Chapter

Advances in Adoptive Cellular Therapy (ACT)

Rajesh Kumar Yadav, Bandana Kumari, Pritanjali Singh, Asgar Ali, Sadhana Sharma and Krishnan Hajela

Abstract

Adoptive T cell therapy (ACT) is getting acknowledged as the Advanced Therapy Medicinal Products (ATMPs) in many countries and it has evolved as one of the newest regimens to treat cancer. Developed gradually by the basic understanding of cells, involved in innate and adaptive immunity, ACT has emerged as one of the successful immunotherapies in recent times. It broadly includes various cell types such as stem cells, T cells, dendritic cells and Natural Killer cells. By the applications of genetic engineering and advanced cell culture techniques, these cells from patients' blood, can be manipulated to train them for better efficacy against specific tumor cells. However, only some cells' subsets have shown promising regression for certain cancer cells types. To understand the reason behind this, technical knowledge about the tumor antigens presentation, tumor microenvironment (TME), hosts' immune responses and possible issues in the manufacturing of adoptive cellular material for infusion in patients are being explored further. This chapter brings together development of immune cells from basic research to clinical use, newer approaches which have been taken to address the resistance of ACT and future promises of this therapy.

Keywords: immunotherapy, advanced therapy medicinal products, adoptive T cell therapy, tumor microenvironment, TCR T cell Therapy, CAR T cell therapy

1. Introduction

Human body has a natural tendency to fight against diseases including cancer, aided by its immune system. So far, the journey of understanding mechanisms of tumor suppression by immune system and immune suppression by tumor cells, has been overwhelming. The ability of immune cells to differentiate between self and non-self is the key for immune response against cancer cells [1]. However, as cancer cell is actually a transformed self-cell, its ability to escape immune recognition is quite probable and a reason of cancer progression.

The treatment of cancer in the new era has shifted its focus from conventional treatment to physiological treatment, which involves the modification of immune system. This has also led to a concept of personalized treatment where an individual

immune system of patient is manipulated rather immune responses obtained based on general population. The efficacy of the immune cells is modified so that its tumor suppression function is improved. This strategy has shown better outcomes and therefore has drawn attention of researchers and clinicians and is now a preferred choice for cancer treatment. The efforts to design universal immune cell-based immunotherapy are also being explored besides personalized immunotherapy [2]. Numbers of approaches have been developed to treat cancer cells using immune-based technology broadly known as cancer immunotherapy (**Figure 1** and **Table 1**).

Among all these immunotherapies, cell-based cancer immunotherapy is getting popular day by day [18–20]. The ability of immune system to inhibit tumor growth and cure it, has been exploited in the development of anti-neoplastic immunotherapy. The immune cells play a key role in adoptive cell therapy (ACT). This is achieved by either expanding the autologous cancer-cognate lymphocytes or empowering them by genetic modifications. These alterations are done *ex-vivo* and then these cells are infused back to patient to fight against the cancer (**Figure 2**).

Cancer treatments by general immunotherapy have their own limitations due to personal variation in the immune response. In such cases, precision medicine through adoptively modified cellular transfers is being preferred lately. The cells to be transferred may be autologous (self-derived) or allogeneic (donor derived) depending upon the availability. These cells undergo various genetic modifications to suit the cancer types. Allogeneic cells are chosen on the basis of haplo-identical donors, or immune-suppressive conditioning to the patient.

This chapter outlines the emergence and evolvement of ACT, advancements particularly with genetic engineering of autologous cells, treatment approaches, evidences for its effectiveness in refractory patients, and future directions of ACT.

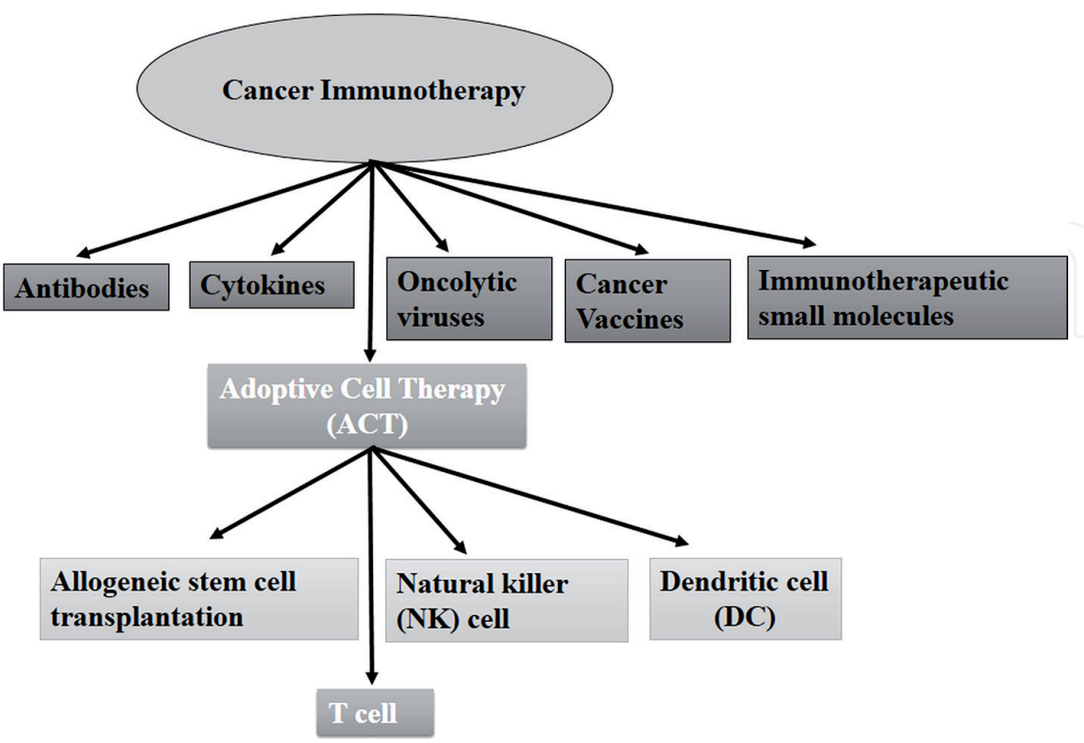

Figure 1.
Different approaches to cancer immunotherapy. Arrow indicates different modes through which immunotherapy can be performed. Application of T cells is the foremost choice of the cellular advancement for ACT.

Types	Salient features	Ref.
Antibody	• Therapeutic use of monoclonal antibodies (mAbs) • Tumor killing by cytotoxicity; Fc mediated immune effector engagements, non-restricted activation of T-cells and blockade of inhibitory signaling.	[3–5]
Cytokines	• Molecular messengers with anti-tumor property, majorly secreted by immune cells • Pro-inflammatory cytokines limit tumor cell growth by stimulating the cytotoxic activity of immune cells against tumor cells. • Recombinant interferon-alpha (IFN-α) and interleukin-2 (IL2)	[6]
Oncolytic Viruses	• Genetically engineered viruses carrying tumor suppressor genes • Work like the gene therapy • When administered, self-replicate in the tumor and induce apoptosis. • Modulate tumor microenvironment and provide anti-cancer immunity	[7]
Vaccine	• Meant to treat cancer as a personalized cancer vaccine • Helps the patient's immune system for cancer killing and relapses. • May consist of dendritic cells, tumor cell lysate, nucleic acids (DNA and mRNA), or neoantigens. • Approaches: dendritic cells engineered to express high levels of tumor-associated target antigens, and delivered to relevant lymph nodes to activate T-cells • The DNA and mRNA-based vaccines: taken up by APCs and present to T-cells to induce their activation • Tumor neoantigen: tumor specific antigens for the development of cancer vaccines without 'off-target' adverse effects.	[8–15]
Small molecules	• Chemotherapeutics as regulator of immune cells • Indoleamine 2,3-dioxygenase-1 inhibitors boost cellular immunity	[16, 17]

The salient features with examples of various approaches toward the cancer immunotherapy have been discussed. The bullets points highlight their primary modes and mechanisms.

Table 1.
Cancer immunotherapy.

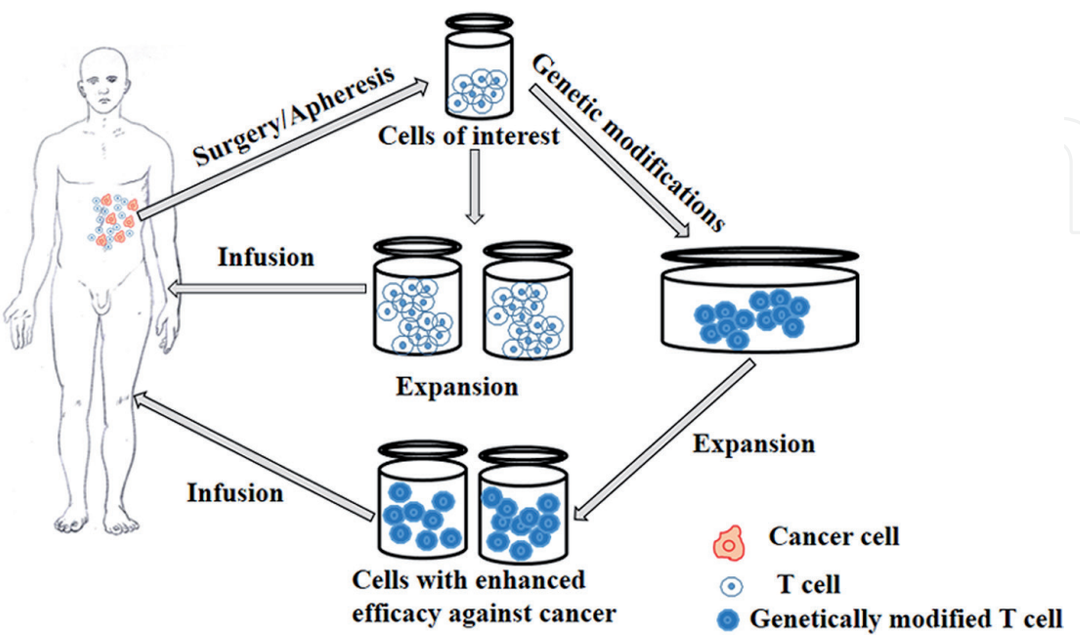

Figure 2.
Approach toward adoptive cellular therapy. Cells of interest to perform ACT may be collected through surgery or apheresis. Next, either these cells can be re-infused to patient after its proper expansion or genetic manipulations depending upon cancer types.

2. Emergence of ACT

The concept of ACT was first developed more than fifty-five years back when immune lymphocytes were successfully used to inhibit growth of sarcoma in an animal model [21].

Further, efforts were made to augment the potential of ACT by inducing activation and proliferation of immune cells in use, which was achieved by the use of recombinant IL2. The IL-2 has been known to potentiate immunological functions and later, its use as T cell growth factor were also recommended [22].

In cell based or cellular therapy, autologous or allogenic stem cells, progenitor or primary cells were the choice of possible cell types, which were modified *ex-vivo* and transfused into the patient for disease regression [2, 18, 19]. Cellular therapy's outcomes were better studied in the case of cancer as it was easy to readout its regression and thus, cancer was chosen first for treatment by cellular therapy. However, lately, this strategy has also been tested for treating cardiovascular, neurological and bone diseases [23]. The choice of cells to develop immunotherapy was from innate and adaptive immune systems as these cells play a key role in controlling cancer progression [24].

Further, initial failures of cell- based therapy were reported due to the role of T cells as the mediators of allograft rejection and also due to the host immune inhibitory factors [2, 25]. Therefore, measures were taken prior to ACT such as use of syngeneic lymphocytes for transfer to minimize the failures of the same [25]. Thus, ACT was developed as a biomedical procedure where the immune cells of the cancer patient, which have high anticancer activity, are expanded, modified and returned to the patients [2, 18, 19].

During eighties, the antitumor activities were reported in cells like natural killer (NK) and lymphokine-activated killer cells [26–30]. These cells directly recognize antigens present on tumor cells and kill them, whereas T cells recognize tumor antigens when presented with major histocompatibility complex (MHC) [31]. However, each cell has different anti-tumor potential. With advancements in immuno-technologies, such as fluorescence activated cell sorter, molecular markers were assigned on various immune cells to define them properly [32, 33]. This led to the classification of lineages and subtypes of immune cells. Thus, selection of cells was made and this followed their manipulation *ex-vivo* for ACT.

So far various cell types such as stem cells, T cells, dendritic cells and NK cells from patients are successfully used and have shown promising results in cancer regression (**Figure 1**). Dendritic cells (DCs) regulate innate immune response and due to its nature of antigen presentation, it may induce adaptive response. Tumor antigen exposed DCs may play a critical role in enhancing cytotoxic activities of immune cells. Therefore, DCs are used as an anti-tumor therapeutic vaccines or to enhance the stimulation of cytotoxic T cells by appropriate antigen presentation [34].

Different subset of T cells (gamma/delta T cells, regulatory T cells, helper and cytotoxic T lymphocytes) respond differently for various tumor subtypes [35]. Interestingly, some T cell subtypes confer advantage over other in reducing tumor volume [36].

3. Evolving modes and mechanisms of ACT

ACT developed in different ways has different mechanisms to target cancer cells. These are the foremost cellular technology adopted for cancer treatment after their validations and regulations through various clinical trials.

3.1 Tumor infiltrating lymphocytes (TILs)

Under the immune surveillance, lymphocytes differentiate between self and non-self-cells and antigens. Any self-cell when gets transformed and starts proliferating as cancer, lymphocytes infiltrate into that site, recognize abnormally growing cells and activate themselves to remove these *not so self*, cancerous cells. These lymphocytes are named as Tumor Infiltrating Lymphocytes (TILs). It so happens sometimes that these TILs fail to perform their function efficiently which may lead to cancer progression. In such cases, it was found that the TILs are not enough in number to show effective cytotoxicity, though have ability to specifically recognize tumor cells, stop their growth and eventually kill them. Thus, to develop any cellular therapy, the first and the foremost approach was to expand TILs, which have infiltrated into the tumor site with anti-tumor potential. These cells can be isolated from cancer origin tissue by resection and expanded *ex-vivo* to a sufficient number to improve their anti-tumor activity. These are then infused back into the patient as ACT therapy (**Figure 3a**). The TILs used in the therapy are autologous lymphocytes as these are derived from the tumor site.

Hence, for the development of ACT, antitumor lymphocytes are grown *in-vitro* up to a number of 10^{11}–10^{12}, followed by a process of selection of specific tumor recognizing cells with effector functions. These cells when infused in the patient, behave like live drug, which proliferate when encounter tumor antigen in the host and help in tumor regression. Though the process of cellular expansion *in-vitro* does not absolutely match with *in-vivo* environment around the tumor, which has certain immune inhibitory responses. Thus, in ACT, a favorable tumor microenvironment is necessary prior to the therapy which should support the anti-tumor immune function of the infused TILs [37].

3.2 Genetic manipulations of T cells

Sometimes in certain cases, tumor infiltrated T cells do not recognize tumor cells and hence they neither get activated nor proliferated *in-vivo*. In such cases, T cells' usefulness becomes redundant. To improve the functional properties of these cells including recognition of antigen on cancer cells, an alternative approach is adopted

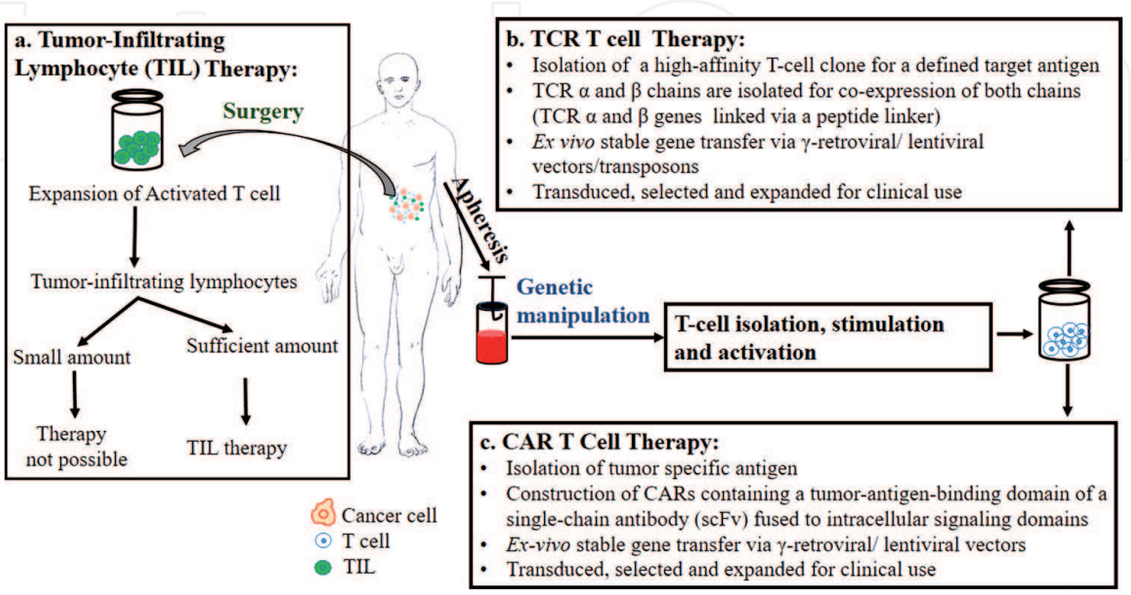

Figure 3.
Leading adoptive cellular therapy. Patients' T cells utilized in TILs, TCR T cells and CAR T cell therapy. Major steps of these therapies discussed in the boxes.

where patient's T cells are genetically manipulated using gene editing technology. This also overcomes the problem of isolating pre-existing tumor-reacting T cells from patients with tumors of other types. Here the cells are made to express tumor antigen-specific TCRs, thus are effective in anti-tumor cytotoxic function [38].

There are two strategies to genetically modify the specificity of T cells. The patient's cells can be genetically modified by integrating genes encoding either conventional alpha-beta TCRs, or Chimeric Antigen Receptors (CARs), specific for tumor antigen(s). To develop ACT by these mechanisms, TCR T cells or CAR T cells are manufactured by autologous T cells which are amended *ex-vivo,* expanded and re-injected in patient to fight against cancer cells (**Figure 3b** and **c**). The only difference remains in the mode of recognition of tumor antigen by these T cells (**Figure 4**).

3.2.1 TCR T cell therapy

The TCR is a specific receptor as well as characteristic marker on T cell surface. TCR complex is a di-sulphide linked membrane anchored heterodimer protein, consisting of two different peptide chains, TCR α and TCR ß encircled by four CD3 chains [39]. These TCR α and ß chains recognize the polypeptide fragment presented by MHC molecule on cancer cells. The principal objective behind TCR T cell technology is to modify TCR binding to tumor antigen which as such shows poor affinity for antigens making them incompetent to recognize and kill tumor cells effectively [40]. Thus, making of high affinity TCR T cell requires identification of specific targets on cancer cells. This way the genetically engineered TCR shows augmented recognition specificity and affinity for tumor cells.

In a study done by Rapoport AP et al., an autologous T cell was engineered to express a high affinity TCR specific to identify naturally processed peptide shared by cancer-testis antigen New York Esophageal Squamous Cell Carcinoma-1 (NY-ESO-1) and L antigen family member 1 (LAGE-1) to be used for multiple myeloma patients showing encouraging clinical response in 16 of 20 (80%) patients with advanced disease [41].

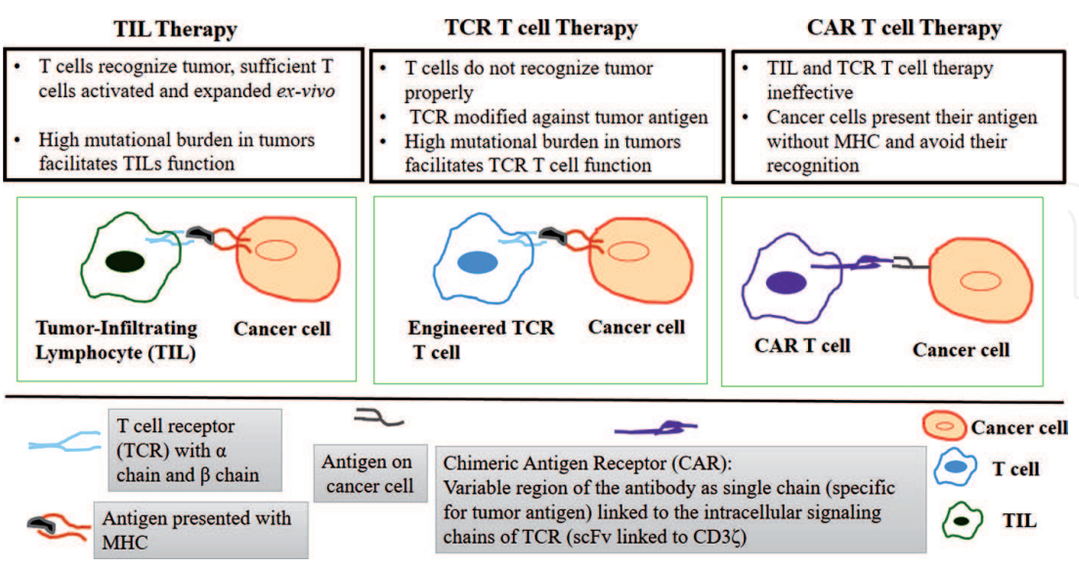

Figure 4.
Molecular insights of cellular therapy. The boxes illustrate the salient features to determine the choice of ACT. In TILs and TCR T cells, the TCR α and ß chains recognize the antigen presented with MHC molecule on cancer cells whereas CAR T cells recognize the tumor antigen independent of MHC. In TCR T cell, genetically engineered high affinity TCR recognizes tumor cells. In CAR T cell, a CAR, a scFv derived from variable regions of heavy and light chains of a monoclonal antibody against tumor antigen recognize tumor cells. CAR also consists of a trans-membrane domain; a hinge; one or more than one intracellular co-stimulatory molecules and a CD3z signaling domain. TCR T cell and CAR T cell therapy highly depend upon identification of unique antigen on cancer cells.

In above mentioned TIL and TCR T cell-based therapies, there may be yet another problem which may be encountered when these expanded/modified cells still do not so efficiently recognize cancer cells. This happens when cancer cells smartly down regulate expression of specific MHC molecules and tumor antigen is presented without MHC complex and thus these T cells fail to recognize the target cancer cells. Fortunately, emergence of recombinant DNA technology and novel cell isolation techniques from blood have paved newer ways to cancer immunotherapy. Hence, to overcome this evading mechanism by cancer cells, T cells are genetically modified in such a way that they recognize cancer cells by a mechanism independent of MHC [2, 42, 43].

3.2.2 CAR T cell therapy

This newer modality of modifying T cells is yet another type of ACT, where T cell are armed with a CAR, which can now make the T cells recognize cancer antigen without MHC molecules. Use of such modified T cells bearing CAR is called as CAR T cell for therapy. The concept of CAR T cell is based on the ability of genetically engineered patient's own T cells to express a CAR, which is specific for a tumor antigen and therefore better in fighting cancer cells. A CAR consists of a scFv derived from variable region of heavy and light chains of a monoclonal antibody against tumor antigen to recognize tumor cells. Apart from this, a trans-membrane domain; a hinge; one or more than one intracellular co-stimulatory molecules and a CD3 zeta signaling domain are the part of CAR construct to make fully activated and functional T cell [2, 44].

Four generations of CARs have been developed, with subsequent generations being better than the previous ones with respect to cytotoxicity and shelf-life. First generation CARs had only single chain variable fragment (scFv) linked to CD3ζ or Fc receptor gamma signaling domain. With the subsequent additions of co-stimulatory domains like CD28, CD 137, or OX40, second and third generation CARs have been created. Fourth generation CARs also called TRUCK (T cell redirected for universal cytokine killing) are armed with immune stimulatory cytokines that ameliorate the performance of CAR T cell with respect to its expansion, persistence, and resistance even in immunosuppressive tumor microenvironment [45, 46]. This therapy harnesses the power of immune system to fight the cancer but in contrast to the regular T cell receptor (TCRs), that recognize Ag, only when presented with MHC, CARs have the aptitude to redirect the effector function of T cell toward any tumor associated antigen (TAA) expressed on the tumor surface even without MHC. Once CAR of the T cell binds to its specific TAA, T cell gets activated through phosphorylation of immune receptor tyrosine-based activation motifs leading to cytokine secretion, T cell proliferation and cytotoxicity [47]. The word chimeric here signifies both, antigen binding, independent of MHC and T cell activation function into a single receptor (**Figure 4**).

Advantage of TCR T cell therapy over CAR T cell therapy is that these can recognize even deep-seated antigens fragment presented on MHC molecule in contrast to CAR T cell that recognize only cell surface proteins. So, TCR T cell therapy offer wider range of application but it is also MHC restricted and recognizes only those antigens presented on MHC molecule, a major drawback of TCR T cell therapy.

4. Opportunities and challenges

Like any other therapy, ACT also has success and failures. The therapy deals with T cells in two different ways, firstly, the natural T cell with antitumor activity

and secondly, the genetically manipulated T cells, either the TCRs engineered or the receptor is chimeric. Thus, T cells' immune responsiveness for tumor targeting functions discretely. Also, during the therapy, responsiveness and un-responsiveness of host is guided by many factors. Similarly, many criteria in preparation of the cells for therapy are the deciding factor for the effectiveness of the therapy, which in turn is decided by extent of tumor regression.

4.1 Tumor regression

Dramatic regression of variety of cancers, like melanoma, cervical cancer, lymphoma, leukemia, bile duct cancer, and neuroblastoma has been reported by the use ACT based approaches [2]. A number of cancer patients showed success using TILs, however, TIL therapy requires surgery and to obtain enough TILs is always a huge challenge technically. TILs expansion approach has been best used for the treatment of metastatic melanoma by Rosenberg et al. [48].

Applications of genetic manipulations in ACT have greatly contributed in improving the remission rate of treatment of various type of cancers [49]. For relapsed and refractory B-cell precursor Acute Lymphoblastic Leukemia in children and young adults, CAR T cell therapy was successful in 52 of 63 patients and three of every four patients did not show relapse in six months [50]. In another trial to treat refractory large B-cell lymphoma, CAR T cell therapy has shown promising results as it completely cured 54% patients, slowed tumor growth in 82% patients and there was no relapse in 40% patient even after 15.4 months [51]. CAR T cell therapy using B cell maturation antigen was tried on multiple myeloma patients and showed remission in 74% of patients [52].

4.2 Challenges

The failures of the therapy in any form have been associated with many factors. The extent to which the tumor cells can evade immune recognition and successfully employ immune suppression mechanisms leads to failure of ACT.

4.2.1 Cell selection

One of the factors which guards the successful use of ACT in humans is the identification of cells that can target antigens selectively expressed on the cancer and not on essential normal tissues. This criterion is the basis of the success of ACT. Also, sometimes immune cells lose their natural tendency to recognize and kill the tumor cells, leads to failure of therapy. Therefore, even though the cell selection is appropriate, sometimes success is not achieved.

4.2.2 Tumor microenvironment

Activities of tumor cells also play a key role in suppressing the effector function of immune cells used in ACT. The tumor cells along with their neighborhood constitute a unique environment which is called as tumor microenvironment (TME). This has an ability to suppress host's immune system by various mechanisms such as a) T cell exhaustion due to continuously changing antigen signatures on them; b) affects the cytotoxic function of T cells at the site of tumor and c) also the T cell trafficking [53]. To counter these immune suppressive mechanisms of TME, there are possible technologies available that locally deliver T cells to the TME and increase their proliferation, thus, could provide a means to treat inoperable solid tumors [54].

4.2.3 Technical glitches

ACT involves different stages to generate clinical grade therapeutic cells and trained personnel to execute the technology. Therefore, it needs great care and precautions to avoid technological pitfalls.

4.2.3.1 Manufacturing

The technical issues are related to the manufacturing process of adoptive cellular material and the delivery platforms which also account for the success or failure of the ACT. During the *ex-vivo* expansion and genetic modification process, factors such as cell culture time, use of cytokines and use of vectors for gene transfer are the major concerns deciding the efficacy and survival of T cells being used in the therapy. Besides sometimes cancer specific T cells may not grow that well and not sufficient for infusion. At the same time efficacy of T cell may also change in *ex-vivo* growth conditions [55].

Major hurdle with regard to TCR T cell technology is related to its expansion which includes identifying of a good target, along with specific TCRs, screening for desirable TCR affinity. Also, TCR T cell therapy is MHC dependent and there is grave peril of hybridization between exogenous and endogenous chain causing recognition of auto-antigens thereby leading to graft-versus host disease [43].

Failures of the therapy with adverse outcomes have been reported due to some chromosomal DNA translocations and rearrangements during the preparation of the cells [56, 57].

4.2.3.2 Delivery system

A major limitation of adoptive T cell therapies is the delivery technology used in the patient. It is noted that the viability and function of the transplanted cells rapidly decline after administration [58]. Hence, different delivery technologies (nanoparticles or scaffolds) have been explored to improve success of ACT. Adjuvant-loaded nanoparticles, chemically conjugated to the surface of T cells to stimulate transplanted cells and minimize the systemic side effects have been designed [59]. Advantages of having a delivery system of T cells which has some immune stimulating mechanisms linked, may help the T cells to enter the tumor site and perform better results of the therapy. Apart from systemic administration route, biomaterials-based strategies have also been explored to locally deliver adoptive T cells to solid tumors [60, 61] as successful targeting of T cells to most solid cancers remain challenging [62]. To overcome the barrier of secluded location of solid tumors, local injection of T cell in brain tumors in mouse model has been tried and has shown better outcome than systemic administration [54].

Overall, biomaterial-mediated local T cell delivery approaches could improve the efficiency of adoptive T cell therapies for treating inoperable solid tumors by overcoming local immunosuppressive barriers. The usefulness of these therapies depends on how quickly T cells can be generated in tumors *in-vivo* using this approach relative to the time it takes to expand T cells *ex-vivo*.

4.2.4 Regulatory guidelines and cost

Regulatory guidelines are other part of the story that limit the use of any therapy where biological samples are used for therapeutic. There are certain considerations to be followed for minimal manipulation and homologous use of human cells, tissues, and cellular and tissue-based products. Cellular & Gene Therapies are complex

products which are regulated by the Food and Drug Administration (FDA) in the United States. The European Union (EU), governs the regulation of all medicinal products for human use, including advanced therapy medicinal products (ATMPs), i.e., medicinal products comprised of cells, genes, or tissues to ensure the quality, safety, and efficacy of medicines placed on the market in the EU. The aims of EU are to ensure the quality, efficacy and most importantly safety of public health.

A very high treatment cost of ACT based immunotherapies has been another concern for its limited use [20, 63]. Cost of CAR-T cell has been curtailed by developing Universal CAR-T cell and CAR NK cell so that this therapy can be a hope for majority of cancer patients [64].

4.3 Advantages

There are many advantages with ACT that outweigh the conventional therapy.

One major advantage of TCR-T cell therapy is that it can target many TAA, even when these lie intracellular and are deep seated. Site directed injection of T cells into tumor giving superior result than systemic administration, is yet another favorable approach in cell-based therapies. A successful example of such application is reported in brain tumors where T cells are injected into CSF directly [43, 54]. Similarly, next generation of CARs have enhanced the ability of T cells to destroy tumor by infiltrating into diseased tissue site and have potential to moderate tumor microenvironment by secreting pro-inflammatory cytokines and expand their own life span *in-vivo* [64].

CARs have another unique ability to recognize not only peptide but also carbohydrate and glycolipid antigens, thus increasing their target antigen number and are also not MHC restricted.

5. Enhancing efficacy of ACT

The journey of development of ACT for cancer treatment has faced success and failures of the therapy in different cancer types. This has led to newer researches and explorations in various domains of the treatment by ACT. Work on improving effectiveness of therapy has contributed enormously and made difference in its outcomes. Following are some areas, which have been mentioned in this section about the efforts made toward enhancing efficacy of ACT.

5.1 Measuring effectiveness

Measuring effectiveness of any therapy is an essential part of it. It is critical to measure of cellular therapy's effectiveness as variations may occur in various steps, starting from isolation of cells to its re-infusion and homing of effective cells to target the cancer cells (**Figure 5**). For this purpose, a therapeutic index (TI) has been developed [65].

TI of a drug is a quantitative assessment of the ratio of a drug dose that produces toxicity to the dose that yields a clinically effective response. ACT has complex biodistribution and also content dependent potency, so for this TI estimation depends on other factors as well. These include functional fitness of the product, vague pharmacokinetics due to trapping, sequestration and extravasations in nearby tissue and inconstant rate of expansion *in-vivo*. Addition factors also influence pharmacokinetics in case of solid tumors like difference in trafficking to benign and cancer tissue, immune suppression and cellular dysfunction due to unfavorable hostile metabolic state.

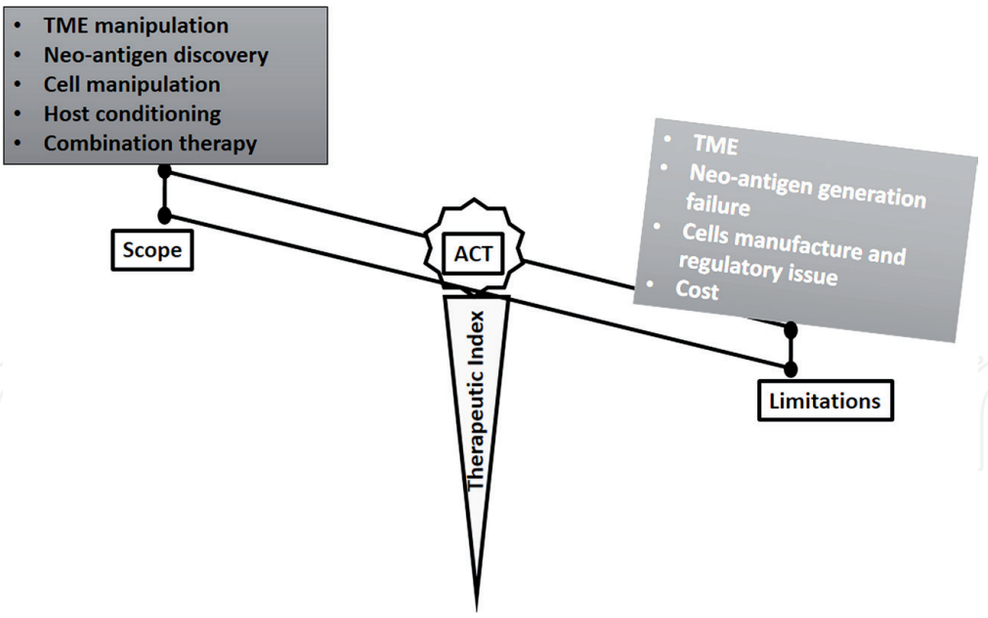

Figure 5.
Scope to enhance therapeutic efficacy of ACT. The scope and limitations are two ends ACT. It may be viewed as the seesaw game. Increasing the scope will determine its future.

Thus, TI of the ACT largely depends on generic factors (T cell potency and fitness, Dispersion, Dysfunction, combinatorial therapies, comorbidities and microbiome) and TME-specific factors (Antigen availability, tumor, immune response related) [65].

5.2 Host conditioning

It is important to understand that anti-tumor efficacy of ACT greatly depends on the persistence of adoptively transferred T cells in the host. This is achieved by an optimal pre-conditioning of the host, an important part of pre-treatment protocol where lymphodepletion by chemotherapy and/or radiation therapy is done prior to therapy. The process of lymphodepletion is important to deplete T regulatory cells and lymphocytes, as these cells compete with the transferred cells for homeostatic cytokines, interleukin 7 and IL15 and this needs to be minimized [66]. This may also be important to avoid excessive cytokine release by lymphocytes which causes adverse effect during the therapy. Host conditioning by either nonmyeloablative chemotherapy or irradiation may induce high levels of IL-1β which increase the number and functionality of adoptively transferred T cells within the tumor and thus improves efficacy of ACT [67]. An FDA-approved reagent, fludarabine has predictable lymphodepleting kinetics and duration of action. Its use in a conditioning regimen, promotes homeostatic upregulation of cytokines and growth signals for T cell persistence [6]. The use of cytokine IL2 has also been recommended for better proliferation of the cells being used in this cellular therapy [2, 6].

5.3 Affinity of T cells

5.3.1 Selection of CARs with moderate affinity

Affinity of the immune cells in ACT is a highly critical criterion while using them for therapy. Optimum affinity is ensured during making of the cells. In TILs based therapy, it may not be that critical as there are no manipulations involved as such except increasing their numbers. However, while doing genetic manipulations,

it needs care especially when a gene fragment is being incorporated into the cell to express a chimeric receptor as in the case of CAR T cell therapy. High affinity CAR bearing cells are *not* the choice of cells as these might recognize the TAA present on the normal cells too and may cause *on target/off tumor* effect, an effect occurs when CAR T cells attack non-tumor cells expressing the target antigen. Thus, CAR selection is very important and ensured to be of low affinity so that it recognizes the antigen when it is present in high number as in case of tumor cells. This helps in recognizing *only* tumor cells and sparing normal cells [68].

5.3.2 Countering loss of antigen on cancer cells

Sometimes problem of therapy arises when the cancer cells start losing CAR-targeted antigen on them and escape their detection by CAR T cells, thus avoid their killing. Such situation is countered by targeting multiple antigens with multiple CARs [69]. For this, anti-tag CARs (AT-CARs) have been developed by adding affinity-enhanced monomeric streptavidin2 (mSA2) biotin-binding domain in the CAR construct. Such novel mSA2CARs have an advantage that the T cells expressing such CARs can bind cancer cells coated with biotinylated antibodies [69]. Binding of such antibodies to cancer cells probably avoids the loss of antigen being targeted on them. Thus, recognition of cancer cells occurs followed by their killing by such CAR T cells without fail.

5.3.3 Formation of synapse

To further improve efficacy of CARs, small sized antibodies (variable heavy homodimers) or nanobodies are recommended to be used with CAR T cell preparation for infusion. These antibodies cause tight synapse formation between the target and effector cell, which is important for the initiation of immune signaling, thus effective T cell mediated killing [70].

5.3.4 CARs expressing Heparanase

It has been discovered that heparanase enzyme expression needs to be upregulated in CAR T cells to penetrated tumor stroma which consists of heparin sulfate proteoglycane. In vitro expanded T cells show reduced heparanase expression as compared to activated immune cell, suggesting their compromised migration [71]. This drawback has been overcome by designing better CAR T cells which were engineered to express heparanase enzyme and therefore show greater capacity to infiltrate tumor stroma with enhanced anti-tumor activity in neuroblastoma xenograft model [72].

5.4 Dose

Next important part of therapy is the dose, i.e., number of the cells in the prepared fraction/dose. The dose frequency and the number of cells per dose to be used for infusion, both play a crucial role in outcome of the therapy. Proportion of immune cells responsible for tumor regression controls the success of ACT. Such as CD8+ enriched "young" tumor infiltrating lymphocytes show better response in the regression of metastatic melanoma compared to the crude fraction containing both CD8+ and CD4+ both proportions [73].

It has also been reported that the number of transfused CAR T cell needed for single transfusion is much less than that needed for TCR T cell therapy to produce equivocal response [43].

5.5 Minimizing toxicities

ACT involves manipulation of immune system to improve its efficacy for specific killing of cancer cells. Such alterations may lead to exaggerated immune response and cause toxicities which are different from other cancer therapies. Thus, depending upon the type of mechanisms involved in these toxicities, discrete approaches are needed to minimize them. The possible toxicities observed and their management are as follow:

a. On target/off tumor recognition develops a toxicity due to shared expression of target antigens by normal tissue leading to varying severity of adverse event from B cell aplasia to death. Hypogammaglobulinemia in B cell aplasia can be treated with intravenous immunoglobulin replacement therapy.

b. Anaphylaxis is seen in patients receiving genetically modified T cell as their antigen recognition domain in derived from murine mAb. Efforts are being made to humanize the expressed protein [74].

c. Graft versus host disease is commonly observed phenomena in immunotherapies. Infusion of isolated autologous TILs is the way to curtail it.

d. Cytokine Release Syndrome (CRS) is associated with overt activation of T cell, which leads to immune activation process with markedly elevated cytokines. It is seen in CAR T cell therapy and called as CAR T cell toxicity. CRS can be minimized by controlling the activity of CAR T cells. For this a bispecific adaptor has been designed which is a cancer specific ligand conjugated with fluorescein. This specifically binds with cancer cells and tag them with fluorescein. CAR is so devised that it recognizes fluorescein and not tumor antigen. Thus, the bispecific adaptor bridges CAR T cell and its tumor target. Thus, availability of bispecific adaptor regulates the killing of tumor cells and can control the CRS. Also, to subdue any CRS, rupture of bridge between CAR T cell to cancer cell can be lifesaving [74]. Other successful approaches to control CRS are immuno-suppression by systemic corticosteroids, IL-6 receptor blockade with mAb or lymphodepleting chemotherapy.

e. Immune effector cell associated neurotoxicity syndrome has also been reported, plausible explanation being elevated cytokine level.

f. Toxicity of T cell activation is also managed by inclusion of an "on switch" in CAR design which can make a hold on functional intensity and T cell activation. This requires selection of two target antigens that are co-expressed on malignant tissue making dual antigen binding a must for complete T cell activation. So normal tissue expressing one target antigen cannot provide complete activation and so limits this toxicity. Conversely, if dual antigens presentation is exclusive to normal tissue, inhibitory signaling in CAR design allow for selective targeting of malignant tissue expressing one antigen while normal tissue is spared.

g. Severe neurological side effects leading to coma and death are reported in patients treated with ACT using autologous T cell when T cells were modified with Melanoma-associated antigen 3 (MAGE-A3) antigen specific TCRs (MAGE-A3 is cancer testis antigen never expressed in normal tissue). These TCRs recognize the different but similar epitopes of especially MAGE-A12 and

possibly MAGE-A1, A8, A9 expressed in human brain. So unfortunately, strategies to enhance TCR affinity to neoantigens on tumor may lead to unanticipated toxicities thus warrants need of improved preclinical testing methods to better enable prediction of TCRs specificity.

6. Conclusions

Cancer is a multifactorial disease with varieties of treatment options available, depending upon the location, drug delivery and stage of cancer. Optimization of immune response to curb the growth, proliferation and containment of cancer has been chosen in TIL, TCR T cell and CAR T cell therapy. Growing technologies in cell biology are improving the future promises for further breakthroughs in the T cell ACT field. Various future domains, which are being explored for further improvements are being discussed below [75]:

a. The TME and other immune escape mechanisms are the challenges to ACT for solid tumors. For this, individualized approaches and strategies combining treatments targeting different immunotherapeutic aspects will be needed in order to expand the applicability and improve the response rates in future.

b. Cross reactivity is seen in antigen specific TCRs, which leads to fatal outcomes, needs newer platforms for preclinical screening, such as X-scan.

c. Newer approaches to manage abnormalities of blood vessels and endothelial cells that hinder T cell infiltration into tumors may be tried with inhibitory molecules.

d. Scope to make ACT more effective in solid tumors can be tested by reducing high interstitial pressure and dense extracellular matrix of solid tumor tissue by angiotensin inhibition.

e. Another strategy to enhance the antitumor immunity of infused T cell may be explored through depletion of tumor-associated macrophages.

f. Also, combination of these therapies is providing newer opportunities to personalized immunotherapy due to individual variation in immune response.

In short, advantages versus toxicities of the anti-cancer therapy have to be considered before deciding the treatment modalities. Ultimately, successful implementation of ACT as the clinical program and cost minimization will determine its success across the globe.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Rajesh Kumar Yadav[1*], Bandana Kumari[1], Pritanjali Singh[2], Asgar Ali[1], Sadhana Sharma[1*] and Krishnan Hajela[3]

1 Department of Biochemistry, All India Institute of Medical Sciences, Patna, India

2 Department of Radiotherapy, All India Institute of Medical Sciences, Patna, India

3 School of Life Sciences, Devi Ahilya Vishwavidyalaya, Indore, MP, India

*Address all correspondence to: biochemrajesh.yadav@gmail.com and drsadhanas@aiimspatna.org

References

[1] Pillai M, Davies MM, Thistlethwaite FC. Delivery of adoptive cell therapy in the context of the health-care system in the UK: challenges for clinical sites. Ther Adv Vaccines Immunother. 2020; 8:2515135520944355.

[2] Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet. 2015; 385(9967):517-28.

[3] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363(8):711-23.

[4] Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013; 369(2):122-33.

[5] Weiner LM, Murray JC, Shuptrine CW. Antibody-based immunotherapy of cancer. Cell. 2012;148(6):1081-4.

[6] Berraondo P, Sanmamed MF, Ochoa MC, Etxeberria I, Aznar MA, Perez-Gracia JL, et al. Cytokines in clinical cancer immunotherapy. Br J Cancer. 2019;120(1):6-15.

[7] Ylosmaki E, Cerullo V. Design and application of oncolytic viruses for cancer immunotherapy. Curr Opin Biotechnol. 2020; 65:25-36.

[8] Chiang CL, Coukos G, Kandalaft LE. Whole Tumor Antigen Vaccines: Where Are We? Vaccines (Basel). 2015; 3(2):344-72.

[9] Guo C, Manjili MH, Subjeck JR, Sarkar D, Fisher PB, Wang XY. Therapeutic cancer vaccines: past, present, and future. Adv Cancer Res. 2013; 119:421-75.

[10] Srivatsan S, Patel JM, Bozeman EN, Imasuen IE, He S, Daniels D, et al. Allogeneic tumor cell vaccines: the promise and limitations in clinical trials. Hum Vaccin Immunother. 2014; 10(1):52-63.

[11] Garg AD, Coulie PG, Van den Eynde BJ, Agostinis P. Integrating Next-Generation Dendritic Cell Vaccines into the Current Cancer Immunotherapy Landscape. Trends Immunol. 2017; 38(8):577-93.

[12] Butterfield LH. Dendritic cells in cancer immunotherapy clinical trials: are we making progress? Front Immunol. 2013; 4:454.

[13] Li L, Goedegebuure SP, Gillanders WE. Preclinical and clinical development of neoantigen vaccines. Ann Oncol. 2017; 28(suppl_12):xii11-xii7.

[14] Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018; 17(4):261-79.

[15] Schreibelt G, Bol KF, Westdorp H, Wimmers F, Aarntzen EH, Duiveman-de Boer T, et al. Effective Clinical Responses in Metastatic Melanoma Patients after Vaccination with Primary Myeloid Dendritic Cells. Clin Cancer Res. 2016;22(9):2155-66.

[16] Liu X, Shin N, Koblish HK, Yang G, Wang Q, Wang K, et al. Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity. Blood. 2010;115(17):3520-30.

[17] Adams JL, Smothers J, Srinivasan R, Hoos A. Big opportunities for small molecules in immuno-oncology. Nat Rev Drug Discov. 2015; 14(9):603-22.

[18] Choi D, Kim TG, Sung YC. The past, present, and future of adoptive T cell therapy. Immune Netw. 2012; 12(4):139-47.

[19] Cohen JE, Merims S, Frank S, Engelstein R, Peretz T, Lotem M. Adoptive cell therapy: past, present and future. Immunotherapy. 2017; 9(2):183-96.

[20] Yadav RK, Ali A, Kumar S, Sharma A, Baghchi B, Singh P, et al. CAR T cell therapy: newer approaches to counter resistance and cost. Heliyon. 2020;6(4):e03779.

[21] Delorme EJ, Alexander P. Treatment of Primary Fibrosarcoma in the Rat with Immune Lymphocytes. Lancet. 1964; 2(7351):117-20.

[22] Vormittag P, Gunn R, Ghorashian S, Veraitch FS. A guide to manufacturing CAR T cell therapies. Curr Opin Biotechnol. 2018; 53:164-81.

[23] Buzhor E, Leshansky L, Blumenthal J, Barash H, Warshawsky D, Mazor Y, et al. Cell-based therapy approaches: the hope for incurable diseases. Regen Med. 2014;9(5):649-72.

[24] Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural innate and adaptive immunity to cancer. Annu Rev Immunol. 2011; 29:235-71.

[25] Thangavelu G, Blazar BR. Achievement of Tolerance Induction to Prevent Acute Graft-vs.-Host Disease. Front Immunol. 2019; 10:309.

[26] Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med. 1982; 155(6):1823-41.

[27] Yron I, Wood TA, Jr., Spiess PJ, Rosenberg SA. In vitro growth of murine T cells. V. The isolation and growth of lymphoid cells infiltrating syngeneic solid tumors. J Immunol. 1980; 125(1):238-45.

[28] Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. Int J Cancer. 1975; 16(2):216-29.

[29] Kiessling R, Klein E, Pross H, Wigzell H. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. Eur J Immunol. 1975; 5(2):117-21.

[30] Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol. 1975; 5(2):112-7.

[31] Davis ZB, Felices M, Verneris MR, Miller JS. Natural Killer Cell Adoptive Transfer Therapy: Exploiting the First Line of Defense against Cancer. Cancer J. 2015;21(6):486-91.

[32] Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol. 2012; 12(3):191-200.

[33] Pitoiset F, Cassard L, El Soufi K, Boselli L, Grivel J, Roux A, et al. Deep phenotyping of immune cell populations by optimized and standardized flow cytometry analyses. Cytometry A. 2018;93(8):793-802.

[34] Sabado RL, Balan S, Bhardwaj N. Dendritic cell-based immunotherapy. Cell Res. 2017; 27(1):74-95.

[35] Wesch D, Kabelitz D, Oberg HH. Tumor resistance mechanisms and their

consequences on gammadelta T cell activation. Immunol Rev. 2020.

[36] Klebanoff CA, Gattinoni L, Torabi-Parizi P, Kerstann K, Cardones AR, Finkelstein SE, et al. Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. Proc Natl Acad Sci U S A. 2005; 102(27):9571-6.

[37] Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol. 2012; 12(4):269-81.

[38] Ikeda H. T-cell adoptive immunotherapy using tumor-infiltrating T cells and genetically engineered TCR-T cells. Int Immunol. 2016; 28(7):349-53.

[39] Xu Y, Yang Z, Horan LH, Zhang P, Liu L, Zimdahl B, et al. A novel antibody-TCR (AbTCR) platform combines Fab-based antigen recognition with gamma/delta-TCR signaling to facilitate T-cell cytotoxicity with low cytokine release. Cell Discov. 2018; 4:62.

[40] Barrett DM, Grupp SA, June CH. Chimeric Antigen Receptor- and TCR-Modified T Cells Enter Main Street and Wall Street. J Immunol. 2015; 195(3):755-61.

[41] Rapoport AP, Stadtmauer EA, Binder-Scholl GK, Goloubeva O, Vogl DT, Lacey SF, et al. NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma. Nat Med. 2015; 21(8):914-21.

[42] Kershaw MH, Westwood JA, Darcy PK. Gene-engineered T cells for cancer therapy. Nat Rev Cancer. 2013; 13(8):525-41.

[43] Zhao L, Cao YJ. Engineered T Cell Therapy for Cancer in the Clinic. Front Immunol. 2019; 10:2250.

[44] Kenderian SS, Porter DL, Gill S. Chimeric Antigen Receptor T Cells and Hematopoietic Cell Transplantation: How Not to Put the CART Before the Horse. Biol Blood Marrow Transplant. 2017; 23(2):235-46.

[45] Sadelain M, Brentjens R, Riviere I. The basic principles of chimeric antigen receptor design. Cancer Discov. 2013; 3(4):388-98.

[46] Zhang Q, Zhang Z, Peng M, Fu S, Xue Z, Zhang R. CAR-T cell therapy in gastrointestinal tumors and hepatic carcinoma: From bench to bedside. Oncoimmunology. 2016; 5(12):e1251539.

[47] Kershaw MH, Westwood JA, Slaney CY, Darcy PK. Clinical application of genetically modified T cells in cancer therapy. Clin Transl Immunology. 2014;3(5):e16.

[48] Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res. 2011; 17(13):4550-7.

[49] Heyman B, Yang Y. New developments in immunotherapy for lymphoma. Cancer Biol Med. 2018; 15(3):189-209.

[50] Ruella M, Kenderian SS. Next-Generation Chimeric Antigen Receptor T-Cell Therapy: Going off the Shelf. BioDrugs. 2017; 31(6):473-81.

[51] Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. N Engl J Med. 2017; 377(26):2531-44.

[52] Cohen AD. CAR T Cells and Other Cellular Therapies for Multiple Myeloma: 2018 Update. Am Soc Clin Oncol Educ Book. 2018; 38:e6-e15.

[53] Lu C, Rong D, Zhang B, Zheng W, Wang X, Chen Z, et al. Current perspectives on the immunosuppressive tumor microenvironment in hepatocellular carcinoma: challenges and opportunities. Mol Cancer. 2019;18(1):130.

[54] Kavunja HW, Lang S, Sungsuwan S, Yin Z, Huang X. Delivery of foreign cytotoxic T lymphocyte epitopes to tumor tissues for effective antitumor immunotherapy against pre-established solid tumors in mice. Cancer Immunol Immunother. 2017; 66(4):451-60.

[55] Grigor EJM, Fergusson D, Kekre N, Montroy J, Atkins H, Seftel MD, et al. Risks and Benefits of Chimeric Antigen Receptor T-Cell (CAR-T) Therapy in Cancer: A Systematic Review and Meta-Analysis. Transfus Med Rev. 2019; 33(2):98-110.

[56] Basecke J, Griesinger F, Trumper L, Brittinger G. Leukemia-and lymphoma-associated genetic aberrations in healthy individuals. Ann Hematol. 2002; 81(2):64-75.

[57] McLean AR, Michie CA. *In-vivo* estimates of division and death rates of human T lymphocytes. Proc Natl Acad Sci U S A. 1995; 92(9):3707-11.

[58] Riley RS, June CH, Langer R, Mitchell MJ. Delivery technologies for cancer immunotherapy. Nat Rev Drug Discov. 2019; 18(3):175-96.

[59] Stephan MT, Moon JJ, Um SH, Bershteyn A, Irvine DJ. Therapeutic cell engineering with surface-conjugated synthetic nanoparticles. Nat Med. 2010;16(9):1035-41.

[60] Smith TT, Moffett HF, Stephan SB, Opel CF, Dumigan AG, Jiang X, et al. Biopolymers codelivering engineered T cells and STING agonists can eliminate heterogeneous tumors. J Clin Invest. 2017; 127(6):2176-91.

[61] Stephan SB, Taber AM, Jileaeva I, Pegues EP, Sentman CL, Stephan MT. Biopolymer implants enhance the efficacy of adoptive T-cell therapy. Nat Biotechnol. 2015; 33(1):97-101.

[62] Fesnak AD, June CH, Levine BL. Engineered T cells: the promise and challenges of cancer immunotherapy. Nat Rev Cancer. 2016; 16 (9):566-81.

[63] Lindenberg M, Retel V, Rohaan M, van den Berg J, Haanen J, van Harten W. Evaluating different adoption scenarios for TIL-therapy and the influence on its (early) cost-effectiveness. BMC Cancer. 2020; 20 (1):712.

[64] Huang R, Li X, He Y, Zhu W, Gao L, Liu Y, et al. Recent advances in CAR-T cell engineering. J Hematol Oncol. 2020; 13(1):86.

[65] Wang E, Cesano A, Butterfield LH, Marincola F. Improving the therapeutic index in adoptive cell therapy: key factors that impact efficacy. J Immunother Cancer. 2020; 8(2).

[66] Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer. 2008; 8(4):299-308.

[67] Lee PH, Yamamoto TN, Gurusamy D, Sukumar M, Yu Z, Hu-Li J, et al. Host conditioning with IL-1beta improves the antitumor function of adoptively transferred T cells. J Exp Med. 2019; 216(11):2619-34.

[68] Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH, et al. Anti-CD22-chimeric antigen receptors targeting B-cell precursor acute lymphoblastic leukemia. Blood. 2013; 121(7):1165-74.

[69] Lohmueller JJ, Ham JD, Kvorjak M, Finn OJ. mSA2 affinity-enhanced biotin-binding CAR T cells for universal tumor targeting. Oncoimmunology. 2017;7(1):e1368604.

[70] Bever CS, Dong JX, Vasylieva N, Barnych B, Cui Y, Xu ZL, et al. VHH antibodies: emerging reagents for the analysis of environmental chemicals. Anal Bioanal Chem. 2016; 408(22):5985-6002.

[71] Tsunekawa N, Higashi N, Kogane Y, Waki M, Shida H, Nishimura Y, et al. Heparanase augments inflammatory chemokine production from colorectal carcinoma cell lines. Biochem Biophys Res Commun. 2016; 469(4):878-83.

[72] Caruana I, Savoldo B, Hoyos V, Weber G, Liu H, Kim ES, et al. Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. Nat Med. 2015; 21(5):524-9.

[73] Dudley ME, Gross CA, Langhan MM, Garcia MR, Sherry RM, Yang JC, et al. CD8+ enriched "young" tumor infiltrating lymphocytes can mediate regression of metastatic melanoma. Clin Cancer Res. 2010; 16(24):6122-31.

[74] Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ. Toxicity and management in CAR T-cell therapy. Mol Ther Oncolytics. 2016; 3:16011.

[75] Magalhaes I, Carvalho-Queiroz C, Hartana CA, Kaiser A, Lukic A, Mints M, et al. Facing the future: challenges and opportunities in adoptive T cell therapy in cancer. Expert Opin Biol Ther. 2019; 19(8):811-27.