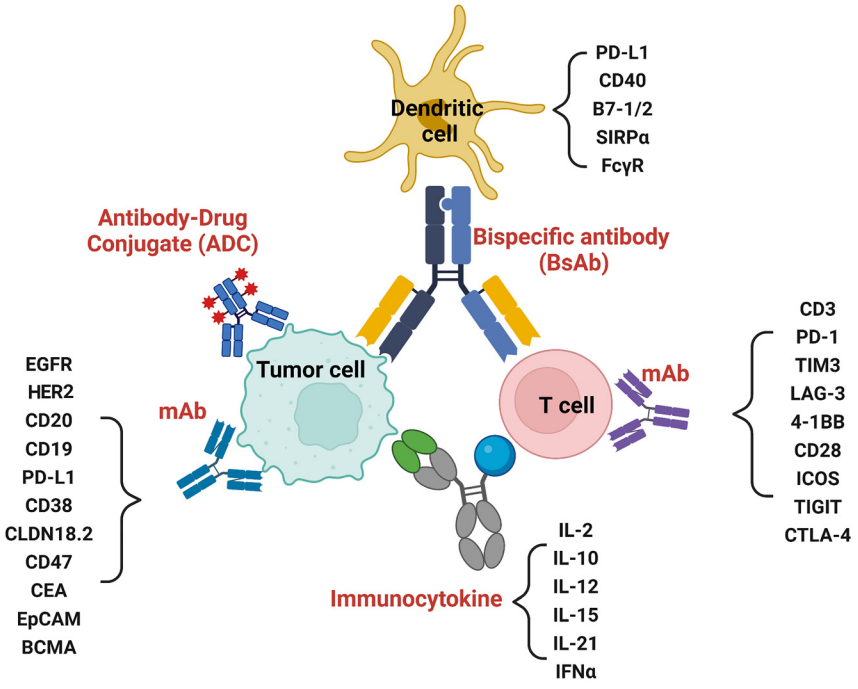

Figure 1: Schematic overview showing current antibody-based therapeutics for the cancer treatment. There are four major categories of antibody therapeutics. First, monoclonal antibody (mAb), which includes anti-tumor associated antigen (TAA) and T-cell costimulatory/coinhibitory molecules. Second, antibody-drug conjugate. Third, bispecific antibodies. Fourth, immunocytokines. IL-10, interleukin-10; IL-12, interleukin-12; IL-15, interleukin-15; IL-21, interleukin-21; EpCAM, epithelial cell adhesion molecule; BCMA, B-cell maturation antigen; EGFR, epidermal growth factor receptor; HER2, epidermal growth factor receptor 2; PD-L1, programmed cell death 1 ligand 1.

Table 4: Advantages and disadvantages of different therapeutic antibodies.

Name	Advantage	Disadvantage
Monoclonal antibody	1. Easy to prepare with high purity 2. Well-defined mechanism of action	1. Drug resistance 2. "On-target off-tumor" side-effect
Antibody-drug conjugate	1. Efficient tumor cell killing 2. Not easy to resistance	"On-target off-tumor" side-effect
Bispecific antibody	1. High efficiency 2. Activate host immune response	"On-target off-tumor" side-effect
Antibody fusion proteins	1. High efficiency 2. Activate host immune response	Undefined mechanism of action

murine and human. Even though the mouse tumor models have been extensively optimized using human peripheral blood mononuclear cell (PBMC) and $CD34^+$ stem cells. The reconstituted human immune system on mouse still have some differences compared with patients [179]. Cetuximab, rituximab, ipilimumab and nivolumab are successful mAbs that are being applied in patients broadly, lessons should be learnt from these drugs on the engineering and evaluation strategies. Some future investigation on antibody therapeutics will help more patients benefit from this therapy. First, more cancer antigens should be screened to identify more specific targets. This will be important for immune checkpoint blockade, antibody-cytokine fusion protein and agonist antibodies development. Second, optimization on the antibody sequence. The Fc region play controversial roles on the function of therapeutic antibodies. On one hand, it can extend the *in vivo* half-life of antibodies. On the other hand, it may also bind to different Fc receptors and mediate ADCC or CDC effect. For tumor cell targeting mAbs, Fc-FcR binding can promote innate immune activation [180]. However, for ICB treatment, Fc may also lead to T cell depletion. In the bispecific antibody design, mutations on Fc have been made to avoid Fc mediated non-specific cross-link of CD3 in the peripheral tissues [181, 182]. Further screening on Fc is essential to improve the efficacy. Third, development of computation protein design to antibody discovery. The generation of antibody repertoires deep-sequencing data and matched functional data can be used to train machine-learning-based models for affinity maturation and humanization [183, 184]. This will also be an important technology to discover ideal mask for prodrugs. Fourth, the application of novel technologies. To improve the tumor targeting ability and antitumor efficacy of BsAbs, several trispecific antibodies have been developed, including the CD3/CD28/CD38 trispecific antibody that can increase the costimulatory signaling and the "2 + 1" platform that can increase tumor targeting [134, 185, 186]. Other tumor-targeting strategies should also be considered for antibody delivery. For example, as tumor tissue usually have a lower pH than other tissues, pH sensitive nanoparticle can be a good platform for

antibody encapsulation and targeted release [187, 188]. Tumor tropic cell and bacteria can also be engineered for antibody delivery [189]. Finally, the combination treatment. Antibody therapeutics may generate synergistic antitumor effect with traditional therapy. For example, neoadjuvant ICB may induce better and systemic antitumor effect with either radiation or surgery [190, 191]. The combination with ICB can also improve the efficacy of BiTE [192]. Blocking the inflammatory cytokines may reduce the irAE from ICB while enhancing the antitumor effect simultaneously [193]. In multiple ongoing clinical trials, antibodies are used as adjuvant therapy to chemotherapy and observed clinical responses. Thus, the combination of either different antibody therapeutics or antibody therapeutics with traditional therapies will be potential strategies to try. In conclusion, we have summarized the highlights of innovation with antibody therapeutics, the progress of antibodies engineering in recent years and discussed the limitation of antibody therapeutics and the potential strategies to overcome the hurdles. With more than 100 FDA approved antibody-based drugs, immunotherapy will benefit more cancer patients in the near future.

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