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Clinical trials of cellular therapies for the treatment of colorectal cancer: a narrative review

A. Hosseini Abgir[1], M.M. Mokhtari Tabar[1], Z. Zahedifard[2], Z. Shokrolahi[3], M.R. Atashzar[2,3]

[1]Department of Biochemistry, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran
[2]Department of Biotechnology, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran
[3]Department of Immunology, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran

Corresponding Author: Mohammad Reza Atashzar, MD; e-mail: mr.atashzar@yahoo.com

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Abstract – Colorectal cancer (CRC) treatment using common chemotherapy approaches has drawbacks such as side effects, costs, and resistance of cancer cells which affects patients' prolonged survival, and quality of life. The immune cells have pivotal roles in regulating tumor progression in the tumor microenvironment (TME).

The most important CRC cellular immunotherapies include the use of tumor-derived cells such as tumor-infiltrating lymphocytes (TILs) and lymph node lymphocytes (LNLs), peripheral blood mononuclear cells (PBMCs), derived cells, including T cells, natural killer (NK) cells, cytokine-induced killer (CIK) cells, and chimeric antigen receptor (CAR) cells.

Although adoptive cell therapy has some advantages, some disadvantages have been reported. TILs cells are strictly directed against tumor-specific antigens; however, they are inefficient due to immune editing. CIK cells have a major histocompatibility complex (MHC)-independent cytotoxic effect and need concurrent high-dose interleukin (IL)-2 administration. In addition, chimeric antigen receptor-T cells (CAR-T cells) are MHC-independent that overcome MHC downregulation by the tumor. They are potent in recognizing any cell surface antigen and are applicable to a broad range of patients and T-cell populations. Here, the researchers present the most popular cancer cellular immunotherapy approaches and discuss their clinical relevance by referring to data obtained from CRC clinical trials.

To date, clinical experience and efficacy suggest that combining more than one immunotherapy intervention, in combination with other treatments like chemotherapy, radiotherapy, and targeted therapy, is promising for cancer therapy.

Introduction

The immune system plays a complex role in all aspects of cancer, from carcinogenesis to treatment[1]. Immunotherapy is considered a promising anti-tumor treatment. Two types of immunotherapies are effective in treating cancer: immunosuppressive inhibitors to enhance normal antitumor activity and the administration of antitumor immune cells by adoptive cell therapy (ACT)[2]. ACT is a type of cancer treatment that potentiates the immune cells' ability to recognize and eliminate cancer cells. The most important event in the evolution of ACT is the discovery of novel high-quality antigens that reduce tedious immune control techniques and contribute to the formation of ACT as a very practical treatment[3]. The ultimate goal of ACT is to produce a strong immune-mediated antitumor response under *in vivo* conditions. Many promising research projects[4] are being conducted with the aim to increase the patients' survival receiving benefits from this treatment and increase ACT efficacy as a standard approach for various cancers. Different sources of cells are used for ACT. One of the important cells of the immune system includes natural killer (NK) cells with high toxicity against

tumor cells and virus-infected cells without prior sensitivity. Utilizing NK cells and lymphokine-activated killer (LAK) cells in ACT is one of the successful and promising methods[5,6]. Moreover, ACT using T lymphocyte cells isolated from patients' tumors tumor-infiltrating lymphocytes (TILs) genetically engineered with T cell receptors (TCRs) or CAR-T cells presents new promises for the treatment of some cancers[7].

Colorectal cancer (CRC) is one of the leading causes of cancer death worldwide. CRCs are a very heterogeneous group of diseases caused by a set of mutations. Since not all CRCs have the same mutations, it is difficult to design an epidemic molecular treatment plan. Surgery is still the mainstay of treatment in the early phase of colorectal cancer, but in the advanced phase, where cancer has metastasized, it is no longer helping, and half of the patients die of the disease eventually. Despite the improvement of immuno-checkpoint or targeted therapy for the treatment of severely mutated tumors, a new treatment is required[8]. The ACT is now becoming one of the most promising strategies in the treatment of CRC. In this narrative review, we discuss the clinical application of adoptive immunotherapy in CRC and outline the disadvantages and future development directions of ACT in CRC treatment.

Current Therapeutic Approaches in CRC

The common types of treatments used for CRC are described below. The treatment options depend on several factors, including the type and stage of cancer, possible side effects, and the patient's preferences and overall health. Surgery is the most common treatment for CRC, but some of the side effects of surgery include pain, diarrhea, constipation, and tenderness in the area of the operation. Radiation therapy is commonly used for the treatment of CRC as well because this kind of tumor tends to recur near where it was originally initiated[9]. Some complications of CRC radiation therapy include bloody stools, upset stomach, and fatigue.

Chemotherapy is the use of drugs to destroy cancer cells and may be given after surgery to eliminate any remaining tumor cells. The Food and Drug Administration (FDA) approves many drugs to treat CRC as a chemotherapy regimen, or schedule, including leucovorin-5, 5-fluorouracil, and oxaliplatin (FOLDFOX) or capstamine with oxaliplatin. Besides, using Folinic acid, fluorouracil, and oxaliplatin (FOLFOX), or 5-Fluorouracil (5-FU) is a standard treatment for metastasis. Chemotherapy of CRC may cause neuropathy, mouth sores, diarrhea, vomiting, nausea, and numbness in the feet or hands.

Targeted therapy by targeting cancer's specific genes and proteins blocks the growth and spread of cancer cells. For the CRC, targeted therapies such as bevacizumab, regorafenib, cetuximab and panitumumab may be suggested. These drugs affect angiogenesis and inhibit epidermal growth factor receptors playing a role in the treatment of CRC[10].

Immunotherapy approaches also boost the body's natural defense to fight such cancers as CRC. By targeting programmed cell death protein 1 (PD-1), a receptor on tumor cells, Pembrolizumab as well as Nivolumab used for microsatellite instability (MSI)-H or mismatch repair deficient (dMMR) metastatic CRC are used as immunotherapy drugs. The most common side effects of targeted therapies and immunotherapy include vomiting, fatigue, rash, nausea, bone pain, abdominal pain, diarrhea, fever, joint pain, itching, cough, and shortness of breath. Because of the limited efficacy of current therapies, there is a strong interest in the development of personalized immunotherapy such as ACT.

ACT is the transplantation of human cells to replace or repair damaged tissue and/or cells. Classification of ACT is based on source and type of cells, including tumor source, TILs and lymph node lymphocytes (LNLs), peripheral blood mononuclear cells (PBMCs), cytokine-induced killer (CIK) cells, NK cells, and T lymphocytes, genetically engineered cells, [CAR-T cells, TCR, chimeric antigen receptor-NK cells (CAR-NK cells)], and stem cells.

Adoptive Cell Therapy of CRC

Tumor Source

Tumor-infiltrating lymphocytes (TILs) and lymph node lymphocytes (LNLs)

TILs are lymphocytes increasing in the tumor microenvironment (TME) and have antitumor activity. One of the TILs is T lymphocytes with antitumor activity. T cells express the T-cell receptor-cluster of differentiation 3 (TCR-CD3) complex, which

can bind with major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells (APC) cells and initiate the activation of the immune system. There are two types of TCRs, including αβ and γδ TCR. In addition, αβ T cells can be classified into CD4+ and CD8+ T cells. Subsets of CD4+ T cells are T-helper (Th) 1, Th 2, Th 17, regulatory T cell, follicular helper T cell, and Th 9[11]. Mixed T cells are used in the ACT[12].

Using TIL for the ACT, these cells were isolated from tumor tissue in large numbers *in vitro* and then re-injected into the host body. TILs can be produced from a variety of tumors, including colon adenocarcinoma. Liver metastases can also be a suitable source of TILs for cellular therapy of CRC without being contaminated by the intestinal flora[12]. In a recent study, Pagès et al[13] examined the role of TILs in CRC and showed that TILs can act as a prognostic indicator in CRC patients. However, several factors, like difficulty in obtaining a sufficient number of TILs from CRC patients, may prevent the effective use of TILs in CRC patients[14]. In 1990, a clinical trial[15] was carried out on patients with CRC. In this experiment, TILs were extracted from liver metastases, incubated with interleukin-2 (IL-2), and re-injected in the patient, but no significant difference was found between the TIL group and traditional treatment. In 2016, CD+8 T lymphocytes targeting Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) were isolated from TILs of CRC patients with lung metastasis. These CD+8 TILs proliferated and re-injected into the patient and showed promising results in the eradication of lung metastases[16].

Despite the role of CD8+ TIL in CRC, Kroemer et al[17] also isolated CD+4 T cells from TILs derived from hepatic metastasis of patients with CRC. In total, high levels of Th 17 cells were associated with a poor prognosis and survival. This suggests a particular role of these Th 17 cells in the CRC area. The results showed that TIL-induced pluripotent stem cell (iPSC) technology had a high potential for advancing TIL-ACT to the next stage and overcoming the problems of conventional TIL-ACT providing regenerated TIL[18]. Some clinical trials using TIL for cellular therapy of CRC are in progress around the world, which are listed in Table 1.

Despite tumor specimens, tumor-draining lymph nodes are used for the isolation of tumor-specific lymphocytes called LNLs. The majority of LNLs are antigen-presenting cells (APC) which present tumor-associated antigens (TAAs) epitopes through MHC molecules to TCR of T cells for the activation of the immune system. Due to better effects, LNLs are suitable sources of a cell for the ACT, especially in CRC. Studies[19,20] have shown the positive effects of *in vitro* expanded LNLs on patients with metastatic CRC. In a pilot study[21] on the application of LNL in advanced CRC, it was shown that the survival rate of patients with stage IV disease significantly increased from 0.8 years to 2.6 years. A clinical trial study[22] on 71 CRC patients (stages of I-IV) revealed that LNLs containing CD3+CD69+ and CD4+CD69+ T lymphocytes are more activated than TILs. The majority of LNLs cells were tumor-specific effectors and central memory T cells, leading to an elevation of overall survival (OS). Although TILs and LNLs have no immunogenicity and adverse effects, further research is necessary to overcome challenges associated with using these cells as the ACT for CRC.

Table 1. Clinical trials using TIL for cellular therapy of CRC are in progress around the world.

Clinical trials identifier	Drugs	Phases	Status	Type of disease	Year
NCT03904537	Anti-PD-1 antibody activated TILs and chemotherapy	I/II	unknown	Colorectal cancer stage III	2019
NCT01174121	Young TIL+ other drugs	II	recruiting	Metastatic colorectal cancer and other cancers	2021
NCT04842812	TILs and CAR-TILs	I	recruiting	Colorectal cancer and other cancers	2021
NCT03935893	Tumor-infiltrating lymphocytes (TILs)+ Fludarabine+cyclophosphamide combination	II	recruiting	Colorectal cancer and other cancers	2021
NCT04426669	Tumor-infiltrating lymphocytes (TILs)+ Fludarabine+cyclophosphamide+Aldesleukin	I/II	recruiting	Colorectal cancer and other cancers	2021

Peripheral Blood Mononuclear Cells (PBMCs)

T Lymphocytes

The majority of PBMC cells are T lymphocytes with antitumor activity, and a combination of CD4+ T cells with chemotherapy or monoclonal antibody demonstrated a promising response to treat advanced or recurrent CRC patients[23-25]. ACT using αβ T cells may be the ideal way to decrease liver metastasis in patients with CRC[12]. In addition, γδ T cells are promising approaches of ACT for CRC. Isolated γδ T cells from PBMC, when expanded *in vitro* with interferon-gamma (IFN-γ), have shown cytotoxic effects against various tumors, including CRC[26,27]. It was reported[28] that γδ T cells in a CRC model prevented tumor growth and metastasis into the liver or lung. Some subsets of γδ T cells can be ideal for ACT of CRC. PBMCs Vδ1+γδ T cells showed better activity in CRC[29]. More research is needed for a better understanding of the mechanisms of therapeutic efficacy of γδ T cells and other T cells in the ACT.

Cytokine-Induced Killer (CIK) Cells

CIKs are natural killer T lymphocytes that are generated by the incubation of cord blood mononuclear cells or PBMCs with IL-1, IL-2, anti-CD3 antibody, and IFN-γ. CIK cells, along with the administration of interleukins, have been used to treat cancers experimentally and in clinical trials. Various immune cells can be obtained for ACT using different protocols of cytokine management[30]. One of the famous and first antitumor effector cells generated from PBMCs is LAK cells. LAKs are a heterogeneous population of cells consisting primarily of NK, Natural killer T cells (NKT), and T cells, which are generated *in vitro* by the culture of PBMCs in the presence of IL-2. Stimulation of antibody-dependent cellular cytotoxicity (ADCC) against monoclonal antibody (mAb)-coated tumor cells by IL-2-activated LAK cells, may be effective in the treatment of cancers[31]. Kim et al[32] used LAK cells and IL-2 to evaluate the therapeutic effects of solid tumors such as CRC. IL-15 is another cytokine that pays attention to cancer immunotherapy and inhibits colitis-associated colon carcinogenesis. It is shown[31] that IL-15 can also increase ADCC, LAK activity, increase tumor necrosis factor (TNF), and IFN signaling that leads to the destruction of cancer. In addition, IL-21 can also suppress tumor growth through the stimulation of NK and CD8+ T cells. It was reported[33] that incubation of IL-2 and IL-21 with NK cells in a culture medium leads to better NK cell function and is better than the combined effect of IL-2, IL-12, and IL-15. Moreover, expression levels of IL-15 were significantly reduced in patients with colorectal cancer. It was shown[34] that IL-15 expression levels were significantly increased in stimulated NK-92 cell lines following IL-2 incubation. A study[35] showed that using IL-2-activated LAK cells and substance P neuropeptide led to high cytotoxicity against fresh colorectal cancer tissues.

In addition to LAK cells, CIK cells showed greater cytotoxicity against cancer cells. One of the important CIK cells is CD3+CD56+ CIK cells, a T-cell subset that acquires NK function and TCR-mediated specific cytotoxicity. Many clinical trials using ACT with CIK cells were performed in CRC. For the first time, CIK cells were transfected with an IL-2 gene-containing plasmid by electroporation, indicating a promising treatment for CRC, renal cancer, and lymphoma patients[36]. CIK cells, in combination with chemotherapy, can promote the survival time of patients with CRC[37].

Research[38-40] has shown that the combination of dendritic cells (DCs) with CIK immunotherapy, in addition to chemotherapy or radiotherapy, can be an effective treatment in controlling tumor progression in CRC patients.

DCs can be pulsed with a tumor-specific antigen to increase the immune response and therapeutic effects of CIK cells. The combination of FOLFOX with DC-CIK was also used to treat CRC patients[41]. In a study, researchers used a combination of CIK cells with recombinant adenovirus KGHV 500, and they showed that CIK cells could carry the recombinant adenovirus containing the anti-p21Ras *single-chain fragment variable antibody* (*scFv*) gene into tumors and could enhance antitumor activity against CRC[42]. However, the definitive effects of DC-CIK treatment are not yet known and need to be studied[43]. Table 2 shows that an increasing number of clinical trials have demonstrated the effectiveness of CIK cells for CRC.

Table 2. Cytokine-induced killer (CIK) cell findings from clinical trials of CRC.

Clinical trials identifier	Drugs	Phases	Status	Type of disease	Year
NCT01929499	Cytokine-induced killer cells	II	unknown	Colon neoplasms	2013
NCT01839539	Dendritic and cytokine-induced killer cells	II	unknown	Colorectal cancer	2013
NCT02280278	Radical surgery+adjuvant chemotherapy +CIK cell therapy	III	unknown	Stage III colon cancer	2014
NCT02202928	DC-CIK plus Radiotherapy and chemotherapy	II	unknown	Colorectal cancer	2015
NCT02419677	Cytokine-induced killer cells	II/III	completed	Colorectal cancer	2015
NCT02886897	D-CIK and anti-PD-1 antibody	I/II	unknown	Colorectal cancer and other cancers	2016
NCT02487992	Combination of CIK plus S-1 and bevacizumab	II	active, not recruiting	Colorectal neoplasms	2016
NCT03220984	Immune cell-LC intravenous infusion using a CIK cell agent	II	unknown	Metastatic colorectal cancer	2017
NCT03084809	CIK+FOLFOX4	IV	completed	Colorectal cancer	2017
NCT03047525	DC-CTL combined with CIK	I/II	unknown	Colorectal cancer and other cancers	2017
NCT04476641	DC-CIK immunotherapy	II	recruiting	Colorectal cancer and other cancer	2020
NCT03329664	Immune-cell therapy with CIK cells	I/II	recruiting	Colon cancer stage IV	2021

Natural Killer (NK) Cells

NK cells are a type of cytotoxic lymphocyte of the innate immune system. NK cells secrete cytokines such as IFNγ and TNF-α, which affect other immune cells, as well as NK cells. They release cytotoxic granules containing granzymes and perforin, which leads to lysis of the target cell, especially cancer cells. The function of NK cells is determined by the inhibitory [CD94/Natural Killer Group 2 (NKG2), Killer cell immunoglobulin-like receptors (KIRs), Leukocyte Ig-like receptors (LIR), …] and activating [natural killer group 2 member D (NKG2D), CD16…] receptors[44]. By distinguishing between healthy cells and target cells, NK cells recognize and kill target cells. The ligands of inhibitory receptors are MHC class I, found on the surface of healthy cells. When NK cells recognize MHC class I molecules through their inhibitory receptors, they trigger the delivery of a 'No-killing' signal to healthy cells, thereby protecting them from NK cell-mediated cytotoxicity. As well as in target cells, such as tumor cells, the downregulation or loss of surface MHC class I molecules and the overexpression of activating ligands on their surface, leads to the activation of NK cells. NK cells identify certain cells as 'missing-self' and then proceed to eliminate them[45]. The antitumor activity of NK cells was first studied in hematopoietic malignancies before solid tumors. NK cells proliferate *in vivo*, and some hematopoietic malignancies exhibit cytotoxicity and strong cytotoxic effects in various CRC cell lines. There are some promising strategies that use improved NK cells against solid tumors, including active NK cells, administration of NK cells along with cytokines, genetic modification with CARs, and nucleic acids, as well as using genes of cytokines or other immune system regulators[46].

One of the first interventions is using cytokines along with NK cells. Systemic administration of cytokines such as IL-2, IL-15, IL-21, IL-12 and IL-18 can increase the antitumor activity of NK cells in the treatment of cancer[47]. IL-2 has the highest level of interest among researchers for its role in NK cell activation and proliferation compared to other cytokines because the co-administration of mAbs and IL-2 improves the antitumor activity of NK cells. Cytotoxic effects of NK cells can also be enhanced by anti-tumor drugs and monoclonal antibodies.

Some studies[48,49] showed that using cytokines such as IL-21, IL-2, and IL-15 together with Cetuximab [an epidermal growth factor recep-

tor (EGFR) monoclonal antibody] could increase NK cell activity in the treatment of CRC. In these studies[48,49], the results showed that activated NK cells effectively killed CRC cells. Cetuximab can mediate the activity of ADCC through NK cells *in vivo* and has been approved for the treatment of CRC-positive EGFR[50]. In addition, this NK cell cytotoxicity may mediate antitumor activity in EGFR-CRCs that do not respond to anti-EGFR therapy[51]. In trastuzumab-treated colorectal cancer patients, a correlation was also obtained between clinical responses, ADCC activity, and NK cell counts. The main interest of this clinical trial[52] was safety assessment. It was observed that NK cell therapy with a combination of antibodies and chemotherapy did not increase the toxic effects. The expanded NK cells from the patients showed an increase in cytotoxicity in combination with trastuzumab *in vitro*.

Moreover, the combination of monalizumab (Anti-KLRC1) and cetuximab increased the stimulation of NK cells. Monalizumab (anti-KLRC1) also increases NK-mediated ADCC using obinutuzumab, an anti-CD-20 antibody[53]. NK cells, after being injected, can change the fate of target cells. It was shown[54] that co-injection of NK cells with Oxaliplatin-resistant CRC into mice leads to reducing the growth of CRC cells by repressing the *WBSCR22* gene *via* upregulating microRNA-146b-5p. Funding these mechanisms could help to serve them as a suitable candidate for targeted therapy against CRC cells.

Genetic engineering of NK cells is another method to enhance the strength of NK cells. Modification of NK cells with a secretory TNF-related apoptosis-inducing ligand (*TRAIL*) gene is an attractive approach for increasing the potency of NK cells. These engineered NK cells were injected into the peritoneal cavity of CRC-bearing mice and induced tumor cell apoptosis[55]. In addition, it was shown[56] that a bispecific recombinant fusion protein of ULBP2 (a ligand of NKG2D on the surface of NK cells) and an antibody-derived single-chain targeting the tumor carcinoembryonic antigen (CEA) on CRC cells could induce the antitumor activity of NK cells against CRC cells. These methods represent a novel immunotherapeutic strategy for clinical applications of solid tumors, especially CRC.

We have summarized some of the clinical trials using NK cell therapies in CRC in Table 3.

Genetically Engineered Cells

CAR-T Cells

Given that TCR signaling has important roles in T cell activation that leads to the activation of the immune system and antitumor functions, efforts have been made to exploit synthetic molecules to mimic TCR signaling. One of the artificial molecules of TCR is called "CAR". CARs contain the extracellular antigen-binding domain, the hinge, the transmembrane domain, the intracellular signaling do-

Table 3. Natural killer (NK) cell findings from clinical trials of CRC.

Clinical trials identifier	Drugs	Phases	Status	Type of disease	Year
NCT00909558	Natural killer T cells Immunotherapy	I	suspended	colon cancer and other cancers	2010
UMIN 000013378	Combination of cetuximab or trastuzumab mediated NK cell with IL-21	I	completed	Gastric or colorectal cancer	2014
NCT02839954	anti-muc1 CAR-pNK cells	I/II	unknown	colorectal cancer and other cancers	2016
NCT0363450	activated NK cells	I/II	recruiting	colon cancer and other cancers	2019
NCT03319459	FATE NK-100 combined with cetuximab	I	active, not recruiting	advanced colorectal cancer	2020
NCT02890758	NK cells therapy in combination with ALT-803	I	active, not recruiting	colon carcinoma and other cancers	2021
NCT05040568	combination of cetuximab with CB-NK cells	I	not yet recruiting	colon cancer (resected stage)	2021

main with costimulatory molecules, and cluster of differentiation 3 zeta (CD3ζ) chain as a T cell activating signaling domain[57]. The antigen recognition domain or extracellular domain that interacts with potential target molecules is typically derived from antibody single-chain variable fragment (scFv). Growth factors, TNF receptors, innate immune receptors, and cytokines have been used as CAR antigen recognition domains. Classification of CAR-T cell generations is based on the intracellular signaling domain. The first generation of CAR-T cells had only a CD3ζ cytoplasmic domain. After that, the second generation of CAR-T cells had co-stimulatory domains of CD28. The third generation of CAR-T cells had several co-stimulatory domains, such as CD28-41BB or CD28-OX40[57,58]. CAR-T cells are used as therapeutic approaches to diseases, especially cancers.

CAR-T cell therapy is a kind of ACT in which T cells are taken from the patient's blood. Then, the gene for recognition of special cancer receptors is added to the T cells. Large numbers of genetically engineered CAR-T cells are grown in the laboratory and infused into the patient. Several clinical trials[59] by different targets of CAR-T cells are globally being conducted for the treatment of cancers.

One of the CAR-T cells, called CART72 cell, which targets tumor-associated glycoprotein (TAG)-72, was used during the clinical phase I of CRC patients[60]. The findings have shown the relative safety of CART72 cells, and there were no signs of tumor toxicity on the target. In another study[61], GUCY2C (a cancer mucosa antigen expressed on the luminal surfaces of the intestinal) targeted CAR-T cells, which were effective in mouse models as well as CRC xenograft transplantation models. The researchers[61] demonstrated reduced tumor number, morbidity, and improved mice survival, as well as observations highlighting the therapeutic potential of GUCY2C-directed CAR-T cells to treat metastatic CRC. One of the CRC antigens is a CEA. Anti-CEA CAR-T cells have antitumor activity and also have the potential to stop immunosuppression. In a phase-I-clinical trial[2], CEA was used as a target CAR-T cell in 10 patients with CEA+ CRC. The results showed the good efficiency and persistence of CAR-T cells in CEA+ CRC patients after high doses of CAR-T cell treatment. Third-generation anti-NKG2D CAR-T cells showed dose-dependent cytotoxicity, higher secretion of IL-2 and IFN-γ than untransduced T cells, inhibition of tumor growth, decreased tumor size, and excellent killing of targets[62]. There are several targets for CAR-T cell therapy in CRC patients, including CD133 (cancer stem cell marker), human epidermal growth factor receptor 2 (HER2), and epithelial cell adhesion molecule (EPCAM) which have showed[59,63,64] antitumor activities. The combination of CAR-T cell therapy with drugs is a suitable approach for the ACT. *Indoleamine 2,3-dioxygenase 1* (*IDO1*) is highly expressed in colorectal tumors. It was demonstrated[65] that miR-153, as a tumor-suppressive miRNA, inhibits *IDO1* expression in CRC cells, and the combinatorial use of CAR-T cells and *IDO1* inhibitors in treating solid tumors was promising. In a case study[66], an attempt to treat metastatic CRC patients with anti-ERBB2 (also known as HER2) CAR-T cells reported serious adverse events and the patient died 5 days after treatment. Results showed increases in IFN-γ, IL-6, IL-10, TNF-α, and granulocyte macrophage-colony stimulating factor (GM-CSF) as a cytokine storm that leads to respiratory failure due to reactivity to lung tissue. Clinical trials using CAR-T cells in CRC patients are listed in Table 4.

CAR-NK Cells

NK cells have been introduced as promising candidates for CAR-based cellular immunotherapy. No MHC restriction and abundant sources make CAR-NK cells potentially available for clinical use. Nonetheless, several problems need to be addressed for the successful implementation of CAR-NK treatment in solid tumors. In addition to designing the optimal CAR structure, it is important to make genetic changes in the inhibitory and activating pathways inherent in NK cells. One of the main barriers to the production of CAR-NK, regardless of the source of NK cells, is that they all need to be activated and expanded before injection[67]. The structure of CAR-NK cells is similar to CAR-T cells. The extracellular antigen binding domain of CAR-NK cells consists of scFv or NKG2D, and the signaling domain can be CD28, inducible T cell co-stimulator (ICOS), 4-1BB, CD27, CD40, CD134 and DNAX-activating protein 10 (DAP10)[68]. Recent studies[69] have shown that CAR-NKG2D-NK cells can increase the cytolytic activity of CRC cells *in vitro*, which can be effective in therapeutic interventions in CRC. A clinical study[70] showed that the treatment of three metastatic colorectal cancer patients with local infusion of the

Table 4. Clinical trials using TIL for cellular therapy of CRC are in progress around the world.

Clinical trials identifier	Drugs	Phases	Status	Type of disease	Year
NCT02959151	CAR-T cell	I/II	unknown	Colorectal cancer metastatic	2016
NCT02617134	Anti-muc1 CAR-T cell	I/II	unknown	Colorectal cancer and other cancers	2016
NCT03152435	EGFR CAR-T cell	I/II	unknown	Metastatic colorectal cancer	2017
NCT02349724	Anti-CEA-CAR_T cell	I	unknown	Colorectal cancer and other cancers	2017
NCT03542799	EGFR-IL12-CAR_T cell	I	unknown	Metastatic colorectal cancer	2018
NCT04107142	Adoptive cell transfer of NKG2D targeting CAR-grafted Gama Delta T cell	I	unknown	Colorectal cancer and other cancers	2019
NCT03638206	CAR-T cell Immunotherapy	I/II	recruiting	Colorectal cancer and other cancers	2019
NCT04513431	Anti-CEA-CAR_T	Early phase I	not yet recruiting	Stage III colorectal cancer and colorectal cancer liver metastatic	2020
NCT03310008	NKG2D CAR-Tcell	I	active, not recruiting	Colon Cancer liver metastatic	2020
NCT03370198	NKG2D CAR-Tcell	I	active, not recruiting	Colon cancer liver metastatic	2020
NCT04348643	CEA CAR-T cells	I/II	recruiting	Colorectal cancer	2020
NCT04550663	KD-025 CAR-T cell	I	not yet recruiting	Colorectal cancer	2020
NCT04503980	Alpha-MSLN-CAR-T cell	Early phase I	recruiting	Colorectal cancer and ovarian cancer	2020
NCT05089266	CAR-T cells	I	not yet recruiting	Colorectal cancer	2021

CAR-NK cells promised therapeutic potential. Two patients reported a decrease in ascites generation and tumor cell number, and other patients showed rapid tumor regression.

Zhang et al[71] conducted a study where they explored the combination therapy of the CAR-NK92 cell line with other therapeutic approaches. In their research, they utilized lentivirus vectors to transduce epithelial cell adhesion molecule (EP-CAM) to NK-92 cells. The engineered CAR-NK-92 cells were able to detect epithelial cell adhesion molecule (EP-CAM)-positive colorectal cancer cells, and release cytokines such as perforin, granzyme B, and IFN-γ. It was also found[71] that the combined effect of regorafenib (a multi-kinase inhibitor drug against metastatic colorectal cancers) and CAR-NK-92 on EP-CAM-positive tumor xenografts was greater than monotherapy with CAR-NK-92 or regorafenib cells, which could increase the therapeutic effect of CRC. Interestingly, the treatment of colorectal carcinoma cell lines with CEA-CAR-NK-92MI cells and histone deacetylase-inhibitor (HDAC), histone deacetylase sodium butyrate (NaB) inhibitors, or 5-azacitidine methylation inhibitor (5-AZA) showed lysed effects on high CEA-expressing tumor cell lines (LS174T) and moderate CEA-expressing tumor cell lines (WiDr). The cytolytic function of anti-CEA-CAR NK-92MI cells was increased after the treatment of CRC cell lines with histone deacetylase (HDAC), neutralizing antibody (NAb) and 5-Azacytidine (5-AZA)[72]. This data indicates that pharmacological modifications can increase the efficacy of anti-CEA-CAR NK-92 MI cells[53,69]. There are some challenges in the field of CAR-based ACT, so some of the important topics are cell source as well as the kind of CAR construct that can improve the efficiency of CAR-T and CAR-NK cells. Given the promising results, but together with some adverse reactions of the CAR-T cells and CAR-NK cells, more investigations need to be carried out.

Conclusions

Despite the increasing ACT methods for various cancers, including CRC, there are still several drawbacks, including immune cell anergy, inadequate

proliferation, or removal of the engineered cells after tumor regression. The use of a cancer combination approach or better manipulating the engineered cells can overcome these problems[12]. Because of the multiple immunosuppressive mechanisms of solid tumors, it is important to present more effective and personalized strategies to strengthen the efficacy of ACT. Difficulty in the identification of TCRs among the repertoire of billions of candidates, tumor-specific epitopes, and designing a suitable CAR molecule are other obstacles or challenges to the successful application of the ACT[10].

There are some other kinds of ACT also which may achieve success in the future. The stem cells have immunosuppressive, anti-tumor, and migratory properties. They can also regulate innate and cellular immune pathways by expressing growth factors and cytokines. These cells could be a suitable candidate for ACT[73]. In a study[74], the researchers investigated umbilical cord blood-derived NK cells (UCB-NK) and allogeneic-activated peripheral blood NK cells (A-PBNK). The results showed that UCB-NK cells had higher cytotoxic effects than A-PBNK cells in colon cancer cells resistant to cetuximab *in vitro*. TCR engineering, or artificial TCRs, is another technique of ACT. In a clinical trial[75], CRC patients were treated with autologous T cells genetically engineered with murine CEA TCRs. The results of this study were not promising, in fact, all patients had severe autoimmune colitis. The data demonstrated that the use of natural TCRs may be promising in the treatment of cancers[75]. In a study[76], TCR was isolated naturally from a CRC patient who was vaccinated with a synthetic TGF-β type II receptor (TGFβRII) peptide. The authors engineered the T cells with the *TCR* gene and showed that the engineered T cells could produce some cytotoxic cytokines following incubation with the target cells. Therefore, developing specific and more powerful approaches such as ACT with fewer side effects is required. ACT, alone or in combination with other therapy methods, can hold great promise for CRC treatment.

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The authors declare no conflict of interest.

ORCID ID:
Mohammad Reza Atashzar: 0000-0002-4135-8178

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