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REVIEW ARTICLE

CAR-modified immune cells as a rapidly evolving approach in the context of cancer immunotherapies

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Abstract

Nowadays, one of the main challenges clinicians face is malignancies. Through the progression of technology in recent years, tumor nature and tumor microenvironment (TME) can be better understood. Because of immune system involvement in tumorigenesis and immune cell dysfunction in the tumor microenvironment, clinicians encounter significant challenges in patient treatment and normal function recovery. The tumor microenvironment can stop the development of tumor antigen-specific helper and cytotoxic T cells in the tumor invasion process. Tumors stimulate the production of proinflammatory and immunosuppressive factors and cells that inhibit immune responses. Despite the more successful outcomes, the current cancer therapeutic approaches, including surgery, chemotherapy, and radiotherapy, have not been effective enough for tumor eradication. Hence, developing new treatment strategies such as monoclonal antibodies, adaptive cell therapies, cancer vaccines, checkpoint inhibitors, and cytokines helps improve cancer treatment. Among adoptive cell therapies, the interaction between the immune system and malignancies and using molecular biology led to the development of chimeric antigen receptor (CAR) T cell therapy. CAR-modified immune cells are one of the modern cancer therapeutic methods with encouraging outcomes in most hematological and solid cancers. The current study aimed to discuss the structure, formation, subtypes, and application of CAR immune cells in hematologic malignancies and solid tumors.

Keywords Chimeric antigen receptor · CAR T cell therapy · Hematological malignancy · Solid tumor · Immunotherapy

Introduction

Malignancies are one of the leading causes of death globally, with nearly 19.3 million new cases and 10 million deaths in 2020. The distribution of cases and cancer according to place, race, and gender vary among all continents and countries. It depends on several different factors, which are mainly associated with the socioeconomic status of countries [1]. Different extrinsic and intrinsic factors are associated with cancer that results in damage to DNA and several mutations. These promote unstoppable cell growth, which elicits abnormal homeostasis and death. In normal individuals, these mutations are detected by the DNA response system (DRS) and, in association with the immune system, are removed, thus preventing the formation of any tumors or cancerous cells. In addition to avoiding cancerous cells, the immune system activates different pathways in fighting tumor cells, such as by triggering the anti-tumor response of the adaptive and innate immune systems through natural killer cells (NK cells) and T lymphocytes. In this pathway, IFN-γ is released by T lymphocytes

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and NK cells. IFN-γ stimulation increases the expression of both MHC I and MHC II molecules that aid in the formation of tumor-associated antigens (TAA) that work as a target for cytolytic CD8 + T lymphocytes (CTLs) and thus induce T cell tumor killing. Thus, the risk of cancerous cells increases in people with ineffective or suppressed immune systems, like patients with autoimmune diseases or chronic infections. In addition to the anti-tumor function of immune system, it can be a part of tumor progression through different mechanisms, such as forming new blood vessels through different factors like V-EGF that aid in tumor nutrition and the formation of myeloid-derived suppressor cells (MDSCs) that suppress CTL anti-tumor activity [2, 3]. Routine therapeutic procedures that apply to both hematologic malignancies and solid tumors include chemotherapy, radiotherapy, and surgical resection. Later, when we understand the importance of the role of the immune system in the prevention and fight against malignancies and the progression of diseases in advanced stages, as we mentioned above, new therapies in the field of immunotherapy are introduced to reduce the progression and increase life expectancy in different types of malignancies. These procedures include monoclonal antibodies as immune checkpoint inhibitors like Pembrolizumab and vaccines such as ipilimumab. These target therapies using new antigens (neoantigens) are restricted because of the complexity of MHC I during treatment. This requirement made clinicians design chimeric antigen receptor (CAR) immune cells. CAR immune cells (e.g., CAR T cells) are a composition of immune cells with CARs. CARs are genetically engineered receptors made up of a combination of an extracellular part (single-chain variable fragment (scFv) obtained from monoclonal antibodies), a transmembrane part, an intracellular domain (CD3ζ chain), and co-stimulators like CD28 or 4-1BB. This receptor (CAR) is transported to T cells through retroviral transduction [4, 5]. The CAR immune cell's mechanism begins by recognizing antigens by the scFv part and starting the stimulation cascade by co-stimulators to induce cytotoxicity. During this process, the significant point is that this recognition process, contrary to previous target therapies, is non-HLA restricted and recognizes the antigens regardless of the patients' HLAs [6]. In the upcoming paragraphs of this paper, we discuss this new method and give the last progression that carried on in this new target therapy. The current study has focused on different aspects of CAR-T cell therapy in cancers by discussing the structure, formation, subtypes, therapeutic functions, and the last progression of CAR-T cells.

Immune cells dysfunction in the tumor microenvironment

The immune system has an excellent ability for the particular destruction of tumors without any adverse effect on normal tissue and for persistent memory that can prevent malignancy recurrence. Tumors are recognized and destroyed through a process called immunosurveillance. Generally, when a normal tissue undergoes mutation, it can have three outcomes: termination, balance, and escape. During termination, immune cells destroy tumor cells by recognizing their surface antigens. Tumor antigens are normal cellular proteins that are unusually expressed on the cell surface due to genetic mutations [7]. The first few mutated cells are recognized by NK cells through their encounter with antigens on the tumor cells. This leads to demolishing some mutated cells and the uptake and destruction of their fragments by macrophages and dendritic cells (DCs) [7]. A balanced state will be established when the immune system cannot eradicate cancer. That means the tumor does not metastasize anymore. Sometimes, by increasing risk factors and establishing suitable conditions, tumors can overcome the immune system and become malignancies. As tumors grow, they form an immunosuppressive microenvironment that aids tumor growth and metastasis and decreases the potential responses of adaptive immunity to tumor antigens. The tumor microenvironment (TME) is structured by cancer and controlled by tumor-induced factors [8]. Once formed, the tumor microenvironment represents a functional barrier to immune cell functions (Fig. 1). The tissue microenvironment of a progressing tumor is made up of proliferating tumor cells, the tumor stroma, vessels, infiltrating inflammatory cells, and a variety of related tissue cells [8]. Immune cells in the TME include those with adaptive control immunity like T lymphocytes, DCs, and irregular B cells, as well as innate immunity cells like macrophages, polymorphonuclear leukocytes, and natural killer (NK) cells [8]. Tumors recruit two strategies for their progression and escaping from the immune system destruction. They either hide from the immune system to avoid identification by immune cells or they disable immune cells and alter their normal function. They escape recognition by immune cells through the downregulation of their HLA antigens. The display of molecules like tumor-associated antigens (TAA), HLA-I molecules, or antigen processing machinery components (APM) is often downregulated or altered in tumor cells due to abnormalities in the APM structure, which might contain their downregulation, absence, or mutation [9]. These alterations in tumor antigens make them poor targets for effector immune cells, especially cytotoxic T cells (CTLs). Another way to alter stimulatory function is by downregulating costimulatory peptides on the cell surface. The B7 family is one of the

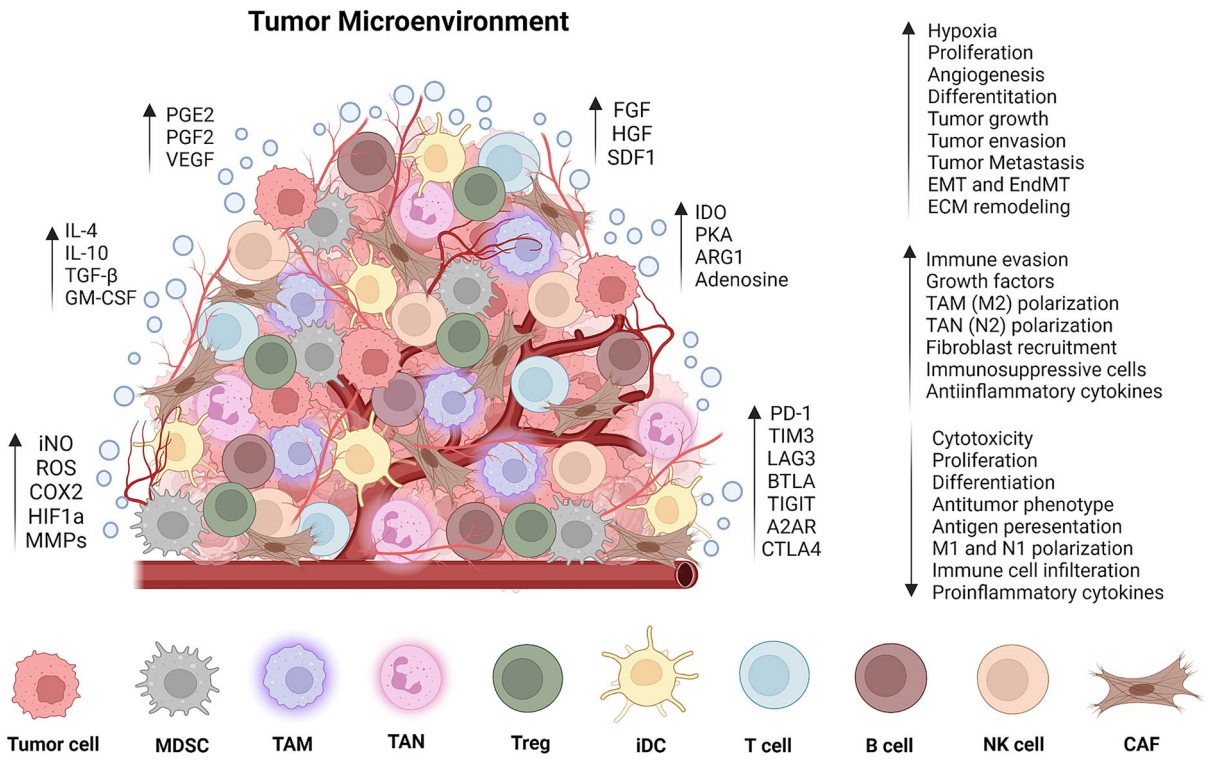

Fig. 1 The tumor microenvironment and associated components. Tumor microenvironment (TME) contains both immunosuppressive cells and factors that contribute to tumor progression and immune response suppression. Immunosuppressive cells include MDSC, TAM, TAN, Treg, and iDC, and immunosuppressive factors include anti-inflammatory cytokines, inhibitory checkpoints, hypoxia, and aberrant metabolic factors, enzymes, as well as tumor stroma and extracellular matrix components

costimulatory molecules that, by changing their function, signal transfer to leukocytes is affected [9]. As mentioned earlier, tumors can avoid immune system destruction by directly affecting the immune system. The assembly and release of immunosuppressive factors alter the function of immune cells or induce their apoptosis. These factors include TGF-β, IL-10, and ROS, which are released by tumor cells. Additionally, immunosuppressive cells, including Treg cells, myeloid-derived suppressor cells (MDSC), tumor-associated macrophages (TAM), and regulatory DCs, play a key role in T cell dysfunction and downregulation of the immune system in TME [9, 10]. One subclass of DCs in TME induces immune suppressive activity and elicits effector T cell dysfunction through the FOXO3 transcription factor [10]. Also, MDSCs have great potential for tolerizing immune cells in innate and adaptive immune responses. On the one hand, they suppress the innate immune response by polarizing macrophages and inhibiting the cytotoxic activity and IFNγ production of NK cells. On the other hand, they inhibit the adaptive anti-tumor response by suppressing the activation and function of effector T cells [11].

CAR structure and CAR-modified immune cells generation process

The new clinical achievements of immunotherapy have developed the field of cancer therapeutics due to the deep understanding of cancer immunobiology and improving this knowledge to eliminate malignant cells functionally. Besides surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, which is considered the fifth method for targeting malignancies in the current decade. Up to now, most immune-based therapies have been immune checkpoint blockades using monoclonal antibodies (mAbs). These mAbs block intrinsic inhibitory factors that shut down the immune system and are often overexpressed in tumors. In need of new techniques in this field, the CAR-based immune cell therapy technique has been established and has obtained United states Food and Drug Administration (FDA) approval for treating some hematological malignancies [12, 13]. CAR-immune cells are genetically engineered to expose nonnatural receptors, called CARs, on their cell surface, enabling recognition of exact tumor antigens and thereby destroying cancer cells [14]. CAR immune cells are composed of three main parts: an extracellular receptor for the recognition of tumor antigens, a hinge (spacer), a transmembrane domain,

and an intracellular tyrosine, which activates the intracellular process required for tumor destruction [15, 16]. The extracellular part of CARs is antigen-targeting fragments derived from a tumor-specific monoclonal antibody, made up of a single-chain variable fragment (ScFv). This receptor binds to tumor antigens and triggers T-cell activation, cytokine production, and cytolytic function in the TME. The intracellular domain is linked to the extracellular domain through a membrane-spanning protein, which controls the quality, strength, and persistence of a T cell reaction to tumor antigens. The intracellular domain yields the space for sufficient CAR T-cell therapies and the outcomes of five generations of CARs. The first generation of CARs is composed of an endodomain with only the CD3- ζ chain or FcεRIγ signaling domain, which supports insufficient T-lymphocyte proliferation, a short life span, and inadequate cytokine secretion. In the second generation, costimulatory endodomains like CD28 or 4-1BB are added to increase T-cell proliferation, life span, and better cytotoxic response. In the third generation, these two costimulatory endodomains are utilized together to achieve high potency and long persistence. To simultaneously activate innate immunity and adaptive immunity, in the fourth generation, the transcription factor expressing cytokines like IL-8, IL-9, IL-12, and IL-18 or other biological factors is inserted into the second generation structure. In the fifth generation of CARs, a domain of the IL-2 receptor β–chain is inserted into the binding site of transcription factor STAT3. This insertion results in the reduction of systemic side effects of CARs and thus leads to the broad use of CAR therapy in other diseases [17].

CARs construction

The construction of CAR-immune cells is carried out by different approaches that generally have common steps (Fig. 2). The first step began with obtaining the WBC of patient through leukapheresis, a process in which WBCs are separated from the blood. During the washing process, red blood cells and platelets are removed through centrifugation in cell washers such as the COBE 2991 Cell Processor [18], the Haemonetics CellSaver [19], and the CaridianBCT Elutra [20]. In this stage, anticoagulants are added, potentially adjusting cell performance during activation [21].

Naïve T cells are activated when they encounter their matching antigens. These antigens are presented by antigen-presenting cells (APC) to the T cells. To avoid this slow activation mechanism, in the second step of CAR manufacturing, T cells are activated by exposing to various activating factors [19], including OKT3/IL-2 [22] and anti-CD3/CD28 antibody beads [20]. The dominant utilized activating strategy is recruiting anti-CD3/CD28 antibody-coated paramagnetic beads [23].

In the third step, the CAR gene is transduced to the T cells that are responsible for the chimeric receptor formation. There are two different gene transduction methods,

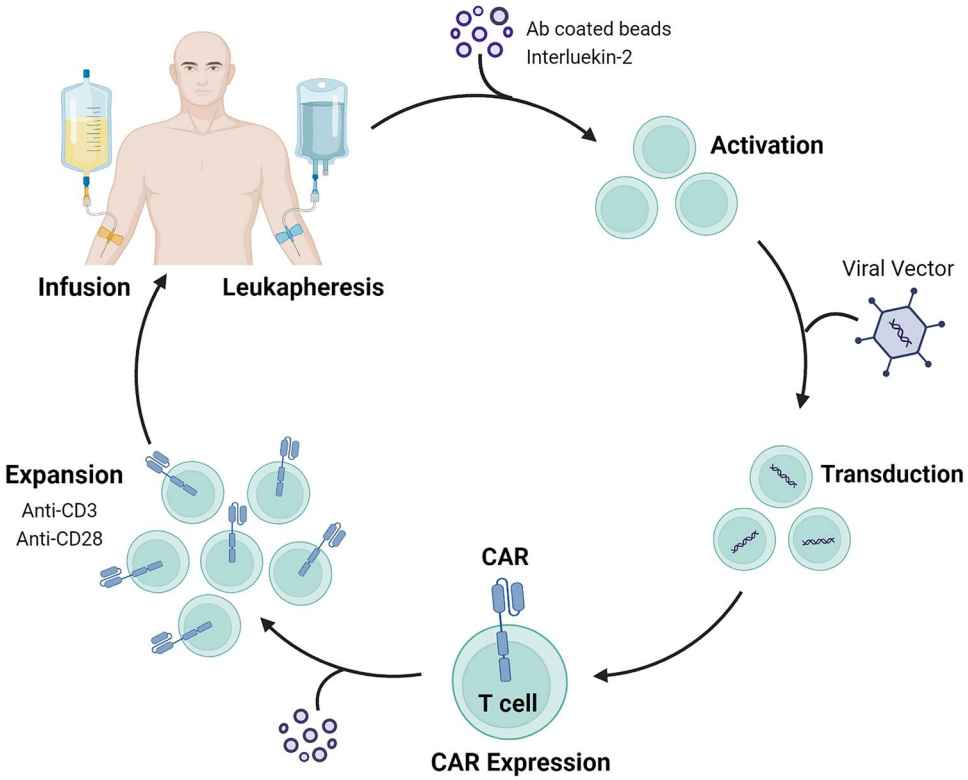

Fig. 2 CAR-T cell manufacturing process. The CAR-T cell production and treatment process are briefly performed in the following steps: 1) Leukapheresis: the autologous T cells of patient are isolated under this process; 2) Activation: Isolated T cells are activated via artificial antigen-presenting cells (aAPCs), antibody coated magnetic beads (anti-CD3/anti-CD28), and growth factors like IL-2 and are harvested in culture medium; 3) Transduction: T cells are transduced with the CAR-encoding viral vectors (lenti- or retroviral vectors) to express CAR on their surface; 4) Expansion: Engineered CAR-T cells are expanded to large numbers in bioreactors via expanding factors like anti-CD3 and anti-CD28; 5) Infusion: the harvested CAR-T cells are washed and concentrated for intravenous infusion of CAR-T cells back to the patient

which are viral and non-viral. The most common strategy is viral transduction via retroviral and lentiviral gene transfer strategies, which can get high efficiencies in the result, but the disadvantage of this method is its high cost [23]. Recently, methods based on transposon/transposase systems with positive results have been introduced to CAR-T cell therapies and can provide significant economic advantages over viral transduction [24].

To acquire an adequate amount of CAR-T cells, ex vivo T cell expansion is needed. One of the methods used for this process is the use of tissue culture plates or flasks [25, 26]. This method requires great effort since flasks need frequent medium changes by trained staff in biosafety cabinets [27]. Using this strategy is improper for large-scale manufacturing as it includes many open-handling steps and is not allowed as an industry standard in modern industries. Another technique for culturing is utilizing static culture bags, which are easier to implement and more appropriate for manufacturing than tissue culture flasks [28]. The rocking motion bioreactor is another culturing technique used in this process. This method eradicates growth-inhibiting substances and provides a constant amount of nutrition, thus allowing cell culture in smaller capacities than with culture bags [29].

Immune cells sources

As mentioned earlier, for CAR immune cell construction, T cells and NK cells should be obtained first. There are different sources to get these immune cells. The main goal that should be considered in this process is to choose the immune cells from the source that is less affected by graft versus host diseases (GVHDs). T cells used in CAR T cell production are generally obtained from peripheral blood mononuclear cells (PBMCs) and rarely from umbilical cord blood (UCB). These T cells could also be obtained from stem cells like induced pluripotent stem cells (iPSCs) or embryonic stem cells. In selecting immune cells for CAR construction, the chance of any alloimmune response should be decreased. Therefore, to obtain T cells from peripheral blood mononuclear cells (PBMCs), a healthy donor would be a better source than the patient. This type of selection provides different subtypes of the human leukocyte antigen (HLA) complex, which increases the chance of selecting HLAs that match the patient. Despite the autologous T cells from patients affected by chemotherapeutic agents and cancer immune impacts, the allogenic T cells selected from healthy donors can provide better immunity and decrease the diversity of the final cell product [30]. Umbilical cord blood (UCB) also could be used as a source for T cells, especially when it decreases the chance of GVHDs by presenting unique antigen-naïve status [31].

In CAR-NK cells, there is a low probability for GVHDs; therefore, this opens a great path for producing "off-the-shelf" allogeneic CAR-NK cells for multiple patients [32, 33]. For this process, there are different sources for obtaining NK cells, such as the NK92 cell line, PBMCs, UBC, $CD34^+$ hematopoietic progenitor cells (HPCs), and iPSCs [34]. Most of the clinicians in the process of constructing CAR-NK cells use the NK92 cell line because of its unlimited expansion ability in vitro [35]. However, because NK92 cell lines were obtained from tumor cell lines, they have a potential tumorigenicity risk. Also, the absence of CD16 and NKp44 expression and the lack of in vivo expansion potential needed before their infusion because of their fatal irradiation [36] mean they can't be an ideal source for CAR-NK cells. Interestingly, CAR-NK cells produced from PBMCs are powerful anti-tumor cells [37, 38]. As we mentioned above, another source for NKCs is UCB, which offers several advantages over PB. This is due to the low number of contaminating T cells in UCB compared to PB, which results in a low risk of GVHD [39, 40].

Similarities and differences between CAR-T cells and CAR-NK cells

As CAR-T cells and CAR-NK cells are considered new approaches in immunotherapy, there are some similarities and differences between them that have been listed in Table 1. For instance, their construction, mechanism of action, sources from which they are obtained, their applications and outcomes, and future side effects. Both CAR immune cells are constructed from three main parts: an outer single-chain antibody variable fragment (ScFv), a transmembrane domain, and intracellular signaling domains [41]. The outer (ScFv) is the antigen-recognition site. It is generally composed of variable regions of a monoclonal antibody's heavy and light chains, combined by a flexible linker. Most ScFvs are of murine sources with the possibility to generate a human anti-mouse antibody (HAMA) or an anti-idiotype immune response [42]. The transmembrane domain is located between the hinge and the intercellular signaling domain. The intracellular domain transmits activation signals to the immune cells. Different generations of CAR-T cells and CAR-NK cells are created depending on the type of intercellular domain. CD3ζ endodomain is decisive for the activation of both NK cells and T cells [43]. In this case, CAR generations are created by adding costimulatory signaling domains such as CD28, IL-2, IL-8, IL-9, IL-12, and IL-18 to the CD3ζ endodomain in CAR T cells, and leading to higher potency among the malignancies. In addition to these signaling molecules used in CAR T cells, scientists discovered other molecules that could serve as the activation signaling domain of CAR-NK cells. Examples of these molecules are CD244 or 2B4 from the signaling lymphocyte activation molecule (SLAM) family, DNAX-activation

Table 1 Comparison between three type of CAR immune cells; T cell, NKT cell, and NK cell

Features	T-cell	NKT-cell	NK-cell	Reference
Population	Heterogeneous	Homogenous	Homogenous	
Maturation	Thymus-dependent	Thymus-dependent	Thymus-independent	
Receptor	TCR	TCR	NCRs, KIRs, LIRs, NKG2	[60, 61]
MHC restriction	MHC-I/II-restriction	Non-MHC restricted	Non-MHC restricted	
Immune response	Adaptive (Cellular)	Innate	Innate	
Cell Sources for CAR engineering	PBMCs, UCB, iPSCs	PBMCs, UCB, iPSCs	PBMCs, UCB, iPSCs, cell line	[62, 63]
Activation	MHC-peptide, Costimulatory signals	$CD1d^{+}$-Antigen, Costimulatory signals	Regulate by activatory and inhibitory signals	
Function	Cell death (perforin and granzyme), apoptosis	Destroy CD1d + cells, Regulate the immune system	ADCC, Cell death (perforin and granzyme), apoptosis	[64, 65]
CAR-armed cytotoxicity	CAR-dependent	CAR-dependent and CAR-independent	CAR-dependent CAR-independent	[66]
Proliferation	High	High	Low	[67]
Persistence	High	High	Low	
Memory	High	High	Low	
Side effects	On target-off tumor, CRS, GVHD, Anaphylaxis, Neurotoxicity, TLS	No significant toxicity, with no reported GVHD or CRS	Limited GVHD and CRS. Fever and fatigue	[5, 68]

CAR Chimeric antigen receptors, *TCR* T-cell receptor, *NCR* Natural cytotoxicity receptor, *KIR* Killer Ig-Like Receptor, *LIR* Leukocyte immunoglobulin-like receptors, *MHC* Major histocompatibility complex, *PBMC* Peripheral blood mononuclear cell, *UCB* Umbilical cord blood, *iPSC* Induced Pluripotent Stem Cells, *ADCC* Antibody-dependent cell-mediated cytotoxicity, *CRS* Cytokine release syndrome, *GVHD* Acute Graft versus host diseases, *TLS* Tumor lysis syndrome

protein (DAP) 12, and DNAX-activation protein (DAP)-10 [44–46].

Infusion of a foreign gene in CAR-NK cells is performed by the same methods of transduction used in CAR-T cells, which are viral transduction (retrovirus-based and lentivirus-based methods) and transfection (electroporation, lipofection, and in combination with transposon systems) [47]. To obtain T cells and NK cells for this process, there are different sources to increase the quality of work and achieve better outcomes in the end. In constructing CAR-T cells, generally, autologous T cells are obtained from the peripheral blood mononuclear cells (PBMCs) of the patient because of the risk of tissue destruction, as seen in autoimmune disorders, graft rejection, and graft-versus-host disease (GVHD). Autologous T cells used in this procedure are T cells of patients that emerged from precursors that are genetically rearranged in germline antigen receptor VDJ genes, and their T cell receptors (TCRs) undergo positive and negative selection in the thymus. This decreases the chance of GVHD and produces T cells with high tolerance among the self-tissues [48]. In addition to the previous process, in manufacturing CAR-NK cells, the same sources of CAR T cells (PBMNCs, UCBs, IPSCs, or HSCs) are used, but considering that allogeneic NK cells are not related to GVHD as are CAR-T cells [49–51]. However, despite the advantages of each source, there are some disadvantages, such as the absence of a single renewable source in PBMNCs and UCB and the lack of essential activation markers in IPSCs [52, 53]. Therefore, in CAR-NK cells, the NK-92 cell line can be used as another source to give more opportunities for treatment and exclude disadvantages that are present in common sources [54]. Another difference is the amount of cytokine produced by CAR-NK cell infusions that is normal [55] in contrast to concentrations seen in mortal cytokine release syndrome (CRS) caused by CAR-T cell infusion. Thus, the administration of CAR-NK cells decreases the chance of any autoimmune disorders compared to CAR-T cells [56]. Eventually, as with any therapeutic procedure in medicine, there are some side effects related to this therapy, such as cytokine release syndrome, tumor lysis syndrome, and "on-target/off-tumor" toxicity [57]. In order to reduce these side effects and achieve better outcomes in CAR-T cell therapy, researchers recommended different safety strategies such as suicide genes, combinatorial target-antigen recognition, synthetic Notch receptors, on-switch CAR, and inhibitory CAR (iCAR) [58]. By contrast, none of the abovementioned suicide systems has been examined in CAR-NK cells because suicide genes are discussed for effector cells with a long life span, as in the case of T cells. Hence, because of the short life span of NK cells in circulation, until now, the necessity of any suicide system has not been given for CAR-engineered NK cells [59].

CAR-modified T cells in hematologic malignancies

In 2017, two CAR T cell products, Kymriah and Yescarta, received FDA approval for treating patients with B-cell acute lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL). So far, this number has increased for five products, with the final product, an anti-BCMA CAR T cell therapy for treating multiple myeloma having been approved [69, 70]. In addition to these products, other CAR T cell products are under investigation for hematological malignancies but have not yet been approved [71]. Table 2 summarized the applied clinical trials on approved CAR T cells [72–74]. In the following sections, the application of CAR immune cells has been reviewed for each type of mentioned blood cancer. Besides, a summary of terminated and completed CAR-T cell therapy clinical trial studies in hematological malignancies has been listed in Table 3.

Lymphoma

Lymphomas are a group of malignancies derived from different subtypes of B-, T-, and natural killer (NK)-cells in various stages of maturation [75]. As with any other malignancy, there are different environmental, infectious, and genetic factors that collaborate together to establish an immunosuppressive environment for the emergence of this type of malignancy. According to morphology and immunophenotype, lymphomas are classified into different groups and subgroups [76]. For treatment, different modalities, including chemotherapy, radiotherapy, and antibody–drug conjugates, are carried out depending on the type of lymphoma. As a new therapeutic procedure, CAR-T cells are used to treat some types of lymphoma. The first CAR-T cell therapy, so-called CD19-directed Kymriah, was approved by the FDA in 2017 for treating B-ALL and DLBCL [77]. In the same year, the FDA also approved Yescarta CAR-T cells according to the ZUMA study, which showed 58% complete responders and 25% partial responders in patients with refractory large B-cell lymphomas who were under treatment with CAR-T cells (Yescarta) [78]. In the following two years, no new products were realized. It was not until 2021 that two new products, Breyanzi (Lisocabtagene marleucel) as a new treatment for refractory large B-cell lymphomas, such as DLBCL, high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma [79], as well as Tecartus (Brexucabtagene autoleucel) for treating mantle cell lymphoma (MCL), were approved by the FDA [80].

Leukemia

In this type of blood cancer, there are two major groups. They are acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL). ALL is caused by the uncontrolled growth of lymphoid cell precursors, especially pre-B cells (80–85%), less frequent T cells (10–15%), and mature B cells (< 5%) [81]. The prevalence of this malignancy in children is higher than in adults, with a better prognosis compared to them [82]. Nowadays, the survival of these patients is improved with the application of multimodal treatments, including stem cell rescue, radiation therapy, chemotherapy, and high-dose chemotherapy [83]. Until now, there has been only one CAR-T cell that has been approved by the FDA for B-ALL, under the name Kymriah [77]. Kymriah attacks CD19 surface antigens expressed on malignant B cells, leading to a defect in the growth of malignant cells as an off-tumor effect [84]. Chronic Lymphocytic Leukemia (CLL) is the second type of this malignancy. According to the WHO, CLL is a type of lymphoproliferative disorder marked by the expansion and accumulation of mature CD5/CD19-positive B cells in peripheral blood, bone marrow, lymph nodes, and the spleen [85]. Now, in Western countries, dominant leukemia among adults is CLL, with an increase in incidences of aging [86]. Till now, CLL has been linked to several risk factors, including a family history of hematological malignancies, farming, exposure to the hepatitis C virus, and immune dysregulation [85, 87]. For this type of leukemia, standard therapeutic procedures

Table 2 Brief summery of applied clinical trails on approved CAR T cells for hematological malignancies

Clinical trial	Car T cell product	Target	No. patients	Age	Best compliment response	Treatment related mortality
ZUMA-1	YESCARTA	CD19	119	23–76	58%	2
JULIET	KYMRIAH	CD19	167	22–76	40%	0
TRANSCEND-NHL-001	BREYANZI	CD19	344	18–86	53%	1
ZUMA-2	TECARTUS	CD19	74	38–79	67%	16
KARMMA	ABECMA	BCMA	140	33–78	33%	44

Table 3 Summary of completed and terminated clinical trials of CAR-T cell therapy in hematological malignancies

Target Antigen	Cancer Type	Intervention	Phase	Reference
CD19	B-cell Malignancy	CD19-CAR-T cell	I	NCT03952923
	Acute Lymphocytic Leukemia	CD19-CAR-T cell	I	NCT02975687, NCT03825718, NCT04888468
			II	NCT02935543, NCT02535364, NCT02030847
			I, II	NCT01195480
		CD19-CAR-NK cell	I	NCT05563545
	Diffuse Large B-Cell Lymphoma	CD19-CAR-T cell	I	NCT02976857, NCT02431988, NCT02431988
	B-cell Leukemia and Lymphoma, Acute Lymphocytic Leukemia, Large Cell Lymphoma, Non-Hodgkin Lymphoma	CD19-CAR-T cell	I	NCT01593696
	Acute Lymphocytic Leukemia, Chronic Lymphocytic Leukemia, Non-hodgkin Lymphoma	CD19-iCasp9-IL15-CAR-NK Cell	I, II	NCT03056339
	Hodgkin Lymphoma	CD19-CAR-T cell	I	NCT02624258, NCT02277522, NCT02624258
	Non-hodgkin Lymphoma	CD19-CAR-T cell	I	NCT03483688, NCT03029338, NCT02706405
			II	NCT02030834
	Mediastinal B-cell Lymphoma, Diffuse Large B-Cell Lymphoma	CD19-CAR-T cells	I, II	NCT00924326
	B-cell Leukemia and Lymphoma	CD19-CAR-T cells	I, II	NCT02132624, NCT03076437, NCT01626495
			II	NCT03068416
	Acute Biphenotypic Leukemia, Acute Lymphoblastic Leukemia, Non-Hodgkin Lymphoma, Small Lymphocytic Lymphoma, Chronic Lymphocytic Leukemia	CD19-CAR-T cell	I	NCT02529813
	Acute Lymphoblastic Leukemia, Diffuse Large B-Cell Lymphoma, Immunoblastic Large Cell Lymphoma, Mantle Cell Lymphoma, Chronic Lymphocytic Leukemia	CMV/CD19 bi-specific-CAR-T cell	I, II	NCT01475058
	B-cell Acute Lymphoblastic Leukemia, Non-Hodgkin's Lymphoma, Mediastinal Large B Cell Lymphoma, Diffuse Large B-Cell Lymphoma, Follicular Lymphoma, Mantle Cell Lymphoma, High-grade B-cell Lymphoma	RPM CD19-mbIL15-CAR-T cell	I	NCT04844086, NCT03579888
		CD19-EGFRt- CAR-T cell	I, II	NCT01865617
CD19/CD20	B-Cell Lymphoma, Non-hodgkins Chronic Leukemia, B-Cell Chronic Leukemia	CD19/CD20-CAR-T cell	I	NCT04160195, NCT03019055, NCT04260932, NCT04260945
	B-cell Acute Leukemia, B-cell Chronic Leukemia, Prolymphocytic Leukemia, Diffuse Large B-Cell Lymphoma, Follicular Lymphoma, Mantle Cell Lymphoma	CD19/CD20-CAR-T cell	I, II	NCT03097770
CD19/CD22	Acute Lymphoblastic Leukemia	CD19/CD22-CAR-T cell	I	NCT03593109
			I, II	NCT03289455
CD20	Diffuse Large B-Cell Lymphoma, Follicular Lymphoma, Mantle Cell Lymphoma, Small Lymphocytic Lymphoma	LUCAR-20S CAR-T cell	I	NCT04176913
CD22	Acute Lymphoblastic Leukemia	CD22-CAR-T cell	I	NCT02588456
CD123	Acute Myeloblastic Leukemia	CD123-CAR-T cell	I	NCT02623582,NCT03672851

Table 3 (continued)

Target Antigen	Cancer Type	Intervention	Phase	Reference
ROR1	Acute Lymphoblastic Leukemia, Mantle Cell Lymphoma, Chronic Lymphocytic Leukemia	ROR1-CAR-T Cells	I	NCT02706392
?	CD4 + T Cell Lymphoma and Leukemia	LCAR-T2C CAR-T cells	I	NCT04219319, NCT04973527
CD7	T/NK-cell hematologic malignancies	CD7-Universal-CAR-T Cell	I	NCT04538599, NCT04572308
CD30	Diffuse Large B-Cell Lymphoma	CD30-CAR-T Cell	I	NCT03049449
CD33	Hematopoietic/Lymphoid Cancer, Acute Myeloid Leukemia	CD33-CAR-T Cell	I	NCT03126864
CD38	Multiple Myeloma	CD38-A2-CAR-T Cell	I	NCT03464916
SLAM7	Multiple Myeloma	SLAM7-CAR-T Cell	I	NCT03958656
BCMA	Multiple Myeloma	BCMA-CAR-T Cell	I	NCT03338972, NCT04650724, NCT03318861, NCT02215967, NCT02546167
			I, II	NCT03448978, NCT03288493, NCT03548207
APRIL	Multiple Myeloma	APRIL-CAR-T Cell	I, II	NCT03287804
CD44v6	Multiple Myeloma, Acute Myeloid Leukemia	CD44v6-CAR T-cell	I, II	NCT04097301
NKG2D	Acute Myeloid Leukemia	NKG2D-CAR-T Cell	NA	NCT05247957
	Acute Myeloid Leukemia, Myelodysplastic Syndrome, Multiple Myeloma	NKG2D-(CM-CS1)-CAR-T Cell	I	NCT02203825

CAR Chimeric antigen receptor, *iCasp9* Inducible Caspase9, *CMV* Cytomegalovirus, *EGFRt* Truncated epidermal growth factor receptor, *ROR1* Receptor-tyrosine-kinase-like orphan receptor 1, *SLAM* Signaling lymphocytic activation molecule 7, *BCMA* B-cell maturation antigen, *APRIL* A proliferation-inducing ligand, *NKG2D* Natural Killer Group 2D, *NA* Not Applicable

are chemotherapy, monoclonal antibodies, and immunotherapy, according to the condition of the disease [88]. So far, no CAR-T cells have been approved for this leukemia, and the results are by far worse in CLL compared to those in ALL or DLBCL. According to a study carried out on a group of patients, there was only 38% overall response and 25% complete response from patients, with a median survival of 17 months [89]. After one year, another study shows a 44% overall response and a 28% complete response, with a median survival of 64 months in the patients [90]. Even with these results, there are some uses of CAR-T cells in the CLL [91].

Multiple myeloma

Multiple myeloma is a cancer of the plasma cells that aggregates in the bone marrow and suppresses the production of stem cells and osteoblast function [92]. This disease has been incurable till now. Still, there are some procedures like chemotherapy, hematopoietic stem cell transplant (HSCT), and immunomodulatory drugs for stabilizing and relieving symptoms in patients [93]. In leukemia and lymphoma, CD19-targeted CAR-T cells are functional because of the expression of CD19 antigen on the malignant cells. But in multiple myeloma, this method is unsuitable because of the low expression of CD19 antigen on the surface of malignant cells [94]. Up to now, clinicians have investigated different targets for CAR-T cells, like BCMA antigens, for treating multiple myeloma. Positive responses from patients in clinical trials using BCMA-targeted CAR-T cells led to FDA approval for this CAR-T cell under the ABECMA name (idecabtagene vicleucel) for treating multiple myeloma [95].

Table 4 Summary of completed and terminated clinical trials of CAR-T cell therapy in solid tumors

Target	Cancer Type	Intervention	Phase	Status	Reference
GD2	Sarcoma, Osteosarcoma, Neuroblastoma, Melanoma	GD2-CAR T cell, AP1903, Cyclophosphamide	I	Completed	NCT02107963
	Neuroblastoma	1RG-CAR T cells, Fludarabine, Cyclophosphamide	I	Completed	NCT02761915
		GD2 CAR modified Tri-virus specific cytotoxic t-cells	I	Completed	NCT01460901
Lewis Y	Advanced solid tumors	LeY CAR T cells	I	Completed	NCT03851146
cMet	Metastatic Breast	cMet RNA CAR T cells	I	Completed	NCT01837602
Glypican-3	Hepatocellular Carcinoma	CAR-GPC3 T Cells	I	Completed	NCT03884751, NCT03980288, NCT02395250
HER2	Glioblastoma Multiforme	HER.CAR CMV-specific CTLs	I	Completed	NCT01109095
MSLN	Metastatic Pancreatic Adenocarcinoma, Epithelial Ovarian Cancer, Epithelial Pleural Mesothelioma	Meso CAR T cells	I	Completed	NCT02159716
	Pancreatic Ductal Adenocarcinoma	Autologous Meso-CAR T cells	I	Completed	NCT01897415
CEA	Liver Metastases	CEA CAR-T cells	I	Completed	NCT02850536
		CEA CAR-T cells, Sir-Spheres	I	Completed	NCT02416466
EGFRvIII	Glioblastoma	CAR T-EGFRvIII T cells, Pembrolizumab	I	Completed	NCT03726515
	Malignant Glioma, Glioblastoma, Brain Cancer, Gliosarcoma	EGFRvIII CAR CD8 plus PBL, Fludarabine, Aldesleukin, Cyclophosphamide	I, II	Completed	NCT01454596
	Glioblastoma, Gliosarcoma	EGFRvIII CAR T cells	I	Terminated	NCT02664363, NCT03283631, NCT02209376
PD-L1	Advanced Lung	PD-L1 CAR-T cell, Fludarabine, Cyclophosphamide	I	Terminated	NCT03330834
MSLN	Epithelial Ovarian	LCAR-M23 cells, Fludarabine, Cyclophosphamide	I	Terminated	NCT04562298
	Cervical Cancer, Pancreatic Cancer, Ovarian Cancer, Mesothelioma, Lung Cancer	MSLN CAR CD8 plus PBL, Fludarabine, Aldesleukin, Cyclophosphamide	I, II	Terminated	NCT01583686
Claudin	Gastric Cancer Pancreatic Ductal Adenocarcinoma	LCAR-C182A cells	I	Terminated	NCT03890198
cMet	Malignant Melanoma, Breast Cancer	cMet RNA CAR T cells	I	Terminated	NCT03060356
CD19 MSLN	Pancreatic Cancer	CART-meso-19 T cells, Cyclophosphamide	I	Terminated	NCT02465983
CEA	Metastatic Pancreatic Carcinoma	CAR2 Anti-CEA CAR-T cells	I	Terminated	NCT03818165
VEGFR2	Metastatic Cancer, Metastatic Melanoma, Renal Cancer	VEGFR2 CAR CD8 plus PBL, Fludarabine Aldesleukin, Cyclophosphamide	I, II	Terminated	NCT01218867
AFP	Hepatocellular Carcinoma Liver Cancer	Autologous ET1402L1-CART cells	I, II	Terminated	NCT03349255

CAR Chimeric antigen receptor, *GD2* Disialoganglioside 2, *CMV* Cytomegalovirus, *PBL* Peripheral blood lymphocytes, *PDL-1* Programmed cell death protein ligand 1, *EGFRvIII* Epidermal growth factor receptor variant III, *Her2* Human epidermal growth factor receptor 2, *MESO* Mesothelin, *APIRL* A proliferation-inducing ligand, *GPC3* Glypian-3, *CEA* Carcinoembryonic antigen, *VEGFR2* Vascular endothelial growth factor receptor 2, *AFP* Alpha-fetoprotein

CAR-modified immune cells in solid tumors

After remarkable achievements of CAR-T cell therapy in treating hematological malignancies, clinicians try to use CAR-T cell as a new therapeutic option for treating solid tumors. Even so, due to the structure and location of solid tumors in the body, there are some challenges and limitations in solid tumors that retards clinicians from using CAR-T cells, such as antigen heterogenity, trafficking, "on-target/off-tumor" effects, and immunosuppressive tumor microenvironment [96]. To overcome these complications, clinicians invented many strategies and approaches, including secretion of cytokines/chemokines by CAR-T cells and using CAR-T cells in combination with other drugs [97]. In this part, we focus on the last updates of CAR-T cell therapies in solid tumor and their application in each type of mentioned cancers. Some

clinical trials that applied CAR-T cells to solid tumors have been listed in Table 4.

Breast cancer

Despite all of those advancements in cancer treatment, breast cancer remains the second leading cause of death among malignancies for women in the world [98]. With the advances in our understanding of the immune system role in cancer progression and inhibition, immunotherapy played a significant role in treating breast cancer and slowing its progression. Immunotherapy for breast cancer includes a variety of therapeutic methods like monoclonal antibodies (mAbs), vaccines, immune checkpoint inhibitors, and adoptive T-cell transfer. By observing the curative role of cytotoxic T cells in several cancers, clinicians started to treat breast cancer with cytotoxic T cells. There are two methods for using cytotoxic T cells as a therapeutic procedure: first is using tumor-infiltrating lymphocytes (TILs), and the second, which we focused on in this review, is CAR-T cells. In breast cancer, several types of antigens are expressed on the cell surface and act as targets in clinical trials for CAR T cells. These target antigens include human epidermal growth factor receptor 2 (HER2), mesothelin, carcinoembryonic antigen (CEA), carbonic anhydrase IX (CAIX), folate receptor α (FRα), disialoganglioside (GD2), epidermal growth factor receptor (EGFRvIII), fibroblast activation protein (FAP), vascular endothelial growth factor receptor 2 (VEGF-R2), and CD171 [99]. Studies are continuing on these antigens, especially on CAR-T cells targeting the HER-2 antigen, due to the high role of this antigen in breast cancer recurrence and survival [100]. There are some investigations on receptor tyrosine kinase-like orphan receptor 1 (ROR1) on 3D models, which is highly expressed in some solid tumors like breast cancer [101].

Ovarian cancer

Ovarian cancer is one of the main causes of death in women [102]. 75% of women with this malignancy are diagnosed in advanced stages (III or IV) of the disease. The chance of recurrence after treatment is 60 to 70% in patients with low residual disease and 80% to 85% in patients with high residual disease, considering that the recurrence of ovarian cancer remains incurable [103]. Therefore, according to these data, we conclude the high mortality rate of this disease. So far, as with any other solid tumor, the therapeutic approaches used for ovarian cancer include conventional surgery, chemotherapy, and radiotherapy. Despite the promising results from these treatments, novel therapeutic strategies are needed to achieve better outcomes using immunotherapy methods like adoptive T-cell immunotherapy, cancer vaccines like Provenge and Vigil, checkpoint inhibitors, immune regulatory cytokines, and mAbs [102]. CAR-T cells received considerable attention for treating solid tumors after successfully treating hematological malignancies. However, clinicians face challenges with the issue of the tumor microenvironment and the difficulty of reaching T cells at the site of the tumor due to abnormal vasculature, reduction of adhesion molecules, and low PH [104]. Therefore, the best way to treat solid tumors using CAR-T cells is to target specific antigens on the malignant cell surface. According to the GEO database (GSE66957), two genes responsible for coding their antigens are (FOLR1) folate receptor 1 and (MSLN) mesothelin, which are highly expressed in ovarian cancer. Based on this data, a clinical trial was performed between tandem CAR-T cells targeting these two antigens in vitro and in vivo and CAR-T cells targeting each antigen in particular. Results indicated that tandem CAR-T cells could destroy antigen-positive OV cells efficiently in vitro and discharge a higher quantity of cytokines than single-target CAR-T cells, as well as decreasing tumor volume and increasing survival in vivo examination in mice [105].

Lung cancer

Lung cancer is still the leading cause of death among all malignancies, with 2·20 million new cases and 1·79 million deaths per year worldwide, as reported by the world health organization (WHO) [106]. According to their histopathological features, lung cancer is classified into two main groups: small-cell lung carcinoma (SCLC), contained 15% of cases, and non-small-cell lung carcinoma (NSCLC), which contained 85% of cases, with the presence of two main subtypes: adenocarcinoma and squamous cell carcinoma. Among these two groups, SCLC is more cancerous than NSCLC, with a chance of 5% for overall survival over five years [107]. Due to the difference between their histopathological features, metastatic rate, and rate of survival, the therapeutic approaches that are undertaken to treat these cancer types are different. In NSCLC, surgical removal is used depending on the TNM stage of NSCLC patients, and radiotherapy is used for patients with non-removable metastatic NSCLC [108]. Also, as with solid tumors, chemotherapy is another conventional therapeutic approach applied to treat this cancer. In this type of lung cancer, Platinum-based double-agent combination chemotherapy is used as the standard treatment regimen for NSCLC [109]. Immune-based therapies for this cancer include fusion gene ALK and ROS1 inhibitors and anti-EGFR monoclonal antibodies (EGFR-TKIs) [110]. In small-cell lung carcinoma, SCLC, conventional therapeutic strategies include radiotherapy and chemotherapy due to the high metastatic rate of SCLC during the diagnosis [111]. In addition to conventional therapies, there are immunotherapy agents like nivolumab

approved by the FDA for the treatment of SCLC patients [112].

After the great success of CAR-T cells as a new immunotherapy agent in hematological malignancies, several clinical trials were done on antigens that are highly expressed in malignant tissues to be targeted by CAR T cells during the treatment of several solid tumors. As one of the solid tumors, there are clinical trials on specific antigens in both of the lung cancer types that can be used as a target by CAR-T cells in the future. These antigens are Epidermal growth factor receptor (EGFR), Mesothelin (MSLN), Receptor Tyrosine Kinase-Like Orphan Receptor 1 (ROR1), Mucin-1 (MUC1), Prostate Stem Cell Antigen (PSCA), Human Epidermal Growth Factor Receptor 2 (HER2), Carcinoembryonic Antigen (CEA), Fibroblast Activation Protein (FAP) and Programmed death-ligand 1 (PD-L1) [113].

Colorectal cancer

Among malignancies, colorectal cancer is in the second order of lethal cancers, and third order of incidence according to research on the 36 types of cancers in 185 countries [1]. As with any cancer, the molecular characteristics of colorectal cancer (CRS) or cancer hallmarks include mutations in some genes that assist in producing some antigens. These antigens have a role in the activation or inactivation of some pathways that are characteristic of cancerous tissue and abnormal in normal tissue. The examples are a mutation in the gene of the APC protein, which leads to familial adenomatous polyposis; a mutation in the T53 gene, which leads to suppression of the immune system; mutations of RAS and BRAF; a mutation in TGF-β; and a mutation in PI3KCA [114]. Therapeutic approaches for colorectal cancer include surgical removal (laparoscopic or invasive) for primary tumors as well as chemotherapy and radiotherapy for higher stages. Targeted therapies for colorectal cancer include anti-EGFR (cetuximab and panitumumab), anti-VEGF-A (bevacizumab), and multi-kinase inhibitors (regorafenib) [115]. As a novel therapeutic strategy, CAR-T cells can be directed toward tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs) that are overexpressed on the tumor tissues. Examples of these antigens are CD133, CEA, EGFRvIII, EpCAM, GUCY2C, HER2, MSLN, NKG2DL, and PLAP. Several preclinical and clinical trials are carried out on these antigens, and studies are ongoing to achieve better results [116].

Pancreatic cancer

Pancreatic cancer is one of the most lethal cancers, with progressive tumor cells, a high mortality rate, and a 5-year survival rate of 9%. Detection of a pancreatic tumor in its early stages is complex, and most patients are asymptomatic throughout tumor development and even during advanced metastases to nearby tissues. Pancreatic ductal adenocarcinoma (PDAC) accounts for most pancreatic cancer cases. Risk factors for pancreatic tumors include infection, age, sex, chronic pancreatitis, diabetes, and family history of pancreatic cancer [117]. Traditional therapeutic procedures for this cancer are surgical removal, sometimes followed by adjuvant and neoadjuvant chemotherapy, and in recent years immunotherapy and targeted therapy have been added to the therapies [118]. As with the previously mentioned solid tumors, pancreatic cancer-specific antigens can be targeted by the CAR-T cell. These target antigens are mesothelin (PSCA), CEA, HER2, MUC-1, and CD133. Several clinical trials were undertaken, which reported positive results of CAR-T cells targeting these antigens on animal models, and several clinical trials are ongoing to reach better results in the future [119].

Glioma

Gliomas are tumors that arise from brain glial cells and are generally divided into circumscribed (benign) and diffuse (malignant) gliomas. These tumors account for most brain tumors. According to the last World Health Organization Classification of Tumors of the Central Nervous System (WHO CNS5), these tumors are divided into four families that are adult-type diffuse gliomas, pediatric-type diffuse low-grade gliomas, pediatric-type diffuse high-grade gliomas, and circumscribed astrocytic gliomas [120]. The initial treatment of gliomas includes safe surgical removal followed by radiotherapy and temozolomide chemotherapy [121]. Moreover, due to advances in technology and genetic science, several mutations in genes have been discovered that are responsible for displaying biomarkers in tumors, e.g., EGFR, PI3K/AKT/mTOR pathway, FGFR, NTRK, retinoblastoma (pRB) pathway, VEGF, TGF-β, CD276, etc., that assist in the better management of gliomas by producing inhibitory factors and monoclonal antibodies against them. Till now, among these biomarkers, CAR-T cells are directed against GD2, EGFR, and CD276 [122, 123]. Clinical trials are ongoing to reach better results that can be added to future therapies.

Limitations and challenges

Despite the many advantages of CAR-immune cells, some impediments limit the functions of CAR-T cells and CAR-NK cells. In CAR-T cells, antigen escape is the main challenge we deal with during our therapies. Antigen escape is the downregulation of CAR-T cell target antigens by the tissue after CAR-T cell function, as seen after the activity of CD19-CAR-T cells in 30–70% of patients and

BCMA-CAR-T cells in the patients of multiple myeloma. Another concern for CAR-T cell therapy is some common toxicities, which include cytokine-release syndrome (CRS), as a result of high activation and production of a vast amount of cytokines, which is known as a major problem in patients with COVID-19 infection and patients treated with CAR-T cells [124, 125], "on-target/off-tumor" toxicity, hemophagocytic lymphohistiocytosis, macrophage activation syndrome, anaphylaxis, and immune effector cell-associated neurotoxicity syndrome (ICANS) [5]. Despite the abovementioned limitations, there are some challenges that we face when targeting solid tumors. First, because of expressing tumor antigens on normal tissues, the risk of the target of tumor effects increases. To eliminate this risk, the antigens should be chosen so that they are specific to tumors and not expressed in normal tissues. Another challenge is the immunosuppressive microenvironment, which decreases CAR-T cell efficacy by producing cytokines, chemokines, and growth factors that aid tumor progression [5, 126]. Physical barriers, such as tumor stroma, altogether limit CAR-T cell infiltration and trafficking to the site of tumors [127]. In CAR-NK cells, the generation of modified cells is the first challenge that we face at the beginning. Till now, several methods, e.g., viral transduction, and mRNA electroporation, as well as several sources, e.g., NK92, PB, and CB, with different techniques, have been performed to achieve better transduction efficiency and to be used in clinical experiments in the future. Another challenge is the risk of infusion of contaminated NK products with T- or B-cells, which increases the chance of GVHD. Furthermore, the sensitivity of NK cells to the freeze and thaw process is another challenge that can reduce their activity and persistence. Additionally, CAR-NK cells cannot survive for just 1–2 weeks without cytokine support; therefore, this could limit their potency. Finally, the high cost of approved CAR-T cells and their analogs remains a substantial challenge for patients, as they haven't reached general production by pharmaceutical companies [42].

Safety and efficacy improvement strategies

The challenges faced by CAR-T cell therapy are those that affect the efficiency and safety of these cells, especially in solid tumors. Tumor heterogeneity, trafficking, and the immunosuppressive tumor microenvironment, including inhibitory cytokines, checkpoint inhibitors, a lack of nutrients, and hypoxia, are among the challenges leading to CAR-T cell malfunction or failure. Therefore, several strategies have been developed to enhance the performance and efficacy of these cells. Among the most important of these are methods based on genetic manipulation using the CRISPR/Cas system, equipping CAR cells, designing smart and programmable cells, and co-administrating CAR cells in combination with other cancer therapeutic approaches. Supra CARs, universal CARs, bispecific CARs, tandem CARs, dual CARs, split CARs, physiological CARs, synotch CARs, and TRUCKs are examples of smart-programmable-equipped CAR-based cells that can recognize one or more antigens simultaneously and function in TME with increased cytotoxicity, proliferation, and persistence [97, 128]. On the other hand, the use of combined treatments of CAR-T cells with chemotherapy, radiotherapy, anti-cancer vaccines, oncolytic viruses, checkpoint inhibitors, cytokines, etc., has resulted in useful and promising outcomes with augmented efficacy, which leads to increased survival and accelerated regression of tumors [129]. Another important concern raised is the low specificity and some significant adverse effects caused by the administration of CAR-T cells, as discussed previously. In this case, gene engineering methods based on knocking out or knocking down genes and the use of switches, including suicide switches [CD20, EGFRt, caspase 9 (iCasp9), and Herpes simplex virus thymidine kinase (HSV-TK)], endogenous switches [Dual and Tandem CARs, Inhibitory CAR (iCAR), and Synthetic Notch (SynNotch) receptor], and exogenous switches [Bispecific T cell engager (BiTE) and On-switch CAR], are the recent development strategies that intensify the safety, efficacy, and specificity of CAR cells in TME [128, 129].

Conclusion and future perspectives

In need of new techniques in immunotherapy to overcome the obstacles of other therapies, CAR-immune cells have emerged as a non-HLA-restricted targeted therapy. In the field of CAR-immune cells, CAR-T cells have shown great response in treating hematological malignancies, as five products have been approved in this field. This positive response was achieved by undergoing several steps that include understanding the nature of the suppressive tumor microenvironment, selecting autologous T cells from different sources, CAR gene delivery to T cells, formation of five different generations, ex vivo expansions of the product, product transfusion, and finally undergoing clinical trials before approval. However, despite the approval, we face several challenges in using CAR-T cells for hematological malignancies. Yet, this product is not approved for treating solid tumors or CAR-NK cells for both hematological and solid malignancies. Substantial challenges related to CAR-T cell therapy include antigen escape, "on-target/off-tumor" toxicity, trafficking, and an immunosuppressive microenvironment. Concerning CAR-NK cells, there are generation problems, the risk of contaminated NK cells with T- or B-cells resulting in GVHD, and the sensitivity of CAR-NK

cells to the freezing process. These challenges require further molecular engineering and high-technology investigations to treat the malignancies successfully. Strategies that are taken in clinical trials for emerging potent CAR-T cells against solid tumors are selecting predominantly expressed antigens by cancer as targets and using CAR-T cells in combination therapy with other drugs. Currently, molecular engineering strategies could also increase tumor-targeted specificity and antigen escape, as well as change the tumor microenvironment, to increase the efficacy of the therapy. Moreover, in the case of CAR-NK cells, some strategies like CRISPR-based genetic modifications are being taken to reach a better results in the future [34]. Consequently, despite CAR-T- and CAR-NK-cells, clinicians are investigating macrophages as another candidate for CAR-immune cell therapies [130].

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