Archives of Advances
in Biosciences

Review Article:

Design, Effectiveness, Limitations and Future Perspectives of CAR-T Cells: A Review Article

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Cite this article as: Chegni H, Entezari S. Design, Effectiveness, Limitations and Future Perspectives of CAR-T Cells: A Review Article. 2022; 13(1):E38054. https://doi.org/10.22037/aab.v13i.38054

https://journals.sbmu.ac.ir/aab/article/view/38054

Article info:
Received: 05 Apr 2022
Accepted: 14 May 2022
Published: 28 May 2022

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Abstract

Context: Targeted anti-cancer approaches bring about individual therapies to combat the complexity of most malignancies and enhance their chances of success. Currently, immunotherapy, which exploits the patient's immune system to fight the disease, has made a significant progress in the success rate of cancer treatment. T lymphocytes are one of the most powerful arms of the immune system against cancer cells; however, many tumor cells can escape by hiding their peptide antigens.

Evidence Acquisition: CAR-T cells can detect tumor cells' HLA without any restrictions. Promising outcomes from CAR-T cell clinical trials have increased hope among cancer patients, making CAR-T cell a prospective treatment for most cancers. However, its unique toxicities and the possibility of recurrence have raised concerns among scientists.

Results: Therefore, in this review, in addition to the design of CAR-T cells, we intend to discuss the process of CAR-T cell therapy in the treatment of malignancies and explore its disadvantages, advantages, and prospects.

Conclusion: Despite extensive studies, it is not yet possible to confirm the role of CAR-T cells, but based on the experience of applying CAR-T cells, a definite treatment is feasible through immunotherapy and strengthening the immune system.

Keywords: Adoptive immunotherapy, Chimeric antigen receptor (CAR), Cytokine release syndrome, Toxicity

1. Context

One of the leading causes of death worldwide is cancer which often leads to treatment failure through several complex and unknown mechanisms. Surgery, chemotherapy, and radiotherapy have been the primary cancer treatment for many years, yet recently targeted therapies have also become available. Targeted anti-cancer approaches assist individual therapies in combating the complexity of most malignancies and increase their chances of success. Although these approaches have improved the outcomes, most malignancies still represent a poor prognosis. Currently, immunotherapy, in which the patient's immune system is exploited to fight the disease, has increased and has made a significant progress in the success rate of cancer treatment. T lymphocytes are one of the most powerful arms of the immune system to fight cancer cells. However, since the function of these cells is limited to peptide antigens, many tumor cells can escape by hiding their peptide antigens from these cells. It is assumed that developing an approach to overcome this restriction can improve the application of immunotherapies. One way is to genetically engineer patients' T cells

so that they express chimeric antigen receptors (CAR) that detect and attack certain tumor cell antigens [1, 2]. Due to the alterations that tumor cells induce to prevent the expression of protein antigens, T lymphocytes must become able to identify their non-protein antigens as well. This idea has led to the development of CAR-T cell therapy. CAR-T cells can detect tumor cells' HLA without any restrictions [3]. Proper transfer of T cells taken from autologous peripheral blood and manipulating them to express CARs have generated a significant clinical response in patients with blood malignancies [4]. CAR-T cell treatment has shown to be effective in treating a range of immunological diseases, including leukemia, autoimmune diseases, solid cancers, asthma, and allergic disorders in recent years [5-7]. In this approach, T cells have been genetically engineered in order to express a receptor on their surface that specifically binds to the tumor antigens. CAR-T cells are then amplified for therapeutic use and reinfused into the patient's body to kill cancer cells resistant to chemotherapy [8].

Despite the advantages, this treatment can lead to tumor relapse in some cases after responding to the treatment. Loss of antigen, unique toxicity, low persistence, and limited tumor penetration are among the negative sides of this treatment [9]. Therefore, in this review, in addition to the design of CAR-T cells, we intend to discuss the process of CAR-T cell therapy in the treatment of malignancies and explore its disadvantages, advantages, and prospects.

Construction

Chimeric antigen (CAR) receptors were first developed in the mid-1980s [10]. Then in 1993, the Escher Group changed its concept [11]. The basic structure of a CAR-T cell is comprised of four components, including an ecto-domain, a hinge domain, a transmembrane domain, and an endo-domain [12, 13].

The extracellular portion, namely ScFv, is the single-strand variable fragment (ScFv) of the monoclonal antibodies that binds to the tumor cell antigens [12, 13]. Unlike natural T cell receptors, CAR ScFvs do not require antigen processing and epitope presentation to detect antigens and can detect antigens and kill tumor cells independent of MHC [14, 15]. The ability of each CAR in binding to its target antigen depends on its extracellular ScFv. For example, in the anti-CD19 CARs that have been approved (kymriah and yescarta), the ScFv domain was created using mouse PMC63 (anti-CD19 antibody) [16]. A ScFv's format usually consists of two modifiable parts linked together by a peptide sequence as a linker and is designed as VH-linker-VL and VL-linker-VH [17]. Currently, most linkers used in CAR-T cells contain polypeptides based on glycine and serine replicates. For example, (Gly4Ser) 3-linker contains three replicates of the pentapeptide Gly -Gly -Gly -Gly -Ser [18-20]. The location of the variable segments in ScFv depends on the structure of ScFv, which may help the expression of CAR on the Tcell surface or target an antigen or signal. Furthermore, the linker variable's length or composition can have a significant impact on the ScFv's stability [17].

The extracellular portion of the CAR structure that separates the junction from the transmembrane region is known as the hinge or the spacer. Except for certain CARs based on the full extracellular part of a receptor, such as NKJ2D, immunoglobulin-like hinges are used in the construction of the majority of CAR-T cells, and these spacers are often used to provide stability for proper CAR expression, flexibility, and function in reaching the target antigen. Many studies have revealed that the optimum hinge length in a CAR is determined by the location of the target epitope [21]. CARs with longer spacers supply more flexibility for CAR and improve access to proximal membrane epitopes or composite glycolyzed antigens [21-23]. In contrast, short hinge CARs are better in binding to the distal membrane epitopes [24, 25]. Therefore, the length of the hinge domain is critical for providing enough intercellular space for the immunological synapses to develop. Besides, the overall function of the CAR-T cell can be influenced by the hinge. For example, a human IgG-derived spacer consists of two IgG-like components (CH3 and CH2) used to detect antigens and are quite useful for determining the level of CAR expression on the surface of T cells. However, many investigations have demonstrated that even after fusion with CAR, IgG-derived spacers retain their ability to bind to the FcgR receptor (gamma receptor) via the CH2 region. CARs that have a spacer with the second Fc bind to IgG Fc gamma receptors (FcγRs), which activate cellular immunity including monocytes and natural killer (NK) cells, that leads to release of a huge number of proinflammatory cytokines. However, Engineered T cells are activated by FcγR binding, which results in cytokine release and monocyte and NK cell destruction. To reduce the binding to FcγR, the second spacer is modified without affecting CAR expression. CAR-modified T cells do not activate in the presence of FcγR (+) cells, thus reducing the likelihood of off-target activation while retaining redirected targeting specificity [26].

The membrane portion consists of an alpha hydrophobic helix that runs along the cell membrane.

Although the membrane's primary role is to bind the CAR to the T cell membrane, some proofs suggest the membrane might be related to the function of the CAR-T cell. CAR signaling nature is not fully understood. Providing that a CAR is to function like TCRs, the signaling action should be accompanied by another molecule as a dimer. Common membrane fragments are usually derived from CD4, CD8α, CD28, and CD3ζ [27-29].

CAR endodomains are often formed from stimulus-assisting molecules of the CD28 family (including ICOS and CD28) or genes of the tumor necrosis factor receptor (TNFR) family (including 4-1BB, CD27, or OX40). The most prevalent stimulants utilised in CARs are CD28 and 4-1BB. Clinical studies with CD28 or 4-1BB in patients with hematologic malignancies have shown similar results, but T cells generated with these two CARs have extremely different life spans [30, 31] Clinical trials for B cell malignancies have shown that CAR-T cells with CD28 are usually undetectable for more than three months; on the other hand, CAR-T cells containing 4-1BB can remain in patients' years following therapy. More comprehensive studies have shown that signaling via CD28-based CARs promotes T cell proliferation, activation, increased glycolysis, and cytolysis, but also reduces T cell viability. Signaling through 4-1BB, on the other hand, causes a delayed T cell response and increases oxidative metabolism, mitochondrial biogenesis, and more extended T cell durability [8, 32-34] (Figure1).

CAR-T cells are classified into four generations depending on their ability to release cytokines and the presence of a co-stimulator. The first generation has extracellular ScFv attached to the intracellular signaling portion of CD3ζ. This generation of CAR-T cells is eliminated immediately due to its low survival rate and inability to elicit a sustained immune response. The second generation includes a co-stimulatory (4-1BB or CD28) and a CD3z signaling portion. In this generation, CAR-T cells have demonstrated high persistence and proliferation, with considerable anti-tumor efficacy at the tumor site. The third generation has two common excitation portions in addition to the CD3ζ signal portion. This type of CAR-T cell exerts high efficiency, substantial proliferation, and better durability against tumor cells in comparison to the first and second generations [35]. The fourth generation of CAR-T cells are called redirected universal cytokine killer (TRUCK) cells, which can release biochemical molecules, cytokines, and enzymes by adding other genes to its genome. The most commonly used cytokines in the production of TRUCK are IL-18, IL-15, and IL-12. TRUCK prolongs the life span of CAR-T in the tumor environment and enhances the strength of the immune response against solid tumors [36, 37] (Figure 2).

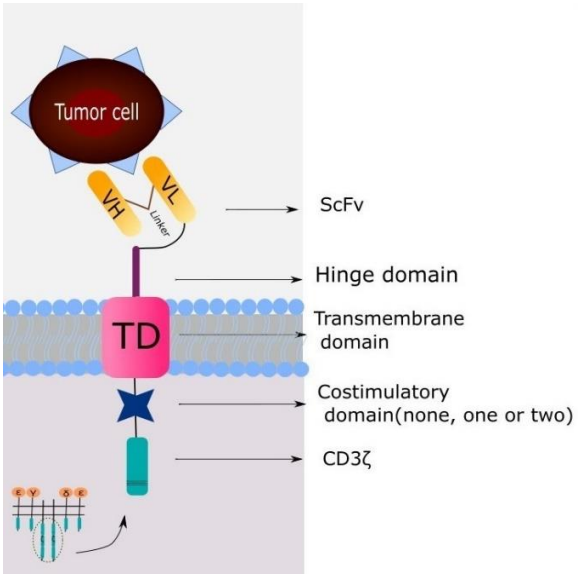

Figure 1. The basic structure of a CAR-T

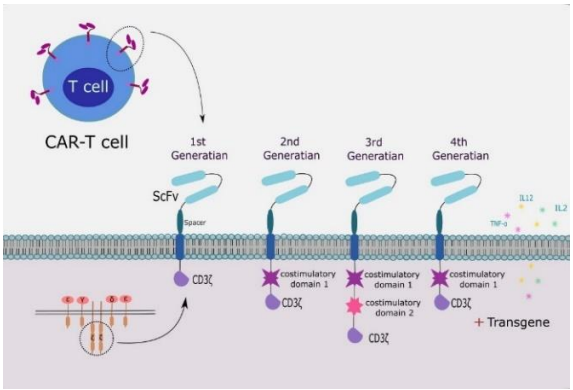

Figure 2. Different generations of CAR-T cells. Divided into four generations based on the presence of a co-stimulator and the ability to secrete cytokines

2. Evidence Acquisition:

Clinical trials for CAR T-cells

Although CARs were initially proposed in the late 1980s, they were not clinically tested as a cancer therapy until 2006. Because of the limited durability of genetically modified lymphocytes, initial results in adult patients were not encouraging [38, 39]. One study represented antitumor effects in four of the eight children with metastatic neuroblastoma after CAR-T cell injection containing only CD3ζ, but the survival of these cells was often short [40]. Despite the fact that CAR-T cells were safe, most early trials showed poor results [41]. The main problem was that CAR-T cells did not spread and persist in the body for more than a few days. Attempts to overcome this challenge were made through increasing the injection dose, but they were unsuccessful, emphasizing the need to increase CAR-T cells' life span and function in vivo [8]. Subsequently, studies on different constructions of CAR-T cells found that using the second costimulatory 4-1BB increases the durability of these cells [32-34].

Some studies have shown that CAR-T cell can lead to the patient's long-term survival without disease and has shown excellent results in clinical trials of recurrent diseases [42-45] Table 1. For example, promising results were obtained in phase 2 single cohort experiment of tisagenlecleucel (anti CD19 CAR), performed in 2018 on children and adolescents with resistant B-ALL or with recurrence of CD19+. In this study, 75 patients with a mean age of 11 years were enrolled. They received 3.1×10^6 T cells per kilogram of their body weight. Patients' overall response to treatment within three months was 81%, and their overall survival at six months was 90%. tisagenlecleucel remained in patients' blood for 20 months, and grade 3 or 4 complications associated with tisagenlecleucel occurred in 73% of patients. CAR-T cell treatment with a single injection of tisagenlecleucel showed long-term benefit with recurrence or resistance in children and adolescents

Table 1. Some studies have shown results in clinical trials of CAR-T cell

Author	Targeted antigen	Disease	Phase	NO. of patients	Outcome
Li et al. [47]	BCMA	RRMM and plasma cel leukemia	Phase 1 clinical trial	30	CRR: 43.3% Objective response rate: 90%
Madduri et al. [48]	BCMA	RRMA	Phase 1b/2 clinical trial	97	CRR: 55.7% Overall survival rate (OSR): 87.7%
Mailankody et al. [49]	B cell maturation antigen (BCMA)	Relapsed refractory multiple myeloma (RRMM)	Phase 1 clinical trial	44	Complete response rate (CRR): 39% Objective response rate: 76%
Lin et al.[50]	BCMA	RRMM	Phase 1 clinical trial	62	CRR: 39% Overall response rate (ORR): 76%
Raje et al. [51]	BCMA	RRMM	Phase 1 clinical trial	33	CRR: 45%
Chen et al. [52]	BCMA	RRMM	Phase 1 clinical trial	17	CRR: 82%
Mailankody et al. [53]	BCMA	RRMM	Phase ½ clinical trial	19	CR: 1
Zhao et al. [54]	BCMA	RRMM	Phase 1 clinical trial	57	ORR: 88% CRR: 68%
Shah et al. [55]	CD19	R/R B-ALL	Phase ½ clinical trial	45	Overall complete remission rate: 45%
Zhou et al. [56]	CD19	R/R B-cell non-Hodgkin lymphoma (B-NHL)	Phase 1 clinical trial	21	CRR: 43% ORR: 67%
Wang et al. [57]	CD19	R/R mantle-cell lymphoma	Phase 2 clinical trial	74	CRR: 59%

Table 1. Continued

Locke et al. [43]	CD19	Refractory large B cell lymphoma	Phase 1-2 clinical trial	108	CRR: 58%
Wang et al. [58]	CD19	R/R mantle-cell lympohma	Phase 2 clinical trial	28	CRR: 57% ORR: 86%
Siddiqi et al. [59]	CD19	R/R CLL/SLL	Phase ½ clinical trial	22	CRR: 45.5%
Yan et al. [60]	CD19	Refractory B-NHL	Phase 1 clinical trial	10	ORR: 100% CRR: 66.7%
Locke et al. [61]	CD19	Refractory DLBCL	Phase 1 clinical trial	9	CRR: 57% ORR: 71%
Lee et al. [62]	CD19	R/R B-ALL	Phase 1 clinical trial	20	CRR: 70%
Zhang et al. [63]	CD20	B-NHL	Phase 2a clinical trial	11	CRR: 54.5%
Shah et al. [64]	CD22	B-ALL	Phase 1 clinical trial	58	CRR: 70%
Ramos et al. [65]	CD 30	R/R Hodgkin lymphoma	Phase ½ clinical trial	41	CRR: 59% 1-year OSR: 94%
Wang et al. [66]	CD30	R/R Hodgkin lymphoma	Phase 1 clinical trial	18	Partial remission [PR]: 38.3%
Davila et al. [67]	CD28	B-ALL	Phase 1 clinical trial	16	CRR: 88%
Wang et al. [68]	CD133	CD133 positive malignancies	Phase 1 clinical trial	25	PR: 3/23 Stable disease: 14/23
Liu et al. [69]	CD19/CD22	Relapsed B-ALL	Phase 1 clinical trial	27	CR rate: 85% OSR: 88.5%
Cordoba et al. [70]	CD19/CD22	R/R B-ALL	Phase 1 clinical trial	15	CR rate: 86% 1-year OSR: 60%
Tong et al. [71]	CD19/CD20	R/R B-ALL	Phase 1/2a clinical trial	33	CRR: 71% ORR: 79%
Sang et al. [72]	CD19 and CD20	R/R diffuse large B cell lymphoma (DLBCL)	Phase 2 clinical trial	21	CRR: 52.4% Median overall survival [os]: 8.1
Yan et al. [73]	BCMA/CD19	RRMM	Phase 2 clinical trial	21	ORR:95% CRR: 43%
Zhang et al. [74]	Epidermal growth factor receptor (EGFR)	R/R non-small cell lung cancer	Phase 1 clinical trial	9	Median overall survival: 15.63 months Progression free survival: 7.13 months
Shi et al. [75]	Glypican-3(GPC3)	Advanced Hepatocellular carcinoma	Phase 1 clinical trial	13	1-year OSR: 42%
Guo et al. [76]	EGFR	EGFR-positive Advanced Biliary tract cancers	Phase 1 clinical trial	19	1 of 17 evaluable patients achieved CR and 10 had stable disease
Beatty et al. [77]	Mesothelin (MSLN)	Pancreatic ductal adenocarcinoma (PDAC)	Phase 1 clinical trial	10	6 patients were treated. 2 of 6 patients had stable disease.
Zhang et al. [78]	CEA	CEA+ Metastatic colorectal cancers	Phase 1 clinical trial	10	Patients having stable disease: 70%
Junghyans et al. [79]	Prostate specific membrane antigen (PSMA)	Prostate cancer	Phase 1 clinical trial	6	5 patients were treated. 2 of 5 patients achieved PR.
Louis et al. [80]	GD2	Neuroblastoma	Phase 1 clinical trial	19	CR: 3/19 PR: 1/19

with B-ALL [42]. Moreover, in the ZUMA-1 clinical trial, which was a single-arm multicenter study, it was found that axicabtagene ciloleucel (autologous anti-CD19 chimeric antigen receptor) could cause persistence in response and overall survival of more than two years. The study was conducted in 22 centers in the United States and Israel in 2018 among 108 patients over 18 who had large B-cell lymphoma. Patients' overall response to treatment was 27% at 27.1 months, and the mean response time was 11.1 months, with 48% of patients having grade 3 complications [43] These results led to the US Food and Drug Administration (FDA) licensing for cilicucel and tisagenlecleucel axicabtagene, as well as obtaining European Medicines Agency approval for both products [46].

Limitations in the application of CAR T-cells

Promising results of CAR-T cell in clinical trials have offered great hopes among patients, which has made CAR-T cells one of the most effective cancer treatment. Nevertheless, antitumor activities of CAR-T cell has unique toxicities as well [81]. Life-threatening problems can emerge as a result of CAR-T cells activating the immune system [82]. The most common toxicities are related to neurotoxicity and cytokine release syndrome (CRS). The most prevalent complication of CAR-T cells is CRS. CRS is a systemic inflammatory response induced by the release of inflammatory cytokines and chemokines such as tumor necrosis factor (TNF) α, interferon (IFN) y , GM-CSF, interleukin (IL) -2, IL-8, and IL-10 subsequent to binding of CAR-T cells to the target antigen [81-83]. Fever is the first sign of CRS. After the injection of CAR-T cell fever can appear in patients within a couple hours to a few weeks. Fever may be above 40°C and may be associated with weakness, headache, anorexia, and nausea, which can rapidly lead to hypoxia, life-threatening tachycardia, and hypotension, associated with elevated serum cytokine levels [84]. CRS often arises within the first week of CAR-T cell injection [42, 44]. The levels of interlukin-6 in serum is related to the severity of CRS following CAR-T cell therapy. Those with a higher tumor burden, other conditions, and those who manifest CRS after three injection doses show more severe symptoms [85-87].

Neurotoxicity is the second most common consequence in individuals treated with CD19 CAR-T cells. Although it is self-limiting, it can be fatal and life-threatening. Neurotoxicity was previously known as CAR-T cell encephalopathy syndrome. However, today it is known as ICANS [50, 53]. ICANS has also been shown in experiments other than those targeting CD19. In one trial, ICANS was seen in 24% of patients with ALL treated with CD22 CAR-T cells [88]. ICANS appears to be less common in CAR-T cells targeting B-cell maturation antigen; however, several severe symptoms such as reversible cerebral edema have been reported [89-91].Whether the difference in toxicity depends on the target antigen or the structure design is not yet known; however, the presence of ICANS reduces the direct targeting of CD19 antigen on central nervous system elements (this is a previously proposed hypothesis.) [81].The clinical signs of ICANS are numerous, including expressive aphasia, encephalopathy, and language dysfunction, low level of consciousness, motor weakness, headache, seizures and rarely cerebral edema [84, 93-96]. Some of these symptoms, such as aphasia, are very specific to ICANS. In one study, the primary neurological symptoms of aphasia, which occurs in 85% of patients with ICANS, included incorrect naming of objects, stuttering, and persistent speech [92]. Other common and early symptoms of ICANS are impaired attention and handwriting changes. Intubation may be required to preserve the airway if symptoms develop to seizures and loss of consciousness [84, 88, 96]. The pathophysiology of ICANS is less understood than CRS. For this reason, in mild cases, supportive care and corticosteroids are often used for more severe cases [84].

Recurrence of the disease after CAR-T cell treatment

Initial CAR-T cell analyses showed excellent performance, leading to FDA approval for two CD19 CAR-T cells (axicabtagene ciloleucel and tisagenlecleucel) [44, 96]. However, according to continuous follow-up of patients with B-cell malignancies, who were treated with CAR-T, had a considerable recurrence rate after treatment due to tumor resistance [97]. Typically, the immune stress caused by CAR-T cells leads cancer cells to evolve below the CAR-T detection threshold by modulating their target antigens' expression, either by losing the target antigen or by reducing the antigen expression [98]. There are two types of disease recurrence following CAR-T cell injection: recurrence with positive antigen in the early stages and recurrence with antigen escape in later stages. Recurrence of the disease with a positive antigen is closely related to the persistence of CAR-T cells, with the antigen still present on the surface of the tumor cell, which can be detected by flow cytometry [97]. Nevertheless, the low persistence of CAR-T cells causes low effectiveness by manipulating their structure. For example, based on studies conducted by Zhao et al., it was found that the use of 4-1BB has a significant effect

on the durability and stability of CAR-T cells [34]. In recurrence of antigen-negative disease (recurrence with antigen escape), there is no antigen on the surface of the tumor cell. For example, in the study by Xinjie Xu in 2019, it was found that about 10-20% of patients had a recurrence of CD19 negative after receiving CD19 CAR-T cells [99]. Some of these reports are mentioned below.

In the first Phase 1 report published by CD19-4-1BB, CAR for B-ALL cancer in children from Philadelphia Children's Hospital in 2014, 3 of 27 respondents (11%) recurred with leukemia lacking CD19. A summary of the patients at this hospital with more extended follow-up than the initial patients showed that 13 of the 55 patients (24%) who presented a complete response (CR) experienced recurrence with CD19 negative [96]. In a clinical trial at the Seattle Children's Research Institute (SCRI), a CAR with a similar CD19-4-1BB, 7 of 40 B-ALL patients (18%) who developed CR later recurred with negative CD19 [101]. [101]. Besides, in a phase 2 trial of novartis's tisagenlecleucel, among 16 recurrent patients, 15 of them had a recurrence of CD19 negative. Thus, at least 15 of the 61 patients (25%) who thoroughly responded were CD19 negative or had reduced CD19 expression [96].

Published data suggest that CD19 antigen loss occurs through two mechanisms: antigen escape or lineage switch [99]. During the antigen escape, the disease recurs with phenotypically similar cells, which lack surface expression of CD19 and can bind to the anti-CD19 antibody embedded in CAR [101]. Evidence suggests that point mutations can cause antigen escape during CAR-T cell therapy. CAR-T and CD19+ B cells were genetically examined by Zhang Z et al in a patient with a high grade of B lymphoma before and after the recurrence. The patient recurred six months after receiving CD19 CAR-T cells (FMC63). A mutation in exon 3 of malignant B cells was observed. Further analysis showed that FMC63 CAR-T cell has anti-tumor capabilities against various CD19 + cells but failed to eradicate these CD19 + mutant cells [102]. Another mechanism for CD19 loss is lineage switch. Lineage switch occurs when the patient recurs with similar genetics but different phenotypes, which often occurs in acute myeloid leukemia. Lineage switch is more common in patients with MLL gene rearrangements in response to CD19 immunotherapy, the phenotype of their leukemia shifted from lymphoid to myeloid. Not only does the developed leukemia population lose CD19 expression, but it also receives other phenotypic characteristics of AML. This was seen in two non-rearranged children with ALL-MLL who were treated with CD19 CAR in the SCRI (Seattle Children's Research Institute) trial and one adult treated in the FHCRC (Fred Hutchinson Cancer Research Center) trial [103, 104].

New strategies have recently been discovered to improve the safety and efficacy of CAR-T cell therapy [105]. Since antigen escape is the primary mechanism for escaping immunotherapy, a combination of CAR cells that target multiple antigens is being tested as a strategy to reduce the recurrence of the disease, called the multi antigen targeted CAR-T cell method [106]. Several major treatments using multiple CAR-t cells include the following: 1-Pooled CAR-T cell: A combination of two modified T cell lines, each with a specific CAR for an antigen. 2-Dual CAR-T cells: Two independent CARs in a T cell that target distinct antigens in a cancer cell. 3-Tandem CAR-T cell CAR-T: In this model, two antigen-binding parts are connected to a single CAR and 4- Trivalent CAR-T cell: Three CARs in an engineered T cell target specific antigen molecules [107]. Zah et al, in one of the first successful preclinical trials, developed CD19-CD20 CAR tandem CAR-T cells and demonstrated that the dual structure might inhibit the spontaneous generation of CD19-negative tumor cell types in immunocompromised mice [108]. In another in vivo study with multiple myeloma, dual CAR-T cells were designed to target CAML interactor (TACI) B cells and transmembrane activator and B-cell maturation antigen (BCMA), which yielded promising results [109].

Other applications of CAR-T cells have been utilised to treat malignancies, and FDA has approved many CAR-T cells for cancer treatment. However, the benefits of CAR-T cell therapy can also be used to treat different sorts of disorders [110]. Today, research is being done to treat diseases such as autoimmune diseases, asthma and allergies, infectious diseases, HIV, and even Covid-19 using CAR-T cell, and despite the difference in the cause, common features among this disease, there is a possibility that they can be treated with CAR-T cell. In many cases, due to the progression of the disease, the immune system has become somewhat dysfunctional, and the CAR-T cell serves as a powerful alternative to the human immune system [111].

3. Results

Given the fact that cancer is one of the main causes of mortality worldwide, finding an effective

treatment for it is one of the most significant concerns among scientists. Due to the increasing percentage of recurrence and resistance of cancer cells against common treatments such as surgery, chemotherapy, radiotherapy, the need to provide new treatment strategies based on changes in the immune system is quite evident. With the advent of CAR-T cell treatment, there is much more hope for the treatment of various cancers. In this treatment, the limitation of antigen detection by T lymphocytes, which performs as the strongest arm of the immune system in the defense against cancer cells, was partially removed, and the results of clinical trials based on this method were highly promising. However, over time and seeing recurrence in treated patients, it was found that cancer cells are highly adaptable and can undergo phenotypic and genotypic changes in different conditions and fight for their survival. Changing the behavior of cancer cells against CAR-T cells led to the formation of new methods of using CAR-T cells, which somehow succeeded in controlling recurrence after treatment. However, for the final conclusion, we have to wait much longer for the new behaviors of cancer cells against new CAR-T cell methods, including the method of using multiple CAR-T cells.

4. Conclusion

The use of CAR-T cell method in the treatment of tumors, despite its success, has limitations such as the possibility of CRS. Recurrence after treatment has posed new challenges for the use of this method, which we hope will prevent recurrence after treatment using the new CAR-T cell methods. Despite extensive studies, it is not yet possible to confirm the role of CAR-T cells, but based on the experience of applying CAR-T cells, a definite treatment is feasible through immunotherapy and strengthening the immune system.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This study received no financial support from public or private agencies.

Author's contributions

All authors have equally contributed to preparation of this article.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors appreciate the contribution of all participants of this study.

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