Review Article

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2020 FDA/EMA approvals for new immunotherapy drug technologies and applications

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Abstract

Immunotherapy is a new approach applied in treatment of infections, autoimmune diseases, or cancer by activating or suppressing the immune system. Pre-clinical and clinical investigations on discovering new products with high efficacy and low side effects are still ongoing. Clinical studies revealed numerous advantages of immunotherapy over chemotherapy, including prolonged progression-free survival and improved overall survival rate. However, immunotherapy may cause occasional severe adverse reactions due to an overactive immune system. This review gives an overview of new immunotherapeutic products approved by FDA/EMA in 2020. Moreover, the technologies used in manufacturing monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), and CAR-T cells are explained. In 2020, mAbs approved for the first time in management of migraine, autoimmune CNS disease, and thyroid eye disorder. In addition, new ADC and CAR-T cell therapeutics were authorized by FDA for mAb-targeted treatment of metastatic breast cancer and multiple myeloma, and mantle cell lymphoma, respectively. Despite the complexity and ambiguity of the technological development, ADCs and CAR-T cell therapy have great potential for further clinical applications in cancer therapy.

Keywords: Immunotherapy, monoclonal antibody, antibody-drug conjugate, CAR-T cell

1. Introduction

Immunotherapy has long become of great interest to both researchers and pharmaceutical companies in management and treatment of several diseases through multiple pathways controlling the immune system (1, 2). In cancer and infectious diseases, immunotherapy are used to activate immune system to eradicated evading cells, while for autoimmune disorders, transplantation, allergies, and wound healing, immunotherapy are designed to suppress immune responses to boost safety or tissue regeneration (3, 4). It can be classified into several categories, including a) immune modulators (e.g., cytokines, interleukins, chemokines, and immunomodulatory drugs), b) monoclonal antibodies, c) checkpoint blockade, d) oncolytic viruses, e) vaccines, and f) cell therapy such as chimeric antigen receptor (CAR) T cell (5).

Immunotherapy regimens can be adopted alone when radiation or chemotherapy does not work or in combination with other therapies to amplify their effects. This therapeutic approach offers fewer side effects, prolonged progression-free survival, and higher overall survival. However, there are still some obstacles limiting its clinical application such as incidence of occasional severe adverse reactions, inadequate durability, unpredictable efficacy, drug tolerance and its high cost. These challenges have drawn the scientists' attention to expand the horizons of immunotherapy through gene editing, synthetic biology, cell manu-

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Table 1. Biological therapeutics received first approvals in 2020 by the US-FDA or the European Medicines Agency (EMA)

International nonproprietary name	Brand name	Target	Type	First approved indication	Date of first EU approval	Date of first FDA approval
Teprotumumab	Tepezza	IGF-1R	Human IgG1 mAb	Thyroid eye disease	NA	21–01-2020
Eptinezumab	Vyepti	CGRP	Humanized IgG1 mAb	Migraine prevention	Review	21–02-2020
Isatuximab	Sarclisa	CD38	Chimeric IgG1 mAb	Multiple myeloma	30–05-2020	02–03-2020
Sacituzumab govitecan	Trodelvy	TROP-2	Humanized IgG1 ADC	Triple-negative breast cancer	Review	22–04-2020
Inebilizumab	Uplizna	CD19	Humanized IgG1 mAb	Neuromyelitis optica spectrum disorders	Review	11–06-2020
Brexucabtagene autoleucel	Tecartus	CD 19	CAR T cell	Mantle cell lymphoma	14–12-2020	24–07-2020
Tafasitamab	Monjuvi	CD19	Humanized IgG1 mAb	Diffuse large B-cell lymphoma	Review	31–07-2020
Belantamab mafodotin	Blenrep	BCMA	Humanized IgG1 ADC	Multiple myeloma	25–08-2020	05–08-2020
Satralizumab	Enspryng	IL-6R	Humanized IgG2 mAb	Neuromyelitis optica spectrum disorders	Review	13–08-2020
Atoltivimab, Maftivimab, and Odesivimab-ebgn	Inmazeb	Ebola virus	cocktail of 3 human IgG1 mAbs	Ebola virus infection	NA	14–10-2020
Naxitamab	Danyelza	GD2	Humanized IgG1 mAb	High-risk neuroblastoma and refractory osteomedullary disease	NA	25–11-2020
Ansuvimab	Ebanga	Ebola virus	Human IgG1 mAb	Ebola infection	NA	21–12-2020
Margetuximab	Margenza	HER2	Humanized IgG1 mAb	HER2+ breast cancer	NA	16–12-2020

abbreviations: mAb, monoclonal antibody; IGF-1 R, insulin-like growth factor 1 receptor; CGRP, calcitonin gene-related peptide; TROP-2, trophoblast cell-surface antigen 2; ADC, antibody-drug conjugate; BCMA, B cell maturation antigen; IL, interleukin; CAR, chimeric antigen receptor; NA, not applicable.

facturing, and material engineering (2, 6).

In 2020, amongst 53 products granted the US Food and Drug Administration (FDA) approval, there are 12 antibody therapeutics and a CAR-T cell therapy. Here, this review aimed to explain new immunotherapy drug applications approved by the FDA/EMA in 2020 (Table 1), which belong to 3 main categories: monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs) and CAR-T cell therapy.

2. Monoclonal antibody

Monoclonal antibodies (mAbs) alone or in combination with other drugs, toxins, radionuclide, etc. have received enormous attention during the last decades (7). There are produced by various technologies (Figure 1). They were first generated in mice using hybridoma technology which utilizes the fusion of B lymphocytes derived from an immunized animal's spleen with an immortal myeloma cell line (8). However, the clinical application of the intact murine mAb is hampered by generation of human anti-mouse antibody, increasing the clearance of murine mAb and undesirable allergic reactions (9). Therefore, murine mAbs are transformed by the chimerization technology in which non-human variable domains was combined with human constant (C) region domain to

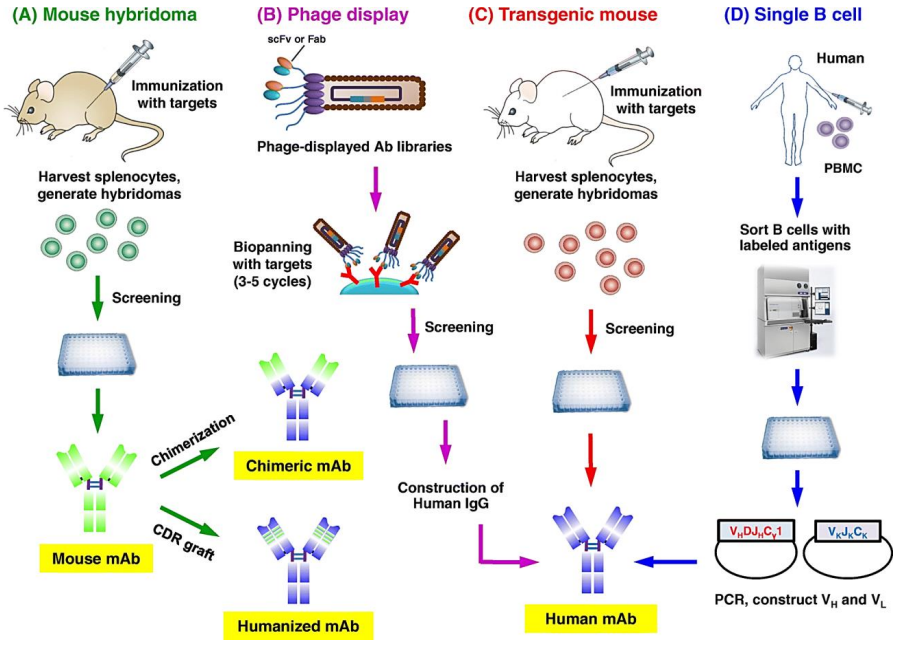

Figure 1. Technologies adopted for generation of therapeutic antibodies: a) murine hybridoma which includes immunization of mice, fusion of harvested splenocytes with myeloma cell line, and screening to select the desired B lymphocyte clone. The selected clones can be used to produce chimeric or humanized antibodies; b) phage display which involves generation of human antibody library in a bacteriophage and selection of single chain antibodies against the desired antigen that can be used for production of human mAb; c) transgenic mouse which is immunized with target antigens, followed by harvesting splenocytes and generation of murine hybridoma for producing human mAb; d) single B cell technique in which the peripheral blood mononuclear cell (PBMC) isolated from the infected or vaccinated donor is screened by flow cytometry, followed by RNA extraction, RT-PCR, and cell-free protein synthesis (CFPS) for production of human mAb (10).

generate molecules with at least 70% human domain content. A great advance in the production of humanized mAbs was through using the complementary-determining region (CDR) grafting technique.

In this technology, non-human antibody domain transferred into a human framework sequence while conserving the target specificity (10). This technology platform has been progressed to obtain fully human monoclonal antibody. In another technique named phage display, exogenous genes are incorporated into bacteriophages to generate a peptide library on the surface of phage virion, allowing screening of phage libraries for binding to the target antigen (11).

Transgenic animals bearing the human antibody repertoire represent another technology to obtain fully human therapeutic antibody after immunizing with target antigen. Abgenix and Medarex are two examples of transgenic mice producing fully human antibodies for oncology indications (7). However, the transgenic mice are unable to exactly imitate a human immune response. Therefore, the single B cell technology has been evolved, which works based on sorting of the patient's isolated B cells labeled with target antigen, and immortalization by transformation with Epstein-Barr virus (EBV) (8). In 2020, 10 mAbs including 4 humanized (Naxitamab, Tafasitamab, Satralizumab, and Inebilizumab), 3 fully human (Ansuvimab, Sacituzumab, and antibody cocktail of Atoltivimab, Maftivimab, and Odesivimab) and 2 chimeric (Margetuximab and Isatuximab) were granted the first approval in the US or European Union (EU) (table 1).

2.1. Eptinezumab in episodic migraine

Eptinezumab (Vyepti^{TM}, Lundbeck) is a humanized IgG1 mAb which blocks calcitonin gene-related peptide (CGRP) alpha and beta. It is

produced in Pichia pastoris yeast cells by the recombinant DNA technology (12). Vyepti™ was approved as the first intravenous (IV) migraine prevention medication for use in USA in February 2020. FDA's approval was based primarily on positive data from two clinical trials (Trial 1/ NCT02559895 and Trial 2/ NCT02974153) of 1741 subjects with chronic or episodic migraine headaches (13). The recommended dose is 100 mg every 3 months although some patients may benefit from a single dose of 300 mg (14).

2.2. Naxitamab in high-risk neuroblastoma

Naxitamab (Danyelza™, Y-mAbs Therapeutics) is a humanized IgG1 mAb against GD2 (hu3F8), a disialoganglioside highly expressed in neuroblastoma. Its binding to GD2 stimulates both complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) (15). Naxitamab is produced in a Chinese hamster ovary (CHO) cell line. The product was first developed at the Memorial Sloan Kettering Cancer Center (MSK) in New York and then commercial rights licensed completely to Y-mAbs therapeutics Inc. for the treatment of neuroblastoma, osteosarcoma and other GD2-positive cancers (16). Naxitamab in combination with granulocyte-macrophage colony-stimulating factor (GM-CSF) was granted an accelerated approval by US FDA in November 2020 for treatment of patients (aged over one year old) with relapsed or refractory high-risk neuroblastoma in bone or in bone marrow (17). The drug approval was based on the results of two open-label trials (Trial 1/NCT03363373 and Trial 2/NCT01757626) in 97 patients with high-risk neuroblastoma in bone or bone marrow. Subjects were received intravenous infusion of naxitamab (3 mg/kg) on days 1, 3, and 5 of each 4-week cycle in combination with subcutaneous injection of GM-CSF at the dose of 250 µg/m2/day on days -4 to 0 and 500 µg/m2/day on days 1 to 5 (18). The results showed an overall response rate of 45% and 34% in the Trial 1 and 2, respectively.

2.3. Tafasitamab and Isatuximab in blood cancers

Tafasitamab (Monjuvi™, MorphoSys Inc.) is a humanized IgG1 anti-CD19 mAbs with a modified Fc domain resulted in increased Fcγ receptor affinity (19). It is produced by the recombinant DNA technology in CHO. In July 2020, Monjuvi™ in combination with lenalidomide received the FDA approval for the treatment of adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) patients who are not eligible for autologous stem cell transplant (20). The efficacy of tafasitamab was evaluated in Phase 2 study (NCT02399085) in 81 patients with relapsed lymphoma. The participants received tafasitamab (12 mg/kg) intravenously in combination with lenalidomide for maximum of 12 cycles. The results demonstrated that the best overall response rate was 55%, with complete responses in 37% and partial responses in 18% of patients (21).

Isatuximab (Sarclisa™, Sanofi), a chimeric IgG1 monoclonal antibody produced in CHO cells using the recombinant DNA technology, is directed against CD38 receptor overexpressed in plasma cells of multiple myeloma patients. In March 2020, intravenous administration of isatuximab in combination with pomalidomide and dexamethasone was approved by the US-FDA for the treatment of adult patients with multiple myeloma who have received at least two prior therapies, including lenalidomide and bortezomib (22). Isatuximab has also received a positive opinion in the EMA in May 2020 for the same indication (19). Sarclisa™ approval was based on the results of multinational, randomized, open-label Phase 3 study (ICARIA-MM, NCT02990338) in 307 patients with relapsed and refractory multiple myeloma. The results showed that the median progression-free survival increased from 6.47 to 11.53 months for the isatuximab- pomalidomide group, compared to the group received pomalidomide and dexamethasone (23).

2.4. Margetuximab in HER-2 positive breast cancer

Margetuximab (Margenza™, MacroGenics) is a chimeric IgG monoclonal antibody against HER2 receptor in breast cancer. Margetuximab binds to the same epitope as trastuzumab, but its Fc domain was engineered to afford increased binding to activating FcγRIIIA (CD16A) and decreased affinity to inhibitory FcγRIIB (CD32B) receptors (24). The FDA approved margetuximab

in combination with chemotherapy for HER2-positive breast cancer in adult patients who have received at least two prior anti-HER2 regimens (25). Efficacy of the margetuximab was evaluated in the phase 3 SOPHIA (NCT02492711) which recruited 536 patients with HER2+ metastatic breast cancer. Results showed that median progression-free survival in the margetuximab-treated patients was 5.8 months compared with 4.9 months in the control trastuzumab group. Moreover, margetuximab reduced the risk of disease progression up to 24% vs. trastuzumab (26).

2.5. Teprotumumab in treatment of thyroid eye disease

Teprotumumab (Teppeza™, Horizon Therapeutics) is a humanized IgG1 antibody against insulin-like growth Factor-I receptor (IGF-IR). It is produced in CHO-DG44 cells. Teppeza™ was designed by Roche Ltd as a therapy for cancers including breast cancer, non-small cell lung cancer, solid tumors, Hodgkin's disease, non-Hodgkin's disease and sarcoma (27). In January 2020, the FDA approved Tepezza™ as the first drug for the treatment of thyroid eye disease, an autoimmune inflammatory disorder characterized with by upper eyelid retraction, lid lag, conjunctivitis, and bulging eyes (19). Tepezza™ approval was based on positive data from two clinical trials (Phase 2, NCT01868997 and Phase 3, NCT03298867) consisting of 170 patients with active thyroid eye disease. Participants received teprotumumab infusions (10 mg/kg on Day 1 and 20 mg/kg every three weeks for the remaining 7 infusions. Results showed that 71% of the drug-treated group in the phase 2 study and 83% in phase 3 clinical trial had more than 2-millimeter reduction in proptosis (eye protrusion), compared to 20% and 10% of people who received placebo, respectively (28).

2.6. Satralizumab and Inebilizumab in autoimmune CNS disease

Satralizumab (Enspryng™, Genentech Inc.) is a humanized IgG2k anti-interleukin-6 (IL-6) receptor monoclonal recycling antibody that produced in CHO cell line by the recombinant DNA technology (29). The antibody was primarily developed in Chugai and licensed to Roche in 2016 (19). In August 2020, the satralizumab granted FDA approval as the first subcutaneous treatment for the neuromyelitis optica spectrum disorder (NMOSD) or Devic disease in adult patients who are anti-aquaporin-4 (AQP4) water channel autoantibody positive. NMOSD is a rare autoimmune disease of central nervous system characterized by damage and acute inflammation of the optic nerve and the spinal cord (30). The safety and efficacy of satralizumab was investigated by two 96-week clinical studies: SAkuraStar (NCT02073279) and SAkuraSky (NCT02028884) which included 95 (64 anti-AQP4 antibody positive) and 76 adult patients (52 anti-AQP4 positive), respectively (31). Results showed the number of NMOSD relapses reduced by 76.5% compared to 41.1% with placebo. Moreover, 91.1% of satralizumab-treated patients were relapse-free after 96 weeks, compared with 56.8% with placebo (21).

Inebilizumab (Uplizna™, Viela Bio) is a humanized anti-CD19 IgG1 mAbs developed for the treatment of autoimmune diseases associated with CD19-expressing B cells. It was produced by the recombinant DNA technology in CHO. Inebilizumab depletes CD19+ B cells and plasmablasts, which are responsible for the production of autoantibodies directed against AQP4. In June 2020, inebilizumab granted FDA approval for the treatment of NMOSD in adult patients who are AQP4-IgG positive. The drug is also undergoing clinical evaluation for kidney transplant desensitization, myasthenia gravis, and IgG4-related disease (32). Uplizna™ FDA approval was based on the positive results of a 28-week Phase 2/3 study (NCT02200770) consisting 230 adult participants with and without the AQP4-IgG antibody. Subjects received two doses of 300 mg Inebilizumab as a monotherapy or placebo at Day 1 and 15. In anti-AQP4 antibody positive patients, the risk of NMOSD relapse reduced by 89% compared to 58% in the placebo group (19).

2.7. Inmazeb TM and Ansuvimab in ebolavirus infection

Atoltivimab, maftivimab, and odesivimab (Inmazeb™, Regeneron) is a combination of three fully human monoclonal IgG1 antibodies - atolt-

ivimab (REGN3470), maftivimab (REGN3479), and odesivimab (REGN3471)–that bind to the glycoprotein on the surface of ebolavirus (EBOV) responsible for the cell entry. Inmazeb™ is produced by the recombinant DNA technology in CHO using VelociSuite® technology in VelocImmune® mice immunized with DNA encoding or purified recombinant EBOV glycoprotein. This antibody cocktail received FDA approval in October 2020 as the first drug for the treatment of infection caused by Zaire EBOV in both pediatric and adult patients (33). In the PALM study (NCT03719586), the effectiveness of Inmazeb was evaluated for 28 days in which the 154 enrolled patients received a single IV infusion of 50 mg of each mAb, and 168 patients received remdesivir as control . During the intervention, 33.8% of Inmazeb-treated patients died vs. 51% of participants who received control drug (34).

Ansuvimab (Ebanga™, Ridgeback Biotherapeutics) is a human IgG1 monoclonal antibody which binds to the glycoprotein on Zaire EBOV, preventing the virus entry into the cell. Ansuvimab is produced in CHO cells by the recombinant DNA technology. In December 2020, Ebanga™ received the FDA approval for the treatment of Zaire EBOV infection in adults, children, or neonates born from a mother who is infected with ebolavirus (35). The safety and efficacy of Ebanga™ evaluated in clinical trial (Trial 1/ NCT NCT03719586) of 342 patients with Zaire EBOV infection. 173 subjects received single IV infusion dose of 50 mg/kg Ebanga™. Among 174 participants who were received Ebanga™, 35.1% died, compared to 49.4% of the 168 patients treated with remdesivir as control (36).

3. Antibody-drug conjugates

Antibody–drug conjugates (ADCs) are designed to deliver antineoplastic agents specifically to the target antigen via conjugation to antibody (37). Design of an ADC relies on an appropriate selection of four key components: target antigen, antibody construct, cytotoxic agent, and linker (Figure 2) (38). Early ADC developments made use of murine mAbs that have not yielded satisfactory results in clinical trials due to their immunogenicity, low potency, suboptimal targeting, and insufficient selectivity for the tumor compared to normal tissue (39). To resolve these issues, murine antibodies are replaced by humanized or fully human antibodies. First-generation ADCs consisting of mAbs linked to conventional chemotherapy agents have not been successful due to low potency of the payload and instability of the ADC in blood circulation resulting in systemic toxicity

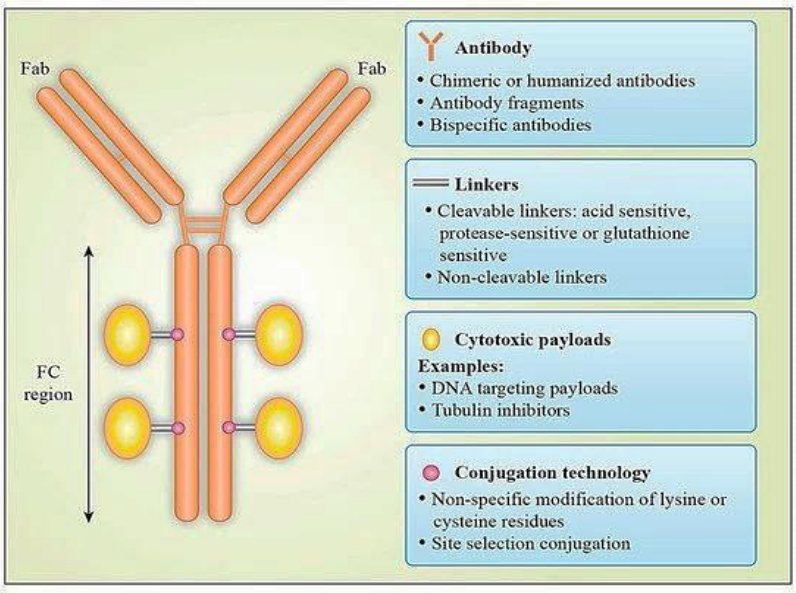

Figure 2. Schematic of an antibody–drug conjugate structure: An antibody–drug conjugate (ADC) comprises three main structural units: the antibody, the cytotoxic payload, and the linker that combines these two parts. Each part should be optimized to enhance potency, safty and efficacy of ADC (44).

(40). Second-generation ADCs utilized potent cytotoxic agents targeting tubulin or DNA with IC50 values in sub-nanomolar range. However, their use can be confronted due to the heterogeneity of the final conjugate, the limited penetration into solid tumors, the development of drug resistance, and the narrow therapeutic index (41). Another issue is the linker chemistry as a crucial part of ADCs defining their stability in the blood circulation and toxicity. The linkers commonly react with the lysine side chains or sulfhydryl groups in the hinge regions of the antibody, resulting in a heterogeneous population of ADCs with an average drug-antibody ratio (DAR) of 3-4 (39). Upon the binding of the antibody to target antigen, the payload can be released in extracellular condition; otherwise, the ADC is internalized and the payload is released due to proteolytic degradation of the entire ADC molecule or the cleavage of the linker in low pH or through proteasome-mediated degradation (38). In the third-generation ADCs, a special concern has been paid to the site-specific conjugation to ensure homogenous ADCs using several technologies such as THIOMAB, ThioBridge, SMARTag, and SMAC-Tag (42). Furthermore, to overcome resistance, novel payloads with a broad spectrum of activity against nonproliferating cancer cells such as topoisomerase inhibitors, pyrrolobenzodiazepine derivatives of tricyclic antibiotics, and duocarmycins are concerned. Mylotarg^{TM} (gemtuzumab ozogamicin) is the first ADC approved by the US-FDA for CD33-positive acute myeloid leukemia (43). Till now, there are eight different ADCs including Gemtuzumab ozogamicin (Mylotarg^{TM}), Brentuximab vedotin (Adcetris^{TM}), Trastuzumab emtansine (Kadcyla^{TM}), Inotuzumab ozogamicin (Besponsa^{TM}), Polatuzumab vedotin (Polivy^{TM}), Enfortumab vedotin (Padcev^{TM}), Trastuzumab deruxtecan (Enhertu^{TM}), and Moxetumomab pasudotox (Lumoxiti^{TM}) approved by the US-FDA, while dozens are in preclinical and clinical development (37). In 2020, Trodelvy^{TM} and Blenrep TM have received US-FDA approval for the treatment of cancer.

3.1. Sacituzumab govitecan in metastatic breast cancer

Sacituzumab govitecan (Trodelvy^{TM}, Immunomedics Inc.) is a third generation ADC comprising of a humanized IgG1 anti Trop-2 antibody which attached to SN-38 as a topoisomerase I inhibitor. SN-38 is covalently linked via a hydrolysable CL2A linker containing a short PEG7 chain for enhancing solubility (Figure 3). After binding to TROP2 receptor, Trodelvy TM is internalized with subsequent release of SN-38, causing DNA damage and cell death (45). In April 2020, sacituzumab govitecan received accelerated FDA approval for the treatment of metastatic triple-negative breast cancer in adult patients who have received at least two prior therapies (46). Effectiveness and safety of Trodelvy^{TM} was demonstrated in multicenter, single-arm clinical trial IMMU-132-01 (NCT 01631552) that enrolled 108 patients with metastatic triple negative breast cancer. Sacituzumab govitecan was administrated at 10 mg/kg intravenously on days 1 and 8 every 21 days. According to results, the assessed overall response rate was 33.3% and the median response duration was 7.7 months (47). Sacituzumab govitecan is undergoing phase III clinical trial for breast cancer and phase II study in urothelial cancer in the US and EU. It is also under investigation for brain metastases, glioblastoma, endometrial cancer and prostate cancer (45).

3.2. Belantamab mafodotin in multiple myeloma

Belantamab mafodotin (Blenrep^{TM}, GlaxoSmithKline) comprises a humanized IgG1 antibody targeting B cell maturation antigen (BCMA) attached to the microtubule inhibitor monomethyl auristatin F (MMAF) through a non-cleavable linkage (Figure 3) (48). The drug is developed using POTELLIGENT technology licensed from BioWa to improve the efficacy of ADC through reducing the fucose content of the carbohydrate structure of mAb produced in fucosyl transferase-knockout CHO cell line. After Blenrep^{TM} binding to BCMA and cellular internalization, the linker is cleaved by lysosomal proteases to provide intracellular delivery of MMAF and subsequently cell cycle arrest. In August 2020, the FDA and the EMA granted approval to Belantamab mafodotin for the treatment of adult patients with relapsed or refractory multiple myeloma who have received at least four prior therapies, including an

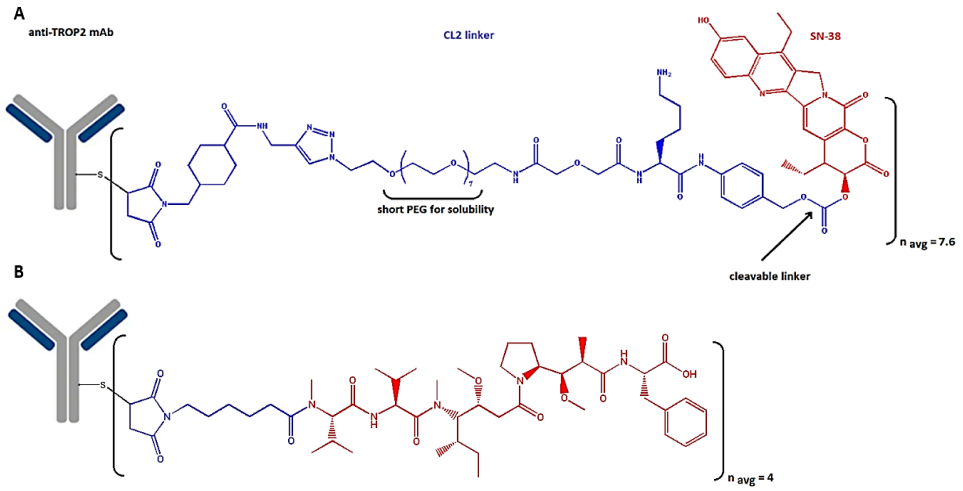

Figure 3. The structure of A) sacituzumab govitecan consisting an anti Trop-2 antibody attached to SN-38 via a hydrolysable CL2A linker containing a short PEG7 chain, and B) belantamab mafodotin consisting an antibody targeting B cell maturation antigen (BCMA) attached to the microtubule inhibitor monomethyl auristatin F (MMAF) through a non-cleavable linkage (50).

anti-CD38 mAbs, a proteasome inhibitor, and an immunomodulatory agent (19). Belantamab mafodotin was evaluated in the open-label, multicenter DREAMM-2 (NCT03525678) study. Patient received the dose of 2.5 mg/kg every 3 weeks revealed the overall response rate of 31% while 73% of responders exhibited response durations more than 6 months (49).

4. CAR-T cell therapy

CAR-T cells are composed of T cells isolated from peripheral blood and CAR (chimeric antigen receptor) (51). For CAR T-cell therapy, T cells are isolated from the patient's blood that are transfected with a plasmid DNA or transduced with a viral vector, presenting a new antigen on the T-cell surface enabling redirection of T cells to specific antigens in tumor cells (52). The molecule of CAR consists of three segments: 1) an extracellular domain containing a single-chain fragment variable directed against a specifically targeted antigen. This domain is connected to 2) a transmembrane domain, part of CD3, CD8, CD28 or FcεRI, which is bound to 3) an intracellular domain, consisting of an activating domain (CD28, CD27, CD134, CDB7, or CD3ζ) with or without a second costimulatory factor (CD28, or 4-1BB) (52). Structure of different CAR generations are characterized by various intracellular signaling domains (Figure 4). The original CAR framework contains an intracellular CD3ζ signaling domain suffering from insufficient signaling power. First-generation CARs can specifically detect tumor antigens, but their therapeutic effect is unsatisfactory in vivo due to reduced proliferative ability. Second-generation CARs contain also costimulatory domains such as CD28 and 4-1BB, exhibiting improved cell proliferation. In third generation CARs, two different costimulation signals such as CD28 and CD137 are involved at the same time. Fourth generation CARs comprises cytokines, improving the tumor cytotoxicity by overcoming the immunosuppressive tumor microenvironment (51). The cytotoxicity of CAR-T lymphocytes is based on two principal pathways: (1) secretion of perforin and granzyme granules and (2) activation of death receptor signaling via Fas or TNF receptor. As CAR specifically binds with the tumor associated antigens (TAAs) on cancer cells, T cells are activated through the phosphorylation of immune receptor tyrosine-based activation motifs (ITAMs), which provokes cytokine secretion, T-cell proliferation, and cytotoxicity (53). Kimria^{TM} and Yeskarta TM are the CAR T-cell drugs approved by the US-FDA in 2017 for the treatment of leukemia and lymphoma (54). In July 2020, the FDA approved the third CAR T-cell drug called Tacartus TM for the treatment of mantle cell

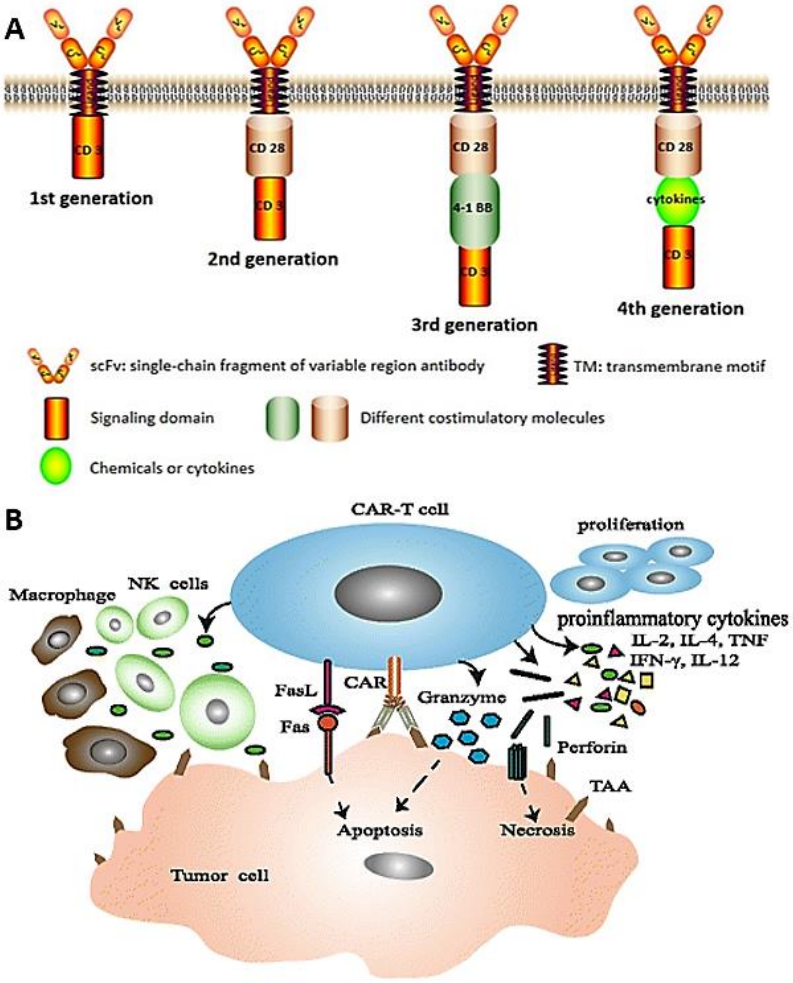

Figure 4. A) The generations of chimeric antigen receptor (CAR). The first-generation CARs contain an intracellular CD3ζ signaling domain that forms the second generation if combined with a costimulatory domain (mostly CD28). Third generation CAR involves additional costimulatory domains, such as CD28 or CD137. Fourth generation CARs incorporate additional gene cassette expressing cytokines (51). B) CAR-T cells mechanism of anti-tumor cytotoxicity: T-cells are activated through the phosphorylation of immune receptor tyrosine-based activation motifs (ITAMs) followed by enhanced cytokine secretion (including IL-2, IL-4, IFN-γ, IL-12, and TNF) and T-cell proliferation. IL-12 reinforces the function of immune cells such as NK cells and macrophages. Activated CAR-T cells kill tumor cells mainly through secretion of perforin and granzyme granules, as well as Fas/Fas-L death receptor pathway (55).

lymphoma (MCL).

Brexucabtagene autoleucel (Tecartus^{TM}, Kite Pharma) is an anti-CD19-transduced CD3+ cells designed for autologous T-cell immunotherapy in adult patients with relapsed or refractory mantle cell lymphoma (MCL). Tecartus^{TM} contains the patient T cells that is genetically reprogrammed by inserting an artificial receptor gene using an MSCV based gamma-retroviral vector to express a CAR consisting of anti-CD19 single chain variable fragment (scFv) bound to CD28 co-stimulatory domains and CD3-zeta signaling domain. The patient receives the modified T cell suspension by intravenous infusion. The modified T cells presenting the CAR on their surface can bind to CD19 protein on cancer cell membrane and subsequently the CD28 co-stimulatory domain and CD3-zeta signaling domain initiate downstream signaling cascades, causing the T-cell activation and secretion of inflammatory cytokines and chemokines (56). This chain of events leads to death of target cells. Each dose of Tecartus^{TM} contains

2×106-2×108 CAR-positive viable T cells/kg of patient body weight in an approximate volume of 68 ml (57). The FDA granted accelerated approval to Tecartus^{TM} in July 2020 based on a multicenter, single-arm trial ZUMA-2 (NCT02601313) enrolling 74 patients with relapsed or refractory MCL who had previously received anthracycline or bendamustine, and a Bruton tyrosine kinase inhibitor chemotherapy. The results showed the complete remission rate of 62% and the response rate of 87% (58).

5. Conclusion

Immunotherapy is a rapidly growing field, with a series of new treatment approved by the FDA/ EMA in 2020. Apart from their therapeutic indications in cancers, mAbs approved this year for the first time in treatment of EBOV infection, migraine, an autoimmune CNS disease, and thyroid eye disorder. This year also brought two ADCs for mAb-targeted treatment of metastatic breast cancer and multiple myeloma. Moreover, the third CAR-T cell therapeutic got FDA approval for the treatment of mantle cell lymphoma. Despite the complexity and ambiguity of the technological developments, ADCs and CAR-T cell therapy have great potential in cancer therapy in future.

Conflict of Interest

None declared.

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