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CAR-T Cell Therapy in Gastrointestinal Cancer, Head and Neck Cancer, and Autoimmune Diseases: Current Advances and Future Perspectives

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ABSTRACT

Introduction: Chimeric Antigen Receptor (CAR) T cell therapy has revolutionized cancer treatment, showing significant success in hematologic malignancies. However, its application in solid tumors like gastrointestinal (GI) and head and neck cancers, as well as autoimmune diseases, remains a challenge. The development of CAR-T cell therapy for these indications presents unique difficulties, including tumor heterogeneity, antigen specificity, and immune suppression within the tumor microenvironment.

Aim of the study: This review aims to provide an in-depth analysis of the current advancements in CAR-T cell therapy for gastrointestinal cancer, head and neck cancer, and autoimmune diseases. We seek to evaluate the therapeutic potential, current challenges, and future directions of CAR-T cell-based interventions in these conditions.

Materials and methods: We performed a comprehensive literature review, analyzing recent clinical trials, preclinical studies, and research published in PubMed and Google Scholar. We focused on CAR-T cell therapy targeting solid tumors and autoimmune diseases, current treatment options, clinical efficacy, and safety concerns across these therapeutic areas.

Conclusions: CAR-T cell therapy has shown promising potential in the treatment of gastrointestinal cancers, head and neck cancers, and autoimmune diseases, but its clinical application remains limited by challenges such as antigen escape, off-tumor toxicity, and insufficient T-cell persistence. Future studies must focus on improving CAR design, enhancing targeting accuracy, and addressing the immunosuppressive tumor environment to unlock the full therapeutic potential of CAR-T cells for these indications.

Key Words CAR-T, Chimeric Antigen Receptor, Gastrointestinal tumors, Head and Neck Cancer, systemic lupus erythematosus, Immunotherapy

Introduction

Chimeric Antigen Receptor T-cell (CAR-T) therapy has emerged as a groundbreaking approach in cancer treatment, particularly for hematological malignancies. By engineering a patient's own T cells to express receptors specific to tumor antigens, CAR-T cell therapy facilitates targeted immune responses against cancer cells[1].

The aim of this review is to explore the current advancements and future potential of CAR-T cell therapy in the treatment of gastrointestinal cancer, head and neck cancer, and autoimmune diseases. This article aims to summarize recent clinical and preclinical studies, assess the efficacy and safety of CAR-T cell therapy in these emerging applications, and discuss

the challenges and innovations needed to optimize its therapeutic impact in solid tumors and autoimmune conditions.

CAR-T cell therapy is a form of adoptive cell transfer that involves genetically modifying a patient's own T-cells to recognize and attack cancer cells. The first step is leukapheresis, during which T-cells of the patient are being collected to later be genetically engineered to express a synthetic receptor—called a chimeric antigen receptor (CAR)—which is designed to recognize a specific tumor-associated antigen[2, 3] CARs are receptors which potentiate T lymphocytes to antigen-binding and change their specificity and function[4].

They are typically comprised of an extracellular single chain variable fragment (scFv) of a monoclonal antibody (mAb) specific for a surface antigen on the tumor cell, a transmembrane domain, signaling modules that trigger T cell effector functions, and a spacer domain that supports resilience and improves T cell and target cell engagement.[5] CAR-T lymphocytes are genetically engineered to recognize any chosen antigen, thus by-passing the restrictions of repertoire limitation and immune tolerance.[6]

This genetic modification is usually achieved using viral vectors, such as lentiviruses or retroviruses. Once modified, the CAR-T cells undergo ex vivo expansion to increase their count before being infused back into the patient. When infused, synthetic CARs recognize and actively seek out tumor-associated antigens on the surface of cancer cells through the single-chain variable fragment domain triggering a significant immune response in T cells, which leads to tumor cell destruction[7].

Compared to other immunotherapies, such as immune checkpoint inhibitors that enhance endogenous T-cell activity by blocking inhibitory pathways (e.g., PD-1/PD-L1), CAR-T cell therapy provides a more direct and personalized approach. The main benefit of CAR-T cell therapy over traditional T-cell receptor (TCR)-based therapies is that CARs recognize antigens directly on the cell surface, independently of major histocompatibility complexes (MHC/HLA)[8] making them effective across diverse patient populations. However, CAR-T cell therapy also comes with unique challenges, including severe side effects like cytokine release syndrome (CRS) and limited efficacy in solid tumors due to tumor microenvironmental barriers, which will be further discussed in this article[9].

CAR-T cell therapy background

CAR-T cell therapy is one of the latest therapies in treating leukemia. Here, we briefly review the historical studies that led to the development of CAR-T cell therapy in cancer treatment. First mentions of CAR-T cells were in 1989 when Dr. Zelig Eshhar and his team had generated and expressed chimeric T-cell receptor (TcR) genes composed of the TcR constant (C) domains fused to the antibody's variable (V) domains. Following transfection into a cytotoxic T-cell hybridoma, expression of a functional TcR was detected. The chimeric TcR exhibited the idiotope of the Sp6 anti-TNP antibody and endowed the T cells with a non-MHC-restricted response to the hapten TNP. The transfectants specifically killed and produced interleukin 2 in response to TNP-bearing target cells across strain and species Barriers[10].

One of the brightest chapters in the history of CAR-T cell therapy was the trial at the University of Pennsylvania Carl June and his team conducted a groundbreaking clinical trial using second-generation CAR-T cells targeting CD19 to treat patients with advanced, chemotherapy-resistant chronic lymphocytic leukemia (CLL). This trial marked a significant advancement in cancer immunotherapy[11].

In 2017, after more than three decades Kymriah (tisagenlecleucel) by Novartis became the first FDA-approved CAR T-cell therapy, for acute lymphoblastic leukemia (ALL). The FDA's approval of CAR-T therapies, such as Kymriah and later that year, Yescarta, marked significant milestones in this field.

Kymriah (tisagenlecleucel) Prescribing information. East Hanover, New Jersey, USA: Novartis Pharmaceuticals Corporation; August 2017.

CAR-T cell therapy has already significantly advanced the treatment of hematologic malignancies, particularly leukemia and lymphomas. In acute lymphoblastic leukemia (ALL), especially B-cell ALL, CAR-T cells targeting the CD19 antigen have demonstrated remarkable efficacy, leading to high rates of complete remission in both pediatric and adult patients. Similarly, for certain types of non-Hodgkin lymphomas, such as diffuse large B-cell lymphoma, CAR-T cell therapies have achieved substantial response rates. For instance, a systematic review reported that CAR-T cell therapies showed some benefit in terms of progression-free survival in patients with relapsed/refractory B-cell lymphoma, although no significant

differences in overall survival were observed compared to standard treatments. These initial successes have positioned CAR-T cell therapy as a promising option for patients with certain hematologic cancers[9].

Challenges in expanding CAR-T therapy to solid tumors on the example of gastrointestinal malignancies.

As mentioned, CAR-T cell therapy in hematology has yielded spectacular results. This success has encouraged researchers to explore the potential expansion of indications for this therapy. Many of them have decided to investigate its effectiveness in solid tumors. Here we would like to analyse the challenges in expanding CAR-T cell therapy to solid tumors on the example of gastrointestinal tumors, having regard to the fact that gastrointestinal cancers are especially common and epidemiologically significant. In 2020, nearly two million patients were diagnosed with colorectal cancer[12], exceeding the population of Latvia[13]. This places colorectal cancer as the third most common malignancy worldwide. Other gastrointestinal malignancies are also among the most frequently diagnosed types of cancer. This underscores the significance of the issue and explains the increasing interest in CAR-T cell therapy as a potential treatment approach for GI tract tumors. However, the treatment of solid tumors presents several challenges. The most evident barrier is the physical structure of the tumor, characterized by a dense tissue architecture and the presence of stromal components that hinder cellular migration. This is further exacerbated by the immunosuppressive microenvironment within the tumor site[14]. CAR-T cells may also encounter difficulties in tumor penetration at the stage of extravasation from blood vessels[14]. Another major challenge is the antigenic heterogeneity of solid tumors, which results in a diverse tumor cell population with varying levels of tumor-associated antigen expression. This heterogeneity can impede the ability of CAR-T cells to accurately recognize and eliminate malignant cells[15]. Addressing these barriers is the focus of extensive research. Some investigators are exploring potential markers of cancer stem cells, which are believed to express antigens associated with tumor cell proliferation, in accordance with the cancer stem cell oncept[16,17]. Identifying such markers could enable specific targeting of cancerous cells, thereby increasing the likelihood of therapeutic success.

One of said markers could be Leucine-rich repeat-containing G-protein (LGR5 or GPR49). LGR5 is a transmembrane protein found on normal stem cells of various tissues, including intestinal crypts[18]. Under normal conditions, the expression of this protein is lost as

stem cells differentiate and simultaneously migrate towards the lumen of the intestine[19]. However, it has been proven that in cancerous changes in the intestine, a population of stem-like cells is present at the base of the tumor, which, like normal stem cells, express LGR5[20]. As a result, this protein has become an interesting target for targeted therapy of colorectal tumors. Currently, there is no registered drug that specifically binds to this protein. A promising one appears to be LGR5-targeted biomimetic magnetoliposomes loaded with oxaliplatin or 5-fluorouracil, as demonstrated in a recently published study[21]. The presence of LGR5 in cancer cells has encouraged researchers to explore the potential of CAR-T cell therapy in treating colorectal cancer. In vivo studies and experiments using mouse models have shown promising results, demonstrating the potential of CAR-T cell therapy[22]. Importantly, the studies have shown that LGR5 expression outside of the mentioned cancer cells is significantly lower, suggesting limited targeting of normal cells by CAR-T cells, and thus reduced side effects[22]. Pancreatic cancer, although less frequently occuring, yet extremely lethal has also been taken into consideration while identifying the use of CAR-T cell therapy. In the case of pancreatic cancer, the main treatment line is surgical intervention, with preoperative neoadjuvant or postoperative adjuvant therapy. However, only 20% of patients with this diagnosis have resectable lesions[23]. This is due to the fact that the tumor is most often diagnosed at an advanced stage of the disease[24].This contributes to the picture of limited therapeutic success[24]. As a result, there is an increasing focus on finding new solutions in the treatment of pancreatic cancer, including the use of CAR-T cell therapy. The first step in this process is to identify tumor-associated antigens. It has been shown that the expression of membrane-associated glucose-regulated protein 78 KD (GRP78) is increased in cancer tissues[25]. Subsequently, researchers used CAR-T cells targeting GRP78, demonstrating significant cytotoxicity against pancreatic cancer cells with high or moderate GRP78 expression, suggesting an effective therapeutic option, particularly for gemcitabine-resistant tumors[25]. High GRP78 expression has been observed not only in pancreatic tumors but also in breast, prostate, liver cancers, and many others, making it a potential target for numerous malignancies[26]. A key challenge in pancreatic cancer remains the possibility of early disease detection. A recently published study proposes a promising test termed "PAC-MANN," which, combined with the clinical biomarker CA 19-9, was 85% sensitive for detecting stage I pancreatic ductal adenocarcinoma (PDAC) with 96% specificity[27].

The examples outlined above represent just a small part of the vast potential of CAR-T cell therapy in gastrointestinal tumors. In theory, for any cancer with an appropriate target, a tumor-associated antigen, CAR-T cells could be used as a potential line of treatment.

Researchers have also focused on hepatocellular carcinoma[28,29], gastric cancer[30,31], and bile duct cancer[31]. The growing interest in this topic within the scientific community raises hopes for the rapid development of new therapeutic options for patients.

CAR-T HEAD AND NECK CANCER

Head and neck cancer is also becoming a major challenge in contemporary otorhinolaryngology practice. This heterogeneous group of diseases collectively ranks as the sixth most common cancer type worldwide, with 90% histopathological diagnoses being head and neck squamous cell carcinoma (HNSCC)[32]. This type of cancerous tumor develops from the epithelial cells of the mucous membranes lining the inside of the oral cavity, pharynx and larynx cavity. The risk factors of HNSCC include Human Papilloma Virus (HPV) infection, ethanol use and smoking. The association with HPV infection is most commonly observed for HNSCC tumors located in the oral cavity and pharynx, while ethanol use and smoking are associated with an increased risk of developing HNSCC in the oral cavity, larynx and subglottic region[32,33]. The choice of the treatment method and its effectiveness depends on the TNM classification of the stage of the disease, with surgery supplemented with chemotherapy as the standard treatment. Despite the continuous improvement of these methods and the progress that is being made in the oncological treatment of cancer, the 5-year survival rate of patients with HNSCC has remained in the range of 40-66% for the past 40 years[34]. For this reason, alternative treatments are now being actively developed to increase the treatment efficacy and improve survival in patients diagnosed with HNSCC. One of the possibilities is CAR-T cell therapy.

In 2019 the study „MUC1 as a target for CAR-T cell therapy in head and neck squamous cell carcinoma" was published. The publication reviewed the efficacy of MUC-1 specific CAR-T cells in the treatment of HNSCC. MUC-1 is a compound present on the surface and in the cytoplasm of epithelial cells. In the study, two tissue samples of 2cm3 were collected from 52 patients, previously untreated with chemotherapy and radiotherapy; the first was a fragment taken from the tumor and the second was a sample of healthy tissue taken 3-5 cm from the tumor border. Tissue samples were analysed for MUC-1 expression by detecting RNA encoding MUC-1. qRT-PCR showed higher expression of MUC-1 in cells harvested from HNSCC tumors compared to healthy cells. MUC-1-specific CAR- T cells showed higher cytotoxicity measured by flow-cytometry against MUC-1-expressing HNSCC cells compared to unmodified

T cells. However, authors of the publication indicate that further studies are needed to verify the efficacy of MUC-1 specific CAR-T cells[35].

As mentioned earlier, HPV infection can promote the development of HNSCC. The infection results in the expression of viral oncoproteins such as E5, E6 and E7 in epithelial cells. These proteins affect the activity of ErbB family tyrosine kinases (HER), which include the epidermal growth factor receptor (EGFR), which play a key role in the regulation of cell growth, proliferation and differentiation. HPV infection, therefore, can cause increased HER activity and promote carcinogenesis. Many studies have shown that 90% of HNSCC cases result in increased HER expression in the tumour cells. Previous attempts to utilise this relationship in oncological treatment have involved the use of inhibitors that block single receptors, e.g. EGFR, but this approach has not been successful because overexpression of other HERs compensated for the function of the blocked EGFR. However, the development of CAR-T cell therapies has opened new treatment options. Currently in phase 1 clinical trials, the new TQE29z therapy is a T4 immunotherapy in which lymphocytes have been enriched with a non-specific ligand that interacts with ErbB dimers on cells, achieving potent cytotoxic activity. The advantage of CAR-T cell therapy over classical HER inhibitors lies in its ability to simultaneously block several types of HER, which is associated with potentially higher efficacy. In addition, HPV+ HNSCC tumours show higher immunogenicity compared to HPV- tumours, which may be a potential factor enhancing the efficacy of CAR-T cell therapy in HPV+ HNSCC[32].

Promising results were also obtained by Papa et al, who investigated the efficacy of ErbB-specific CAR-T cells injected into the HNSCC tumors in 15 patients with advanced disease, achieving stabilisation of the disease process 6 weeks after injection in 60% of subjects[36].

The CD70 antigen protein may be another potential target for CAR-T cell therapy in HNSCC; it was previously considered to be a protein only expressed on activated B and T lymphocytes. However, increased expression of CD70 has also been observed in various tumor types including HNSCC. CD70 overexpression has been demonstrated in tumours of the oral cavity, tongue and larynx; but CD70 overexpression has not been detected in tumours of the hard palate, lips and pharynx. Research has shown that CD70-specific CAR-Ts can be an effective therapeutic option in up to 10-20% of HNSCC patients whose tumor cells show high CD70 expression. Nevertheless, the authors of the publication point out that CD70 overexpression in HNSCC is characterised by high variability between patients with this diagnosis, which may affect the limited use of CD70-specific CAR-Ts in some patients without increased CD70 expression[37].

CD98hc antigen is associated with T-cell activation, moreover systemic treatment-resistant HNSCC cells are characterised by high expression of this antigen[38]. CAR-T cells targeting CD98hc showed efficacy in destroying tumour cells in a 3D tumour spheroid model of HNSCC[38].

Haist et al. in a 2021 study verified the cytotoxic activity of CD44v6 specific CAR-T cells against HNSCC cells. CD44v6 is an isoform of the hyaluronan receptor CD44 that shows increased expression in HNSCC cells. Using fluorescently labelled antibodies by flow cytometry, the increased expression of CD44v6 was demonstrated in tested tumour cells. Subsequently, the cytotoxic activity of CD44v6 specific CAR-T cells was tested against cells of the HNSCC cell lines used in the study, using cytotoxicity assays and observing the interaction dynamics of specific CAR-T and tumour cells by fluorescence microscopy. The high specificity of CD44v6-sensitive CAR-T cells against HNSCC cells expressing this antigen was demonstrated; the cytotoxic effect developed within 2-3 hours of co-incubation of CAR-T and HNSCC cells and depended on the level of CD44v6 expression. CD44v6-specific CAR-T cells have shown to have the potential to be a novel therapy for HNSCC. Nevertheless, it requires refinement and further studies on the potential systemic toxicity of the treatment, as CD44v6 is also present in some healthy tissues[39].

Currently, CAR-T cell therapy represents a promising tool that can significantly improve treatment outcomes and prognosis in patients with HNSCC. Nevertheless, further experimental studies are still needed to verify its safety of use and the technology itself needs to be improved, which should include, among other things, the search for highly specific tumor cell markers in order to reduce possible systemic toxicity of the treatment.

CAR-T CELL THERAPY IN AUTOIMMUNE DISEASES

Autoimmune diseases are characterized by chronic inflammation, organ damage and elevated mortality[40]. They are usually treated with immunosuppressive drugs and blocking antibodies, which can control disease progression but are rarely curative. This underscores the need for more effective treatment strategies, with cell therapies being currently explored as a potential long-term solution.

In 2019, Kansal et al.[41] published the first in vivo study on CAR-T cell therapy targeting CD19 in mouse models for systemic lupus erythematosus (SLE). Treatment with anti-CD19 CAR-T cells resulted in reduction of native DNA autoantibodies, decreased lupus-associated

tissue damage and a significantly extended lifespan—doubling the typical mice lifespan to over 12–18 months. Beyond the depletion of CD19+ B cells, including autoantibody-producing autoreactive cells, the study also revealed effects on the CD4 T cell population and a reduction in double-negative CD4-CD8- cells suggesting CAR-T cell therapy may also modulate autoreactive T cells. Additionally, treated mice retained a population of central memory T cells expressing the CAR receptor, indicating a self-renewing cell subset that could contribute to long-term efficacy. These encouraging findings have paved the way for considering CAR-T cell treatment as a potential option for patients with SLE.

The first successful application of CAR-T cell therapy in patient with SLE was documented by Mougiakakos et al.[42] They reported the case of a 20-year-old woman with severe, treatment-resistant lupus. After multiple failed conventional treatments, the patient underwent autoCAR-T cell therapy. Within five weeks of infusion, the patient achieved both clinical and serological remission, demonstrated by dsDNA seroconversion (from >5000 U/mL to 4 U/mL), resolution of proteinuria (from 2000 mg/g creatinine to <250 mg/g), and normalization of C3 and C4 levels. The treatment was well tolerated, with no reports of neurotoxicity, cytokine release syndrome (CRS), or prolonged cytopenia. Following CAR-T cell expansion post-infusion, sustained B-cell depletion was observed.

Another study was conducted by Mackensen et al.[43] in which five patients with refractory SLE were treated with CAR-T cell therapy. By three months, all five patients had achieved drug-free remission according to the Definition of Remission in Systemic Lupus Erythematosus (DORIS) criteria. Notably, one patient experienced a resolution of cardiac valve fibrosis and lung involvement. Mild cytokine release syndrome (CRS) was reported in three patients, with only one requiring tocilizumab.

Taubmann et al.[44] conducted a study involving six females and one male, aged 19 to 39 years with severe, treatment-resistant lupus who underwent CAR-T cell therapy. Following CAR-T cell infusion, a significant expansion of T cells was observed, accompanied by sustained B-cell depletion lasting a median of 120 days. All patients achieved drug-free remission, as defined by the DORIS criteria, which was sustained for at least 22 months despite B-cell reconstitution.

The study published in 2024 by Müller et al.[45] provides compelling evidence on the efficacy of CD19 CAR-T cell therapy in the treatment of refractory systemic autoimmune diseases, including SLE, idiopathic inflammatory myositis, and systemic sclerosis. A cohort of

15 patients (8 with SLE, 3 with idiopathic inflammatory myositis, and 4 with systemic sclerosis) underwent a single infusion of CD19 CAR-T cells following preconditioning with fludarabine and cyclophosphamide. All patients achieved significant clinical improvement and were able to discontinue immunosuppressive therapy without disease relapse. While mild adverse effects, including grade 1 cytokine release syndrome, were observed in the majority of patients, no severe complications were reported. It highlights the potential for CAR T-cell therapy to induce long-term remission.

These findings support CAR-T cell therapy as a potentially transformative approach for autoimmune diseases, necessitating further investigation in larger clinical trials.

Future Perspectives and Emerging Strategies

Future perspectives and emerging strategies in CAR-T cell therapy promise significant advancements in the treatment of both hematologic malignancies and solid tumors, as well as autoimmune diseases. Next-generation CAR-T cell technologies, such as armored CAR-T cells and dual-targeting CAR-Ts, are being developed to address the limitations of conventional CAR-T cell therapies. Armored CAR-Ts incorporate additional genetic modifications to improve their persistence, resistance to immune suppression, and efficiency within challenging tumor microenvironments[46]. Dual-targeting CAR-Ts, which are designed to simultaneously target two distinct antigens, aim to overcome antigenic heterogeneity in tumors, improving the specificity and potency of treatment[47]. Additionally, gene-editing technologies, particularly CRISPR-based modifications, are advancing CAR-T cell therapies by enabling precise alterations of T cells to enhance their anti-tumor activities and reduce unwanted adverse effects. These innovations hold great promise for optimizing CAR-T cell therapy and extending its applications to more patients, including those with complex or refractory diseases[48,49]. Combining CAR-T cell therapy with checkpoint inhibitors, such as PD-1/PD-L1 inhibitors, is another promising strategy, which seeks to overcome the immunosuppressive tumor microenvironment that often limits CAR-T cells[50]. These combination approaches could increase CAR-T cell persistence, boost anti-tumor immune responses, and enhance overall treatment outcomes. However, several challenges remain for the widespread adoption of CAR-T cell therapy, particularly the production of personalized CAR-T cells is resource-intensive, requiring specialized facilities and expertise, and the cost remains a significant barrier to

accessibility. Moreover, issues related to scalability, consistency of product quality, and standardization of protocols across different institutions present obstacles that need to be addressed for CAR-T cell therapy to become a global treatment option[51,52]. Despite these challenges, the future of CAR-T cell therapy looks promising, with ongoing research focusing on improving the durability of responses, expanding indications to include solid tumors, and addressing safety concerns, such as cytokine release syndrome and neurotoxicity[53].

In conclusion, while CAR-T cell therapy has already revolutionized the treatment of hematologic malignancies like leukemia and lymphomas, its full potential across various disease settings, remains an area of intense investigation. Current limitations, such as the high cost, manufacturing difficulties, and immune-related toxicities, underscore the need for further research into improving efficacy, safety, and accessibility. The future outlook on CAR-T cell therapy seems to be very promising, with new strategies and innovations on the horizon poised to improve patient outcomes and broaden its therapeutic applications.

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