A Comprehensive Review of 2 Types of Cancer Immunotherapies: Adoptive Therapies and Immune Checkpoint Blockades

Mahika Reddy

Received July 04, 2024
Accepted October 02, 2024
Electronic access October 31, 2024

Cancer is a common and often deadly disease caused by the uncontrolled proliferation of cells. It can arise in many different parts of the body including the breast, kidney, and lungs. Widely-used treatments for cancer, typically chemotherapy, radiation therapy, and/or surgery, are often effective in slowing disease progression, but usually dramatically reduce the patient's quality of life and involve severe side effects. Immunotherapy is a new approach that looks to treat cancer by manipulating the body's own immune system to fight off the cancer. Immunotherapies generally focus on a type of immune cell called T cells, a vital part of the immune system known for their inherent killing abilities. Although numerous forms of immunotherapies have been developed, the two most prevalent methods are adoptive therapies and immune checkpoint blockades. Adoptive therapies involve isolating T cells from the patient, altering them to attack cancer cells, expanding them, and then reintroducing them into the patient. In chimeric antigen receptor (CAR) T cell adoptive therapy, the T cells are transformed to express CARs on their surface, which primes the T cells to recognize and attack cancer cells. Immune checkpoint blockades work by blocking natural immunosuppressive pathways, which cancers exploit to mitigate antitumor responses. Current research overwhelmingly focuses on downregulating the PD-1/PD-L1 and CTLA-4 interactions to keep more T cells alive and active. Unfortunately, both adoptive therapies and immune checkpoint blockades have their many challenges and limitations, including immune escape and toxicity. To overcome this, many promising proposals to tweak drugs in development or administer combinations of immune therapies have been made. Additionally, successes in clinical studies create much hope for the future of cancer immunotherapy.

Keywords: Cancer, Immunotherapy, CARs, TCR, Immune Checkpoint Blockade

Introduction

Cancer is a disease characterized by the uncontrolled growth and proliferation of cells forming a mass of tissue called a tumor. A common and deadly disease, in 2020, over 1.6 million new cancer cases were reported in the United States and over 602,000 people died of the disease. This correlates to 403 new cancer cases and 144 deaths per 100,000 people[1]. Many different types of cancer exist, affecting different parts of the body. This includes, but is not limited to, breast, ovarian, kidney, lung, skin (melanoma), and blood cancers such as lymphoma. Worldwide, breast and lung cancers are the most common, with breast cancer being the most common among women[2]. Current treatment options vary depending on the type of cancer, but typically include surgery, chemotherapy, and/or radiation. Chemotherapy involves using chemicals in the form of drugs to kill cancer cells. It is given with the aim of either eliminating the cancer, prolonging the life span, or reducing carcinogenesis. Radiation therapy delivers radiation in high doses to patients to kill cancer cells. Both of these treatments have saved many lives, but risk severe negative side effects that can dramatically affect quality of life. Surgery aims to cut the tumor, along with nearby healthy tissues out of the body, but it may be unable to remove every cancer cell, and surgery is often not offered if the cancer resides very close to delicate tissues or vital organs. For chemotherapy, these unwanted side effects may include intense nausea, fatigue, and shortness of breath, since chemotherapy can kill healthy cells in addition to the cancer cells[3]. Radiation therapy also causes side effects such as fatigue, vomiting, appetite changes, nausea, and even lung issues[4].

In light of these limitations, scientists have begun to develop cancer treatments that more specifically target the cancerous cells by using the patient's own immune system. This approach is known as cancer immunotherapy and has shown promise in early research. Although numerous approaches have been taken to use the body's immune system to eliminate cancers, the most commonly studied forms of cancer immunotherapy are adoptive cell therapies and immune checkpoint blockades[5].

Cancer and the Immune System

The immune system consists of various proteins, cells, and organs working together to protect the body from foreign pathogens and antigens. Generally, even though they disrupt nor-

mal bodily function, tumors can often evade the immune system because they are not recognized as foreign pathogens, as they consist of the body's own cells. However, tumors may avoid being destroyed by promoting immunosuppressive pathways which exist for the purpose of avoiding excessive inflammation. For example, the immune system relies on specific recognition proteins comprising the major histocompatibility complex (MHC) to identify self versus non-self antigens. MHC class I (MHC 1) is expressed by all cells in the human body to present endogenous, self-peptides for immune surveillance. MHC class II (MHC II) is expressed by specialized antigen-presenting cells (APCs) to present and recognize exogenous, processed antigens[6]. Tumors may downregulate MHC 1, leaving CD8+ T cells unable to identify and eliminate them.

There are two main "arms" of the immune system, the innate immune system and the adaptive immune system. The innate immune system is responsible for immediate recognition and clearance of pathogens, usually taking only minutes to hours to do its job[7]. The adaptive immune system takes longer to work, usually days to weeks, but provides longer-term protection by forming memory against specific pathogens. The adaptive immune system is able to accomplish this primarily through two cell types: B cells and T cells. B cells (CD19+) are responsible for the production of antigen-specific antibodies. These antibodies bind selectively to surface receptors on pathogens, marking them for elimination by T cells. There are two main types of T cells (CD3+): cytotoxic T cells (CD8+) and helper T cells (CD4+). CD8+ T cells are responsible for the direct killing of antibody-tagged pathogens[8]. CD4+ T cells play a role in priming the immune response by increasing antibody secretion by CD19+ cells and activating CD8+ T cells[8]. A subset of CD4+ T cells, called regulatory T cells (Tregs) are responsible for suppressing immune responses. Tumors may recruit Tregs by secreting cytokines to activate them[9]. Both CD19+ and CD3+ cells are activated by the presentation of MHC 2 peptides by dendritic cells, the most common APCs in the body that work to bring foreign pathogen proteins to the lymph nodes.

Additionally, tumors can manipulate the immune system by modifying metabolic pathways. They often upregulate Indoleamine 2,3-dioxygenase (IDO), an enzyme responsible for metabolizing tryptophan. Tryptophan is essential for T cell proliferation, so depleting the tryptophan supply weakens the immune system. MHC 1 downregulation, cytokine secretion, and upregulation of certain enzymes represent only a fraction of the ways in which tumors interact with the immune system to promote their own growth and survival, creating extremely complex tumor-immune relationships. Immunotherapy approaches aim to overcome these evasion tactics by re-training the patient's immune system to recognize and eliminate cancerous cells. Adoptive therapies rely on engineering specialized immune killer cells that can specifically target receptors on the patient's cancer, overcoming the lack of MHC 1 surface expression. Immune checkpoint blockades look to increase immune cell activity by preventing the upregulation of immunosuppressive proteins[9]. While each method has its limitations, both are promising treatments with the potential to address the shortcomings of current treatments and ultimately provide viable cancer treatment options in the future.

Adoptive Cell Therapies

Chimeric antigen receptors (CARs)

Adoptive cell transfer can be described as the isolation and expansion of the cancer patient's own T cells outside the body (ex vivo), followed by reintroduction into the patient along with growth factors to promote their survival and proliferation in the body (*in vivo*) (Fig 1a). It was theorized that modification of these T cells may lead to better results, leading to the introduction of chimeric antigen receptor T cells (CAR T cells)[10]. CARs are receptor proteins that enable T cells to target specific proteins and are derived from monoclonal antibodies, which are laboratory-produced molecules that mimic natural immunostimulatory antibodies. T cells are removed from the patient and engineered to express synthetic CARs on their cell membrane surface (Fig 1b). These engineered CAR T cells can then bind to CD19-expressing cancer cells, activating the T cells to release perforins and granzymes, killing the tumors (Fig 1c).

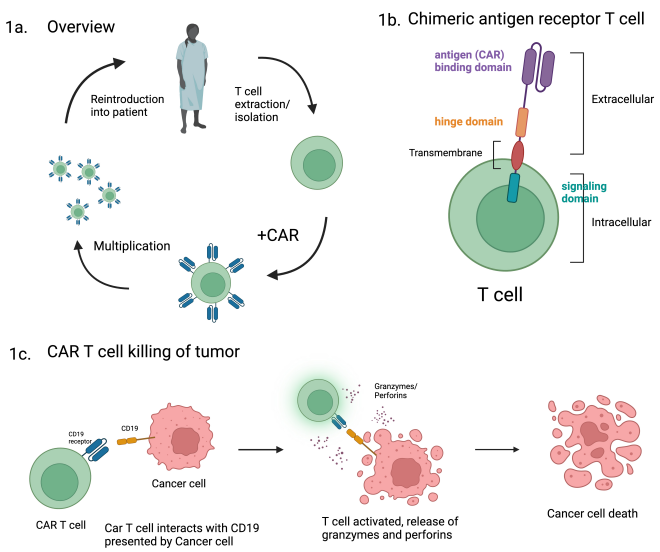

Fig. 1 Overview of adoptive therapy. (a) T cells are isolated from patient blood samples, modified to express CAR, and then reintroduced back into the patient. (b) Chimeric antigen receptors contain a signaling domain and a binding domain. (c) The CAR binding domain recognizes CD19 on the surface of cancer cells. CAR T cell binding to CD19 initiates tumor killing. Created with BioRender.com

T cell receptors (TCRs)

T cell receptors (TCRs) recognize short peptide sequences called epitopes that are part of an antigen, which are processed and presented by the MHC and are where an antibody attaches itself[11]. Three different parameters are thought to govern this peptide-MHC complex (pMHC) and subsequent T cell activation: TCR affinity, avidity, and functional avidity. Affinity indicates the sensitivity of T cells towards an antigen and is defined as the strength of the interaction of the T cell receptors and peptide-MHC complex on an antigen-presenting cell. Avidity measures the strength of multiple TCR-pMHC engagements and considers the effect of other molecules such as TCR co-receptors. Functional avidity depicts T cell fitness and activity at different concentrations of epitope and predicts the ability of a TCR-engineered T cell to induce a tumor-specific reaction, even when the number of pMHC is poor due to downregulation of MHC by the tumor in the immunosuppressive TME[11].

TCRs obtain specificity as they develop and can cause TCRs that recognize naturally expressed self-antigens. Some are called tumor-associated antigens (TAAs) and are targeted in adoptive T cell therapies because of their extreme overexpression in cancerous cells. However, because they are self-antigens, they can also be present in healthy tissues. Therefore, selection mechanisms usually eliminate T cells that have high affinity towards self-antigens, so the frequency of TCRs that have high affinity towards TAAs is low. In response to this challenge, affinity maturation *in vitro* is a possible tool to increase the affinity of T cells, or their ability to recognize lower expression of peptide epitopes[11]. One way of accomplishing this is using the CRISPR-Cas9 system to genetically engineer T cells to include extra disulfide bonds, increasing the avidity of the interaction[11]. However, it has been shown that affinity-matured TCRs may improve the speed at which T cells respond without equipping them to respond to a low density of pMHC. Therefore, this may not always solve the problem of low recognition of lowly present epitopes. Additionally, a published study has shown that avidity is not an important factor in reducing the size of a tumor or reducing tumor burden[12]. This experiment showed both high and low-affinity TCRs destroyed small tumors but could not do the same to larger tumors. Therefore, some suggest that epitope density rather than TCR affinity or avidity might be more important to evoke a cancer-specific T cell response. On the contrary, another report argued that avidity is a major factor in eliminating leukemic cells *in vivo* instead of epitope density or peptide-MHC affinity, and supported the idea that there is some sort of threshold of affinity and avidity above which further enhancement doesn't create a more effective response[13]. Many more contradictory studies like these highlight the complexity of TCR-pMHC interactions related to cancer recognition and underscore the risk of oversimplification. Despite the divergences in correlation between TCR affinity and T cell activity, the selection of high-affinity TCRs or *in vitro* affinity maturation continues to be pursued as a way to improve antitumor responses[11]. Selecting cancer-specific TCRs is another important step of the process which is achieved in multiple ways. One of these ways to find cancer-specific TCRs is to isolate them from patients who responded after a treatment with dendritic cells that have been engineered to express the tumor antigen. In patients where it is hard to isolate cancer-specific TCRs, T cells from healthy donors are a possible alternative, but patients should be accurately matched with donors and the potential off-target reactivities caused by donor TCRs cannot be overlooked[11].

While the potential of TCR therapies is exciting, challenges remain regarding efficacy and safety before they can be widely implemented. Unfortunately, affinity-enhanced TCRs have demonstrated off-target recognition, causing neurotoxicity in mice due to the recognition of melanoma-associated antigen 12 (MAGE-A12) in brain cells[14]. Combined approaches including immune checkpoint inhibitors may improve T cell activity without the need for affinity maturation that can cause severe adverse effects. This method of reinfusing tumor-infiltrating lymphocytes (TIL), known as TIL therapy, has been shown to have a positive effect. However, a major disadvantage of TIL therapy is finding and deriving high-affinity T cells, which can be further complicated by the immunosuppressive TME. This highlights the main advantage of engineered T cells like CAR T cells, where regular T cells are engineered into a highly activated form. Therefore, it is not necessary to seek out T cells that are naturally more useful against tumors, which may be a complicated and technically difficult task[10].

One positive of CARs compared to TCR therapy is that they work independently of MHC, which means they can overcome MHC downregulation in the TME[10]. However, immune escape remains a limit on the effectiveness of CAR T cell therapy. In this case, immune escape means a full or partial loss of the target antigens on cancer cell surfaces[10]. Additionally, there are many restraints when it comes to solid tumors. The success of CAR T cell therapy in hematological malignancies is attributed to high expression of surface markers and easy accessibility of immune cells to lymphoid cells. Solid tumors, which grow in organ systems and can appear anywhere in the body, may not have such factors. For example, there is increased difficulty of CAR T cell delivery to tumor sites and infiltration into the tumor's functional tissue in solid tumors. This is because of a combination of factors in the solid TME. For example, the extracellular matrix (ECM) of solid tumors is full of stroma cells, which provide a physical barrier to CAR T cells. Additionally, immunosuppressive cells such as Tregs, myeloid-derived suppressor cells, and tumor associated macrophages are prevalent inside solid tumors, further hindering CAR T cell efficiency[15]. One solution to this involves insertion of chemokine receptors in CAR T cells so that they can sense tumor-secreted chemokine gradients that lead

them into the tumor. However, there are many drawbacks to this idea, including interfering chemokine gradients produced by other organs during inflammation or injury, immune escape due to chemokine loss or down-regulation, and that the factors secreted by tumors are heterogeneous, varying both intratumorally and between individuals. Additionally, the immunosuppressive TMEs of solid tumors limit the potential of cancer immunotherapy by secreting immunosuppressive cytokines such as PD-L1. To solve this issue, the idea of combining and co-administering CAR T cell therapy and immune checkpoint blockades, especially regarding the PD1/PDL-1 pathway, has been studied for potential to enhance CAR T cell efficacy [10].

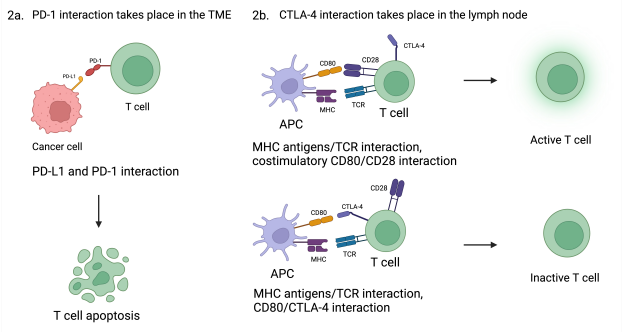

Fig. 2 Overview of PD-1 and CTLA-4 mechanisms and functions. (a) PD-1 and PD-L1 interaction induces apoptosis in T cells. (b) When costimulatory molecule CD80 binds to CTLA-4 instead of CD28, the T cell is not activated. Created with BioRender.com

Immune Checkpoint Blockade *(anti-PD1 & anti-CTLA4)*

Tumors evade immune responses by taking advantage of negative feedback mechanisms that the body has evolved to control the immune system from self-recognition, including inhibitory receptors: programmed cell death protein 1 (PD-1) and anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) [16]. During chronic inflammation, T cells are known to become exhausted and then upregulate inhibitory receptors like CTLA-4 and PD-1, leading to loss of proliferative potential and effective functioning [16]. This is a challenge for anti-tumor responses because it allows the tumors to evade immune cell responses.

The PD-1 gene participates in apoptosis when the PD-1 receptor interacts with PD-L1 or PD-L2 ligands on the tumor. This interaction occurs in the TME, where PD-1 is highly expressed on active T cells, B cells, dendritic cells, regulatory T cells (Tregs), and natural killer cells, and its ligand is expressed on some types of tumor cells and antigen-presenting cells [17]. The binding of PD-1 to PD-L1 results in the phosphorylation of tyrosine residues, setting off a chain reaction that eventually inhibits downstream signaling and T cell biological functions. These downregulated pathways include lymphocyte proliferation, cytokine secretion, and CD8+ T cell cytotoxicity. This results in tumor-specific T cell exhaustion and apoptosis so that the tumor cells can evade immune surveillance (Fig 2a).

In many cancers, PD-1 is expressed on a large amount of tumor-infiltrating lymphocytes, and enhanced PD-1 expression is always observed on Tregs. Therefore, activated Tregs indicate that the TME is in an immunosuppressive state. The accompanying PD-L1 is commonly upregulated in tumor cells. Under normal conditions, the PD-1/PD-L1 interaction serves to prevent excessive tissue inflammation and autoimmune disease. However, during tumor growth and development, a combination of PD-1 and PD-L1 limits the host's antitumor immune response by inhibiting tumor-infiltrating lymphocyte activation and inducing their apoptosis. It may also decrease secretion of inflammatory cytokines while increasing the secretion of inhibitory cytokines [16].

The development of anti-PD-1 antibodies, and immune checkpoint blockade in general, aims to target the tumor indirectly rather than interfering directly with cell growth and survival, like chemotherapy and radiation. Instead, they boost anti-tumor immune responses by inhibiting the immunosuppressive mechanisms that the tumor utilizes to evade detection by the immune system.

The FDA has already approved many anti-PD-1 antibodies. For example, Nivolumab (brand name: Opdivo) and Pembrolizumab (brand name: Keytruda) were approved in 2014, and Cemiplimab (Brand name: Libtayo) was approved in 2018. Nivolumab is a high-affinity anti-PD-1 that blocks PD-1 binding to its cognate ligand PD-L1, which is upregulated on tumor cells. It has demonstrated efficacy in melanoma, non-small cell lung cancer, certain types of lymphoma, and other cancers. Another high-affinity anti-PD-1 antibody is Pembrolizumab. A phase I clinical trial showed it to be safe and effective for melanoma treatment [18] while a phase II trial showed a significant effect on advanced melanoma when directly compared to another immune checkpoint inhibitor involving CTLA-4 [19]. Cemiplimab, another high affinity anti-PD-1 antibody, showed no durable response and no disease recurrence more than 16 months after treatment in a phase I study involving advanced cutaneous squamous cell carcinoma patients [20]. Despite these strides, much remains to be learned about the signaling pathway. The mechanism by which PD-1 regulates Tregs and the acts on tumor cells in the TME is largely unknown. Increased knowledge in this area could lead to the development of more efficient inhibitors based on this mechanism. One concern with treatment administration is that not all cancer patients have high expression of PD-1 [17]. Therefore, it is important to find a method of selecting PD-1/PD-l1 positive patient groups to receive this treatment. An option for this is measuring the expression of PD-L1 within the TME, but studies have shown this to be correlated with treatment efficacy in melanoma patients [21] and not in patients with squamous cell carcinoma [22] and non-small cell lung cancer [23]. Generally, studies suggest that immune checkpoint inhibitors are most effective

in patients who already display anti-tumor immune processes before therapy[24].

Normal T cell activation requires co-stimulation of the TCR to the pMHC and the binding of the T cell costimulatory molecule, CD28, to APC-expressed CD80/86. CTLA-4 inhibits T cell functions and causes immunosuppression by competitively binding to ligands CD80/86 on the APC, diminishing signaling through CD28. Even though both receptors bind CD80/86, CTLA-4 has higher affinity and outcompetes CD28 for its ligands, which increases the activation threshold of T cells (Fig 2b). Unlike the PD-1/PD-L1 interaction that occurs at the tumor site, CTLA-4 inhibits T cells in the lymph node[25]. In cancer, CTLA-4 can be expressed on infiltrating Tregs or exhausted T cells and tumor cells themselves. However, the association with cancer is unclear, and few studies have described the predictive value of measuring CTLA-4 levels in TME. It is known that CTLA-4 blockades are more effective in tumors that have been infiltrated by T cells and are more immunogenic. An anti-CTLA-4 antibody, ipilimumab, was the first checkpoint inhibitor to be tested and approved for cancer patients[16].

Generally, both PD-1 and CTLA-4 checkpoint inhibitors have resulted in increased survival in many studies on melanoma[21,26], squamous cell carcinoma[22], and non-small cell lung cancer[23] when compared to conventional chemotherapies. Anti-PD-1 antibodies were proven to be more effective for survival rates compared to anti-CTLA-4 antibodies in a Phase III clinical trial. Furthermore, combined administration resulted in higher response rates and survival at 11.5 months[21]. This combination is possible because PD-1 and CTLA-4 act independently, and it has been shown to have clinical efficacy, but checkpoint combination therapies often have much higher toxicity and side effects than individual administration30. The side effects of both CTLA-4 and PD-1 inhibition tend to resemble autoimmune reactions, but the rates of severe side effects vary by study and treatment[25]. There are often more side effects with anti-CTLA-4 compared to anti-PD-1 inhibitors. Almost all patients treated with either anti-CTLA-4 or anti-PD-1 antibodies had mild side effects like diarrhea, fatigue, nausea, and decreased appetite, while there were some reports of patients experiencing aggravation of pre-existing autoimmune diseases or even developing new ones[27].

While the potential of the positive effects of checkpoint inhibitors is promising, there are possibilities of negative effects that may arise from treatment. Due to selection pressure caused by treatments, many patients may relapse and experience tumor progression, with the rise of tumor cells that have found alternative pathways to evade immune recognition and response. It may also cause the upregulation of other inhibitory receptors. For example, patients with melanoma exhibited upregulation of the T cell activation inhibitory reception VISTA after anti-CTLA-4 treatment[25].

Limitations & Future Directions

Unfortunately, the limitations of immunotherapies remain a huge challenge for researchers. The largest issue of CAR T cell therapy administration remains toxicity, including over-activation-based general toxicity caused by CRS,[10] usually accompanied by neurotoxicity[28]. Neurotoxicity can also be caused by TCR therapy[14]. CRS can have mild to severe effects on the patient, the most severe of which include respiratory failure and multi-organ system failure. The second type of toxicity is off-target-based toxicity, which arises when CAR T cells attack healthy cells that express target antigens[10]. To address this, strategies such as multi-targeting of different tumor-specific antigens to avoid off-target-based toxicity are being implemented. Additionally, the idea of inhibitory CARs that trigger negative signaling after recognizing normal cell-specific antigens is being explored.

Like adoptive therapies, there are certain drawbacks to immune checkpoint blockades that limit their usage. Besides the autoimmune-reminiscent reactions that treatment causes in patients[27], selection pressure caused by treatments may lead to the rise of tumor cells that rely on alternate pathways to perform immune escape. In response, researchers are looking at different immune receptor pathways that can be targeted without causing as many off-target effects. Continued work in this promising field can lead to treatments that are highly effective and widely administered to improve the quality of life and promote survival rates of cancer patients. CAR-based therapies may even have potential beyond oncology, including treatments for autoimmune diseases, fibrosis, etc[29].

Conclusions

Cancer immunotherapy is an exciting branch of medicine with much potential to transform the current standards for treating cancer. Rather than aiming to directly kill cancerous cells, immunotherapy aims to either train the immune system to recognize and kill the tumor, or diminish cancer's ability to evade detection and suppress the immune system. Adoptive therapy involves improving the immune system's anti-tumor responses by removing a patient's own T cells, altering them ex vivo, and then reintroducing them to the patient. Initially, this approach included just isolation and expansion of T cells, but the idea of engineering these T cells to further enhance efficiency led to the development of chimeric antigen receptor (CAR) T cell therapy and T cell receptor (TCR) therapy. While both of these methods show clinical promise, CAR T cells encounter significant issues such as toxicity and complexity of solid TMEs, while TCR therapy faces challenges such as MHC downregulation. Immune checkpoint inhibitors aim to boost the immune system by blocking checkpoints that negatively regulate the immune system. Anti-PD-1 and anti-CTLA-4 immune checkpoint in-

hibitors have shown efficacy in various cancers, but there is potential for relapse if the tumor finds alternate evasion mechanisms. Generally, while immunotherapy approaches have had clinical success, many critical issues remain, all of which require much more innovation and study to overcome.

References

1 U.S., *Cancer Statistics: Highlights from 2020 mortality and incidence with comparison to 2019 incidence to assess the effect of the COVID-19 pandemic*, https://www.cdc.gov/cancer/uscs/about/data-briefs/no35-USCS-highlights-2020.htm.

2 *Cancer facts for women: Most common cancers in women*, https://www.cancer.org/cancer/risk-prevention/understanding-cancer-risk/cancer-facts/cancer-facts-for-women.html#:~:text=Breast%20cancer%20is%20the%20most,breast%20cancer%20in%20their%20lifetime.

3 A. Pearce, M. Haas, R. Vieny, S. Pearson, P. Haywood, C. Brown and R. Ward, *Incidence and severity of self-reported chemotherapy side effects in routine car: A prospective cohort study*.

4 H. Majeed and V. Gupta, *Adverse Effects of Radiation Therapy*.

5 D. Stanculeanu, Z. Daniela, A. Lazescu, R. Bunghez and R. Anghel, *Development of new immunotherapy treatments in different cancer types*.

6 M. Wieczorek, E. Abualrous, J. Sticht, M. Álvaro Benito, S. Stolzenberg, F. Noe and C. Freund, *Major histocompatibility complex (MHC) class I and MHC class II proteins: Conformational plasticity in antigen presentation*.

7 B. Aristizábal and A. González, *Autoimmunity: From Bench to Bedside: Chapter 2 Innate immune system*.

8 Y. Liu, J. Luo and C. Bennett, *Adaptive immunity: Based on the dual recognition responses of t cells*.

9 S. Kim and S. Cho, *The evasion mechanisms of cancer immunity and drug intervention in the tumor microenvironment*.

10 M. Akhoundi, M. Mohammadi, S. Sahraei, M. Sheykhhasan and N. Fayazi, *CAR T cell therapy as a promising approach in cancer immunotherapy: challenges and opportunities*.

11 D. Campillo-Davo, D. flumens and E. Lion, *The quest for the best: how TCR affinity, avidity, and functional avidity affect TCR-engineered T cell antitumor responses*.

12 G. Segal, S. Prato, D. Zehn, J. Mintern and J. Villadangos, *Target density, not affinity or avidity of antigen recognition, determines adoptive T cell therapy outcomes in a mouse lymphoma model*.

13 K. Vincent, M. Hardy, A. Trofimov, C. Laumont, D. Sriranganadane, S. Hadjmimoune, I. Fourati, H. Soudeyns, P. Thibault and C. Perreault, *Rejection of leukemic cells requires antigen-specific T cells with high functional avidity*.

14 R. Morgan, N. Chinnasamy, D. Abate-Daga, A. Gros, P. Robbins, Z. Zheng, M. Dudley, S. Feldman, J. Yang, R. Sherry, G. Phan, M. Hughes, U. Kammula, A. Miller, C. Hessman, A. Stewart, N. Restifo, M. Quezado, M. Alimchandani, A. Rosenberg, A. Nath, T. Wang, B. Bielekova, S. Wuest, N. Akula, F. McMahon, S. Wilde, B. Mosetter, D. Schendel, C. Laurencot and S. Rosenberg, *Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy*.

15 E. Zhang, J. Gu and H. Xu, *Prospects for chimeric antigen receptormodified T cell therapy for solid tumors*.

16 Y. Jiang, M. Chen, H. Nie and Y. Yuan, *PD-1 and PD-L1 in cancer immunotherapy: clinical implications and future considerations*.

17 M. Keir, M. Butte, G. Freeman and A. Sharpe, *PD-1 and its ligands in tolerance and immunity*.

18 O. Hamid, C. Robert, A. Daud, S. Hodi, W. Hwu, R. Kefford, J. Wolchok, P. Hersey, R. Joseph, J. Weber, R. Dronca, T. Gangadhar, A. Patnaik, H. Zarour, A. Joshua, K. Gergich, J. Elassaiss-Schaap, A. Algazi, C. Mateus, P. Boasberg, P. Tumeh, B. Chmielowski, S. Ebbinghaus, X. Li, S. Kang and A. Ribas, *Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma*.

19 C. Robert, J. Schachter, G. Long, A. Arance, J. Grob, L. Mortier, A. Daud, M. Carlino, C. McNeil, M. Lotem, J. Larkin, P. Lorigan, B. neyns, C. Blank, O. Hamid, C. Mateus, R. Shapira-Frommer, M. Kosh, H. Zhou, N. Ibrahim, S. Ebbinghaus and A. Ribas, *Pembrolizumab versus Ipilimumab in advanced melanoma*.

20 G. Falchook, R. Leidner, E. Stankevich, B. Piening, C. Bifulco, I. Lowy and M. Fury, *Responses of metastatic basal cell and cutaneous squamous cell carcinomas to anti-PD1 monoclonal antibody REGN2810*.

21 J. Larkin, V. Chiarion-Sileni, R. Gonzalez, J. Grob, C. Cowey, C. Lao, D. Schadendorf, R. Dummer, M. Smylie, P. Rutkowski, P. Ferrucci, A. Hill, J. Wagstaff, M. Carlino, J. Haanen, M. Maio, I. Marquez-Rodas, G. McArthur, P. Ascierto, G. Long, M. Callahan, M. Postow, K. Grossmann, M. Sznol, B. Dreno, L. Bastholt, A. Yang, L. Rollin, C. Horak, F. Hodi and J. Wolchok, *Combined nivolumab and ipilimumab or monotherapy in untreated melanoma*.

22 R. Ferris, G. Blumenschein, J. Fayette, J. Guigay, A. Colevas, L. Licitra, K. Harrington, S. Kasper, E. Vokes, C. Even, F. Worden, N. Saba, L. Docampo, R. Haddad, T. Rordorf, N. Kiyota, M. Tahara, M. Monga, M. Lynch, W. Geese, J. Kopit, J. Shaw and M. Gillison, *Nivolumab for recurrent squamous-cell carcinoma of the head and neck*.

23 J. Brahmer, K. Reckamp, P. Baas, L. Crino, W. Eberhardt, E. Poddubskaya, S. Antonia, A. Pluzanski, E. Vokes, E. Holgado, D. Waterhouse, N. Ready, J. Gainor, O. Frontera, L. Havel, M. Steins, M. Garassino, J. Aerts, M. Domine, L. Paz-Ares, M. Reck, C. Baudelet, C. Harbison, B. Lestini and D. Spigel, *Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer*.

24 N. Rizvi, M. Hellmann, A. Snyder, P. Kvistborg, V. Makarov, J. Havel, W. lee, J. Yuan, P. Wong, T. Ho, M. Miller, N. Rekhtman, A. Moreira, F. Ibrahim, C. Bruggeman, B. Gasmi, R. Zappasodi, Y. Maeda, C. Sander, E. Garon, T. Merghoub, J. T. Schumacher and T. Chan, *Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer*.

25 J. Seidel, A. Otsuka and K. Kabashima, *Anti-PD-1 and anti-CTLA-4 therapies in cancer: mechanisms of action, efficacy, and limitations*.

26 C. Robert, L. I. Bondarenko, S. O'Day, J. Weber, C. Garbe, C. Lebbe, J. Baurain, A. Testori, J. Grob, N. J. Richards, M. Maio, A. Hauschild, W. Miller, Jr, P. Gascon, M. Lotem, K. Harmankaya, R. Ibrahim, S. Francis, T. Chen, R. Humphrey, A. Hoos and J. Wolchok, *Ipilimumab plus dacarbazine for previously untreated metastatic melanoma*.

27 Y. Chae, L. Chiec, N. Mohindra, R. Gentzler, J. Patel and F. Giles, *A case of pembrolizumab-induced type-1 diabetes mellitus and discussion of immune checkpoint inhibitor-induced type 1 diabetes*.

28 C. Chou and C. Turtle, *Insight into mechanism associated with cytokine release syndrome and neurotoxicity after CD19 CAR-T cell immunotherapy*.

29 H. Aghajanian, J. Rurik and J. Epstein, *CAR-based therapies: opportunities for immuno-medicine beyond cancer*.