CAR T-cell therapy for gastric cancer: Potential and perspective (Review)

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Abstract. Gastric cancer (GC) is one of the most frequently diagnosed digestive malignancies and is the third leading cause of cancer-associated death worldwide. Delayed diagnosis and poor prognosis indicate the urgent need for new therapeutic strategies. The success of chimeric antigen receptor (CAR) T-cell therapy for chemotherapy-refractory hematological malignancies has inspired the development of a similar strategy for GC treatment. Although using CAR T-cells against GC is not without difficulty, results from preclinical studies remain encouraging. The current review summarizes relevant preclinical studies and ongoing clinical trials for the use of CAR T-cells for GC treatment and investigates possible toxicities, as well as current clinical experiences and emerging approaches. With a deeper understanding of the tumor microenvironment, novel target epitopes and scientific-technical progress, the potential of CAR T-cell therapy for GC is anticipated in the near future.

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1. Introduction

Immunotherapies utilize monoclonal antibodies (mAbs), immunological checkpoint blockade (ICB) agents, cytokine-induced killer cells, tumor-infiltrating lymphocytes (TILs) and T-cell receptors (TCRs). In recent years, the rapid development of immunotherapies has produced novel treatment options for many different types of cancer (1,2). The most attractive feature of tumor immunotherapy is the ability to control or eliminate tumors by restarting and maintaining the tumor-immune cycle *in vivo*, as well as stimulating and restoring the body's normal anti-tumor immune response (3). However, in contrast to other adoptive cell transfer therapies, chimeric antigen receptor (CAR) T-cells recognize tumor surface-associated antigens directly, independent of the major histocompatibility complex (MHC) restriction (4). The use of anti-CD19 CAR T-cells for the treatment of chemotherapy-refractory hematological malignant tumors has revealed encouraging results, including effective targeting, killing and persistence (5). Furthermore, its use has provided novel solutions for immune cell therapy, demonstrating the tremendous potential for the development and clinical application of CAR T-cell therapy (6,7). Significant improvements in the efficacy of CAR T-cell therapy for hematological malignancies have prompted its development for use in solid tumors (8).

Gastric cancer (GC) is one of the most frequently diagnosed digestive malignancies and is the third leading cause of cancer-associated death worldwide (9). According to the CONCORD-3 (10) statistical data of GC obtained from 62 countries in 2010 to 2014 revealed that 29 countries exhibited a 5-year survival rate <30%, occupying 46% of all countries studied. Furthermore, existing conventional treatments, including surgery, chemotherapy and radiotherapy, have limited efficacy in GC; thus, there is an urgent need for novel therapeutic strategies. In contrast to TCR and ICB immunotherapy, the study of CAR T-cells is still in its infancy and appears less efficacious for GC. However, producing an effective CAR T-cell treatment for GC (11,12)

may be possible as the Food and Drug Administration have approved two second-generation CAR T-cell therapies, for the treatment of relapsed/refractory B-cell lymphoma: Kymriah (CD28/CD3ζ costimulatory domain) and Yescarta (4-1BB/CD3ζ costimulatory domain). Preclinical studies have demonstrated the anti-tumor efficacy and persistent activity of CAR T-cells against GC *in vitro* and *in vivo* using an animal xenotransplantation model (13-17).

The current review assessed the potential of CAR T-cell immunotherapy for patients with GC and discussed the history of its development, its current status and toxic side effects, as well as the management of these toxicities.

2. The development and characteristics of CAR T-cell therapy

Tumor immunotherapy has been prevalent for >100 years, with CAR T-cell therapy being developed in the last ~30 years. The first-generation CAR, derived from a chimeric TCR, was pioneered and constructed by Eshhar *et al* in 1993 (18,19). First-generation CARs are modular in nature, containing a single-chain variable fragment (ScFv) and CD3ζ domains, and they inhibit tumor cell escape by downregulating the expression of MHC on the surface of tumor cells (20). To address the poor cytokine production and T-cell expansion observed in first-generation CARs (21), Finney *et al* (22) constructed a second-generation CAR that incorporated a costimulatory domain. The superiority of this second-generation CAR in cytokine-secretion and in T-cell expansion and persistence has been demonstrated in several studies (23-26) (Fig. 1A). Using second-generation CAR as a foundation, a third-generation CAR was created, which contained two tandem costimulatory molecules. The third-generation CAR exhibited enhanced effector functions and persistence *in vivo* (27). However, to further enhance targeted anti-tumor and trafficking activities of CARs in solid tumors and to reduce off-target toxicity and immunosuppression, multiform fourth-generation CARs were constructed using novel mechanisms, for example, T-cells redirected for universal cytokine-mediated killing, armored CARs, switchable CARs, bispecific CARs and CARs incorporating a suicide gene have been created (28). In addition, scientists are working to uncover a universal CAR structure to act against all target cells with an optimal outcome.

CAR is an artificially synthesized membrane protein composed of three domains: An extracellular antigen-recognition domain, a transmembrane domain and an intracellular signaling domain (29) (Fig. 1A). The single-chain variable fragment (ScFv) is a recombinant polypeptide derived from the heavy and light chains of a monoclonal antibody, which binds directly to the tumor surface-associated antigens, independently from MHC restriction (30). The hinge region provides ScFv flexibility and is associated with the target-binding capacity of the CAR (31). The transmembrane domain, primarily consisting of CD8 or immunoglobulin G4 molecules, enhances CAR stability and provides a connection between the ectodomain and endodomain (32). In the intracellular domain, CD3ζ or Fc receptor γ provides the first signal for T-cell activation (33). Although the B7-CD28 pathway provides essential signals for T-cell activation, further studies have revealed that CD3ζ has a more optimal signaling efficacy (34,35). Additionally, the endodomain commonly contains costimulatory signal domains that promote T-cell proliferation, lymphokine secretion and effector function, including CD28 (36), inducible T-cell costimulator (34), DNAX-activating protein 10 (DAP10) (37), CD134 (OX40) (38) or CD137 (4-1BB) (39), which have also been studied successively in different generations of CARs (27). CD28 promotes the multiplication of naïve and $CD4^+$ T-cell subsets, whereas costimulatory CD137 promotes the proliferation of memory and $CD8^+$ T-cell subsets preferentially, improving persistence (40). CD28 has been demonstrated to promote the ability of CARs to enhance the resistance of modified T-cells against regulatory T-cells and to reduce antigen-induced cell death (41). However, CD137 enhances the metabolic adaptability and memory potential of CAR T-cells to a greater extent than CD28 (42,43). Despite the aforementioned costimulatory molecules exhibiting antigen-dependent immune-cytolysis *in vitro*, there is still debate over which costimulatory molecule is most optimal (44). Previous evidence has suggested that the functional activity induced by T-cell-expressed CARs depends on the interaction of endogenous signaling moieties (45).

3. A novel and promising choice of immunotherapy

Based on previous clinical applications of adoptive immunotherapies, including TILs, CAR T-cell therapy was designed for the treatment of various types of cancer. CAR T-cell therapy is a complex and rigorous multi-step adoptive cell transfer therapy as indicated in Fig. 1B (46).

Following a decade of study, the curative effect of CAR T-cells in hematological malignancies has provided valuable information. First-generation anti-CD19 CAR T-cells were demonstrated to persist for 6 months at high levels in peripheral blood and bone marrow. Kochenderfer *et al* (47) first reported that a chemotherapy-refractory patient with stage IV B-cell non-Hodgkin lymphoma (B-NHL) achieved partial remission lasting for 8 months after receiving anti-CD19 CAR T-cell therapy. Subsequently, a patient with refractory chronic lymphocytic leukemia achieved a 10-month complete remission (CR) (48). CD20, a second form of CAR T-cell treatment administered to patients with B-NHL also demonstrated similar results (32,49). However, a phase II trial of anti-CD20 CAR T-cell therapy achieved promising effects without inducing severe toxicities, with an overall objective response rate (ORR) of 81.8% (8/11) and six patients with B-NHL demonstrating CR (50). The curative efficacy of CAR T-cells in hematological malignancies has improved, with ORR rates increasing from 52 to 92% and CR rates ranging from 43 to 90% (51-55). Furthermore, encouraging results from the use of CAR T-cells for the treatment of B cell malignancies has resulted in the application of this therapy to solid tumors.

The first CAR T-cell therapy clinical trials were performed two decades ago in the USA for the treatment of patients with ovarian cancer and metastatic renal carcinoma (56,57). To date, a total of 692 clinical trials have been registered worldwide on ClinicalTrials.gov, which is over three times the total number of registrations recorded at the end of 2016 (Fig. 2A). Of these clinical trials, >400 are associated with cancer therapy. Currently, the majority of clinical trials are in phase I or II, where appropriate dosage, safety and efficacy

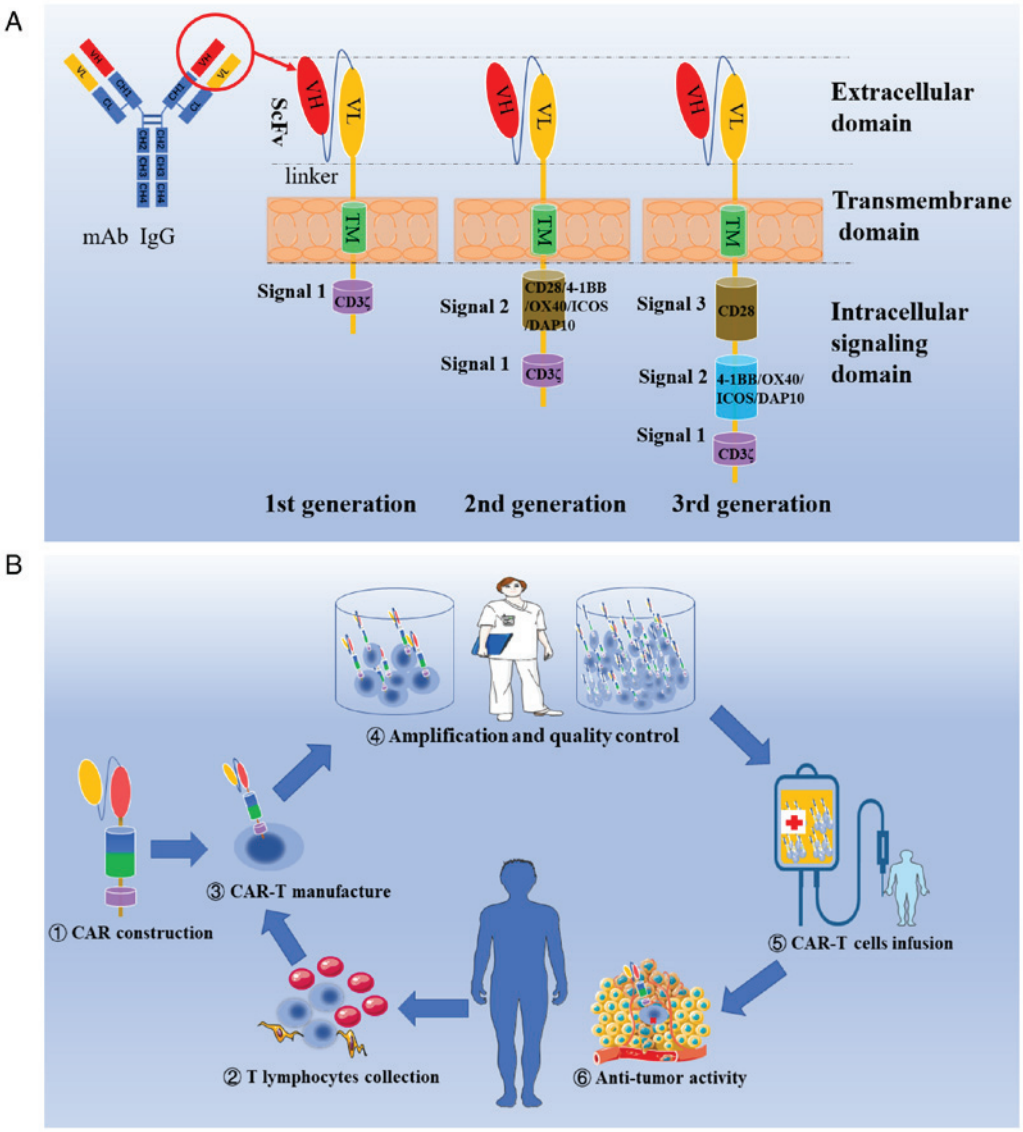

Figure 1. CAR-T cell structure and the clinical application process. (A) The construction of 1st, 2nd and 3rd generation CARs. (B) The clinical treatment is as listed: i) CAR construction: ScFv is used as the ligand-binding domain to mediate tumor cell recognition with heavy chain variable and light chain variable, and is connected to the transmembrane and intracellular domains with a flexible linker; ii) T lymphocyte collection: T lymphocytes are isolated from the PBMCs of patients with cancer; iii) CAR T-cell manufacturing: CAR genes are retrovirally transduced into T lymphocytes; iv) CAR T-cell amplification and screening *in vitro*; v) Quality control: Evaluation of the expansion level, T cell quality, cytokine secretion and infectious contamination; vi) CAR-T cells are infused back into patients; vii) Anti-tumor activity: CAR-T cells are transported to the tumor site and perform their function in the tumor microenvironment. CARs, chimeric antigen receptors; ScFv, single-chain variable fragment; VH, heavy chain variable; VL, light chain variable; PMBC, peripheral blood mononuclear cells; TME, tumor microenvironment; mAb, monoclonal antibody; IgG, immunoglobulin G; TM, transmembrane domain; ICOS, inducible T-cell costimulator; DAP10, DNAX-activating protein 10.

is being established. Only 8% of CAR T-cell therapy clinical trials have been completed (Fig. 2B).

4. Promising preclinical results for future clinical investigation

A single high fidelity target antigen is the most critical factor for the successful clinical application of CAR T-cell therapy (58). Previous literature has indicated that an ideal specific antigen must be expressed on the extracellular surface of cancer cells and be preferentially selected for its density and differential expression in tumors rather than in normal tissues (59). If this does not occur, severe or lethal off-target toxicity, in addition to poor curative effects, may occur (59). The expression of surface antigens in GC is highly heterogeneous, providing tumor cells with the ability to escape host immune surveillance (60). Therefore, the design of CAR T-cell immunotherapy for GC poses a great challenge.

However, promising results have been obtained using preclinical models of first-generation CAR T-cells for the treatment of ovarian cancer (57), renal cell carcinoma (57,61) and neuroblastoma (62). Furthermore, the durable efficacy of CAR T-cell therapy has been high in patients with recurrent or end-stage glioblastoma, demonstrating anti-tumor activity with acceptable toxicities in subsequent GD2-targeting trials (62,63). In murine GC models and *in vitro* experiments, the anti-tumor activity and persistence of CAR T-cells targeting

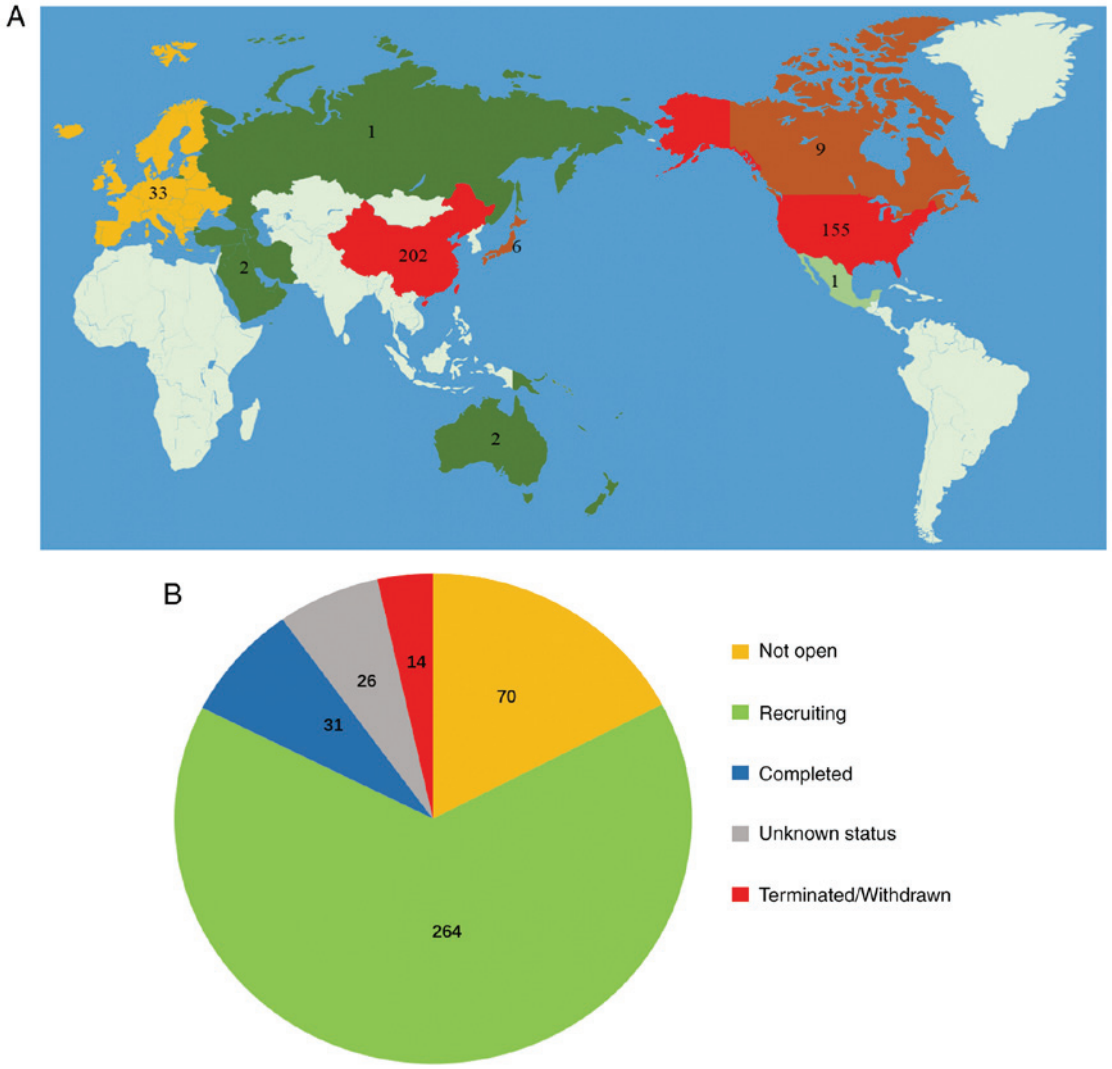

Figure 2. Registered CAR-T cell clinical trials. (A) The geographical distribution of registered clinical trials for CAR T-cell therapy. (B) The status of CAR T-cell clinical trials performed for cancer therapy. CAR, chimeric antigen receptor.

folate receptor 1 (FOLR1), 3H11 and human epidermal growth factor receptor 2 (HER2) has been validated (13-17).

Kim *et al* (15) constructed a second-generation CAR T-cell consisting of FOLR1-scFv, CD28 and CD3ζ signaling domains. The cytotoxicity of this CAR T-cell construct against GC cells was assessed using a luciferase assay. Furthermore, Western blot analysis and ELISA demonstrated, elevated levels of apoptosis-associated proteins and cytokines, respectively. These proteins and cytokines, including interferon (IFN)-γ, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor and granzyme B are crucial for T-cell activation, proliferation and differentiation in target GC cells (15).

In a xenograft subcutaneous mouse model, significant tumor-killing abilities of CAR-T cell have been demonstrated in MKN1 cells (16). An additional HER2-specific CAR T-cell construct has exhibited specific and persistent anti-tumor efficacy, along with a strong homing ability against xenografts derived from $HER2^+$ GC cell lines in mice (16). Similarly, specific tumor-killing abilities and high affinities were also verified in primary patient-derived GC cells through intravenous infusion, which also occurred during HER2 expression knockdown, and these positive outcomes were further investigated by constructing humanized chA21-4-1BBz CAR T-cells (13). Additionally, striking tumor inhibition was observed in an established and advanced intraperitoneal metastatic GC model (13). As a major component of the ErbB2 (CD340) family, HER2 is highly expressed on gastrointestinal epithelial cells and has been extensively investigated as a potential immunotherapy target for various solid tumors (64). The monoclonal antibody, trastuzumab, has been approved as first-line treatment following its successful clinical application against advanced GC (65). Furthermore, following the intravenous injection of HER2-directed CAR T-cells, the tumorigenicity of cancer stem cells (CSCs) derived from patients with GC was markedly inhibited in a tumor-bearing mouse model and was efficiently phagocytized and degraded *in vitro* via a sphere-forming assay (16). Previous studies have indicated that HER2 signaling serves an important role in maintaining CSC populations in GC (66-68). Thus, the eradication of CSCs that possess a capacity for clonal tumor initiation and contribute to carcinogenesis, tumor invasion, recurrence, metastasis and drug resistance, has been identified as a promising immunological approach

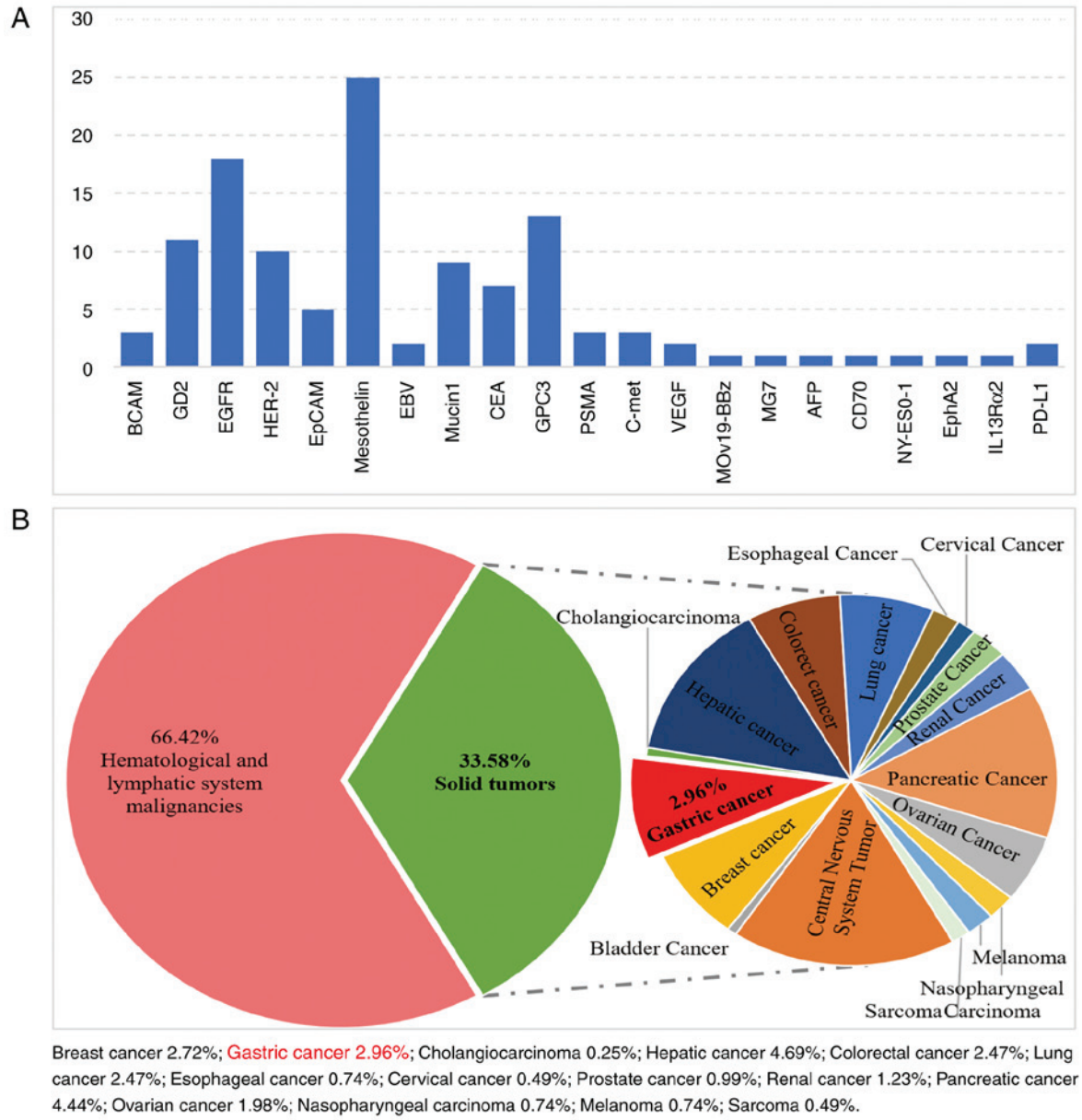

Figure 3. (A) CAR-T target antigen selection and treatment information of different tumors. Target antigens used in the construction of chimeric antigen receptor T-cells for solid tumor therapy. (B) The percentage of solid tumors compared with hematological and lymphatic system malignancies, as well as the percentage of solid tumors that are gastric cancers.

for cancer treatment (69). Luo *et al* (17) constructed a bifunctional αHER2/CD3 RNA-engineered CAR T-cell with a more effective and specific tumor-killing capacity to reduce the possibility of tumor antigen escape and to transfer these attributes to bystander T-cells, which exhibited similar effects against $HER2^+$ GC cells. Additionally, the persistence duration of this bispecific αHER2/CD3 CAR T-cell *in vivo* was 6 days, outlasting other conventional bispecific CAR T-cells (70). Third-generation 3H11-directed CAR T-cells also exhibited similar cytotoxicity and secretion *in vitro* and *in vivo*, while poor trafficking was observed by tail intravenous injection (14). The HER2-directed CAR T-cell therapeutic approach has been continually developed and validated in different types of cancer, including breast cancer (71), renal cancer (72) and osteosarcoma (73). It is worth noting that adverse toxicities may occur unnoticed due to the evaluation of therapeutic effect being implemented on diverse tumor-bearing mouse models. However, CAR T-cell therapy is still considered to have great potential in GC treatment and therefore warrants further clinical development.

5. Exploration of GC treatment in the clinic

A major priority for the development of GC CAR T-cell immunotherapy is the discovery and validation of authentic and specific antigens which minimize potential life-threatening complications. Clinically, various antigens have been targeted for CAR T-cell therapy in solid tumors. These include: Epidermal growth factor receptor, mesothelin, GPC3, GD2 and HER2 (Fig. 3A). On account of the constraints applied to the selection of optimizing antigens (74), only 38% of trials are performed on solid tumors, of which 2.96% are for GC

Table I. CAR-T cell therapy trials for gastric cancer registered in ClinicalTrials.gov.

Targeted antigen	Study phase	Age (years)	Estimated no. of patients	Status	Study institution	Estimated end date	ClinicalTrials number
EPCAM	II	≤75	19	Recruiting	Anhui Province Hospital, Hefei, China	2019 Nov	NCT02725125
EPCAM	I	18-75	40	Recruiting	West China Hospital, Chengdu, China	2022 Dec	NCT03563326
MUC1	I	18-80	20	Unknown	PersonGen Bio Therapeutics, Suzhou, China	2018 Nov	NCT02617134
CEA	I	18-80	75	Recruiting	SHTMMU, Chongqing, China	2019 Dec	NCT02349724
HER2	I/II	18-80	60	Recruiting	SHTMMU, Chongqing, China	2019 Sep	NCT02713984
EPCAM	I/II	18-80	60	Recruiting	ICE of Chengdu Medical College, Chengdu, China	2022 Dec	NCT03013712
Mesothelin	I/II	4-70	73	Recruiting	TFAHZZU, Zhengzhou, China	2023 Mar	NCT03638206
CEA	I	≥18	18	Recruiting	Rutgers Cancer Institute, New Jersey, USA	2019 Sep	NCT03682744
CEA	I	≥18	8	Not recruiting	RWMC, Rhode Island, USA	2019 Jan	NCT02416466
HER2	I	≥18	39	Not open	Baylor College of Medicine, Texas, USA	2037 Jan	NCT03740256
BPX-601	I /II	≥18	138	Recruiting	Moffitt Cancer Center Tampa, Florida, USA	2020 Dec	NCT02744287
EGFR	I /II	18-65	20	Recruiting	Shanghai International Medical Center, Shanghai, China	2018 Mar	NCT02862028

Male and female patients were recruited into each listed study. CAR, chimeric antigen receptor; EPCAM, epithelial cell adhesion molecule; MUC1, mucin 1 cell surface associated; CEA, carcinoembryonic antigen; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; SHTMMU, Southwest Hospital of the Third Military Medical University; ICE, The First Affiliated Hospital of Chengdu Medical College; TFAHZZU, The First Affiliated Hospital of Zhengzhou University; RWMC, Roger Williams Medical Center Providence.

(Fig. 3B). There are still no published clinical outcomes of CAR T-cells used for GC treatment. Therefore, the current review summarized the clinical trials registered on ClinicalTrial.gov. As presented in Table I, a total of 12 registered clinical trials, utilizing seven different antigens, are distributed in China and the USA, the majority of which are in the recruitment phase. The eligibility criteria for participants were as follows: Individuals aged between 18 to 75 years, without restrictions of sex or nationality. A good physical condition was required, which was quantified as an Eastern Cooperative Oncology Group score of ≤2 or a Karnofsky score of ≥60 (75,76). Currently, the majority of trials are conducted for orthotopic GC sites via intravenous injection, while only two ongoing trials (trail nos. NCT03563326 and NCT03682744) have investigated the risk and potential benefits of CAR T-cell intraperitoneal infusion for patients with epithelial cell adhesion molecule- and carcinoembryonic antigen-expressing GC with peritoneal metastasis. Despite the support of previous research, each clinical trial is conducted discreetly, with strictly controlled input dosages, interval times and monitoring indicators, to minimize potentially life-threatening accompanying side effects.

6. Severe side effects

CAR T-cell therapy has produced a durable remission in a subset of patients with relapsed or refractory hematological malignancies (5); however, its efficacy in GC is yet to be fully elucidated. Severe toxicity is a main restriction to the promotion and development of CAR T-cell therapy for patients with GC (47,51). The most common and serious toxicity is cytokine release syndrome (CRS), a non-antigen-specific toxicity that leads to respiratory distress syndrome and multiple organ dysfunction syndrome (MODS). This toxicity occurs due to the rapid and excessive activation of various cytokines, including TNF-α, interleukin (IL)-1, IL-6, IL-8, IL-12, IFN-α, IFN-β and IFN-γ (77). Lymphocyte-depleting chemotherapy regimens, including fludarabine or cyclophosphamide, enhance the activation of CAR T-cells in the human body and are associated with CRS and neurotoxicity (78). In one instance, a patient with colon cancer immediately developed rapid respiratory distress and ultimately died of MODS 5 days following treatment. The death resulted from normal cardiopulmonary tissue with slight HER2 expression being recognized and attacked by high-affinity targeting CAR T-cells (79). Additionally, a clinical trial was suspended due to manufactured anti-CD19-redirected CAR T-cells inducing CRS, resulting in two deaths (80). Clinical symptomatology of CRS, on-target off-tumor toxicity and neurotoxicity of CAR T-cells are summarized in Table II (81-83). The majority of complications are reversible and self-healing. However, fatal complications as a result of CRS and neurotoxicity emphasizes the importance of assessing the preclinical safety of CAR T-cell therapy (79,84,85). Biological informatics analyses that predict target protein distributions in human organs are incomplete and the superior penetrability of CAR T-cells in

Table II. Toxicities of CAR-T cell therapies.

Toxicity	Organ system	Clinical symptomatology
Cytokine release syndrome	Constitutional	Fever, rigors, fatigue, arthralgias, anorexia, myalgias and malaise
	Hematologic	Anemia, lymphopenia, thrombocytopenia, febrile neutropenia, B-cell aplasia, elevated d-dimer, hypofibrinogenemia, prolonged prothrombin time and activated partial thromboplastin time
	Cardiovascular	Tachycardia, arrhythmias, hypotension, Q-T prolongation, widened pulse pressure and variable cardiac output
	Pulmonary	Hypoxia and tachypnea
	Hepatic	Transaminitis and hyperbilirubinemia
	Renal	Acute kidney injury, hyponatremia, hypokalemia, hypophosphatemia, tumor lysis syndrome and azotemia
	Gastrointestinal	Nausea, emesis, vomiting, diarrhea and elevated creatine kinase
	Musculoskeletal	Weakness and elevated creatine kinase
Neurotoxicity	Brain	Headache, mental status changes, confusion, delirium, aphasia, hallucinations, tremor, seizures, somnolence and weakness
	Limbs	Focal motor and sensory defects and altered gait
Off-target/on- target toxicities	Multi-organ	Hepatic, gastrointestinal, respiratory, cardiovascular, endocrine, and neurological dysfunctions, fatal pulmonary complications and B cell aplasia
Tumor lysis syndrome	Multi-organ	Fatigue, fever, rigors, diaphoresis, anorexia, nausea and diarrhea

CAR, chimeric antigen receptor.

solid tissue limits the use of safety-associated conclusions drawn from studies with mAbs (86). A patient with chronic lymphoid leukemia was diagnosed with tumor lysis syndrome on day 22 following anti-CD19-redirected CAR T-cell infusion. However, the kidney and hepatic function of the patient recovered after fluid resuscitation and rasburicase treatment (trail no. NCT01029366) (32). Therefore, accumulating evidence has indicated that CAR T-cell-associated toxicities may be minimized or controlled using preventive or protective interventions (87). Furthermore, well-controlled liver toxicity may be achieved by blocking antigenic sites in tumors that are distant to the tumor (88).

7. Toxicity management and guidelines for future clinical applications

Cancer immunotherapy aims to eradicate malignant cells by harnessing the power of the human immune system. While CAR T-cells attack targets on the surface of tumor cells to exert its therapeutic effect, they also cause inevitable harm to normal tissues in other organs of the body. Therefore, early recognition, vigilant monitoring and timely intervention are necessary to reduce CAR T-cell-associated toxicity (82,89). Thus, based on the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0), toxicity grading systems are considered to be an important measure for standardized treatment (90). Furthermore, according to the Experimental Transplantation and Immunology Branch of the National Cancer Institute (NCI), a normal cardiovascular system and a healthy bone marrow function may reduce the incidence of potential adverse toxicities, demonstrating the necessity for adequate patient condition assessment before receiving CAR-T therapy (82). It has been reported that IL-6 and C-reactive protein can be used as highly sensitive biomarkers for the diagnosis and potential quantification of CRS severity (90,91). Previous studies have also indicated that the IL-6 receptor antagonist, tocilizumab, can attenuate or eliminate CRS toxicities without affecting the efficacy of CAR T-cell infusion (44,92). In addition, corticosteroids and other immunosuppressive drugs (including etanercept, siltuximab and anakinra) have been effectively applied to reduce CRS-associated toxicities (93). However, due to the inhibition of CAR T-cell anti-tumor efficacy and persistence, these drugs are administered second to tocilizumab (93). Neurotoxicity, which may be associated with the increased permeability of cerebrospinal fluid, often occurs concurrently with CRS due to the blood-brain barrier, resulting in the wide usage of dexamethasone and corticosteroids instead of tocilizumab (94).

Despite clinical practice experience being derived from the use of CAR T-cells or treatment against hematological malignancies, previous studies are valuable for the future management of CAR T-cell-associated toxicities in GC therapy.

8. Emerging approaches against GC treatment

Although CAR T-cell therapy is promising, several challenges must be overcome to improve its efficacy for the clinical treatment of GC. Due to the ubiquitous expression of CD19 in the B cell lineage, infections associated with B cell deficiency or hypoplasia can be prevented or

alleviated by immunoglobulin intervention, providing the rationale for the use of CD19 CAR T-cells against hematological tumors (95,96). Similarly, the efficacy of CAR T-cell therapy largely depends on the selection of an ideal epitope target unique to GC that will also prevent off-target effects. A single GC-associated surface neo-antigen is optimal but time-consuming. Thus, a multi-targeted approach is advocated as a promising solution for CAR T-cell efficacy and safety *in vivo* (97). An additional issue to overcome is the limitation of complex tumor microenvironments (TME): GC cells generate a physical and metabolic barrier characterized by hypoxia, nutrient starvation and cytokine secretion, contributing to tumorigenesis and facilitating CAR T-cell tolerance (98). It has been indicated that combined pre-condition treatment, including chemotherapy, radiotherapy, immune checkpoint molecules and other drugs involving small molecules, may contribute to the removal of regulatory T lymphocytes. This makes the TME permissive for immunotherapy and for the improvement of antitumor effects (99,100). However, compared with traditional cell experiments, GC organoids can simulate the GC microenvironment *in vitro* and accurately assess the specific efficacy and toxicities of CAR T-cells for GC *in vitro* (101). Traditional subcutaneous tumor implant and patient-derived xenograft models have the disadvantage of not simulating human immunity and human-derived tumors, resulting in different preclinical and clinical study outcomes (102).

Further study assessing GC CAR T-cell therapy should focus on the following aspects: i) Seeking ideal CAR T-cell therapeutic targets with higher positive expression rates in GC tissues; ii) clarifying the specific role of other combined precondition treatments used in CAR T-cell therapy for GC; and iii) developing a novel GC organoid model and humanized tumor implantation model to improve the reliable evaluation of CAR T-cell efficacy and toxicity in preclinical research. Additionally, the development of a generic CAR structure may lead to an increase in the number of patients with GC benefiting from CAR T-cell therapy, causing a reduction in medical costs.

9. Conclusion and perspective

CAR T-cell immunotherapy is confronted with many challenges and difficulties; however, it is still recognized as the most potent cure for GC (103). Although GC CAR T-cell research is in its infancy, the positive results of preliminary trials provides a rationale for the further exploration of its use in clinical practice. This indicates that CAR T-cell therapeutic models are advancing and may eventually improve with continued exploration. Combined with a deeper understanding of the TME, novel target epitopes and scientific-technical progress, CAR T-cell therapy may improve its current standing in the near future. Improving the tumor-killing effect and prolonging the survival time of patients should also be readily solved with future study. Furthermore, combining CAR T-cell therapy with precondition treatment may address its current ineffectiveness. In conclusion, the available evidence strongly supports the potential of CAR T-cells in the treatment of patients with GC.

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Availability of data and materials

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Authors' contributions

ZJ and BL conceptualized the present review. ZY, LQ and QL drafted the manuscript. BZ and HY designed and finalized the figures. LW and GZ collected and analyzed the data. XJ and ZY designed and finalized the tables. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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