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Modified Therapeutic Antibodies: Improving Efficacy

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ABSTRACT

The prosperity of the biotherapeutics market reflects the feasibility and effectiveness of therapeutic antibodies for the treatment of cancers, inflammatory disorders, and refractory infections. As drawbacks emerge in clinical trials and practice, such as impeded binding, reduced effector functions, and frequent adverse reactions, modifications of therapeutic antibodies are unprecedently burgeoning in research and development (R&D). These modifications include: ① modified glycosylation; ② fragment of crystallizable domain (Fc) amino acid alterations; ③ cross-isotype or cross-subclass exchanges; ④ antibody–drug conjugates (ADCs); ⑤ single chain of variable region fragment (scFv) for chimeric antigen receptor T (CAR-T) cells; and ⑥ bispecific antibodies (bsAbs) in order to promote binding affinity, half-life in circulation, effectiveness toward target cells and, ultimately, to achieve overall improved efficacy. While many achievements have been made around the world in the past decades, China has been playing an active role in this realm, with its great demand for biotherapeutics with R&D potential. This review recapitulates the international progress that has been achieved with modified therapeutic antibodies, and then focuses on that of China in an independent section.

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1. Introduction

With an indispensable role in modern therapeutics for diseases such as infection, inflammatory diseases, and cancers, therapeutic antibodies contribute to the incomparably rapid research and development (R&D) of biopharmaceuticals. As of 31 December 2020, 106 therapeutic antibodies have been approved by the US Food and Drug Administration (FDA). Furthermore, in the past two decades, global sales of antibody products have increased around 450-fold—from 0.31 billion USD in 1997, to 37 billion USD in 2008, and then to approximately 135 billion USD in 2018. Despite the prevalent application of therapeutic antibodies, drawbacks have been occurring, including unsatisfactory efficacy, which is attributed to low affinity of antibodies; weak potency of inducing/induced effector functions; unexpected degradation in circulation or intracellular catabolism; severe systemic toxicity caused by inevitable binding to non-target cells expressing targeted antigens; and exposure to large dosages of therapeutics. Therefore, antibody engineering is indispensable in order to modify developing and developed therapeutic antibodies through approaches such as glycoengineering technology and mutagenesis, thereby optimizing therapeutic efficacy to delay the progression of diseases.

As the molecular mechanisms of disease onset and subtle regulatory networks are unveiled, scholars have embarked on modifications of therapeutic antibodies for precise and industrialized treatment and magnified effectiveness. Modified therapeutic antibodies refer to antibodies based on the specific binding of variable regions; they show overall improved efficacy to indications, which is reflected by reduced mortality (in protective therapy) or morbidity (in preventative therapy), impeded relapse, protection from adverse reactions, and so forth. At the molecular level, even though the engagement of antibodies and antigens is fundamental to the efficacy of therapeutic antibodies, interactions between constant regions and corresponding receptors on host cells are nonnegligible, since they directly modulate the effector functions of immune responses by recruiting and activating effector cells and through the intracellular degradation and recycling of therapeutic antibodies. Each biological process can be regarded as a target for the

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modification of therapeutic antibodies to promote efficacy. This review mainly focuses on the progress that has been achieved in six common approaches to therapeutic antibody modification. The accomplishments of China in this realm are also summarized.

2. An overview of the evolution of antibody theory and structure-based functions

In 1888, antibodies were applied to therapy for the first time by Emile Roux [1], who administered polyclonal antisera to cure diphtheria. In 1897, Ehrlich hypothesized that antibody binding with specific chemicals, metaphorized as a "magic bullet," could be utilized for targeted therapy, considering the specificity of antibodies [2]. Von Behring and Kitasato then put forward humoral immunity theory based on antibodies in the early 19th century [3]. The structure of the antibody was discovered by Porter in 1959 [4]; Tonegawa et al. [5] then illustrated the genetic basis of antibody variability in 1974. Meanwhile, the revolutionary technology of hybridoma cells was invented by Köhler and Milstein, providing access to the design and manufacturing of therapeutic antibodies [6]. These milestone achievements laid the foundation of antibody engineering for therapy. Since the first therapeutic antibody, orthoclone OKT3, was approved by the FDA in 1986, an increasing number of antibody drugs have entered the biopharmaceutical market and have significantly motivated the biopharma industry. R&D toward modified therapeutic antibodies has been emerging in recent years, evoked by the limitations of current treatment and the assistance of biomedical techniques such as next-generation DNA sequencing (NGS), proteomics, gene editing, and computer-aided screening. In the foreseeable future, modified therapeutic antibodies will play a greater role in therapies and occupy a larger portion of the biopharma industry (Table 1).

As important effectors of immune response, antibodies can recognize, neutralize, and clear pathogenic antigens. These functions are regulated by structure-determined properties such as binding affinity and pharmacokinetics. In structural and functional research, an antibody can be hydrolyzed by papain under certain conditions to yield two fragments of antigen binding (Fab) and one fragment of crystallizable domain (Fc). More specifically, a Fab consists of one variable region of heavy chain (VH), one variable region of light chain (VL), one constant region of light chain (CL), and one heavy chain constant region 1 (CH1). In VH and VL, respectively, there are three different variable amino acid sequences that contribute to the spatial conformation complementary to the epitopes of antigens. These sequences are called the complementary determining regions (CDRs), and the rest is called the framework region (FR). The Fab domain specifically discriminates and binds antigens or targeted cells in a monovalent pattern. As Fab modification, the site-directed mutagenesis of histidine (with $-\lg K_a$ around 6, K_a is the dissociation constant of the acid) residues into CDRs or FR has yielded pH-dependent binding antibodies that dissociate the bound antigen in acidic endosomes and can be recycled back to plasma to bind antigens more than once [7–9]. This modification strategy has been applied in satralizumab against interleukin-6 receptor (IL-6R) for neuromyelitis optica spectrum disorder (NMOSD), and demonstrated an association with a lower risk of relapse [10]. The Fc portion, which consists of CH2 and CH3 domains without the activity of binding antigens, interacts with the receptors (FcRs) on target cells and subsequently initiates multiple immunological effects, such as antibody-dependent cellular cytotoxicity (ADCC). Fc modification, such as engineered afucosylation, enhances ADCC and consequently improves the therapeutic potency of antibodies such as the marketed mogamulizumab against C–C chemokine receptor

Table 1
List of modified therapeutic antibodies and recent-five-year approved therapeutic antibodies on market by FDA.

Name	Trade name	Company	Target	Indications	Modification	Time
Brentuximab vedotin	Adcetri	Seattle Genetics	CD30	Lymphoma	ADC	2011
Mogamulizumab	Poteligeo	Kyowa Hakko Kirin	CCR4	T cell leukemia	Afucosylation	2012
Obinutuzumab	Gazyva	Roche	CD20	CLL	Afucosylation	2013
Ado-trastuzumab emtansine	Kadcyla	Genentech	HER2	HER2-positive metastatic breast cancer	ADC, Fc amino acid alterations	2013
Vedolizumab	Entyvio	Takeda Pharmaceuticals	α4β7 integrin	Ulcerative colitis, Crohn disease	Fc amino acid alterations	2014
Ramucirumab	Cyramza	Eli Lilly and Co.	VEGFR2	Gastric cancer	Fc amino acid alterations	2014
Pembrolizumab	Keytruda	Merck Sharp & Dohme Corp	PD1	Melanoma	Fc amino acid alterations	2014
Nivolumab	Opdivo	Bristol-Myers Squibb	PD1	Melanoma, NSCLC	Fc amino acid alterations	2014
Blinatumomab	Blincyto	Amgen	CD19 + CD3	ALL	bsAb	2014
Secukinumab	Cosentyx	Novartis	IL-17	Plaque psoriasis, RA	—	2015
Dinutuximab	Unituxin	United Therapeutics	GD2	Neuroblastoma	—	2015
Daratumumab	Darzalex	Johnson & Johnson	CD38	MM	—	2015
Alirocumab	Praluent	Sanofi	PCSK9	High cholesterol	—	2015
Evolocumab	Repatha	Amgen	PCSK9	High cholesterol	—	2015
Necitumumab	Portrazza	Lilly	EGFR	NSCLC	Fc amino acid alterations	2015
Elotuzumab	Empliciti	BMS Abbvie	SLAMF7	MM	—	2015
Mepolizumab	Nucala	GSK	IL-5	Asthma	—	2015
Idarucizumab	Praxbind	BI	Dabigatran	Reverse anticoagulant Pradaxa's blood thinning effects	—	2015
Obiltoxaximab	Anthim	Elusys Therapeutics	*Bacillus anthracis* anthrax	Inhalational anthrax	—	2016
Ixekizumab	Taltz	Eli Lilly and Co.	IL-17A	Plaque psoriasis	Fc amino acid alterations	2016
Reslizumab	Cinqair	Teva Respiratory LLC	IL-5	Asthma	—	2016

(*continued on next page*)

Table 1 (*continued*)

Name	Trade name	Company	Target	Indications	Modification	Time
Atezolizumab	Tecentriq	Genentech, Inc.	PD-L1	Urothelial carcinoma, metastatic NSCLC	Fc amino acid alterations	2016
Daclizumab	Zinbryta	Biogen	CD25	Multiple sclerosis	—	2016
Ustekinumab	Stelara	Janss en Biotech, Inc	IL-12/IL-23	Psoriatic arthritis	—	2016
Olaratumab	Lartruvo	Eli Lilly And Co.	PDGFRα	Sarcoma	—	2016
Bezlotoxumab	Zinplava	Merck Sharp & Dohme Corp.	Toxin	Clostridium difficile infection	—	2016
Avelumab	Bavencio	Merck KGaA & Pfizer	PD-L1	MCC	—	2017
Ocrelizumab	Ocrevus	Roche	CD20	MS	Fc amino acid alterations	2017
Dupilumab	Dupixent	Regeneron & Sanofi Genzyme	IL-4Rα	Eczema	Fc amino acid alterations	2017
Sarilumab	Kevzara	Sanofi & Regeneron	IL-6R	RA	—	2017
Durvalumab	Imfinzi	AstraZeneca	PD-L1	Urothelial carcinoma	Fc amino acid alterations	2017
Brodalumab	Siliq	Valeant	IL-17	Plaque psoriasis	—	2017
Gusekumab	Tremfya	J&J	IL-23 p19	Plaque psoriasis	—	2017
Inotuzumab ozogamicin	Besponsa	Pfizer	CD22	r/r B-ALL	ADC, Fc amino acid alterations	2017
Benralizumab	Fasenra	AstraZeneca	IL-5R	Asthma	Afucosylation	2017
Gemtuzumab ozogamicin	Mylotarg	Wyeth-Ayerst	CD33	ALL	ADC	2017
Emicizumab	Hemlibra	Chugai (Roche)	Factor IXa, X	Hemophilia A	bsAb, Fc amino acid alterations	2017
Tildrakizumab	Ilumya	Sun Pharm	IL-23 p19	Plaque psoriasis	—	2018
Ibalizumab	Trogarzo	TaiMed	CD4	HIV	—	2018
Burosumab	Crysvita	Ultragenyx	FGF23	XLH	—	2018
Erenumab	Aimovig	Amgen	CGRP	Migraine	—	2018
Mogamulizumab	Poteligeo	Kyowa Hakko Kirin	CCR4	r/r Mycosis fungoidesor Sézary syndrome	Afucosylation	2018
Lanadelumab	Takhzyro	Shire	Kallikrein	Angioedema	—	2018
Moxetumomab	Lumoxit	AstraZeneca	CD22-immunotoxin	HCL	—	2018
Fremanezumab	Ajovy	Teva	CGRP	Migraine	Fc amino acid alterations	2018
Galcanezumab	Emgality	Eli Lilly	CGRP	Migraine	Fc amino acid alterations	2018
Cemiplimab	Libtayo	Regeneron	PD-1	CSCC	Fc amino acid alterations	2018
Ravulizumab	Ultomiris	Alexion	C5	PNH	Fc amino acid alterations	2018
Caplacizumab	Cablivi	Genzyme Corporation	vWF	aTTP	Nanobody	2019
Romosozumab	Evenity	Amgen	Sclerostin	Osteoporosis	—	2019
Risankizumab	Skyrizi	AbbVie	IL-23	Plaque psoriasis	Fc amino acid alterations	2019
Polatuzumab vedotin	Polivy	Genentech	CD79b	r/r DLBL	ADC, Fc amino acid alterations	2019
Brolucizumab	BEOVU	Novartis	VEGF-A	Macular degeneration	—	2019
Crizanlizumab	Adakveo	Novartis	P-selectin	Sickle-cell disease	Fc amino acid alterations	2019
Enfortumab vedotin	Padcev	Seattle Genetics and Astellas	Nectin-4	Urothelial cancer	ADC	2019
[Fam]-trastuzumab deruxtecan	Enhertu	Astra Zeneca and Daiichi Sankyo	HER2	HER2-positive breast cancer	ADC, Fc amino acid alterations	2019

CD: cell differentiation molecular; Fc: fragment of crystallizable domain; ADC: antibody–drug conjugate; CCR: CC chemokine receptor; ALL: acute lymphatic leukemia; HER: human epidermal growth factor; VEGFR: vascular endothelial growth factor receptor; PD-1: programmed cell death receptor-1; IL: interleukin; GD: disialoganglioside; PCSK: proprotein convertase subtilisin/kexin; EGFR: epidermal growth factor receptor; SLAMF: signaling lymphocytic activation molecule family; PD-L1: programmed cell death-ligand 1; PDGFRα: platelet-derived growth factor receptor alpha; HIV: human immunodeficiency virus; FGF: fibroblast growth factor; CGRP: calcitonin gene-related peptide; VEGF: vascular endothelial growth factor; aTTP: acquired thrombotic thrombocytopenic purpura; CLL: chronic myeloid leukemia; CSCC: cutaneous squamous cell carcinoma; HCL: hairy cell leukemia; MCC: Merkel cell carcinoma; MM: multiple myeloma; MS: multiple sclerosis; NSCLC: non-small cell lung carcinoma; PNH: proxysmal nocturnal hemoglobinuria; RA: rheumatoid arthritis; r/r B-ALL: relapsed or refractory B cell precursor acute lymphoblastic leukemia; r/r DLBL: relapsed or refractory diffuse large B cell lymphoma; XLH: X-linked hypophosphatemia; vWF: von Willebrand factor.

type 4 (CCR4) for T cell leukemia. In addition, the antibody receptor is crucial for binding and for the downstream biological process. The neonatal Fc receptor (FcRn) on the target cells regulates immunoglobulin G (IgG) catabolism which relies on pH [11,12]. At low pH (pH < 6.5), binding is regulated by the protonation of histidine 310 (His310), His435, and His436 in the Fc region [13]. Protonation results in residues with a positive charge that can bind to glutamic acid 117 (Glu117), Glu132, and aspartic acid 137 (Asp137) with a negative charge in the FcRn [14]. In the physiological extracellular environment, the weak affinity of IgG to FcRn leads to its release from the receptor into circulation [15]. These structural characteristics can not only be naturally regulated, but also be artificially modified, in order to ensure the clinical effectiveness of therapeutic antibodies as biopharmaceuticals.

3. Progress in modified therapeutic antibodies around the world

As the diversity of innovative antibody drugs is continually increasing around the world, modifications of therapeutic antibodies have become a hot topic in biomedical research. In terms of

function, antibodies recruit immune components from patients to carry out effector functions, the major mechanisms of which include ADCC, complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP) [16]. The involvement of Fc gamma receptors (FcγRs) is necessary for ADCC and ADCP, as the complement protein 1q (C1q) of the complement system bound to the antibody CH2 domain activates CDC. Moreover, IgG is endocytosed by cells, where it can be shuttled from endosomes to lysosomes for degradation with low FcRn binding affinity [15] or recycled back to the extracellular membrane [17,18]. Determined by the antibody–FcRn binding, the circulation and half-life can be improved by modification to ameliorate the disadvantages in pharmacodynamics. Based on researchers' efforts to modify therapeutic antibodies, modifications can be categorized as follows: ① modified glycosylation; ② Fc amino acid alterations; ③ cross-isotype or cross-subclass exchanges; ④ antibody–drug conjugates (ADCs); ⑤ single chain of variable region fragment (scFv) for chimeric antigen receptor T (CAR-T) cells; and ⑥ bispecific antibodies (bsAbs). Progress that has been achieved in modified therapeutic antibodies is summarized below, according to this categorization (Fig. 1).

3.1. Modified glycosylation

ADCC is triggered by the interactions of Fc with the corresponding FcγRIIIa (CD16a) expressed on natural killer (NK) cells, which is

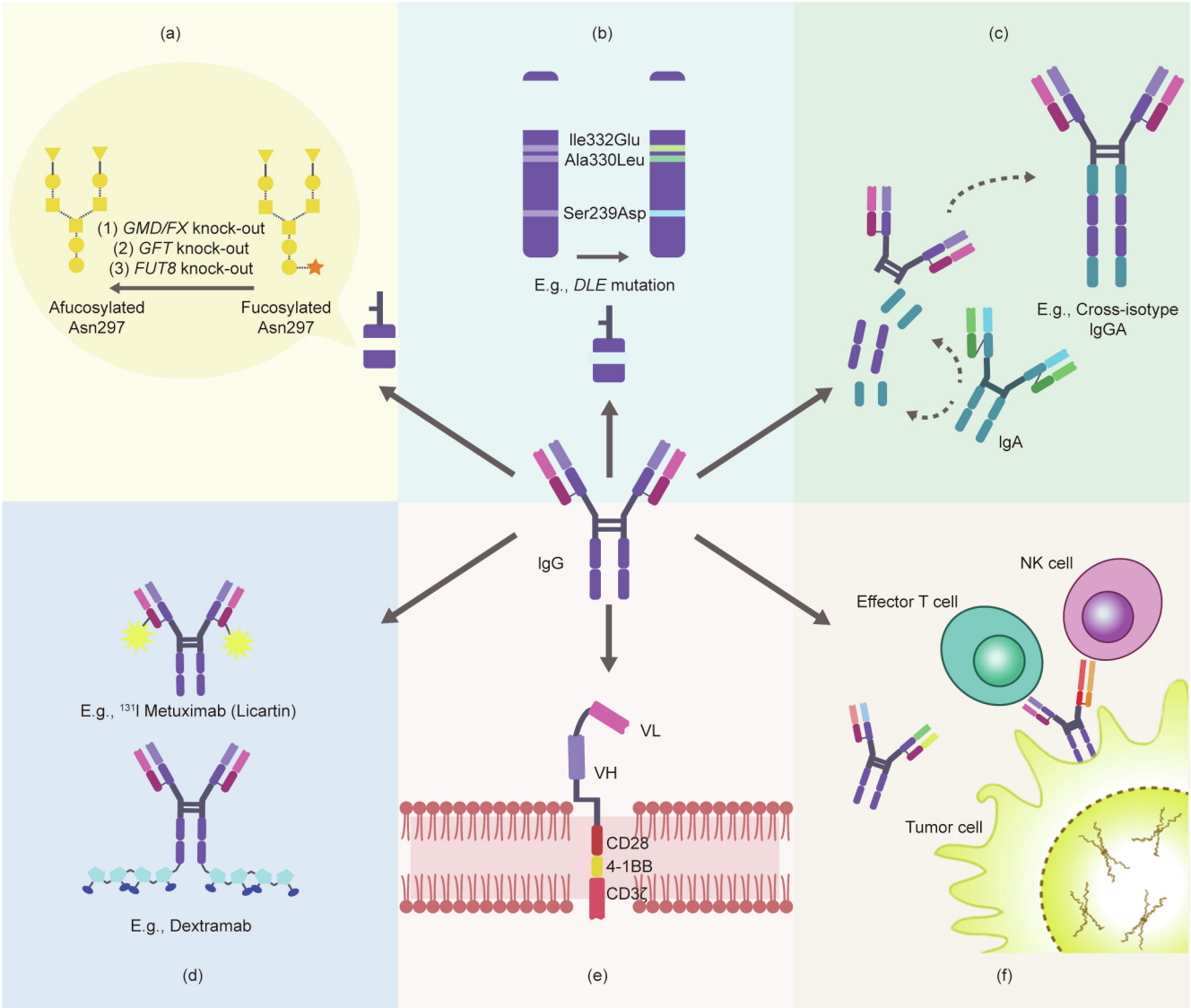

Fig 1. Common modification approaches to modify therapeutic antibodies. (a) Modified glycosylation targets the fucosylation site asparagine 297 (Asn297) in the CH2 domain of the Fc region. The key enzymes and transporters are major regulators of fucosylation, including 4,6-dehydratase (GMD), guanosine diphosphate (GDP)-keto-6-deoxymannose 3,5-epimerase/4-reductase (FX), α-1,6-fucosyltransferase (FUT8), and GDP-fucose transporter (GFT). Using a loss-of-function strategy toward them is a routine afucosylation modification. The dashed line represents variable glycosylation, while the solid line represents core glycosylation in the Asn297 glycans of the Fc region. (b) Fc amino acid alterations can change the affinity and effector functions of target cells and the half-life of antibodies. The serine 239 aspartic acid (Ser239Asp)/isoleucine 332 glutamic acid (Ile332Glu)/alanine 330 leucine (Ala330Leu) termed *DLE* mutation is shown as an example. (c) A cross-isotype antibody can integrate different effector functions elucidated by different isotypes of immunoglobulins. In the fusion regions in the Fc domain of the illustrated example, the cross-isotype IgGA consists of CH1a–CH2g–CH3g–CH3a. (d) An ADC is a specific antibody-guidance-based cytotoxic drug. Here, the ten-angled star represents radioisotope ^{131}I, the pentagon in light blue represents the dextran polysaccharide scaffold, and the oval in dark blue represents the cytotoxic agent. (e) The CAR illustrated here is a third-generation CAR with both costimulatory domains CD28 and anti-human-tumor necrosis factor receptor superfamily member 9 (4-1BB) in the intracellular membrane. The VH and VL depend on specific antibodies targeting the corresponding antigens. (f) bsAbs vary considerably in terms of component and format, and have integrated functions. NK: natural killer.

sensitive to the state of Fc glycosylation [19,20]. The Fc region of IgG includes an N-linked glycosylation site at position asparagine 297 (Asn297), where the oligosaccharide chain is usually composed of two *N*-acetylglucosamine (GlcNAc), three mannose in a "V" pattern, and two GlcNAc linked to the mannose to form a biantennary complex glycan [21]. Additional fucose, GlcNAc, sialic acid, and galactose can be connected to the core glycan structure. With direct effects on FcγR binding, the Asn297 glycan (which is actually fucose at this site) can clash with glycans on CD16a and influence hinge region conformations, which attenuates the engagement of the effector cells in ADCC [22–24]. Although the majority of mammalian IgG is fucosylated at Asn297 [25–27], strategies have been put forward based on a comprehensive understanding of this site-specific fucosylation in the Fc region. The fucosylation process contains three steps: ① Guanosine 5′-diphospho-β-*L*-fucose (GDP-fucose) synthesized in the cytoplasm is the substrate of the first step, and is derived from GDP-mannose. The conversion to GDP-fucose is catalyzed by 4,6-dehydratase (GMD) and GDP-keto-6-deoxymannose 3,5-epimerase/4-reductase (known as FX) as the major anabolic source, known as the *de novo* pathway [28]. ② As the substrate of fucosylation reactions, the synthesized GDP-fucose must be transported to the endoplasmic reticulum (ER) or Golgi apparatus. The GDP-fucose transporter (GFT) encoded by the *Slc35c1* gene is responsible for transportation to the Golgi apparatus [29]. ③ FUT8 is the sole α-1,6-fucosyltransferase to transfer fucose via an α-1,6 linkage to the innermost GlcNAc on *N*-glycans for core fucosylation [30]. Furthermore, the generation of bisecting GlcNAc catalyzed by β-1,4-mannosyl-glycoprotein 4-β-*N*-acetylglucosaminyltransferase (GnT-III) is effective to induce afucosylation because a GlcNAc is attached through a 1,4-β-linkage to the β-linked mannose of the *N*-glycan trimannosylated core [31]. Thus, targeting the abovementioned enzymes to regulate the fucosylation process is theoretically feasible for afucosylation to enhance the ADCC effect.

To modulate the enzymes related to fucosylation, modification of the production cell lines serves as a common approach by means of gene editing such as knock down/out or zinc-finger nucleases (ZFNs). Louie et al. [32] reported an *FX*-knockout Chinese hamster ovary (CHO) cell line that could be used to produce antibodies with completely afucosylated *N*-glycans. Inactivation of the *Slc35c1* and *FUT8* gene by ZFNs in an industrial protein-production host cell line CHO yielded afucosylated antibodies without detrimental effects on cell growth, viability, or product quality [31]. Two of the three marketed glycoengineering antibodies—namely, mogamulizumab (Poteligeo) and benralizumab (Fasenra, MEDI-563)—are produced in *FUT8*-knockout CHO cells. Furthermore, the combination of GnT-III and Golgi resident enzyme α-mannosidase II (αManII) overexpression was found to bring about the highest level of bisecting and afucosylated glycans on IgG antibodies, and has been translated into the commercialized anti-CD20 antibody obinutuzumab (GA101). Obinutuzumab has enhanced FcγR affinity and is the first line of treatment for chronic lymphocytic leukemia (CLL) in combination with chlorambucil [33–35]. Thus, using glycoengineering to modulate key enzymes of fucosylation serves as a mature technology to modify antibody therapeutics with acknowledged effectiveness.

In addition to Asn297 afucosylation, a potential application of antibody glycosylation has been discovered in regard to digalactosylated modification as a principal modulator in the selective transfer of antibodies across the placenta. Martinez et al. [36] revealed that the efficiency of antibody transfer across the placenta in human immunodeficiency virus (HIV)-infected women was affected by maternal IgG characteristics, such as binding to placentally expressed FcγRIIa and FcγRIIIa, as well as Fc region glycan profiles. Furthermore, the bisected and disialylated modification of glycans in the Fc region was associated with transfer efficiency, and a lower fucose level was demonstrated to be a favorable factor in this process. In addition, Jennewein et al. [37] focused on neonatal vaccination and investigated the Fc profiles of neonatal and maternal antibodies. They found that digalactosylated Fc-glycans, which are characterized by selective binding to FcRn and FcγRIIIa, resulted in preferential antibody transfer to efficiently leverage innate immune cells—mainly NK cells on the very first day of life—and thus contributed to enhanced NK cell degranulation and cytokine secretion [38,39]. These findings provide insights for next-generation maternal vaccines to elicit antibodies for neonate aid.

3.2. Fc amino acid alterations

Since sequence variations in the Fc region affect specificities and affinities to FcRs as well as complement protein C1q, modification of this constant region sequence by means of amino acid mutations makes it possible to alter the effector functions. The modification strategy depends on the effects of binding to FcγR, in that FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa, and FcγRIIIb are activating receptors [40], whereas FcγRIIb is the only one to exert inhibitory effects [21]. Much has been done to regulate FcγRIII binding, given its ADCC-initiating role. With the mutations of serine 239 aspartic acid (Ser239Asp)/isoleucine 332 glutamic acid (Ile332Glu)/alanine 330 leucine (Ala330Leu) (referred to as DLE), Herceptin was found to recruit more NK cells, which then killed target cells upon first contact regardless of cancer antigen expression levels [41,42]. The anti-integrin antibody MEDI-522 and Rituximab showed similar improved efficacy with the *DLE* mutations [43]. Furthermore, asymmetric Fc mutation has inspired researchers to combine distinct mutation formats in one antibody Fc region. The combination of leucine 234 tyrosine (Leu234Tyr), glycine 236 tryptophan (Gly236Trp), and Ser298Ala (referred to as *YWA* mutations) in one Fc heavy chain was introduced to another heavy chain with *DLE* mutations, which resulted in more potent ADCC *in vitro* [44]. As for ADCP by macrophages via FcγRIIa binding, Ser239Asp/Ile332Glu/Gly236Ala was found to promote FcγRIIa-dependent ADCP and FcγRIII-dependent ADCC activity *in vitro* [45]. Nevertheless, this mutation inevitably led to a 13-fold greater binding affinity to FcγRIIb in view of the 90% sequence similarity between FcγRIIa and inhibitory FcγRIIb [46]. In contrast, Margetuximab targeting human epidermal growth factor 2 (HER2) with the optimized mutations phenylalanine 243 leucine (Phe243Leu)/arginine 292 proline (Arg292Pro)/Tyr300Leu/valine 305 isoleucine (Val305Ile)/Pro396Leu to obtain a reasonable ratio showed better ADCC activity compared with that of Herceptin [47,48].

When it comes to CDC-related modification, the influence on other effector functions such as ADCC and ADCP should not be neglected, since the triple mutation Ser267Glu, His268Phe, and serine 324 threonine (Ser324Thr) has been found to largely improve CDC at the expense of reduced ADCC and ADCP via increasing the affinity to inhibitory FcγRIIb [41]. Therefore, the ratio of the bound activating and inhibitory FcγRs should be taken into account in Fc region sequence alteration.

As for half-life prolongation, the quadruple mutation Ser298Ala, Glu333Ala, lysine 334 alanine (Lys334Ala), and Asn434Ala was found to enhance binding with both FcRn and FcγR [49]. The Glu294 deletion resulted in higher sialylation of the Asn297 glycan on the Fc and showed an increased antibody half-life *in vivo* [50]. It can be concluded that serum half-life regulation is not limited to FcRn binding, but includes sialylation as well.

3.3. Cross-isotype or cross-subclass exchanges

Considering the binding of different Fc isotypes to their corresponding FcRs in order to induce respective immunological

responses, this category of modified antibodies aims to enhance ADCC and/or CDC by recombining and/or replacing sections of natural Fc domains with those from different isotypes/subclasses to form cross-isotype antibodies. Researchers have attempted to engage multiple Fc sections. As neutrophils engage the Fc of immunoglobulin A (IgA) antibodies via the Fc alpha receptor I (FcαRI) [51,52], Chintalacharuvu et al. [53] appended single domains of IgA2 to the end of the γ1 constant region and substituted the CH1 domain of γ1 with that of α1 to create a four-domain constant region (CH1a–CH2g–CH3g–CH3a) termed IgGA. This cross-isotype antibody was shown to be less affected by pH in comparison with IgG1, and was able to mediate the complement-dependent lysis of sheep red cells. Borrok et al. [54] reported the tandem cross-isotype IgG/IgA, which was created by fusing the hinge, CH2, and CH3 of IgA2 to the C-terminus of IgG1. It was able to bind FcαRI, FcγRI, FcγRII, FcγRIIIa, and FcRn with the approximate affinities of wild-type IgA and IgG, respectively, *in vitro*, such that it could mediate ADCC through both neutrophils and NK cells, whereas C1q binding was compromised three-fold in comparison with IgG1.

In view of its high affinity to C1q *in vitro*, IgG3 was introduced to create IgG1/G3 cross-subclass antibodies with enhanced IgG3 effector functions [55]. The more effective chimeric format is the fusion of CH1 and the hinge region of IgG1 to the IgG3 Fc region named 1133. The CDC activity of 1133 is better than that of either IgG1 or IgG3, even with low antigen levels [55,56]. In addition, subtle modification could be utilized to accomplish gain-of-function in a subclass without the expected properties. IgG2 and IgG4 are incapable of inducing CDC as IgG1 and IgG3 do [57]. In accordance with this strategy, CDC activity can be endowed by replacing IgG2 CH2 domain with that of IgG3. Furthermore, changing the IgG4 residue at position 331, where C1q binds IgG, in order to elicit CDC activity to match IgG1, gave rise to a moderate level of CDC [56]. Hence, residues associated with the specific functions of antibodies can be introduced to obtain gain-of-function in corresponding Fc domains in order to confer effector functions.

3.4. Antibody–drug conjugates

Having evolved from Paul Ehrlich's "magic bullet" concept, monoclonal antibodies (mAbs) can be conjugated with various effector molecules, such as cytotoxic agents, radiopharmaceuticals, and immunotoxins, to generate a new pattern of target therapy: ADCs [58]. In general, an ADC consists of three components: the first component is a mAb, which is armed with the second component, effector molecules as the ultratoxic payload (or warhead) to induce the death of target cells, which occurs through the third component, a stable and conditionally biodegradable linker [59]. The availability of ADCs in clinical practice relies on every component having appropriate characteristics, which include strengthened stability of the linker, outstanding potency, sufficient payload release, and—more importantly—humanized or human antibodies as a framework to attenuate immunogenicity and improve selectivity. Progress has been made in this area, with a total of ten ADCs having been approved for clinical application. Moreover, glembatumumab vedotin (CDX-011), which was developed by Celldex/Seattle Genetics to target glycoprotein nonmetastatic melanoma protein B (gpNMB) against advanced melanoma, has demonstrated modest activity and an acceptable safety profile in a phase II study [60], and holds promise to be approved by the FDA as an ADC for clinical practice.

The components of ADCs can be regarded as modification targets for modifying ADCs to promote their therapeutic effects. As the pivotal determinant of ADC efficacy, the cytotoxic payload mainly targets either DNA (e.g., calicheamicins and duocarmycins) or tubulin (e.g., maytansines and auristatins). Szot et al. [61] reported a potent monomethyl auristatin E (MMAE)-linked ADC that elicited anticancer activity through a prodrug activated by stroma cells in a tumor microenvironment (TME), which was termed drug activation and release through stroma (DAaRTS). Tumor-associated stromal cells released active free drug MMAE, killing nearby proliferating tumor cells in a target-independent manner. This design has overcome the problem of conventional ADCs being limited to the treatment of antigen-positive patients.

Loading a large amount of toxins often unexpectedly results in impaired hydrophilicity, which compromises the ADC's biophysical properties, including decreased solubility, aggregation reactions, and a low drug-to-antibody ratio (DAR) of no more than 3–4 [62–64]. Schneider et al. [59] developed a novel class of hybrid ADC named dextramabs. The therapeutic antibody trastuzumab was equipped with a multivalent dextran polysaccharide, which achieved an elevated DAR, remarkable hydrophilicity, efficient toxic agent load, and high toxicity *in vitro*, as proved by $HER2^+$ breast cancer cell lines.

With regard to linker modification, as the linker on an ADC can also be associated with detectable labels such as fluorophores [65], methods to label antibodies usually rely on the modification of interchain disulfides by means of genetically encoded amino acids such as free cysteine residues [66]. These conventional methods have the following drawbacks: ① Modification of the cysteine residues after disulfide reduction is difficult and may bring out heterogeneous mixtures [67]; and ② genetically inserted noncanonical amino acids in antibodies could risk altering the native sequence of the antibody and sacrificing binding affinity to the target antigen [68]. Matos et al. [69] developed a region-selective lysine methodology through a core organic chemical reaction in which methyl 2-(sulfonyl methyl) acrylate (abbreviated as 1c) directly modified a single lysine residue on the native protein sequences of the antibody without genetic engineering. The modification of trastuzumab not only achieved site-specific fluorescent labeling, resulting in trastuzumab-1c-fluorescein isothiocyanate (FITC), but also retained the specificity of trastuzumab toward its target antigen (HER2/c-erb-2) at a concentration range identical to that of its unmodified counterpart [70]. This method was further validated for the construction of a stable and functional ADC through the conjugation of the kinase inhibitor crizotinib, which was approved for the treatment of anaplastic lymphoma kinase (ALK)-rearranged non-small-cell lung carcinoma (NSCLC), to the acrylate present in trastuzumab-1c [71]. The ADC trastuzumab-1c-crizotinib maintained antigen binding properties, and the secondary structural content retained its specificity toward SKBR3 cells expressing high levels of HER2 antigen.

When modifying antibody drugs by introducing additional elements, including special linkers and effective drugs, the properties of ADCs such as hydrophilicity, half-life, and receptor affinities should be comprehensively considered.

3.5. scFv for chimeric antigen receptor T cells

A CAR is a protein that consists of the scFv from the specific antibody fused with the intracellular signaling transducers, the ζ subunit of CD3 (CD3ζ) and costimulatory domains such as CD28 or anti-human-tumor necrosis factor receptor superfamily member 9 (4-1BB) [72]. Autologous T cells from patients are genetically engineered to express CARs and are termed as CAR-T cells; they are subsequently infused back into the patients to recognize the specific antigens and kill the antigen-expressing cells, thereby serving as a cellular therapy. CARs can be regarded as a peculiar format of modified antibodies expressed by T cells. It can be inferred that CARs derived from certain antibodies determine the specificity of targets and therapeutic effectiveness. In 2017, Kymriah (tisagenlecleucel-T, CTL019), developed by Novartis

Pharmaceuticals Corporation (Switzerland), and Yescarta (axicabtagene ciloleucel), developed by Kite Pharma Inc. (USA), were approved for relapsed or refractory large B cell lymphoma after two or more lines of systemic therapy such as diffuse large B cell lymphoma (DLBCL). As of 31 December 2020, over 900 clinical studies associated with CAR-T have been registered as clinical trials[†], China is the most active area of clinical research (420 items) and has the highest rate of CAR-T research among all clinical trials, followed by the United States (261 items).

Although CAR-T therapy has shown remarkable efficacy [73,74] through the enforcement of the immune system and the redirection of specific immunocytes, this strategy presents three issues:

(1) Safety issues may follow treatment. Caused by the recognition and killing of antigen-expressing cells [75], the most epidemic toxicities brought about by CAR-T are cytokine-release syndrome (CRS) [76] and neurotoxicity reflected by cerebral edema [77]. Lowering the doses of CAR-T cells was shown to decrease toxicities, albeit at the cost of decreasing clinical effectiveness [78]; therefore, IL-6R antibody (Tocilizumab), IL-6 antibody (Siltuximab), Janus kinase (JAK) inhibitors, and corticosteroids have been utilized to block pro-inflammatory IL-6 signaling, in order to reverse fever, hypotension, and hypoxia [79,80]. The mechanisms of both toxicities remain elusive, partially due to a lack of informative animal models for preclinical studies.

(2) The specificity and selection of the target antigen can be problematic. "On-target, off tumor" toxicity gave rise to unexpected damage to patients, since noncancerous cells expressed the targeted antigens as well [81]. Worse still, antigen escape has been continuously observed in treated patients [82]. Therefore, the discovery of highly cancer-specific antigens is greatly needed to prevent extra events in patients in poor condition. Furthermore, multiple targets including different antigens and spliced variants of single antigens should be covered in CAR-T therapy.

(3) There may be overestimation of autologous T cells efficacy. T cell immunity is regulated by complicated networks in the TME, especially in solid tumors, including but not limited to immune checkpoints such as programmed cell death-ligand 1 (PD-L1) and metabolic alterations such as hypoxia and oxidative stress. As a result, T cell immunity efficacy can be compromised [83]. In addition, immune defects related to cancer treatments worsen the immune status and functions of the patients. Thankfully, genome-editing techniques such as transcription activator-like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 have been applied to T cell engineering for the functional enhancement of CAR-T cells [84,85]. Moreover, in ongoing studies, universal CAR-T cells from healthy donors have been shown to potentially overcome the impairment of immune status [84–86] if the inherent barriers of major histocompatibility complex (MHC) are eliminated [87,88]. Thus, much remains to be explored in immunoregulatory networks, optimal antigen selection, and sophisticated affinity modulation, particularly by genetic engineering, for the specific modified antibody format, the CAR.

3.6. Bispecific antibodies

bsAbs refer to a large family of recombinant molecules that are designed to recognize two disease-related targets or distinct epitopes within one molecule, which breaks up the natural IgG architectural format [89], since inhibiting a single target may fail to achieve significant efficacy. In general, constructive strategies of bsAbs aim to achieve advantages such as desired clinical efficacy, favorable physicochemical properties, minimal or no immunogenicity risk, and scalable manufacturability, even without intellectual property issues. The efforts that have been put into constructive strategy exploration have resulted in around 100 different formats in the bsAb family [89], all of which can essentially be divided into two major categories: those bearing an Fc region and those lacking an Fc region. Among these constructive formats, it is acknowledged that engineered-antibody fragments, such as scFv, the single domain antibodies from Llama (VHH), and Fab, have mostly been used as building modules—that is, as basic blocks to design and construct bsAbs [90–92]. These fragments range from tandem scFv/VHH to fragments-attached IgG-like molecules against anticipated targets [89,93,94]. Here, we describe the potential of bsAb candidates to become the next wave of modified antibody-based therapies, with a main focus on the moieties in clinical development.

To date, over 85 bsAbs are in clinical development, and more than 20 commercialized technology platforms are available for bsAb therapeutics creation and development [89]. Among the developed bsAbs, two have been marketed: blinatumomab (CD3 × CD19, where × denotes a combination of two antigen specificities) [95], a fragment-based bispecific T cell engagers (BiTCEs) for acute lymphatic leukemia (ALL) and B cell-ALL; and emicizumab (coagulation factor IXa (cFIXa) × coagulation factor X (cFX) and/or coagulation factor Xa (cFXa)), a full-size bispecific IgG for the routine prophylaxis of hemophilia A patients.

bsAbs can be categorized according to the engaged cells—that is, as BiTCEs, which activate T cells ($CD3^+$) to initiate T cell receptor (TCR) signaling; or as CD16a-associated bsAbs, which activate NK cells ($CD16a^+$) and dendritic cells (DCs) to induce ADCC. To date, there are 158 BiTCEs in total, and the targets mainly concentrate on CD19 (13 BiTCEs), epidermal growth factor receptor (EGFR) (12 BiTCEs), HER2 (11 BiTCEs), B cell maturation antigen (BCMA) (10 BiTCEs), and prostate-specific membrane antigen (PSMA) (8 BiTCEs). The redirected optimized cell killing (ROCK) technology platform developed CD16a-engaged bsAb AFM24 (EGFR × CD16a), AFM26 (BCMA × CD16a), and AFM13 (CD30 × CD16a). All of them showed similar affinity to different isotypes of CD16a encoded by CD16-158V and CD16-158F, which overcame the lower affinity of CD16-158F to the Fc region. AFM24 and AFM26 were more potent to kill target cells *in vitro*, while AFM13 was found to have acceptable safety and tolerability, as well as a high overall response rate (ORR) in phase I/II clinical studies.

It may also be practical to classify bsAbs by their mechanisms, as follows: ① Combinatorial bsAbs are designed as a mixture of antibodies, which may lessen the patients' economic burden. However, the ratio of each component, which is fixed in the development procedure, cannot be personalized in accordance with different patients' conditions. Similarly, there are difficulties in optimizing the pharmacodynamics and tolerability [89]. ② Obligate bsAbs are those in which the bodily linkage of binding domains generally creates a novel functionality; that is, a characteristic which can't be implemented by using an antibody mixture. Temporal obligate bsAbs mediate the sequential binding of the components. For example, binding the first domain facilitates the second domain's function by making the distal target accessible. Spatial obligate bsAbs exert functions that are dependent on the simultaneous binding of the domains. Consequently, targeted antigens are redistributed in space and facilitate downstream effects following this bsAb-dominated positioning. The interaction pattern of spatial obligate bsAbs is similar to that between effector cells and antigen presentation cells or enzymes and their substrates. The phases and progress of the bsAb clinical studies registered[†] are shown in Fig. 2 according to this classification.

The ongoing innovations with the new concepts that are arising may guide future directions in this field. It is attractive that the non-protein delivery of therapeutic bsAbs by mRNA or DNA coding

[†] https://clinicaltrials.gov.

shortens manufacturing duration to attain pharmaceutical-grade nucleic acid products. Stadler et al. [96] described a fragment-based BiTCE (CD3 × tight-junction protein claudin 6 (CLDN6)) encoded by an optimized, nucleoside-modified mRNA with sustainable production *in vivo*. Targeting and translation were ensured in the liver after intravenous administration through polymer-based and/or lipid-based formulation. This system was proven to be as effective as the corresponding purified protein-delivered bsAb. With better thermostability, the DNA-encoded format of bsAbs delivery enables less rigorous transfer conditions and relatively long-term storage. Relevant studies have been accomplished: Petal et al. [97] and Digiandomenico et al. [98] demonstrated a DNA-encoded symmetric bsAb format with sustained *in vivo* expression targeting the pathogenic components such as the V-antigen of *Pseudomonas aeruginosa* (PcrV) and pslexopolysaccharide (Psl) of *Pseudomonas aeruginosa*. This creation displayed minor differences in potency compared with the protein-delivered version. Furthermore, Keyt and colleagues made use of a J chain, which affiliated with the natural framework of IgA and IgM, and attached it to the effector cell targeting arms. This innovation was shown to permit bispecific formats with a 1 + 4 design, as in the case of dimeric IgA, or with a 1 + 10 design, as in the case of pentameric IgM, which could induce the targeting of pathogenic drivers with higher avidity being expressed at low levels. Flexible and less constrained structures endow bsAb with incomparable advantages in exploiting functions and promoting efficacy for clinical management. With the prosperity and improvement of commercialized technology platforms, new therapeutic targets should be explored to take advantage of bi-specificity.

4. Progress in modified therapeutic antibodies in China

China has achieved breakthroughs in the R&D of therapeutic antibodies, based on the introduction and absorption of advanced technology in recent years. Immune checkpoint antibodies for tumors are one example, with 25 therapeutic antibodies having been registered at the Center for Drug Evaluation (CDE), National Medical Products Administration (NMPA) of China. Among these, Toripalimab (JS-001), which was developed by Shanghai Medipharm Biotech Co., Ltd. (China), is the first mAb against programmed cell death receptor-1 (PD-1) that has been conditionally approved by the NMPA of China for the treatment of unresectable or metastatic melanoma in clinical trials in China [99]. In May 2019, Camrelizumab (SHR-1210), a humanized IgG4-κ anti-PD-1 mAb being developed by Jiangsu Hengrui Pharmaceuticals Co., Ltd. (China), received conditional approval in China for the treatment of relapsed or refractory classical Hodgkin's lymphoma [100]. These domestically developed products represent China's potential and capability in the innovation and development of biotherapeutics.

In the field of modified therapeutic antibodies, the National Key R&D Program of China called Precision Medical Research initiated projects on modified antibodies and immune cells. These projects align the strategic construction of modified therapeutic antibodies for clinical precision medicine and the establishment of criteria for biologics-based individualized treatment with associated genetic phenotype detection reagents and methods. Therefore, R&D on domestically developed modified therapeutic antibodies has been extensive and prosperous in recent years. The modified therapeutic

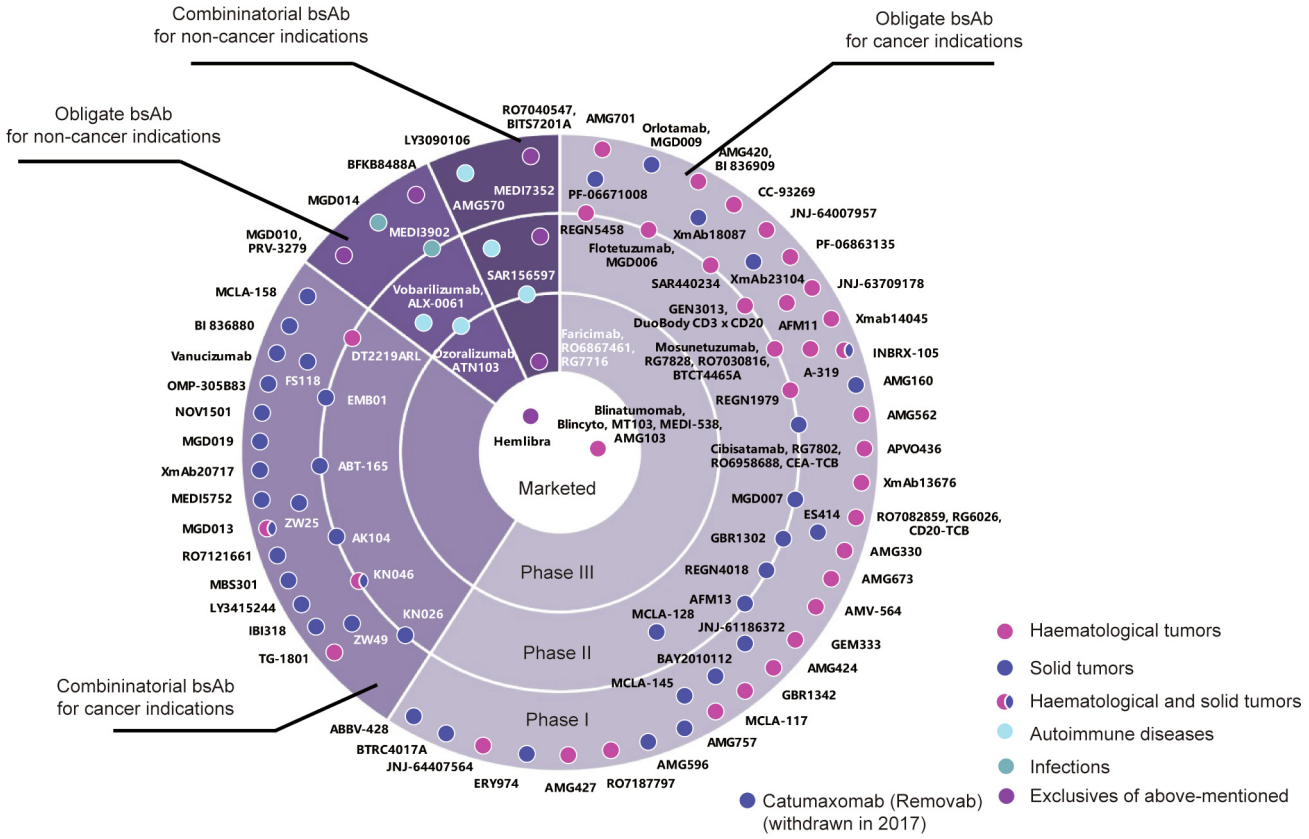

Fig 2. An updated summary of the clinical trial stage of bsAbs. Following the categorization reviewed by Labrijn et al. [89], the bsAbs registered are classified according to indications (data was accessed in September 2019). Tumors are the major approved investigational diseases for bsAbs with a proportion of 85.2% (75/88). Among them, 45 bsAbs are for solid tumors and 37 bsAbs are for hematological tumors. It is notable that MGD014 (CD3 × HIV-1 Env) for HIV infection and MEDI3902 (pslexopolysaccharide (Psl) × the V-antigen of *Pseudomonas aeruginosa* (PcrV)) for the prevention of *Pseudomonas aeruginosa* pneumonia have sparked hope for the management of refractory infections. Catumaxomab (CD3 × epithelial cell adhesion molecule (EpCAM)) was withdrawn in 2017 for commercial reasons and was associated with fatal toxicity at low doses.

antibodies shown in Table S1 in Appendix A were developed by Chinese scholars and have been approved by the CDE, NMPA of China for clinical trials

4.1. Modified glycosylation

During the outbreaks of Ebola virus disease (EVD) in 2014–2015, the cocktail of mAbs called MIL77, which was developed by Beijing Mabworks Biotech Co., Ltd., cured two Ebola virus (EBOV)-infected patients from the United Kingdom and Italy[†]. The CHO cells for MIL77 expression were glycoengineered to prevent fucosylation, thereby demonstrating the role of afucosylated modification in efficacious antibody production [101,102]. Furthermore, the efficient construction and manufacturing of MIL77 was performed in a timely manner, within three months. This event and the production of MIL77 displayed China's speed and competence in the biopharma industry. MIL77 was issued clinical approval by the NMPA in 2017, and a phase I clinical study was carried out.

In regard to cancer-related therapies, Zhang et al. [103] developed Metuzumab (HcHAb18), an affinity optimized and afucosylated human–mouse chimeric IgG1 mAb against CD147 with enhanced ADCC. This achievement was ranked in the "Top 10 Progress in Chinese Medicine Biotechnology" listed by the China Medical Biotech Association in 2016, and the novel mAb HcHAb18, which is based on Metuximab followed by modifications such as humanization and afucosylation, has been used for NSCLC patients as an independently innovative biologic product. By targeting CD147, which is highly expressed in NSCLC, Metuzumab enhances ADCC and sensitizes tumor cells to chemotherapy in combination with chemotherapeutics [103].

4.2. Antibody–drug conjugates

In early September 2019, OBI Pharma, Inc., a biopharmaceutical company in Taiwan Province, China, announced that the FDA had accepted the investigational new drug (IND) application of their novel first-in-class ADC named OBI-999. With proprietary linker technology maintaining a consistent DAR for cancer treatment, this ADC is based on the targeting of Globo-H, a glycosphingolipid that is highly expressed in up to 15 epithelial cancers. In preclinical studies, OBI-999 triggered apoptosis and suppressed metastasis in breast cancer cells and xenograft models [104]. These findings provided insight into the aberrant glycosylation of tumor cells and the original therapeutic targets of solid tumors, and OBI-99 is currently in phase I/II clinical trials.

As a kind of ADC, radioimmunotherapy (RIT) is a crucial part of targeting therapy, since the therapeutics make use of the guidance of specific parent antibodies to target the cells in foci and exert killing properties by radiolabeling. ^{131}I-labeled mouse/human chimeric mAb chTNT, which was developed by Shanghai Medipharm Biotech Co., Ltd. (China), is a radionuclides-strengthened ADC that is based on tumor necrosis therapy antibodies targeting all degenerating cells with antigens [105,106]. The ^{131}I-chTNT-mediated RIT of 43 patients exhibited significant therapeutic effects in advanced lung cancer [106]. The ^{131}I Metuximab injection named Licartin™ is an ^{131}I conjugated anti-CD147 antibody Metuximab that was the ADC modified by Zhang et al. [103] and Chen et al. [107]. It demonstrated safety and activity in the treatment of primary hepatocellular carcinoma (HCC) patients. In advanced HCC, Licartin™ is effective in the prevention of post-orthotopic liver transplantation (OLT) tumor recurrence and has shown a significantly decreased rate of tumor recurrence of 30.4%, as well as a 20.6% increased survival rate [108]. Another novel anti-CD147 ADC conjugates a potent cytotoxic drug tubulin inhibitor maytansinoid derivative 1 (DM1) via a non-cleavable thioether linker (SMCC) named HcHAb18-DM1. For NSCLC, disturbed mitotic spindle formation and antiproliferative activity of HcHAb18-DM1 treated groups were observed in comparison with the control *in vitro*. Meanwhile, decreased tumor weight and volume in A549 xenograft nude mice administered with HcHAb18-DM1 supported the promising role of HcHAb18-DM1 for clinical treatment against CD147-positive NSCLC [109].

4.3. CAR-T

After two CAR-T therapeutics were approved by the FDA, the CDE, NMPA of China started to accept applications for investigation of new drugs (INDs) for CAR-T products at the end of 2017. As of 2019, 32 IND applications have been accepted for CAR-T and 13 of them have been approved for clinical studies in China. In terms of targets, 27 of the 32 applications target CD19, four target BCMA, and one targets glypican 3 (GCP3) for solid tumors. Therefore, new targets may need to be exploited, especially for solid tumors and universal CAR-T.

4.4. Bispecific antibodies

At present, there are 13 ongoing clinical trials for five bsAbs approved by the NMPA of China. Among them, KN046 (PD-L1 × cytotoxic T lymphocyte antigen-4 (CTLA-4)), the first of its class, which was developed by Alphamab Co., Ltd. (China), has shown effective PD-1/PD-L1 and CTLA-4 blockade, with potential immune checkpoint inhibitory and antineoplastic activity. This antibody restores immune function and activates a sustained cytotoxic T lymphocyte (CTL)-mediated immune response against tumor cells. Clinical trials have also been approved for HER2 × HER2 bsAb (Alphamab Co., Ltd., China) and epithelial cell adhesion molecule (EpCAM) × CD3 bsAb (Wuhan YZY Biopharma Co., Ltd., China). Hence, the ongoing clinical trials of bsAbs developed by China demonstrate its anticipated capability and potential to design and manufacture multiple functional antibodies in this field.

5. Prospects and conclusion

Considering the extraordinary achievements that have been made with therapeutic antibodies in the treatment of cancers, autoimmune diseases, infectious diseases, and many other disorders, we can expect the generation of more effective antibody therapeutics with higher specificity, enhanced efficacy, lower toxicity, and better pharmacokinetic profiles. The modification of therapeutic antibodies represents a promising and irreversible tendency to edit antibodies and overcome potential limitations of candidate molecules in the R&D pipeline. In this article, we described six categories of modified therapeutic antibodies: ① modified glycosylation, ② Fc amino acid alterations, ③ cross-isotype or cross-subclass exchanges, ④ ADCs, ⑤ scFv for CAR-T cells, and ⑥ bsAbs. Numerous modified biotherapeutics have been granted market approval and are still in accelerated research or undergoing clinical trials. China has achieved breakthroughs in this field, including the development of therapeutic entities of ADCs, CAR-T products, and the implementation of clinical studies for modified therapeutic antibodies.

The construction and updating of new technology platforms are in progress and are paving the way for innovations in and modifications of therapeutic antibodies. For example, the HexaBody technology provided a generation of therapeutics with enhanced effector functions [110,111]. Heavy-chain antibodies (HCAbs) include the camelid single-domain antibody (sdAb, also

[†] http://1712130038.pool1-site.make.yun300.cn/product/3.html.

known as nanobody) and the variable region of the nurse shark antigen receptor (immunoglobulin novel antigen receptor (IgNAR), also called variable domain of IgNAR (VNAR)) [112,113]. Both of these have been found to have a relatively small molecular weight (about 15 kDa), low immunogenicity, broad antigen spectrum, and high penetration ability [114,115]. The potential translation of nanobodies (for thrombotic thrombocytopenic purpura (TTP)) and VNAR products (for nerve system diseases, autoimmune diseases, etc.) displays the advantages and promising future of therapeutic HCAbs [114–116]. Researchers may achieve a breakthrough in the routine administration of therapeutic antibodies, considering their thermal stability (up to 70 °C) [117], proteolytical stability (in gastric and intestinal juice), and solubility [118]. It is still important to prolong the half-life in circulation and maintain an effective concentration of sdAb in order to avoid excess clearance caused by the low molecular weight. The therapeutic effects of sdAb must be observed and followed up in order to enable global marketing and allow sdAb to serve as a first-line treatment. Furthermore, attaching the polymer polyethylene glycol (PEG)—that is, PEGylation—onto the surface of antibody fragments prolongs the antibody half-life in circulation and reduces immunogenicity. Fc-fusion proteins are derivatives of antibodies that do not necessarily retain the variable region, which is not contained in modified therapeutic antibodies, so that Fc-fusion proteins are not covered [119]. Antibody humanization remains a standard procedure for therapeutic mAbs and has been explored worldwide as an independent process in the R&D of therapeutic antibodies. Thus, it is not summarized as a modification approach in this review.

The major obstacles confronting the R&D of modified antibodies and their corresponding strategies can be grouped into the following four issues. First, more detailed revelations of concrete molecular interactions and subtle regulatory mechanisms are still urgently required, since appropriate therapy targets underpin the design, manufacture, modification, and clinical efficacy of antibody therapy. Second, preclinical studies are required in order to resolve the emerging drug resistance of biologics, including but not limited to ADCs and CAR-T therapy [120–123]. Third, bioinformatics fuels the R&D of modified therapeutic antibodies, and many approaches based on high-throughput screening (e.g., phage display and alanine scanning mutations) have been utilized in antibody modification, such as the Fc region alteration of therapeutic antibodies [49,124–126]. Last but not least, precision medicine could be extended to biopharmaceuticals, which would include the exploration of site-specific targets at the amino acid level [127], and development and validation for the immunogenicity assessment of modified antibody products [128].

In short, the modification of therapeutic antibodies enables the control of diseases that are difficult to address and provides reasonable therapeutic choices by achieving accurate targeting, elevated specificity, the elimination of adverse reactions, and improved efficacy. The enhanced efficacy of modified therapeutic antibodies motivates growth and progress in the biopharma industry, which will yield more effective and accessible therapeutics in the long run.

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Authors' contributions

Ji-Min Dai wrote the original draft, while Xue-Qin Zhang and Jing-Yao Dai were involved in the writing in terms of reviewing and editing. Xiang-Min Yang and Zhi-Nan Chen were involved in the conceptualization, editing, and collection of statistical data, and Zhi-Nan Chen was involved in funding acquisition.

Compliance with ethics guidelines

Ji-Min Dai, Xue-Qin Zhang, Jing-Yao Dai, Xiang-Min Yang, and Zhi-Nan Chen declare that they have no conflict of interest or financial conflicts to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2020.06.030.

References

[1] Ramon G. The discovery of diphtheria toxic and its consequences: homage to Emile Roux. Bull Acad Natl Med 1953;137(30–31):516–9.
[2] Ehrlich P. Die Wertbemessung des Diphterieheilserums und deren theoretische Grundlagen. Klinisches Jahrbuch 1897;6:299–326. German.
[3] Von Behring E, Kitasato S. Ueber das Zustandekommen der Diphtherie-Immunitat und der Tetanus-Immunitat bei Thieren. Dtsch Med Wochenschr 1890;16(49):1113–4. German.
[4] Porter RR. The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain. Biochem J 1959;73(1):119–26.
[5] Tonegawa S, Steinberg C, Dube S, Bernardini A. Evidence for somatic generation of antibody diversity. Proc Natl Acad Sci USA 1974;71(10):4027–31.
[6] Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256(5517):495–7.
[7] Igawa T, Ishii S, Tachibana T, Maeda A, Higuchi Y, Shimaoka S, et al. Antibody recycling by engineered pH-dependent antigen binding improves the duration of antigen neutralization. Nat Biotechnol 2010;28(11):1203–7.
[8] Chaparro-Riggers J, Liang H, DeVay RM, Bai L, Sutton JE, Chen W, et al. Increasing serum half-life and extending cholesterol lowering *in vivo* by engineering antibody with pH-sensitive binding to PCSK9. J Biol Chem 2012;287(14):11090–7.
[9] Igawa T, Mimoto F, Hattori K. pH-dependent antigen-binding antibodies as a novel therapeutic modality. Biochim Biophys Acta 2014;1844(11):1943–50.
[10] Yamamura T, Kleiter I, Fujihara K, Palace J, Greenberg B, Zakrzewska-Pniewska B, et al. Trial of satralizumab in neuromyelitis optica spectrum disorder. N Engl J Med 2019;381(22):2114–24.
[11] Burmeister WP, Huber AH, Bjorkman PJ. Crystal structure of the complex of rat neonatal Fc receptor with Fc. Nature 1994;372(6504):379–83.
[12] Sockolosky JT, Szoka FC. The neonatal Fc receptor, FcRn, as a target for drug delivery and therapy. Adv Drug Delivery Rev 2015;91:109–24.
[13] Raghavan M, Bonagura VR, Morrison SL, Bjorkman PJ. Analysis of the pH dependence of the neonatal Fc receptor/immunoglobulin G interaction using antibody and receptor variants. Biochemistry 1995;34(45):14649–57.
[14] Martin WL, West AP, Gan Lu, Bjorkman PJ. Crystal structure at 2.8 Å of an FcRn/heterodimeric Fc complex: mechanism of pH-dependent binding. Mol Cell 2001;7(4):867–77.
[15] Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol 2007;7(9):715–25.
[16] Weiner LM, Surana R, Wang S. Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nat Rev Immunol 2010;10(5):317–27.
[17] Ober RJ, Martinez C, Vaccaro C, Zhou J, Ward ES. Visualizing the site and dynamics of IgG salvage by the MHC class I-related receptor. FcRn J Immunol 2004;172(4):2021–9.
[18] Rodewald R. pH-dependent binding of immunoglobulins to intestinal cells of the neonatal rat. J Cell Biol 1976;71(2):666–9.
[19] Nimmerjahn F, Ravetch JV. Fcγ receptors: old friends and new family members. Immunity 2006;24(1):19–28.
[20] Malphettes L, Freyvert Y, Chang J, Liu PQ, Chan E, Miller JC, et al. Highly efficient deletion of FUT8 in CHO cell lines using zinc-finger nucleases yields cells that produce completely nonfucosylated antibodies. Biotechnol Bioeng 2010;106(5):774–83.
[21] Saunders K. Conceptual approaches to modulating antibody effector functions and circulation half-life. Front Immunol 2019;10:1296.
[22] Shields RL, Lai J, Keck R, O'connell LY, Hong K, Meng YG, et al. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcγ RIII and antibody-dependent cellular toxicity. J Biol Chem 2002;277(30):26733–40.
[23] Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, et al. The absence of fucose but not the presence of galactose or bisecting *N*-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 2003;278(5):3466–73.
[24] Niwa R, Shoji-Hosaka E, Sakurada M, Shinkawa T, Uchida K, Nakamura K, et al. Defucosylated chimeric anti-C–C chemokine receptor 4 IgG1 with enhanced

antibody-dependent cellular cytotoxicity shows potent therapeutic activity to T-cell leukemia and lymphoma. Cancer Res 2004;64(6):2127–33.
[25] Hamako J, Matsui T, Ozeki Y, Mizuochi T, Titani K. Comparative studies of asparagine-linked sugar chains of immunoglobulin G from eleven mammalian species. Comp Biochem Phys B Comp Biochem 1993;106(4):949–54.
[26] Jefferis R, Lund J, Mizutani H, Nakagawa H, Kawazoe Y, Arata Y, et al. A comparative study of the N-linked oligosaccharide structures of human IgG subclass proteins. Biochem J 1990;268(3):529–37.
[27] Raju TS, Briggs JB, Borge SM, Jones AJ. Species-specific variation in glycosylation of IgG: evidence for the species-specific sialylation and branch-specific galactosylation and importance for engineering recombinant glycoprotein therapeutics. Glycobiology 2000;10(5):477.
[28] Tonetti M, Sturla L, Bisso A, Benatti U, De Flora A. Synthesis of GDP-L-fucose by the human FX protein. J Biol Chem 1996;271(44):27274–9.
[29] Song Z. Roles of the nucleotide sugar transporters (SLC35 family) in health and disease. Mol Aspects Med 2013;34(2–3):590–600.
[30] Miyoshi E, Noda K, Yamaguchi Y, Inoue S, Ikeda Y, Wang W, et al. The α1-6-fucosyltransferase gene and its biological significance. Biochim Biophys Acta 1999;1473(1):9–20.
[31] Pereira NA, Chan KF, Lin PC, Song Z. The, "less-is-more" in therapeutic antibodies: afucosylated anti-cancer antibodies with enhanced antibody-dependent cellular cytotoxicity. mAbs 2018;10(5):693–711.
[32] Louie S, Haley B, Marshall B, Heidersbach A, Yim M, Brozynski M, et al. *FX* knockout CHO hosts can express desired ratios of fucosylated or afucosylated antibodies with high titers and comparable product quality. Biotechnol Bioeng 2017;114(3):632–44.
[33] Ferrara C, Brünker P, Suter T, Moser S, Püntener U, Umaña P. Modulation of therapeutic antibody effector functions by glycosylation engineering: influence of Golgi enzyme localization domain and co-expression of heterologous β1, 4-*N*-acetylglucosaminyltransferase III and Golgi α-mannosidase II. Biotechnol Bioeng 2006;93(5):851–61.
[34] Laurie HS, Sarit EA, Douglas AS, Mangel J, Randy DG, Fine G, et al. A phase 1 study of obinutuzumab induction followed by 2 years of maintenance in patients with relapsed CD20-positive B-cell malignancies. Blood 2012;119(22):5118–25.
[35] Salles G, Morschhauser F, Lamy T, Milpied N, Thieblemont C, Tilly H, et al. Phase 1 study results of the type II glycoengineered humanized anti-CD20 monoclonal antibody obinutuzumab (GA101) in B-cell lymphoma patients. Blood 2012;119(22):5126.
[36] Martinez DR, Fong Y, Li SH, Yang F, Jennewein MF, Weiner JA, et al. Fc characteristics mediate selective placental transfer of IgG in HIV-infected women. Cell 2019;178(1):190–201.
[37] Jennewein MF, Goldfarb I, Dolatshahi S, Cosgrove C, Noelette FJ, Krykbaeva M, et al. Fc glycan-mediated regulation of placental antibody transfer. Cell 2019;178(1):202–15.
[38] Vanessa LH. Innate immunity of neonates and infants. Front Immunol 2018;9:1759.
[39] Lee YC, Lin SJ. Neonatal natural killer cell function: relevance to antiviral immune defense. Clin Dev Immunol 2013;2013(2):427696.
[40] Nimmerjahn F, Jeffrey VR. Fcγ receptors as regulators of immune responses. Nat Rev Immunol 2008;8(1):34–47.
[41] Moore GL, Chen H, Karki S, Greg A. Engineered Fc variant antibodies with enhanced ability to recruit complement and mediate effector functions. Mabs 2010;2(2):181–9.
[42] Romain G, Senyukov V, Rey VN, Merouane A, Kelton W, Liadi I, et al. Antibody Fc engineering improves frequency and promotes kinetic boosting of serial killing mediated by NK cells. Blood 2014;124(22):3241–9.
[43] Dall'Acqua WF, Kiener PA, Wu H. Properties of human IgG1s engineered for enhanced binding to the neonatal Fc receptor (FcRn). J Biol Chem 2006;281(33):23514–24.
[44] Mimoto F, Igawa T, Kuramochi T, Katada H, Kadono S, Kamikawa T, et al. Novel asymmetrically engineered antibody Fc variant with superior FcγR binding affinity and specificity compared with afucosylated Fc variant. Mabs 2013;5(2):229–36.
[45] Richards J, Karki S, Lazar GA, Chen H, Dang W, Desjarlais JR. Optimization of antibody binding to FcγRIIa enhances macrophage phagocytosis of tumor cells. Mol Cancer Ther 2008;7(8):2517–27.
[46] Rankin CT, Veri MC, Gorlatov S, Tuaillon N, Burke S, Huang L, et al. CD32B, the human inhibitory Fc-γ receptor IIB, as a target for monoclonal antibody therapy of B-cell lymphoma. Blood 2006;108(7):2384–91.
[47] Nordstrom JL, Gorlatov S, Zhang W, Yang Y, Huang L, Burke S, et al. Anti-tumor activity and toxicokinetics analysis of MGAH22, an anti-HER2 monoclonal antibody with enhanced Fcγ receptor binding properties. Breast Cancer Res 2011;13(6):R123.
[48] Bang YJ, Giaccone G, Im SA, Oh DY, Bauer TM, Nordstrom JL, et al. First-in-human phase 1 study of margetuximab (MGAH22), an Fc-modified chimeric monoclonal antibody, in patients with HER2-positive advanced solid tumors. Ann Oncol 2017;28(4):855–61.
[49] Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, et al. High resolution mapping of the binding site on human IgG1 for FcγRI, FcγRII, FcγRIII, and FcRn and design of IgG1 variants with improved binding to the FcγR. J Biol Chem 2001;276(9):6591–604.
[50] Bas M, Terrier A, Jacque E, Dehenne A, Pochet-Béghin V, Beghin C, et al. Fc sialylation prolongs serum half-life of therapeutic antibodies. J Immunol 2019;202(5):1582–94.
[51] Braster R, O'Toole T, van Egmond M. Myeloid cells as effector cells for monoclonal antibody therapy of cancer. Methods 2014;65(1):28–37.
[52] Alyssa DG, Houghton AM. Tumor-associated neutrophils: new targets for cancer therapy. Cancer Res 2011;71(7):2411–6.
[53] Chintalacharuvu KR, Vuong LU, Loi LA, Larrick JW, Morrison SL. Hybrid IgA2/IgG1 antibodies with tailor-made effector functions. Clin Immunol 2001;101(1):21–31.
[54] Borrok MJ, Luheshi NM, Beyaz N, Davies GC, Legg JW, Wu H, et al. Enhancement of antibody-dependent cell-mediated cytotoxicity by endowing IgG with FcαRI (CD89) binding. Mabs 2015;7(4):743–51.
[55] Natsume A, In M, Takamura H, Nakagawa T, Shimizu Y, Kitajima K, et al. Engineered antibodies of IgG1/IgG3 mixed isotype with enhanced cytotoxic activities. Cancer Res 2008;68(10):3863–72.
[56] Natsume A, Shimizu YY, Satoh M, Shitara K, Niwa R. Engineered anti-CD20 antibodies with enhanced complement-activating capacity mediate potent anti-lymphoma activity. Cancer Sci 2010;100(12):2411–8.
[57] Sensel MG, Kane LM, Morrison SL. Amino acid differences in the N-terminus of C_H2 influence the relative abilities of IgG2 and IgG3 to activate complement. Mol Immunol 1997;34(14):1019–29.
[58] Weiner GJ. Building better monoclonal antibody-based therapeutics. Nat Rev Cancer 2015;15(6):361–70.
[59] Schneider H, Deweid L, Pirzer T, Yanakieva D, Englert S, Becker B, et al. Dextramabs: a novel format of antibody-drug conjugates featuring a multivalent polysaccharide scaffold. ChemistryOpen 2019;8(3):354–7.
[60] Ott PA, Pavlick AC, Johnson DB, Hart LL, Infante JR, Luke JJ, et al. A phase 2 study of glembatumumab vedotin, an antibody-drug conjugate targeting glycoprotein NMB, in patients with advanced melanoma. Cancer 2019;125(7):1113–23.
[61] Szot C, Saha S, Zhang XM, Zhu Z, Hilton MB, Morris K, et al. Tumor stroma-targeted antibody-drug conjugate triggers localized anticancer drug release. J Clin Invest 2018;128(7):2927–43.
[62] Lambert JM, Berkenblit A. Antibody-drug conjugates for cancer treatment. Annu Rev Med 2018;69:191–207.
[63] Lambert JM, Morris CQ. Antibody-drug conjugates (ADCs) for personalized treatment of solid tumors: a review. Adv Ther 2017;34(5):1015–35.
[64] Chen B, Gianolio DA, Stefano JE, Manning CM, Gregory RC, Busch MM, et al. Design, synthesis, and *in vitro* evaluation of multivalent drug linkers for high-drug-load antibody-drug conjugates. ChemMedChem 2018;13(8):790–4.
[65] Krall N, da Cruz FP, Boutureira O, Bernardes GJ. Site-selective protein-modification chemistry for basic biology and drug development. Nat Chem 2016;8(2):103–13.
[66] Chudasama V, Maruani A, Caddick S. Recent advances in the construction of antibody-drug conjugates. Nat Chem 2016;8(2):114–9.
[67] Lyon RP, Bovee TD, Doronina SO, Burke PJ, Hunter JH, Neff-LaFord HD, et al. Reducing hydrophobicity of homogeneous antibody-drug conjugates improves pharmacokinetics and therapeutic index. Nat Biotechnol 2015;33(7):733–5.
[68] Zhang C, Welborn M, Zhu T, Yang NJ, Santos MS, Van Voorhis T, et al. π-Clamp-mediated cysteine conjugation. Nat Chem 2016;8(2):120–8.
[69] Matos MJ, Oliveira B, Martínez-Sáez N, Guerreiro A, Cal PMSD, Bertoldo J, et al. Chemo- and regioselective lysine modification on native proteins. J Am Chem Soc 2018;140(11):4004–17.
[70] Merlin JL, Barberi-Heyob M, Bachmann N. *In vitro* comparative evaluation of trastuzumab (Herceptin) combined with paclitaxel (Taxol) or docetaxel (Taxotere) in HER2-expressing human breast cancer cell lines. Ann Oncol 2002;13(11):1743–8.
[71] Cui JJ, Tran-Dubé M, Shen H, Nambu M, Kung PP, Pairish M, et al. Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal–epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). J Med Chem 2011;54(18):6342–63.
[72] June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science 2018;359(6382):1361–5.
[73] Ali SA, Shi V, Maric I, Wang M, Stroncek DF, Rose JJ, et al. T cells expressing an anti-B-cell-maturation-antigen chimeric antigen receptor cause remissions of multiple myeloma. Blood 2016;128(13):1688–700.
[74] Garfall AL, Maus MV, Hwang WT, Lacey SF, Mahnke YD, Melenhorst JJ, et al. Chimeric antigen receptor T Cells against CD19 for multiple myeloma. N Engl J Med 2015;373(11):1040–7.
[75] Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Mol Ther 2010;18(4):843–51.
[76] Kalos M, Bruce LL, David LP, Katz S, Stephan AG, Bagg A, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med 2011;3(95):95ra73.
[77] Gust J, Hay KA, Hanafi LA, Li D, Myerson D, Gonzalez-Cuyar LF, et al. Endothelial activation and blood–brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR–T cells. Cancer Discovery 2017;7(12):1404–19.
[78] Ahmed N, Brawley VS, Hegde M, Robertson C, Ghazi A, Gerken C, et al. Human epidermal growth factor receptor 2 (HER2)-specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma. J Clin Oncol 2015;33(15):1688–96.
[79] Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Maric I, et al. B-cell depletion and remissions of malignancy along with cytokine-

associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 2012;119(12):2709–20.
[80] Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med 2011;365(8):725–33.
[81] Cor HL, Stefan S, van Steenbergen S, van Elzakker P, van Krimpen B, Corrien G, et al. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. Mol Ther J Am Soc Gene Ther 2013;21(4):904–12.
[82] Orourke DM, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrissette JJD, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med 2017;9(399):eaaa0984.
[83] Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. Science 2015;348(6230):74–80.
[84] Qasim W, Zhan H, Samarasinghe S, Adams S, Amrolia P, Stafford S, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. Sci Transl Med 2017;9(374):eaaj2013.
[85] Di Stasi A, Tey SK, Dotti G, Fujita Y, Kennedy-Nasser A, Martinez C, et al. Inducible apoptosis as a safety switch for adoptive cell therapy. N Engl J Med 2011;365(18):1673–83.
[86] Gornalusse GG, Hirata RK, Funk SE, Riolobos L, Lopes VS, Manske G, et al. HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. Nat Biotechnol 2017;35(8):765–72.
[87] Zakrzewski JL, Suh D, Markley JC, Smith OM, King C, Goldberg GL, et al. Tumor immunotherapy across MHC barriers using allogeneic T-cell precursors. Nat Biotechnol 2008;26(4):453–61.
[88] Torikai H, Reik A, Liu PQ, Zhou Y, Zhang L, Maiti S, et al. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. Blood 2012;119(24):5697–705.
[89] Labrijn AF, Janmaat ML, Reichert JM, Parren PWHI. Bispecific antibodies: a mechanistic review of the pipeline. Nat Rev Drug Discovery 2019;18(8):585–608.
[90] Suurs FV, Lub-De HM, de Vries E, de Groot D. A review of bispecific antibodies and antibody constructs in oncology and clinical challenges. Pharmacol Ther 2019;201:103–19.
[91] Alibakhshi A, Abarghooi Kahaki F, Ahangarzadeh S, Yaghoobi H, Yarian F, Arezumand R, et al. Targeted cancer therapy through antibody fragments-decorated nanomedicines. J Controlled Release 2017;268:323–34.
[92] Ahmad ZA, Yeap SK, Ali AM, Ho WY, Alitheen NBM, Hamid M. scFv antibody: principles and clinical application. Clin Dev Immunol 2012;2012:1–15.
[93] Bezabeh B, Fleming R, Fazenbaker C, Zhong H, Coffman K, Yu XQ, et al. Insertion of scFv into the hinge domain of full-length IgG1 monoclonal antibody results in tetravalent bispecific molecule with robust properties. mAbs 2017;9(2):240–56.
[94] Schmohl JU, Felices M, Taras E, Miller JS, Vallera DA. Enhanced ADCC and NK cell activation of an anticarcinoma bispecific antibody by genetic insertion of a modified IL-15 cross-linker. Mol Ther 2016;24(7):1312–22.
[95] Gökbuget N, Dombret H, Bonifacio M, Reichle A, Graux C, Faul C, et al. Blinatumomab for minimal residual disease in adults with B-precursor acute lymphoblastic leukemia. Blood 2018;131(14):1522–31.
[96] Stadler CR, Bähr-Mahmud H, Celik L, Hebich B, Roth AS, Roth RP, et al. Elimination of large tumors in mice by mRNA-encoded bispecific antibodies. Nat Med 2017;23(7):815–7.
[97] Patel A, Digiandomenico A, Keller AE, Smith TRF, Park DH, Ramos S, et al. An engineered bispecific DNA-encoded IgG antibody protects against *Pseudomonas aeruginosa* in a pneumonia challenge model. Nat Commun 2017;8(1):637.
[98] Digiandomenico A, Keller AE, Gao C, Rainey GJ, Warrener P, Camara MM, et al. A multifunctional bispecific antibody protects against *Pseudomonas aeruginosa*. Sci Transl Med 2014;6(262):262ra155.
[99] Keam SJ. Toripalimab: first global approval. Drugs 2019;79(5):573–8.
[100] Mo H, Huang J, Xu J, Chen X, Wu D, Qu D, et al. Safety, anti-tumour activity, and pharmacokinetics of fixed-dose SHR-1210, an anti-PD-1 antibody in advanced solid tumours: a dose-escalation, phase 1 study. Br J Cancer 2018;119(5):538–45.
[101] Qiu X, Audet J, Lv M, He S, Wong G, Wei H, et al. Two-mAb cocktail protects macaques against the Makona variant of Ebola virus. Sci Transl Med 2016;8(329):329ra33.
[102] Li H, Yu F, Xia S, Yu Y, Wang Q, Lv M, et al. Chemically modified human serum albumin potently blocks entry of ebola pseudoviruses and viruslike particles. Antimicrob Agents Chemother 2017;61(4). e02168-16.
[103] Zhang Z, Zhang Y, Sun Q, Feng F, Huhe M, Mi L, et al. Preclinical pharmacokinetics, tolerability, and pharmacodynamics of metuzumab, a novel CD147 human–mouse chimeric and glycoengineered antibody. Mol Cancer Ther 2015;14(1):162–73.
[104] Chuang PK, Hsiao M, Hsu TL, Chang CF, Wu CY, Chen BR, et al. Signaling pathway of globo-series glycosphingolipids and β1,3-galactosyltransferase V (β3GalT5) in breast cancer. Proc Natl Acad Sci USA 2019;116(9):3518–23.
[105] Epstein AL, Chen FM, Taylor CR. A novel method for the detection of necrotic lesions in human cancers. Cancer Res 1988;48(20):5842–8.
[106] Yu L, Ju DW, Chen W, Li T, Xu Z, Jiang C, et al. ^{131}I-chTNT radioimmunotherapy of 43 patients with advanced lung cancer. Cancer Biother radiopharm 2006;21(1):5–14.
[107] Chen ZN, Mi L, Xu J, Song F, Zhang Q, Zhang Z, et al. Targeting radioimmunotherapy of hepatocellular carcinoma with iodine (^{131}I) metuximab injection: clinical phase I/II trials. Int J Radiat Oncol Biol Phys 2006;65(2):435–44.
[108] Xu J, Shen ZY, Chen XG, Zhang Q, Bian HJ, Zhu P, et al. A randomized controlled trial of Licartin for preventing hepatoma recurrence after liver transplantation. Hepatology 2007;45(2):269–76.
[109] Huhe M, Lou J, Zhu Y, Zhao Y, Shi Y, Wang B, et al. A novel antibody-drug conjugate, HcHAb18-DM1, has potent anti-tumor activity against human non-small cell lung cancer. Biochem Biophysical Res Commun 2019;513(4):1083–91.
[110] Gulati S, Beurskens FJ, de Kreuk BJ, Roza M, Zheng B, Deoliveira RB, et al. Complement alone drives efficacy of a chimeric antigonococcal monoclonal antibody. PLoS Biol 2019;17(6):e3000323.
[111] De Jong RN, Beurskens FJ, Verploegen S, Strumane K, van Kampen MD, Voorhorst M, et al. A novel platform for the potentiation of therapeutic antibodies based on antigen-dependent formation of IgG hexamers at the cell surface. PLoS Biol 2016;14(1):e1002344.
[112] Arbabi-Ghahroudi M. Camelid single-domain antibodies: historical perspective and future outlook. Front Immunol 2017;8:1589.
[113] Muyldermans S, Atarhouch T, Saldanha J, Barbosa JARG, Hamers R. Sequence and structure of VH domain from naturally occurring camel heavy chain immunoglobulins lacking light chains. Protein Eng 1994;7(9):1129–35.
[114] Greenberg AS, Avila D, Hughes M, Hughes A, McKinney EC, Flajnik MF. A new antigen receptor gene family that undergoes rearrangement and extensive somatic diversification in sharks. Nature 1995;374(6518):168–73.
[115] Dooley H, Flajnik MF, Porter AJ. Selection and characterization of naturally occurring single-domain (IgNAR) antibody fragments from immunized sharks by phage display. Mol Immunol 2003;40(1):25–33.
[116] Mazepa MA, Masias C, Chaturvedi S. How targeted therapy disrupts the treatment paradigm for acquired TTP: the risks, benefits, and unknowns. Blood 2019;134(5):415–20.
[117] Liu JL, Zabetakis D, Brown JC, Anderson GP, Goldman ER. Thermal stability and refolding capability of shark derived single domain antibodies. Mol Immunol 2014;59(2):194–9.
[118] Harmsen MM, van Solt CB, van Zijderveld-van Bemmel AM, Niewold TA, van Zijderveld FG. Selection and optimization of proteolytically stable llama single-domain antibody fragments for oral immunotherapy. Appl Microbiol Biotechnol 2006;72(3):544–51.
[119] Liu L. Pharmacokinetics of monoclonal antibodies and Fc-fusion proteins. Protein Cell 2018;9(1):15–32.
[120] Vernieri C, Milano M, Brambilla M, Mennitto A, Maggi C, Cona MS, et al. Resistance mechanisms to anti-HER2 therapies in HER2-positive breast cancer: current knowledge, new research directions and therapeutic perspectives. Crit Rev Oncol/Hematol 2019;139:53–66.
[121] Qin H, Ramakrishna S, Nguyen S, Fountaine TJ, Ponduri A, Stetler-Stevenson M, et al. Preclinical development of bivalent chimeric antigen receptors targeting both CD19 and CD22. Mol Ther Oncolytics 2018;11:127–37.
[122] Schneider D, Xiong Y, Wu D, Nölle V, Schmitz S, Haso W, et al. A tandem CD19/CD20 CAR lentiviral vector drives on-target and off-target antigen modulation in leukemia cell lines. J Immunother Cancer 2017;5(1):42.
[123] Ruella M, Barrett DM, Kenderian SS, Shestova O, Hofmann TJ, Perazzelli J, et al. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. J Clin Invest 2016;126(10):3814–26.
[124] Lazar GA, Dang W, Karki S, Vafa O, Peng JS, Hyun L, et al. Engineered antibody Fc variants with enhanced effector function. Proc Natl Acad Sci USA 2006;103(11):4005–10.
[125] Ljungars A, Svensson C, Carlsson A, Birgersson E, Tornberg UC, Frendéus B, et al. Deep mining of complex antibody phage pools generated by cell panning enables discovery of rare antibodies binding new targets and epitopes. Front Pharmacol 2019;10:847.
[126] Ghetie V, Popov S, Borvak J, Radu C, Matesoi D, Medesan C, et al. Increasing the serum persistence of an IgG fragment by random mutagenesis. Nat Biotechnol 1997;15(7):637–40.
[127] Hwang LA, Phang BH, Liew OW, Iqbal J, Koh XH, Koh XY, et al. Monoclonal antibodies against specific p53 hotspot mutants as potential tools for precision medicine. Cell Rep 2018;22(1):299–312.
[128] Mojtahed PS, Ulshöfer T, Gabriel LA, Henke M, Köhm M, Behrens F, et al. Immunogenicity assay development and validation for biological therapy as exemplified by ustekinumab. Clin Exp Immunol 2019;196(2):259–67.