



Therapeutic antibodies in oncology: an immunopharmacological overview

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Abstract

The biotechnological development of monoclonal antibodies and their immunotherapeutic use in oncology have grown exponentially in the last decade, becoming the first-line therapy for some types of cancer. Their mechanism of action is based on the ability to regulate the immune system or by interacting with targets that are either overexpressed in tumor cells, released into the extracellular milieu or involved in processes that favor tumor growth. In addition, the intrinsic characteristics of each subclass of antibodies provide specific effector functions against the tumor by activating antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cellular phagocytosis, among other mechanisms. The rational design and engineering of monoclonal antibodies have improved their pharmacokinetic and pharmacodynamic features, thus optimizing the therapeutic regimens administered to cancer patients and improving their clinical outcomes. The selection of the immunoglobulin G subclass, modifications to its crystallizable region (Fc), and conjugation of radioactive substances or antineoplastic drugs may all improve the antitumor effects of therapeutic antibodies. This review aims to provide insights into the immunological and pharmacological aspects of therapeutic antibodies used in oncology, with a rational approach at molecular modifications that can be introduced into these biological tools, improving their efficacy in the treatment of cancer.

Keywords Monoclonal antibodies · Cancer therapy · Immunotherapy · Immunobiotechnology · Bispecifics · Antibody-drug conjugates · Bispecific T-cell engagers (BiTEs)

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Introduction

The pharmaceutical industry has grown along with the development of biotechnology to generate innovative drugs [1]. Among these, monoclonal antibodies (mAbs) to treat diseases, including cancer, are distinguished: of a total of 213 mAbs globally approved or under review up to May 15, 2024, 99 are indicated for cancer treatment, a highly prevalent and deadly pathology worldwide [2, 3]. Among these anti-cancer mAbs, 76 are currently approved by the World Health Organization (WHO)-Listed Authorities (WLA) in several country of the world [4].

Over time, the technologies used for the development of therapeutic mAbs have evolved from the first therapeutic molecules of murine origin to completely human antibodies, which included modifications in their amino acid sequences, further optimizing the exceptional characteristics of these

drugs. The engineering of antibodies has enabled the development of mAbs with enhanced binding affinity to therapeutic targets and prolonged serum half-life, even far exceeding 20 days. Moreover, they have achieved increased capacity to activate mediators that contribute to either eliminate tumor cells, modulate antibody effector functions, or facilitate the entry of cytotoxic drugs into the cells of interest [5].

Here, we provide an overview of the mAbs approved for oncology treatment by the WLA, focusing on their therapeutic mechanisms of action; we also examine the rational design of modifications applied to antibodies in order to improve their therapeutic efficacy. Anti-cancer mAbs that received approval by the WLA, but have been withdrawn or marketing discontinued, will not be covered in this review.

Structure and function of antibodies

Antibodies (Abs) or immunoglobulins (Igs) are soluble glycoproteins responsible for the specific and selective recognition of antigens. Structurally, the basic unit of Abs is a monospecific monomer composed of four polypeptide chains: two identical heavy chains (HC) with a molecular mass of 50–75 kDa each and two identical light chains (LC) of approximately 25 kDa each (Fig. 1A). The modular domains that compose each HC consist of one variable heavy domain (V_H) and three or four constant heavy domains (C_{H1} , C_{H2} , C_{H3} , C_{H4}), depending on the antibody isotype. The modular domains that form each LC consist of one variable light domain (V_L) and one constant light domain (C_L). The combination of a V_H domain with a V_L domain forms the antigen-binding region, which can also be referred to as the Fv region. Each variable domain contains three hypervariable regions called complementary-determining regions (CDRs). The amino acids of CDRs are different in sequence and structure between different antigen-specific antibodies. Upon protein folding, in each V_H and V_L chain, the CDR regions give rise to three loops that connect to β -strands; the six loops are in proximity to each other; together, they form the antigen-binding site, also known as the paratope. The strands of the two β -sheets and the non-hypervariable loops are called framework regions (FRs) [5]. Although the paratope aligns harmoniously with the antigen's epitope, only a few residues on the paratope contribute to the binding energy. The antibody-antigen binding strength, measured in K_D (dissociation constant in the order of nM to pM), as well as the antibody specificity, is useful properties to design therapeutic monoclonal Abs (TmAbs) for treating several diseases, including cancer. In addition, the selection of targets that discriminate between a tumor and a non-tumor cell contributes to the ability of the TmAb to neutralize or selectively block molecules that facilitate tumor growth or even induce cell death.

The two HCs are linked by disulfide bonds between their hinge regions; the V_H and C_{H1} domains, together with the LCs, form the antigen-binding fragment (Fab), and the C_{H2} , C_{H3} , and C_{H4} domains of both HCs form the crystallizable fragment (Fc), located at the carboxyl-terminal end of each chain. Both Fab and Fc fragments are linked by the hinge region, which provides conformational flexibility to the antibody [5]. In a given antibody, the two HCs are identical, as are the two LCs; therefore, an Ab is a bivalent molecule, having two identical combinations of V_H and V_L domains. Although most mammals have the conventional IgG subclass structure, which is composed of four polypeptide chains (2 HCs and 2 LCs), camelid species and sharks contain smaller Ig fractions lacking the LCs and the first HC constant domain (HC-only antibodies or HCAbs), accounting for 25–75% of their total serum IgG [6] (Fig. 1A). In this case, the term “VHH” is used to distinguish the variable domains of camelid from the conventional VH domains [7]. Due to its biological properties, IgG is the most widely used Ig as the basis for therapeutic antibody development, a topic that will be discussed in detail in this article.

Of the 76 TmAbs currently approved by the WLA, 49 are human IgG1, 3 are IgG2, and 19 are IgG4 [4]. The IgG HC constant region determines the antibody subclass. Human IgG1, IgG2, IgG3, and IgG4 carry the $\gamma 1$, $\gamma 2$, $\gamma 3$, and $\gamma 4$ chains, respectively. The Fc fragment of IgG (IgG-Fc) accounts for the antibody's effector functions, which include complement-dependent cytotoxicity (CDC) [8], antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and antibody-dependent cellular trogocytosis (ADCT) [9–12] (Fig. 1B). These effector functions depend, respectively, on IgG-Fc binding to complement factor 1 (C1q), specific Fc-gamma receptors ($Fc\gamma R$ s) on phagocytes, such as macrophages, $Fc\gamma R$ s on Natural Killer (NK) cells, and $Fc\gamma R$ s on neutrophils (Table 1). The IgG-Fc region can also bind to the neonatal Fc receptor (FcRn); this receptor promotes antibody recycling through rescue from normal lysosomal degradation by increasing the affinity of these molecules for the acidic pH of endosomes–lysosomes, which prolongs the antibody plasma half-life for more than 21 days for IgG1, IgG2, and IgG4, thus contributing to the pharmacokinetics of TmAbs [13, 14]. The binding of Fc regions to different receptors depends on allotypic variations on the $Fc\gamma RIIa$ (CD32a), such as H131/R131, and $Fc\gamma RIIIa$ (CD16a) such as F158/V158, with effect, respectively, on the ADCP and ADCC activities (Table 1). In addition, the type of post-translational modification to the Ab, such as N-glycosylation (addition of carbohydrates to the arginine 297 residue in the C_{H2} domain of IgG), also influences Fc-binding to specific receptors [5].

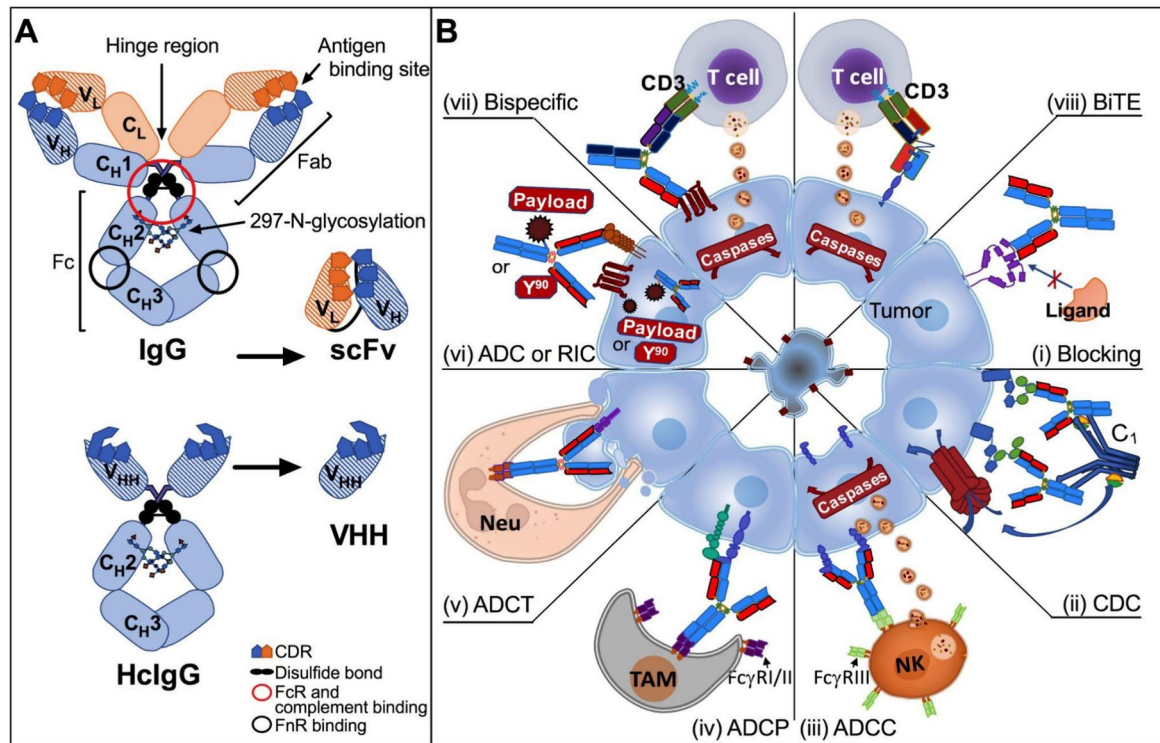


Fig. 1 A Structure of therapeutic monoclonal antibodies (TmAbs). Human IgG (top) is formed by two heavy chains and two light chains linked to each other by disulfide bonds, which originate Fab and Fc fragments. Fab fragment contains the antigen-binding site formed by complementary-determining regions (CDRs). The Fc fragment is responsible for effector functions and binds to the neonatal receptor. The single-chain fragment variable (scFv) structure is composed of the variable domains of heavy and light chains. The structure of heavy chain-only antibodies (HcIgG) from camelids (bottom) includes their respective variable domains (VHH), which are being used in the design of TmAbs. B Mechanism of action of TmAbs. i Blockage of immunoregulators (CTLA-4, PD-1, or PDL-1), cytokines or growth factors. The antibody binds to its target expressed on the tumor cell membrane and prevents its interaction with its ligand; ii complement activation-dependent cytotoxicity (CDC). Complement molecule C1q directly interacts with target-bound IgG C_{H2} domain, resulting in the activation of the complement cascade and the formation of the membrane attack complex, which induces cell lysis. iii Antibody-dependent cellular cytotoxicity (ADCC). FcγRIIIa (CD16a), a low-affinity activation receptor present on Natural Killer (NK) cells, binds to target-bound IgG C_{H2} domain. Activation of CD16 triggers the release of perforin and granzymes,

resulting in target cell death. iv Antibody-dependent cellular phagocytosis (ADCP). ADCP is mediated through the interaction of the Fc domain of target cell-bound IgG with FcγRs on phagocytic cells, such as tumor-associated macrophages (TAM). FcγRI (CD64) and FcγRII (CD32) recognize overlapping but not identical sites in the lower hinge region of IgGs, which promotes tumor cell phagocytosis. v Antibody-dependent cellular trogocytosis (ADCT). Neutrophils can remove tumor cell surface content in the presence of TmAbs targeting specific cell membrane proteins through Fc binding to FcγR on the effector immune cell, which may trigger tumor cell lysis. vi Antibody–drug conjugate (ADC) and radioimmunoconjugate (RIC). Antineoplastic drugs or radioactive elements can be conjugated to the antibody through a peptide linker to be directed to the target tissue, increasing its cytotoxic concentration in situ and decreasing its unwanted effects on other tissues. vii, viii Bispecific molecules. vii Bispecific antibodies. They have dual binding sites that bind two different antigens. viii Bispecific T-cell engager (BiTE). They are recombinant proteins composed of two scFvs linked by a short flexible linker. These bispecific molecules are designed to target tumor antigens and CD3, thereby creating a link between tumor cells and T cells. Created with Microsoft® PowerPoint for Mac. Version 15.32 and Prism 6 for Mac OS X

Development of TmAbs in oncology

Throughout history, the technology used for the production of mAbs has involved the application of techniques that seek to resolve the quantity and the design of essential fragments of Ab structure [15]. The hybridoma platform based on the fusion of murine B cells and myeloma cells, established by George Kohler and César Milstein in 1975 [16], allowed the in vitro production of a large number of mAbs. Muromonab, the first murine anti-CD3 TmAb, used

to eliminate T lymphocytes, was approved in 1986 to treat acute graft rejection [17]. The only two therapeutic anti-cancer full-length murine mAbs were approved by the WLA in 2002 (ibritumomab tiuxetan, an anti-CD20 TmAb radioimmunoconjugate (RIC) with Itrium-90 (Y⁹⁰), which was used for the treatment of non-Hodgkin's lymphoma [18]) and by the National Regulatory Authorities in Cuba (CECMED) in 2013 (racotumomab, a murine anti-GM3 mAb used for the treatment of non-small cell lung cancer). Nevertheless, the administration of murine antibodies to humans can evoke, in

Table 1 Structural, biological, and functional properties of IgG subclasses

Characteristics	IgG1	IgG2	IgG3	IgG4
Structural				
Molecular weight (kDa)	150	150	155–165	150
Disulfide bond between heavy chains	2	4	11	2
H chain type	γ 1	γ 2	γ 3	γ 4
Ig H constant gene	IGHG1	IGHG2	IGHG3	IGHG4
Glycosylation site	297	297	297	297
N-linked glycans per H chain	1	1	2 *	1
O-linked glycans per H chain	0	0	3	0
Biological				
Biological half-life (days)	21–24	21–24	7–8	21–24
Mean adult serum level (g/L)	6.98	3.8	0.51	0.56
Placental transfer	+++	++	+/++++*	++
Functional (binding—activation)				
C1q- CDC	++	+	+++	–
Fc γ RI (CD64) on monocytes—ADCP	+++	–	+++	++
Fc γ RIIa (CD32a)-H131 on monocytes and neutrophils—ADCP (ADCT‡)	++	++	++	+
Fc γ RIIa (CD32a)-R131 on monocytes and neutrophils—ADCP (ADCT‡)	++	–	++	+
Fc γ RIIb (CD32b) on monocytes and neutrophils—ADCP	+	–	++	+
Fc γ RIIIa (CD16a)-F158 on NK cells—ADCC	++	–	+++	–
Fc γ RIIIa (CD16a)-V158 on NK cells—ADCC	+++	+	+++	+
Fc γ RIIIb (CD16b)NA1 on neutrophils and monocytes—ADCP (ADCT‡)	+++	–	+++	–
Fc γ RIIIb (CD16b)NA2 on neutrophils and monocytes—ADCP (ADCT‡)	+++	–	+++	–
FcRn on epithelial, endothelial, macrophages/monocytes and dendritic cells—homeostasis—pH 7,4 / < 6,5	+/++++	+/++++	+/++++	+/++++

Number of (+) symbols indicates the affinity/interaction magnitude of each one of the immunoglobulin subclasses. *IgG allotype-specific. ‡Mechanism demonstrated for IgG1-TmAb. CDC: Complement-dependent cytotoxicity. ADCP: Antibody-dependent cellular phagocytosis. ADCC: Antibody-dependent cellular cytotoxicity. ADCT: Antibody-dependent cellular trogocytosis. FcRn: Neonatal Fc receptor. N-linked glycans connect the sugar to an asparagine residue in the glycosylation process, whereas O-linked glycans connect a serine or threonine residue

some patients, the production of human anti-mouse antibodies (HAMA); these are endogenous antibodies with specificity for antigenic determinants present in the Fc region of mouse Igs [19]. Production of HAMA accelerates mAb clearance, thus reducing its serum half-time; on the other hand, repeated mAb administration could result in undesirable allergic reactions [20, 21].

The limitations in using mAbs of murine origin have been overcome by the development of engineering techniques, such as the recombination of murine variables with human constant regions, thus resulting in the production of chimeric antibodies. In 1997, the Food and Drug Administration (FDA) agency of the USA approved the first chimeric TmAb, rituximab, an anti-CD20 mAb for treating non-Hodgkin's lymphoma [22, 23]. Later, humanized antibodies were generated, which contained human framework sequences surrounded by murine antigen-binding CDRs on the light and heavy variable chains. In 1998, the first humanized TmAb, trastuzumab, a mAb targeting the human epidermal growth factor receptor 2 (HER2), was approved for the treatment of breast cancer

[24]. Currently, there are 10 chimeric and 40 humanized TmAbs, with an expected significant growth in the development of antibodies with a greater proportion of human amino acid sequences (Supplementary Fig. 1A). In 2021, enavofolimab, the first humanized anti-programmed death ligand 1 (PD-L1) single-variable domain camelid antibody (VHH), was approved in China for the treatment of adult patients with previously treated microsatellite instability-high (MSI-H) or deficient Mismatch Repair (dMMR) advanced solid tumors. Unlike other immune checkpoint inhibitors that are administered intravenously, which can be uncomfortable and lead to discontinuation of therapy protocols, enavofolimab is injected subcutaneously (SC) to improve patient tolerance [25, 26].

Another strategy to avoid HAMA while using murine-derived antibodies that provide high specificity and affinity for antigens with low immunogenicity is the use of scFv antibodies [27]. These antibodies are fusion proteins of the variable regions of V_H and V_L chains connected with a short linker peptide of 10–25 amino acids [27, 28]. In 2014, the WLA approved blinatumomab, a bispecific molecule that

includes the scFv structure [29] (Supplementary Fig. 1A and Fig. 2E).

In 1990, Gregory P. Winter introduced phage display, a groundbreaking technique that integrates human antibody genetic sequences into the genome of filamentous bacteriophages. These sequences are further expressed on the phage surface, enabling subsequent screening processes, and this technology also constitutes the basis for scFv production [30–32]. This opened new frontiers in the development of fully human antibodies. In 2002, the first fully human antibody, adalimumab, a phage display-derived mAb that neutralizes tumor necrosis factor- α (TNF- α), was approved for the treatment of rheumatoid arthritis. In addition, since 1994, transgenic mice engineered to carry human Ig genes have been used to develop fully human antibodies [33, 34]. In 2006, the first fully human antibody derived from a transgenic mouse platform, panitumumab, which inhibits epidermal growth factor receptor (EGFR) signaling, was approved for treating colorectal cancer [2, 22, 35]. Currently, there are 23 fully human TmAbs, most of which derive from transgenic mice. The use of the single B cell technique has allowed the isolation of antigen-specific B cells from human blood for the generation of human natural antibodies, which have been applied in the search for antiviral antibodies. More recently, the Next Generation Sequencing (NGS) technology has also been useful in discovering new antibodies and their optimization through glycoengineering [15].

The development of antineoplastic drug conjugation has also improved the efficacy of TmAbs; indeed, 19.7% of the antibodies approved in oncology are antibody–drug conjugates (or ADC) and chemical conjugates. In 2000, the first conjugated antibody was approved; this is gemtuzumab ozogamicin, an anti-CD33 mAb conjugated to the anti-tumor antibiotic calicheamicin. Over the last seven years, the WLA has approved 10 monospecific conjugated antibodies (Supplementary Fig. 1B). In September 2020, the Japanese government approved cetuximab sarotalocan, the first antibody-photosensitizer conjugate, to treat unresectable advanced or recurrent head and neck cancer [36].

The development of recombinant bispecific antibodies has strongly increased in recent years, which goes hand in hand with the increment in the number of uncovered molecular targets to which each therapeutic antibody is designed. These account for 13% of the total antibodies approved in oncology (Supplementary Fig. 1C); 80% of them were approved between 2022 and 2023. Most bispecific antibodies are full-length IgG that can target two different antigens on the same cell or on two different cells [37]. In 2021, amivantamab, a fully human, full-length bispecific antibody against EGFR and the cell survival-related tyrosine kinase receptor cMET, was approved for the treatment of non-small cell lung cancer (NSCLC). In 2022, tebentafusp, the first humanized T-cell receptor-fused scFv bispecific anti-gp-100 and CD3,

expressed in *E. coli* bacteria, was approved for treating metastatic uveal melanoma.

Nomenclature of therapeutic antibodies

The first generation of TmAbs (murine, chimeric, humanized, and fully human) gave rise to the nomenclature used until 2017. With the generation of bispecific and conjugated TmAbs, a new nomenclature was adopted. The nomenclature of TmAbs is now standardized according to the International Nonproprietary Names (INN) assigned by the WHO. The names consist of a prefix (arbitrarily created by the registrant) followed by two infixes, and a suffix. The first infix indicates the target (e.g.: “tu” for tumor, such as trastuzumab; “li” when the antibody regulates the immune system, such as inebilizumab, a TmAb that targets CD19 [2]), while the second infix includes the source used for producing the antibody (e.g.: “u” for human or “o” for mouse, or their modifications: “xi” for chimeric, as cetuximab, “zu” for humanized, as gemtuzumab). However, the WHO discontinued the second infix for antibodies created after mid 2017 to avoid inconsistencies [38]. For all the TmAb developed before 2022, the suffix used was “mab”; however, in 2021, four new suffixes were introduced to provide information about modifications to the Fc structure; these suffixes include: “tug,” for full-length unmodified Igs that recognize a single epitope (monospecific); “bart,” for full-length monospecific Igs with engineered constant regions or any point mutation introduced by engineering; “mig,” bispecific or multispecific Igs with any structure, and “ment” for monospecific Ig variable region fragments [39].

Classification of TmAbs targets in oncology

The current WLA-approved anti-cancer TmAbs were developed for different target molecules (Fig. 2). The most common target is programmed cell death protein 1 (PD-1) (15), followed by CD20 (9), PD-L1 (7), HER2 (7), and EGFR (6). The target molecules can be classified according to their main biological function, biochemical structure, site where they are expressed and release mechanisms, among others. In this review, we used the following target classification:

(1) *Molecules that modulate cells of the immune system*, such as cytotoxic T lymphocyte antigen 4 (CTLA-4), PD-1, PD-L1, and lymphocyte activation gene 3 (LAG-3 or CD223) (Fig. 2E, F). The TmAbs directed toward these molecules block the inhibition of the immune system and are used in various types of tumors; these include ipilimumab and nurulimab, anti-CTLA-4 TmAbs for the treatment of melanoma; durvalumab and atezolizumab, two anti-PD-L1 TmAbs for bladder cancer treatment; avelumab, an

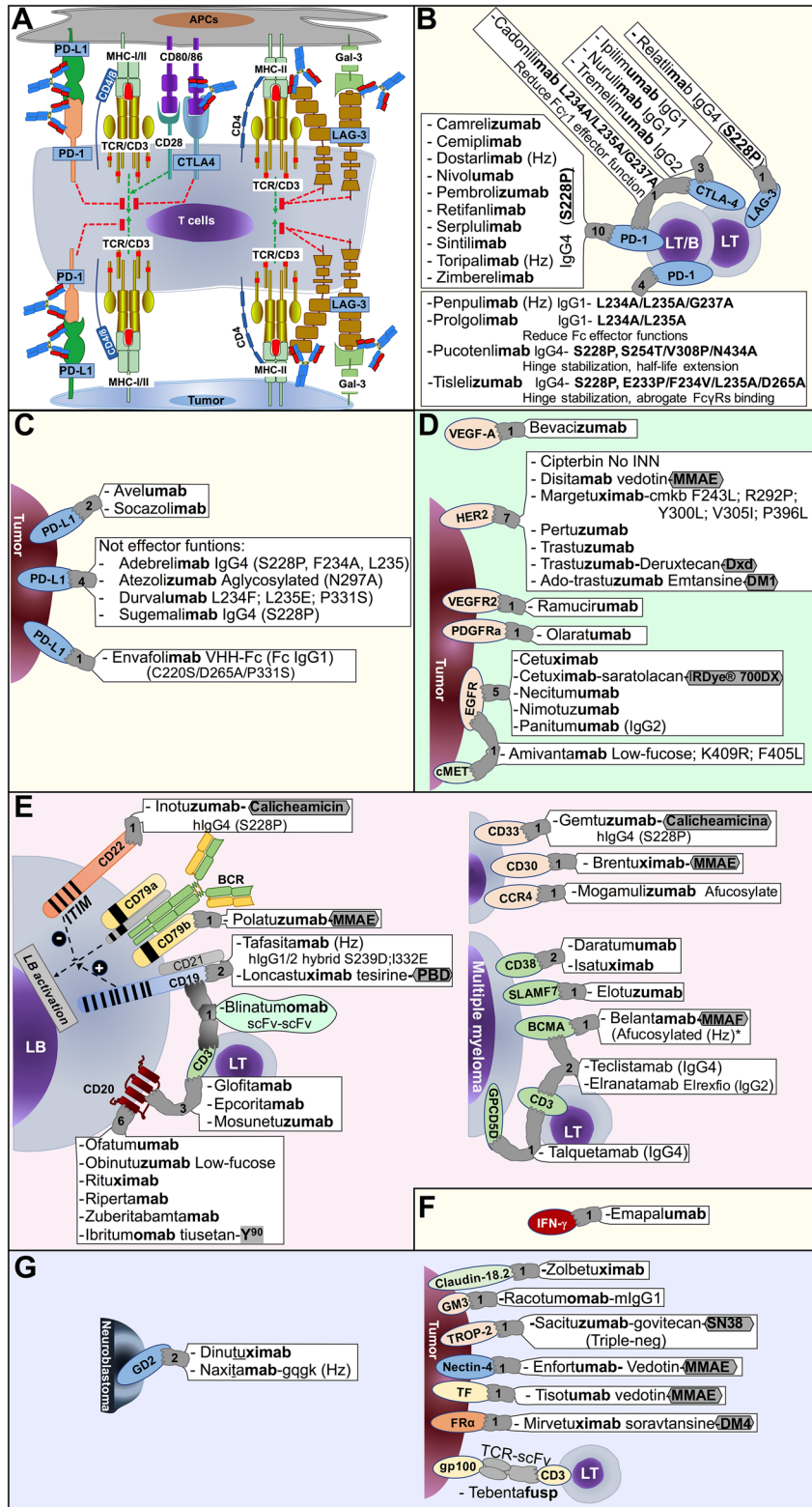


Fig. 2 WLA-approved TmAbs and their targets for the treatment of solid and hematological tumors. **A** Cell-membrane molecules on T lymphocytes (LT), antigen-presenting cell (APC) and tumor cell associated with T cell activation (TCR, CD3, CD4/8, MHC-I/II, CD80/86), T cell inhibition (immune checkpoint molecules CTLA-4, PD-1, PD-L1, Gal-3, LAG-3), and therapeutic antibodies that block immune checkpoint. **B** and **C** Specific TmAbs that target immune checkpoint molecules that regulate T cell function. **D** TmAbs that bind to tyrosine kinase receptors of the ErbB family proteins. **E** TmAbs and their corresponding targets expressed on B lymphocytes (LB), multiple myeloma cells, Hodgkin lymphoma, systemic anaplastic large cell lymphoma (CD30), acute myeloid leukemia (CD33), and mycosis fungoides or Sézary syndrome (CCR4). **F** IFN- γ , a soluble target involved in hemophagocytic lymphohistiocytosis (HLH) syndrome, can be targeted by a TmAb. **G** TmAbs that bind to cell surface molecules expressed on neuroblastoma cells and other types of solid tumors. The number in each target indicates the number of WLA-approved therapeutic antibodies for that target. The origin-dependent suffix letter is indicated in bold: Antibody is fully murine (-omab), chimeric (-ximab), humanized (-zumab), or fully human (-umab or -mab). IgG subclasses and modifications incorporated into TmAb design and their respective conjugated drugs are indicated. Afucosylated or low-fucose antibodies improve Fc γ RIIIa binding and ADCC; aglycosylated (N297A) block Fc effector functions; hinge stabilizing (S228P) is a IgG4 chain mutation to increase the binding between heavy chains (HCs), similar to IgG1. Antibody-conjugated molecules are indicated, including monomethyl auristatin E and F (MMAE and MMAF, respectively), calicheamicin, pyrrolbenzodiazepines (PBD), deruxtecan (DXd), emtansine (DM4), and govitecan (SN38). The radioactive compound yttrium-90 (Y^{90}) is a radioimmunotherapeutic (RIT) drug. Bispecific antibodies, such as epcoritamab bind, concomitantly, to tumor cell targets and CD3 on T lymphocytes (LT). CD: Cluster of differentiation; PD-1: Programmed cell death protein 1, PD-L1: Programmed cell death ligand 1, CTLA-4: Cytotoxic T lymphocyte antigen 4, MHC: Major histocompatibility complex, Gal-3: Galectin-3 protein, LAG-3: Lymphocyte activation gene 3, VEGFA: Vascular endothelial growth factor A, HER2: Human epidermal growth factor receptor 2, VEGFR2: Vascular endothelial growth factor receptor 2, PDGFR α : Platelet-derived growth factor receptor α , BCMA: B cell maturation antigen, expressed primarily on B cells, but especially on multiple myeloma plasma cells; IFN- γ : interferon-gamma; SLAMF7: CD319 surface antigen. FR α : Folate receptor alpha; Hz: Humanized; scFv: Single-chain fragment variable; TCR: T cell receptor. Created with Microsoft® PowerPoint for Mac. Version 15.32 and Prism 6 for Mac OS X

anti-PD-L1 TmAb for the treatment of Merkel cell carcinoma; pembrolizumab and nivolumab, which are anti-PD-1 TmAbs used for patients with melanoma; cemiplimab, an anti-PD-1 TmAb used in advanced cutaneous squamous cell carcinoma (SCC); nivolumab and sintilimab, anti-PD-1 TmAbs against NSCLC; toripalimab and penpulimab, anti-PD-1 TmAbs used for nasopharyngeal carcinoma; tislelizumab, an anti-PD-1 TmAb used for esophageal squamous cell carcinoma, and dostarlimab, an anti-PD-1 TmAb for endometrial cancer. Retifanlimab, also an anti-PD-1 TmAb, as well as nivolumab, pembrolizumab, and avelumab, is used against anal canal carcinoma [40], while pembrolizumab, cemiplimab, durvalumab, atezolizumab, avelumab, nivolumab, and balstilimab (an anti-PD-1 TmAb) are also

used for the treatment of cervical cancer [14, 41]. A LAG-3 blocking antibody, relatlimab, is indicated for melanoma treatment; it interferes with the binding of LAG-3 to major histocompatibility (MHC) class II molecules on antigen-presenting cells, an interaction that induces T cell exhaustion and prevents effector T cell function in the tumor microenvironment [42, 43]. Recently, it has been indicated for advanced melanoma protocols in combination with nivolumab [42].

(2) *Tyrosine kinase receptors of the ErbB family proteins*, which are overexpressed in solid cancers (Fig. 2D). ErbB receptors are pivotal for regulating cellular processes, such as proliferation, survival, differentiation, and migration; disruptions in their signaling are linked to cancer development [44, 45]. Some TmAbs bind to cell membrane receptors, thus altering their functions and potentially inducing receptor degradation [46]. This targeted approach offers improved therapeutic options for cancer patients. Combinations of tyrosine kinase inhibitors, which block receptor phosphorylation, can hinder cancer cell growth; therefore, antibodies are often employed to maximize treatment efficacy, addressing diverse aspects of cell signaling and survival [47, 48]. EGFR and HER2 (also known as the neu oncogene or ErbB2) were the first ErbB family receptors used as therapeutic targets, as they are involved in cell growth control and differentiation. TmAbs against EGFR include necitumumab, used in NSCLC, and cetuximab and panitumumab, used to treat colorectal cancer. On the other hand, trastuzumab, pertuzumab, and margetuximab, TmAbs that block HER2, are used in breast cancer, while ramucirumab, which blocks the vascular endothelial growth factor receptor 2 (VEGFR2), is used in gastric cancer [44]. Olaratumab blocks the platelet-derived growth factor receptor α (PDGFR α) and is used to treat soft tissue sarcoma [49]. The vascular endothelial growth factor (VEGF), a family of proteins involved in the regulation of angiogenesis, an essential step supporting tumor growth and metastasis [50, 51], is targeted by bevacizumab in colorectal, cervical, and ovarian cancers [41, 52, 53].

(3) *Molecules that are preferentially expressed on the lymphoid and/or myeloid cell lineage*, which are the targets for most of the currently approved TmAbs used for treating hematological cancers (Fig. 2E–G). One of these therapeutic targets is CD19, a cell membrane protein that is expressed on all B cell lineages and that plays two major roles in human B cells: On the one hand, it acts as an adaptor protein to recruit cytoplasmic signaling proteins to the membrane; on the other, it works within the CD19/CD21 complex to decrease the threshold for B cell receptor (BCR) signaling pathways [54]. Another significant target is CD20, a receptor involved in calcium transport [55–57]. It is a highly restricted cell surface antigen with abundant, stable, and highly characterized

expression on B cells [58]. Additional targets are CD22, a receptor that regulates B lymphocyte survival and signal transduction [59], and CD79b, an Ig β transmembrane signaling subunit that, together with Ig α , forms part of the multimeric BCR complex; both subunits are necessary for the expression and function of the BCR [60] (Fig. 2E).

In multiple myeloma (MM), the malignant clonal plasma cells overexpress the B cell maturation antigen (BCMA); it is expressed preferentially by mature B lymphocytes, with minimal expression on hematopoietic stem cells or non-hematopoietic cells, and is essential for the survival of long-lived bone marrow plasma cells, but not for overall B cell homeostasis [61–64]. The overexpression and activation of BCMA is associated with the progression of MM [65]; therefore, this molecule represents an interesting therapeutic target for the treatment of this disease. Another target is CD38, a cell surface glycoprotein that functions in cell adhesion, signal transduction, and calcium signaling. It is found at relatively low levels on normal lymphoid cells, including T cells, B lymphocytes, and NK cells, as well as myeloid cells, and some tissues of non-hematopoietic origin [66]. CD38 is highly and uniformly expressed on MM cells [66]. The G-protein-coupled receptor family C group 5 member D (GPRC5D) is a transmembrane receptor expressed in cells with a plasma-cell phenotype, including cells from patients with MM. However, aside from plasma cells and B cells, it is not found at appreciable levels on normal hematopoietic cells and bone marrow progenitors [67]. The signaling lymphocytic activation molecule family member 7 (SLAMF7) is a glycoprotein expressed on myeloma and NK cells but not normal tissues (Fig. 2E). The development of TmAbs targeting molecules associated with MM, whether used alone or in combination therapy, has shown promising results in the treatment of naive and relapsed/refractory MM, even in advanced-stage patients [68, 69]. One of the most revolutionary immunotherapy in MM is the use of ADCs, which will be reviewed below. Belantamab mafodotin was the first ADC approved for treating MM [70].

CD30, a member of the tumor necrosis factor receptor superfamily, is overexpressed on neoplastic cells and Reed-Sternberg cells typical of Hodgkin's lymphoma; therefore, this molecule also constitutes an interesting therapeutic target [71, 72]. CD33, a transmembrane receptor expressed on cells of the myeloid lineage, is implicated in the inhibition of cellular activity [73]; it is a target for gemtuzumab ozogamicin, used in the treatment of acute myeloid leukemia. Another target is C-Cg chemokine receptor type 4 (CCR4), as it is often expressed on leukemic cells in cutaneous T-cell lymphoma (CTCL), which encompasses a heterogeneous collection of non-Hodgkin lymphomas that arise from skin-tropic memory T lymphocytes. Among them, mycosis fungoides (MF) and Sézary syndrome (SS) are the most common malignancies [74] (Fig. 2E).

(4) *Molecules that are released into the extracellular milieu in soluble forms.* In hemophagocytic lymphohistiocytosis (HLH), an acute and rapidly progressive systemic inflammatory disorder that can be triggered by infection or malignancies, including leukemia and lymphoma, the excessive production of IFN- γ contributes to the life-threatening hyperinflammation that characterizes this condition [75, 76]. Emapalumab, approved for use in this syndrome [77], neutralizes IFN- γ by forming immune complexes, thus facilitating cytokine elimination and decreasing its serum concentration [78] (Fig. 2F).

(5) *Miscellaneous targets.* These include disialoganglioside (GD2), a target for high-risk neuroblastoma and refractory osteomedullary disease (naxitamab-gqgk in combination with granulocyte-macrophage colony-stimulating factor) [79]; GM3 ganglioside, a trophoblast cell surface antigen-2 (TROP-2) targeted for the treatment of triple-negative breast cancer (sacituzumab); Nectin-4, a target in urothelial cancer (enfortumab-vedotin linked to monomethyl auristatin E, MMAE); folate receptor α (FR α), a target in ovarian cancer (mirvetuximab); gp100 in metastatic uveal melanoma (tebentafusp scFv-TCR fusion protein) and Claudin 18.2, a tight-junction molecule, become accessible on the tumor cell surface in advanced-stage gastric cancer [80] (Fig. 2G).

Mechanisms of action of TmAbs

TmAbs can either block immunoregulatory molecules, cytokines or growth factors, tag cells to induce an immune response, or transport chemotherapeutics, toxins or radioactive elements into the tumor cells (Fig. 1B). TmAbs exert direct and indirect anti-tumor mechanisms of action (MoA) [81]. Most TmAbs can induce direct apoptosis, interfere with receptor signaling, neutralize soluble ligands, or block cell-surface receptors. TmAbs can also destroy tumor cells indirectly through the recruitment and activity of immune response mechanisms [82]. In this sense, once TmAbs bind to its target on the tumor cell, they can activate the classical pathway of the complement cascade and mediate CDC [8]; in addition, they can trigger ADCC, ADCP, ADCT, or a combination of these [10, 12].

Complement-dependent cytotoxicity (CDC)

The efficacy of TmAbs to induce CDC depends on several factors related to IgG subtype, antigen density [83, 84], antibody hexamerization [85], the specific orientation of the bound antibody molecules with each other, and the proximity of the epitope to the target cell membrane [84].

Under physiological conditions, complement molecules are continuously produced as inactive zymogens; these are cleaved into their active products by specific complement proteases, which generate effector molecules that ultimately promote cell lysis or phagocytosis [86]. To this end, the complement cascade can be activated through three distinct pathways that converge to generate the same set of effector molecules; these are the alternative, the lectin and the classical pathways. Distinct subclasses of IgG can activate the classical pathway: IgG1, IgG2, and IgG3, while IgG4 is unable to activate complement [87, 88].

Binding of IgG molecules to their specific target on the cell membrane is followed by aggregation and hexamerization of the antibodies, which permits the activation of the classical pathway of the complement cascade [8]. It is initiated by the C1 complex, a soluble multimolecular protease comprising a recognition subunit (C1q) and two modular serine proteases (C1r and C1s) [89]. The binding of C1q to the Fc region of TmAbs activates its associated proteases, which initiates the proteolytic cascade of complement [89–92]. Fc-bound C1q leads to the autoactivation of C1r for cleavage and activation of C1s; activated C1s cleaves soluble C4 into C4a and C4b; C4b fragments attach to the cell membrane and bind soluble zymogen C2, forming the C4bC2 proconvertase complex. Once associated with C4b, C2 is cleaved by C1s into C2a and C2b, generating the classical pathway C3 convertase C4bC2a, which binds and cleaves soluble C3 into C3a and C3b. The latter associates with C4bC2a, forming the C5 convertase C4bC2aC3b. C5 is further cleaved into C5a and C5b; C5b, attached to the cell membrane, binds C6, C7, C8, and multiple pore-forming C9 molecules, which polymerize and insert into the cell membrane, leading to complement-dependent cell lysis [93]. C3b alone can also deposit on the cell surface, leading to amplification of complement activation through the alternative pathway; in addition, C3b on the cell membrane can act as an opsonin, as it binds to specific receptors on the surface of phagocytes, thus promoting phagocytosis [88]. In turn, soluble fragments C3a and C5a are anaphylatoxins that induce chemotaxis of macrophages, neutrophils, and dendritic cells, among others, to the tumor microenvironment, which generates inflammation [94].

Several studies have reported classical pathway activation with therapeutic mAbs. One example is HuMab-7D8, a fully human IgG1 that binds to the extracellular loops of CD20, leading to potent complement-mediated B cell lysis [95]. Through cryo-electron microscopy analyses, Ugurlar and cols showed that the amino acid residues in IgG1 that are important for direct C1q binding are located on the two Fc-C_H2 domains, which is near the Fab-Fc hinge [96]. Rituximab, a chimeric human-mouse IgG1 TmAb used for the treatment of non-Hodgkin's B cell lymphoma [97], binds to CD20 on malignant B cells, which triggers B cell lysis

through the classical pathway [98]. Idusogie et al. demonstrated that the binding site of C1q on rituximab is centered around amino acid residues D270, K322, P329, and P331 of the Fc region [98]. Interestingly, it was further demonstrated that C1q binds a distinct site in each of the two C_H2 domains of IgG1: One binding site includes the loop formed by residues 325–331, while the other binding site consists of two loops formed by residues 266–272 and residues 294–300 of the second C_H2 domain [96, 98]. The addition of a positive charge at residues E269, E294, and Y300 abolished CDC, and mutations N297Q and S298K decreased CDC due to the absence of glycosylation; on the contrary, mutations of Y300D and G236D enhanced CDC [96, 99]. The knowledge of residues involved in the interaction between TmAbs and C1q is useful for designing therapeutic antibodies with improved CDC activity.

Antibody-dependent cellular cytotoxicity (ADCC)

A dominant component of the *in vivo* activity of antibodies against tumors involves ADCC, a mechanism that implies the binding of the Fc regions of target cell-bound TmAbs to functional activating Fc receptors for IgG (FcγR) on cytolytic cells, which triggers target cell death [100, 101]. The majority of antibodies, upon opsonizing target cells, exert their effector functions through FcγR on NK cells, mainly via FcγRIIIa (CD16a). These cells induce cytotoxicity through the exocytosis of perforin, a glycoprotein that forms pores on the membranes of target cells, and granzymes, a family of structurally related serine proteases. As perforin and granzymes are previously synthesized and stored in intracellular granules, they can rapidly trigger apoptosis of target cells [102]. The granule-exocytosis pathway potently activates cell-death mechanisms via the activation of apoptotic cysteine proteases (caspases) [103].

The IgG subclasses used in the design of TmAbs (IgG1, IgG2, and IgG4) differ from each other in their capacity to bind FcγR on immune cells [104], the strength of ADCC activity they trigger, the presence of N297-linked glycosylation on the antibody, and FcγR polymorphisms [105]. The FcγRIIIa (V158F) polymorphism has been reported to correlate with the response and efficacy of TmAbs, as its isoforms display higher affinity for the Fc region of IgG, resulting in a greater immunological response. These polymorphisms may even be useful as molecular markers to predict clinical outcomes in patients with metastatic colorectal cancer treated with cetuximab [106] or in patients bearing early ErbB2/HER2-positive breast cancer who receive treatment with trastuzumab [107].

Although ADCC has been studied mainly in NK cells, neutrophils can also participate in this mechanism in the presence of TmAbs. The antibody-related cytotoxic activity of neutrophils is associated especially with FcγRIIIa

[108, 109] and the presence of polymorphisms, such as the Fc γ RIIIa-131H variant, which has shown higher killing capacity in comparison with the Fc γ RIIIa-131R [110].

Antibody-dependent cellular phagocytosis (ADCP)

TmAbs can also promote tumor cell elimination through phagocytosis. The mechanism, named antibody-dependent cellular phagocytosis (ADCP), is mediated through the interaction of the Fc domain of target cell-bound IgG with Fc γ Rs on phagocytic cells, such as macrophages [111, 112]. TmAbs can opsonize the tumor cell to promote its phagocytosis and degradation through phagosome acidification. Currently, some researchers have implemented novel in vitro models to demonstrate the internalization of antibody-coated target cells into macrophages [113, 114], or the capture of antigen-coated fluorescent beads bound to specific antibodies to measure antibody-dependent neutrophil phagocytosis [115]; such methodologies would certainly be useful tools to improve the development of therapeutic antibodies.

Fc γ Rs that participate in ADCP preferentially interact with IgG1, which shows the best high binding affinity for activating Fc γ Rs that trigger ADCC and/or ADCP [116]. On the other hand, Fc γ Rs levels on the cell membrane can be modulated by cytokines. For instance, Fc γ RI expression can be upregulated on polymorphonuclear cells [117], macrophages [118], and monocytic cells [119] in response to IFN- γ [117], a cytokine released by activated NK cells [120], suggesting that the interplay of immune cells with cytokines may enhance the mechanisms of ADCC and/or ADCP.

For some TmAbs, the ADCP contributes significantly to their anti-tumor activity [121]. This is the case for brentuximab vedotin, a chimeric IgG1-drug conjugate used for Hodgkin's lymphoma and systemic anaplastic large cell lymphoma [122]. Not only have its effects been associated with the expected cytotoxicity triggered by mAb-conjugated drugs; its anti-tumor effect also relies on ADCP mediated by macrophages [121]. Similarly, rituximab is able to induce a potent ADCP response in in vitro and in vivo models, and depletion of macrophages results in the attenuation of the anti-tumor activity of this TmAb [123].

It has also been demonstrated that macrophage-mediated ADCP contributes to the anti-tumor activity of daratumumab, an IgG1 antibody against CD38 used for the treatment of MM [124]. ADCP may also be relevant for other hematological tumors [125]. Although some researchers have shown that target molecule expression is correlated with ADCP [126], this may not be the case for some TmAbs, including rituximab, as the expression level of CD20 on B cell-chronic lymphocytic leukemia (B-CLL) cells does not affect the efficacy of the antibody's mediated ADCP [126]. This mechanism involves the participation of Fc γ RIIIa

(CD32a) expressed on macrophages (Table 1). Another TmAb that exerts ADCP is elotuzumab, a humanized IgG1 directed toward SLAMF7 in MM [112, 127]. This antibody induces activation of macrophages and mediates ADCP, thus contributing to its antitumor response [112].

Antibody-dependent cellular trogocytosis (ADCT)

A novel MoA of TmAbs, known as antibody-dependent cellular trogocytosis (ADCT), refers to the capacity of immune cells, such as monocytes, macrophages, and neutrophils, to remove cell surface proteins from the tumor cell membrane through an endocytic process [128]. This mechanism is enhanced by the Fc region of amivantamab [12]. This therapeutic antibody binds simultaneously to cMET and EGFR, cell surface receptors that promote tumor cell proliferation and survival [129]. Such binding leads to Fc γ RIIIa-mediated monocyte and macrophage-dependent downmodulation of these proteins by trogocytosis; deprivation of tumor cell membrane cMET and EGFR leads to an improved antibody-mediated tumor cell killing, both in vitro and in vivo [11, 130]. Neutrophils can also perform ADCT in the presence of TmAbs. Indeed, it has been demonstrated that rituximab can mediate trogocytosis of CLL B cells by neutrophils in vitro, as detected by CD20 downmodulation on these tumor cells. Although this response does not trigger tumor cell death, it will be important to uncover whether it has functional consequences in vivo, as downmodulation of CD20 has been associated with resistance to anti-CD20 treatment in CLL [131–133]. On the other hand, ADCT mediated by neutrophils can precede tumor cell lysis when associated with mechanical disruption of the tumor cell membrane, a process known as trogoptosis. After in vitro incubation of neutrophils with SKBR3 human breast cancer cells, previously labeled with the cytosolic dye calcein and opsonized with trastuzumab, tumor cells displayed significant loss of membrane integrity, which coincided with target cell lysis, as detected by ⁵¹Cr release assay. Therefore, these data provide evidence that neutrophil-mediated ADCT can directly cause tumor cell death [134].

Antibody–drug conjugate (ADC)

An antibody–drug conjugate (ADC) consists of a monoclonal antibody that targets a tumor-specific antigen and is covalently bound to a cytotoxic drug or payload through a chemical linker [135]. ADCs are designed to specifically deliver drugs or payloads into the vicinity of tumor cells, thereby mitigating the side effects of their cytotoxicity [135]. Currently, 13 monospecific ADCs have been approved by WLA (discussed later) (Supplementary Fig. 2A–1I). These ADCs are primarily used to treat hematological malignancies (6 out of 13) and breast cancer (3 out of 13). Additionally,

ADC approvals extend to the treatment of ovarian, cervical, urothelial, and gastric cancer [4].

The primary advantage of ADCs in clinical use is their ability to extend the therapeutic window of cytotoxic drugs. This allows for higher local concentration at the target site while minimizing the risk of systemic toxicity compared to the cytotoxic drug without conjugate [135].

The efficacy of an ADC's payload depends on various factors, including adequate cytotoxicity, low immunogenicity, high stability, and modifiable functional groups [136]. As a result, the drugs or payloads used in the conjugation process have undergone modifications over the years, leading to three generations of innovation fueled by emerging technologies [137]. They mainly include substances that disrupt the cellular cycle, such as tubulin inhibitors and DNA-damaging agents. Next, we describe the ADCs currently used in the clinic to treat cancer.

- Payloads of the ADCs approved by the WLA

Monomethyl auristatin E (MMAE) and F (MMAF) are tubulin inhibitors that are linked to more than half of the approved ADCs, including belantamab mafodotin, an anti-BCMA antibody for MM treatment; brentuximab vedotin, an anti-CD30 antibody to treat Hodgkin lymphoma and systemic anaplastic large cell lymphoma treatment; disitamab vedotin, an anti-HER2 antibody for gastric cancer treatment; enfortumab vedotin, an anti-Nectin-4 antibody for urothelial cancer treatment; polatuzumab vedotin, an anti-CD79b antibody for diffuse large B-cell lymphoma treatment, and tisotumab vedotin, an anti-tissue factor antibody for cervical cancer treatment [4]. These substances are water-soluble synthetic analogs of dolastatin [138, 139], whose mechanisms of action involve binding to tubulin dimers and disrupting microtubule assembly, leading to cell cycle arrest and apoptosis [140].

Calicheamicins are a class of enediyne natural products that are isolated from the bacterium *Micromonospora echinospora* [141]. These natural compounds are among the most cytotoxic DNA-damaging products ever discovered, with unique molecular and cellular mechanisms of action [142]. Calicheamicin y11, a member of this family, was identified for its strong cellular activity, as it binds double-helical DNA within the minor groove, leading to site-specific double-stranded break [143–146]. Due to their robust antitumor activity, calicheamicins have been included in the clinical development of ADCs for treating hematological malignancies: gemtuzumab ozogamicin, which targets CD33 for acute myeloid leukemia (AML) treatment, and inotuzumab ozogamicin, which targets CD22 for acute lymphoblastic leukemia (ALL) treatment [147]. CD33 and CD22 are endocytic receptors ideal for delivering the cytotoxic drug into the cell [147].

Emtansine (DM1) and ravtansine (DM4) are maytansinoid drugs derived from maytansine, a compound originally isolated from the shrub *Maytenus ovatus*. They bind to the β -subunit of tubulin, inhibiting its polymerization and inducing its aggregation. Tubulin polymerization is crucial for spindle fiber formation and subsequent cell division. DM1 is a component of ado-trastuzumab emtansine (Supplementary Fig. 2F), a humanized anti-HER2 IgG1 used in breast cancer treatment. In turn, DM4 is utilized in mirvetuximab soravtansine, an antibody that targets folate receptor alpha (FRA), which is indicated for treating ovarian cancer [148]. DM1 exhibits both HER2-dependent and independent mechanisms of action. In the HER2-dependent pathway, upon internalization of the HER2-ADC complex, the payload activates caspase 3/7, inducing apoptosis; it also binds the microtubule to initiate mitotic arrest. In the HER2-independent pathway, when DM1 is conjugated to trastuzumab (T-DM1), it interacts with the cytoskeleton-associated protein 5 (CKAP5), causing cell membrane damage, increased calcium influx, and subsequent microtubule disruption [148, 149]. This cytotoxicity mechanism could also be extrapolated to DM4, but further studies are needed to unravel this possibility [150].

Camptothecin analogs, such as DXd, a derivative of exatecan, and SN-38, the active metabolite of irinotecan, are topoisomerase I inhibitors that interrupt DNA replication, resulting in cell death [151]. DXd has been reported to be more potent than SN-38 [152]. These components are conjugated to a humanized antibody targeting HER2 (fam-trastuzumab deruxtecan) (Supplementary Fig. 2D) [153] and to an antibody against the intracellular calcium signal transducer TROP-2 (sacituzumab govitecan), respectively [154]; these TmAbs are indicated for HER2-positive metastatic breast cancer and triple-negative breast cancer treatment, respectively.

Pyrrolobenzodiazepine (PBD) dimers are powerful synthetic payloads developed for targeting low antigen expression and to overcome resistance to auristatins or maytansinoids [136]. An example of the dimeric derivative is SG3199, which is incorporated into loncastuximab tesirine, an anti-CD19 IgG1 humanized antibody for treating diffuse large B-cell lymphoma (Supplementary Fig. 2B). SG3199 produces covalent cytotoxic DNA interstrand cross-links mainly in human hematological tumor cell lines, but also in solid tumors. One of the main advantages of SG3199 over other DNA cross-linking drugs is its persistence in cells due to its ability to cause minimal distortion of the DNA. This property contributes to evading excision repair mechanisms that are initiated after repair enzymes detect distortion or perturbations in the DNA. Thus, PBD dimer avoids drug resistance [155].

- Linkers of ADCs approved by WLA

The linker utilized in ADC conjugation plays a critical role in determining the efficacy and safety of these TmAbs. Ideally, the linker should exhibit stability in the bloodstream and facilitate the controlled release of the drug at the target site [156]. There are two main types of linkers used in ADC development: cleavable (chemically or enzymatically) and non-cleavable (thioether and maleimidocaproyl linkers) [157]. Most approved ADCs employ cleavable linkers, such as inotuzumab ozogamicin and brentuximab vedotin.

The chemically cleavable linkers can be pH-sensitive or reduction-sensitive types. An acid-labile hydrazone linker is stable in alkaline environments, but highly sensitive to acidic environments; thereby, it is hydrolyzed in the endosome and lysosome to release the payload. This chemical principle is applied to the conjugation of gemtuzumab ozogamicin or inotuzumab ozogamicin with calicheamicin (Supplementary Fig. 2I), which utilizes hydrozone as a linker; in turn, sacituzumab govitecan (Supplementary Fig. 2E) employs an ester linker enabling the conjugation of the antibody with SN-38 [136].

The group of enzymatically cleavable linkers used in the ADCs are peptide-based, also known as lysosomal protease-sensitive linkers. A prime example is valine-citrulline (Val-Cit), employed in conjugating brentuximab vedotin with MMAE (Supplementary Fig. 2A); this linker relies on cathepsin B, an intracellular protease, to cleave a dipeptide bond and release the payload. Loncastuximab tesirine (Supplementary Fig. 2B) also utilizes the same linker to conjugate PBD dimers. Another class of enzymatically cleavable linkers is based on phosphatase-cleavable, with enzyme targets pyrophosphatase and acid phosphatase in lysosomal compartments. Disitamab vedotin, an anti-HER2 antibody, enfortumab vedotin, an anti-Nectin-4 antibody, polatuzumab vedotin, an anti-CD79b antibody, and tisotumab vedotin, an anti-tissue factor antibody utilizes this strategy to conjugate MMAE through maleimidocaproyl-valine-citrulline-p-aminobenzyloxycarbonyl (PABC) (Supplementary Fig. 2A). This latter, a self-cleavage spacer between the Val-Cit dipeptide and the drug, improves cathepsin B access for the cleavage site [158, 159].

Other cleavable linkers include mirvetuximab soravtansine, an anti-FR α antibody conjugated to DM4 through a cleavable sulfo-SPDB linker (Supplementary Fig. 2H), and fam-trastuzumab deruxtecan, an anti-HER2 antibody conjugated to DXd via Glycine-Glycine-Phenylalanine-Glycine (GGFC) (Supplementary Fig. 2D) [136].

Non-cleavable linkers can be grouped into thioether or maleimidocaproyl (MC) categories. ADCs that use this type of linker have greater plasma stability; in addition, drug release depends on lysosomal enzymatic degradation of the antibody upon internalization into the cell target. An

example of the first group is ado-trastuzumab emtansine (Supplementary Fig. 2F), an anti-HER2 antibody in which trastuzumab-lysine is conjugated with thioether linker to DM1. On the other hand, the MC linker is used in the design of belantamab mafodotin (Supplementary Fig. 2C), an anti-BCMA antibody that conjugates with MMAF (described previously) [136].

Photoactivatable antibody–drug conjugates

Another innovative cancer treatment strategy is photoimmunotherapy, which involves an antibody conjugated with a light-sensitive compound. One example is cetuximab sarotalocan, a chimeric anti-EGFR IgG1 conjugated with the dye IRDye® 700DX. This antibody is specifically designed to treat head and neck cancer. Upon administration, the antibody is activated by laser illumination (690 nm), which damages the cell membrane, increases transmembrane water flux, and leads to tumor cell rupture and necrosis [160, 161].

Radioimmunoconjugate (RIC)

Radioimmunotherapy (RIT) is a highly effective modality in cancer therapy that uses radioisotope-conjugated mAbs to target cancer cells selectively. In 2002, the FDA approved the first radioimmunoconjugate (RIC). It was ibritumomab, a murine anti-CD20 IgG1 mAb that is bound to the chelator tiuxetan and covalently linked through a stable thiourea covalent bond to the β -emitting radionuclide ^{90}Y (Supplementary Fig. 2G). This RIC is specifically indicated for the treatment of non-Hodgkin lymphomas. Upon administration, the emitted radiation directly damages the DNA of cancer cells, ultimately destroying them [162].

Bispecific molecules

Bispecific molecules have dual binding sites that bind two different antigens; these molecules belong to either of three groups: bispecific antibodies (BsAs), scFv-TCR fusion proteins, or *bispecific T-cell engagers* (BiTEs).

- Bispecific antibodies (BsAs)

Between 2021 and May 2024, the WLA approved 8 BsAs for cancer treatment [4, 163] (Supplementary Fig. 1B), as the biological features of these Abs are suitable for this type of clinical application [164, 165]. Such characteristics include increased specificity, as they target two distinct antigens, inhibition of activation of intracellular signals associated with drug resistance, activation of the immune system, and reduction of side effects [164–169]. They also have the potential to target various types of cancer, allow treatment customization, and may reduce disease recurrence [164].

Most BsAs simultaneously engage with an antigen present on the surface of tumor cells and CD3 on T cells, making them particularly effective in hematological cancers [170, 171] (Fig. 1B (vii, viii)). The interaction between T cells and cancer cells promotes the immune response against the tumor [172]. Examples of BsAs include: elranatamab, an anti-BCMA and anti-CD3 antibody to treat patients with MM; epcoritamab, an anti-CD20 and anti-CD3 antibody for the treatment of relapsed or refractory diffuse large B-cell lymphoma; glofitamab, an anti-CD20 and anti-CD3 antibody for the treatment of diffuse large B-cell lymphoma or large B-cell lymphoma; mosunetuzumab, an anti-CD20 and anti-CD3 antibody for the treatment of follicular lymphoma; talquetamab, an anti-GPCR5D and anti-CD3 antibody for the treatment of MM; teclistamab-cqyv, an anti-BCMA and anti-CD3 antibody for the treatment of relapsed or refractory MM [4, 163].

On the other hand, only 2 approved BsAs are directed against tumor-expressed antigens (Fig. 2B,D): they are amivantamab, an anti-EGFR and anti-cMET antibody, and cadonilimab, an anti-PD-1 and anti-CTLA-4 antibody; these TmAbs are used to treat patients with NSCLC bearing EGFR exon 20 insertion mutations and with cervical cancer, respectively [4, 163].

- scFv-TCR fusion proteins

Included in the roster of WLA-approved bispecific molecules is tebentafusp-tebn (approved in 2022), an anti-tumor-associated antigen gp100 and anti-CD3 scFv-TCR fusion protein for the treatment of metastatic uveal melanoma. This molecule is also known as the first immune-mobilizing monoclonal TCR against cancer (ImmTACs) due to its MoA and engineering. The design involves the fusion of two proteins. The first protein arm contains a soluble high-affinity TCR that recognizes a complex formed by a specific peptide of the gp100 protein presented by HLA-A*02:01, which is expressed in approximately 45% of patients with uveal melanoma in the USA and Europe. As a result, the therapeutic response to tebentafusp-tebn is allele-specific. The second protein arm comprises an anti-CD3 scFv to recruit killer T cells to the tumor microenvironment, where they can become activated [173, 174].

- Bispecific T-cell engagers (BiTEs)

BiTEs are recombinant proteins that consist of two scFvs linked by a short flexible linker. They are a promising class of bispecific molecules directed to tumor antigens and CD3, thus establishing a bridge between tumor cells and T cells. In this platform, the activation of T cells is independent of MHC haplotype [175] or TCR recognition and costimulation [176]. This strategy is advantageous, as loss or

downregulation of MHC is a common mechanism in tumor cells [177]. Moreover, BiTEs contribute to T cell proliferation, amplifying the T cell population and enhancing the efficacy of the treatment [178].

Blinatumomab was the first approved BiTE. It is composed of two fragments of scFv targeting CD19 and CD3; therefore, it is indicated for treating B cell malignancies, such as philadelphia chromosome-negative relapsed or refractory B-cell precursor acute lymphoblastic leukemia (Fig. 2E). The fragments are linked by a short linker composed mainly of serine/lysine (G4S) [179]. The proximity between both cells, provided by blinatumomab, structurally forms a typical cytolytic immune synapse, inducing the activation of T cells and triggering the migration of intracellular vesicles containing granzyme B and perforins into the synapse space. Perforins create pores on the membrane of tumor cells, allowing granzyme B to enter the target cell and induce its apoptosis [177, 180].

Rational design of TmAbs

The functions of TmAbs can be optimized by rational designs of different antibody subclasses used in the clinic, such as IgG1, IgG2, and IgG4. Although IgG3 binds with high affinity to most FcγRs, it is not usually chosen for therapeutic purposes because of its long hinge region, which is accessible to proteolysis, and polymorphisms, which may generate immunogenicity [116]. It is important to take into consideration that the variability in the flexibility and length of the hinge region between the different IgG subclasses affects the spatial arrangement of the Fab arms. This feature facilitates the ability of the Fab arms to bind to different targets, while allowing autonomous interactions of the Fc region with effector components of the immune system [181]. TmAbs can also be modified, radiolabeled, conjugated to drugs or toxins, or made bispecific.

IgG subclass selection

There are no strict criteria for selecting the IgG subclass for the design of TmAbs. The target cell type and aspects related to pharmacokinetics, such as molecular half-life, affinity for neonatal receptors and clearance, should be considered. In addition, the pharmacodynamic features, such as the ability of the therapeutic antibody to activate, reduce, or eliminate Fc effector functions, will also depend on its affinity for the different FcγRs expressed on immune cells and the glycosylation status in the Fc region (Table 1). To this end, it is essential to remember that each IgG subclass has different structural, functional, and biological characteristics [116]; therefore, the appropriate design of TmAbs must consider

these characteristics to stimulate or inhibit immune cell functionality.

In general, IgG1 and IgG4 subclasses have been chosen for the design of most TmAbs (49 and 19, respectively) due to their functional characteristics and longer half-life in the serum, as they bind to FcRn [13, 182]. However, the instability of the γ_4 chains of IgG4 due to the presence of S228 at the hinge region implies the need to introduce a mutation at this amino acid for proline to stabilize the binding between the two heavy chains, as it occurs at the junction between the γ_1 - γ_1 chains in IgG1 [183]. The function and type of immune cells must be considered when selecting the appropriate IgG subclass. IgG4, in particular, does not trigger target cell lysis, making it a good candidate for blocking immune checkpoint molecules expressed on effector immune cells, such as PD-1 or LAG3, as maintaining the viability of these cells is crucial [184]. Additionally, IgG4 has been incorporated into the design of ADCs for drug delivery (Fig. 2). Structurally, both IgG1 and IgG4 have two disulfide bridges in their hinge region, serving as available sites for their conjugation with drugs. Although fewer sites than IgG2, this feature potentially enables more efficient drug conjugation to IgG1 and IgG4, as these sites are more readily reducible, rendering them favorable options for ADC design [185, 186].

On the other hand, IgG1 is more suitable for targets expressed on immunosuppressive cells, as IgG1 can trigger target cell lysis [116, 181]. For an immunostimulatory function, it is recommended to design IgG antibodies that favor more significant interaction with the Fc γ RIIB receptor and efficient recognition of specific epitopes [187].

Modifications to the IgG-Fc region

Among the five Fc gamma receptors expressed in humans, IgG1 and IgG3 display higher affinity for Fc γ Rs present on effector cells compared to IgG2 and IgG4 (Table 1). IgG4 is known to have reduced affinity for Fc γ RI, attributed to differences in specific amino acids compared with IgG1 and IgG3. On the other hand, IgG2, in its monomeric form, binds solely to Fc γ RIIa, possibly due to its short hinge lacking G236 [181]. Although the precise mechanisms dictating these affinity differences are largely unknown, advancements in the X-ray crystal structures of mAbs have significantly contributed to understanding the influence of individual residues, as well as their combination, in their effector function, allowing for the selective elimination or enhancement of specific residues as needed [188].

The IgG-Fc region can be modified to improve their therapeutic parameters, such as efficacy, pharmacokinetics, and safety, which are rationally designed as extensively reviewed by Chiu et al. [5] Wang et al. [189], and Liu et al.

[187]. In this section, we will review the major modifications that have been incorporated into WLA-approved TmAbs to modify TmAbs effector functions that influence their anti-tumor response.

To decrease effector functions of antibodies

The IgG-Fc-Fc γ RIII interaction is significantly affected by the glucan located in the conserved N-glycosylation site N297 in each of the C_H2 domains (Fig. 1A). Mutations in the C_H2 domains, which destroy this N-glycosylation motif, give rise to an aglycosylated IgG-Fc and result in complete loss of binding to most Fc γ R, except Fc γ RI [190]. An example is anti-PD-L1 antibodies used to treat bladder cancer, such as atezolizumab and durvalumab [191, 192]. The Fc functions of IgG1 were removed from these TmAbs through mutations of residues N297A in atezolizumab [193], and mutations in L234F, L235E, and P331S in durvalumab (Supplementary Fig. 3) [187].

Regarding most of the antibodies designed to block the inhibition of the immune response mediated by PD-1/PD-L1 axis, they lack the effector functions associated with the Fc region, since their mechanism of action is to block immune checkpoint molecules, thus maintaining the activation of immune cells, but not lysing them. Indeed, 19 out of the 76 TmAbs approved for oncology treatment are IgG4, which does not activate CDC or ADCC. The same occurs with ADCs, such as gemtuzumab and inotuzumab (Fig. 2E). As previously mentioned, this TmAb carries the chemotherapeutic agent calicheamicin; instead of inducing CDC or ADCC, this TmAb facilitates calicheamicin internalization and activity within the tumor cell [194]. The design of bispecifics directed to recognize an oncotarget and approximate T cells also involves the incorporation of mutations into IgG-Fc to reduce their effector functions. For instance, cadonilimab, a tetravalent PD-1/CTLA-4 bispecific antibody [195], decreased Fc-mediated ADCC and CDC effects after introducing L234A and L235A mutations, as well as the mutation G237A (Supplementary Fig. 3), which has also been reported to reduce TmAb binding to Fc γ receptors [196].

To optimize antibody effector functions and their antitumor efficacy

Several approaches have been used to increase the affinity between TmAbs and Fc γ RIIIa. These include engineering of the Fc region through amino acid mutations and glycoengineering of the N-glycan Fc to reduce core fucose, which is implicated in immune regulation. This strategy was applied to tafasitamab, an anti-CD19 TmAb used in diffuse large B-cell lymphoma. The increase in binding strength to Fc γ RIIIa, which increased ADCC capacity, was achieved through changes in residues S239D and I332E

(Supplementary Fig. 3) [197, 198]. Genetically modified Chinese Hamster Ovary (CHO) cell lines or rat myeloma YB2/0 cells have also been used to produce recombinant afucosylated or low-fucose proteins. Obinutuzumab and ublituximab, two anti-CD20 TmAbs used for the treatment of chronic lymphocytic leukemia, are examples of these novel molecular modifications [199, 200] (Fig. 2E). Amivantamab, a bispecific full human IgG1 antibody against EGFR and cMET, includes modifications at residues K409R of cMET and F405L on EGFR, and both are produced with low fucose content to increase interaction with Fc γ RIIIa, thus improving ADCC [201]. Therefore, amivantamab induces a more potent ADCC than cetuximab, a bivalent anti-EGFR mAb with normal fucose content [11]. Other modifications that enhance binding to Fc γ RIII are the F243L, R292P, Y300L, V305I, and P396L mutants into the Fc domain of margetuximab, an anti-HER2 antibody (Supplementary Fig. 3). These mutations are strategically designed to promote ADCC while simultaneously reducing affinity for the low-affinity human inhibitory Fc γ RIIb receptor (CD32B), thereby augmenting its antitumoral activity [5, 202].

Conclusions and future perspectives

The therapeutic value of manipulating the immune system in oncology through immunotherapy, by either improving or suppressing its response through TmAbs, has been revolutionary, scientifically and clinically recognized.

The biotechnological development of TmAbs and the current scenario of their functional optimization through rational engineering have made it possible to considerably improve the effectiveness of oncological therapies, even positioning them as first-line therapies in some types of cancers. The novel modifications introduced to their design, which have improved their pharmacokinetics and pharmacodynamics, are crucial for optimizing therapeutic schemes and, simultaneously, reducing the adverse effects that were more severe in the first generations of therapeutic antibodies. In this context, the era of multispecific antibodies, designed to recognize multiple epitopes located on the same or distinct antigens, has transformed cancer immunotherapy. Indeed, VHH, scFv, and Fab-based technology platforms are helpful in their design due to their flexibility in building blocks against different molecules, thus facilitating the assembly of multispecific structures. Additionally, the affinity of these antibodies is carefully balanced for efficient binding to their respective antigens, which contributes to improving their pharmacokinetics and pharmacodynamics profiles. Several clinical trials are currently enrolling participants in phase I/II studies to evaluate the safety, tolerability, pharmacokinetics, and efficacy of trispecific antibodies in various

types of cancer. Some examples of these new molecules include the GB263T antibody against EGFR and 2 distinct epitopes on cMET, which is being tested in advanced NSCLC and other solid tumors (NCT05332574), and the NM21-1480 antibody against PD-L1, 4-1BB, and human serum albumin (HSA), which is considered a new generation of immune checkpoint inhibitors that block PD-L1/PD-1 signaling and co-stimulates 4-1BB, a member of the tumor necrosis factor receptor superfamily expressed on tumor-infiltrating T cells (NCT04442126). Development of T cell-redirecting trispecific antibodies is also being tested in hematologic malignancies, including the JNJ-79635322 antibody targeting CD3 on T lymphocytes, as well as BCMA and GPRC5D on tumor cells; this TmAb is being evaluated in relapsed and/or refractory multiple myeloma (NCT05652335); the JNJ-80948543 antibody targeting CD3, CD79b, and CD20 in patients with non-Hodgkin lymphoma and CLL (NCT05424822); and the PIT565 antibody against CD3, CD2, and CD19 for relapsed and/or refractory B-cell non-Hodgkin lymphoma or B-ALL (NCT05397496).

TmAbs are currently used as transporters to target radioactive molecules, toxins, and antineoplastic drugs. They aim to improve the delivery of these substances, achieve a better antitumor response, and reduce unwanted toxicity in healthy cells and tissues. Their innovative design also considers directing therapies to more than one molecular target, aiming to enhance recognition of the tumor cell, thus favoring its elimination.

The understanding of the molecular basis of cancer development and progression currently goes hand in hand with the discovery and development of novel and more efficient therapeutic approaches to treat this disease. In this sense, we envision that the challenge for the future is to continue improving the effector functions of TmAbs to obtain the best clinical outcome.

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Declarations

Conflict of interest The authors declare no competing interests.

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