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Research Article

The Potential Success and Setbacks of CAR-T Cell Therapy

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Abstract

CAR-T cells prove to be a potential treatment for cancer. However, some clinical trials have not shown complete success, with complications regarding the growth and recognition of cancer cells. There is also an evolution of CAR-T cells, from the first to fourth generations, and there will likely be more as researchers continue to conduct studies to overcome the disadvantages of CAR-T cell therapy. There are many factors present in the tumor microenvironment that create a problem for the successful treatment of cancer. However, there are studies being conducted to provide solutions for an effective treatment.

Introduction

In recent years, researchers recognized cancer as a major cause of death throughout the world [1]. There are many studies being conducted to find an effective treatment in addition to cytotoxic immunotherapies and conventional therapies [2]. One promising area of research for potential treatment is chimeric antigen receptor (CAR)-T cell therapy [3]. Essentially, T cells that are acquired from patient blood are altered in vitro to express artificial receptors that recognize a tumor antigen, therefore targeting the antigen [4]. CAR-T cell therapy has been proven to successfully treat blood cancer, as well as change the scope of treatment for lymphoid malignancies. The potential for this new therapy could change the course of cancer [5].

There are several steps of performing CAR-T therapy, but the process is not the only component; there are other things to consider, such as the individual response from the patients, and most importantly, the outcome [6]. The first step of the process is to extract blood from the patient in order to obtain the T-cells from the blood [7]. Leukapheresis is used to return the red blood cells to the patient. Next, the T-cells will receive new genetic instructions to produce chimeric antigen receptors (CAR) and some other molecules that are engineered to target the malignancies [8]. Then, the researchers cultivate and induce the CAR-T cells until there are enough to successfully target the cancer cells [9]. The patient must receive chemotherapy since it is possible for the immune system to reject the new cells [10]. Afterwards, doctors will infuse the new CAR-T cells into the bloodstream; the advanced CAR-T cell receptors will now be able to recognize cancer cells and attach to the antigens, which are proteins in the malignant cells [11]. Essentially, the new cells collaborate to eliminate the cancer cells. As a result, the CAR-T cells will continue to multiply to protect the patient from any possible new cancer cell that contains the antigen that the CAR-T cells were engineered to identify [12].

Besides the facts that there are many successful cases in using CAR-T cell therapy and that the U.S. Food and Drug Administration (FDA) approved of the practice due to accomplished clinical trials, there are instances of failure with this new treatment [13]. For example, there are reports of the relapse of cancer after receiving the treatment [14]. There could be many reasons behind this; one instance could be if the chimeric antigen receptors are not activated, so the T cells do not have the ability to identify cancer cells [15]. Another cause is the lack of induction of the T cells, meaning that there are not sufficient cells to successfully eradicate all cancer cells [16]. In turn, the malignancies will replicate rapidly, spreading throughout the body [17]. In fact, the cancer cells could mutate, changing the antigen and causing the chimeric antigen receptors to be useless since it will not be able to recognize the mutated antigen [18].

These are the risks and benefits of CAR-T cell therapy, but there are many more details that will be in the sections that follow [2]. For a completely effective treatment, researchers need to ensure

that all the cancer cells are certainly eliminated without a doubt to erase the possibility of a relapse [19]. In addition, they need to verify that the CAR-T cells are reproducing properly [20]. There are several other components to check before CAR-T therapy can be completely triumphant over cancer cells with great ability to replicate and differentiate [21].

Generations from Clinical Trials

Scientists have been changing the design of the chimeric antigen receptors to adapt to the breakthroughs found throughout clinical trials [22]. There are four main generations, and researchers applied adjustments for more effective identification of malignant cells in each succeeding generation [23]. It is important to know that each individual generation consisted of a crucial element that is used for the following generations and contributes to overall research of CAR-T cells [24].

The key transmitter of endogenous T-cell receptors (TCR) signals of the first-generation CAR-T model is a CD3ζ chain [25]. However, they were only designed with the CD3 domain [26]. Although there was absence in activation which resulted in insufficient attacks on cancer cells, the tumor microenvironment (TME) had consistent exposure and allowed a therapeutic consequence for patients [27]. However, these patients specifically had either B-cell lymphoma infused with α-CD20-CD3ζ or are patients diagnosed with neuroblastoma and were tended with scFv-CD3ζ CAR T cells [28]. The constructional components of scFv, an antibody or B cell receptor, includes light and heavy chains; these parts are merged with the T cell that activates the TCR ζ chain or the CD3ζ domain for the purpose of creating activating receptor molecules that are not restricted by MHC [29]. The drawback of the first-generation CARs (Chimeric Antigen Receptors) was their restricted signaling ability because of either the persistent cytokine release or the ability to fill the resting T cells or control the lasting T-cell responses [30]. Despite the disadvantages, researchers drew the successful components from the first-generation CARs and modified it to construct the second-generation CAR-T cell therapy. The experiments with first-generation CARs took place in the first phase of clinical trials [31].

After the phase I clinical trials, the first-generation models of CAR-T cells had to possess a more successful anti-leukemic response [32]. There were complete remission rates varying up to 90% of the patients that had recurring B-cell acute lymphoblastic leukemia (B-ALL) [33]. From this observation, researchers created the second-generation anti-CD19 T cells combined with either a CD24 co-stimulatory domain or a 4-1BB connected to the domain of CD3 [34]. Predicting the problems, the second-generation CARs were primarily created to merge intracellular signaling domains from various co-stimulatory molecules [35]. Examples of these molecules include 4-1BB, CD137, and CD28, and others that can increase the intensity of the signal [36]. Virtually, the second-generation CARs carry a CD3ζ chain with a sole costimulatory molecule, hence the classification of the second-generation CAR [37]. For instance, the CARs recognizing CD19 includes the CD28 or 4-1BB signaling domains, which produced remarkable complete response, or CR rates [38]. This reaction is specific to patients who have recurrent B-cell malignancies [39]. Fundamentally, the second-generation receptors work as "living drugs." This is because the CD28-based CARs can multiply quickly, which increases the function of T effector cells [40]. Essentially, the agglomeration of T cells was partly because of the CARs based on 4-1BB [41]. After taking the innovations of the first-generation CARs, the second generation offered a better accumulation rate for T cells, which is an enormous success for researchers [42]. Despite the accomplishments, there are still pieces that are in question or have the potential to be improved to be more effective [43].

Building off the second-generation CARs, the third-generation CARs expand the eradication of cancer cells [44]. The third-generation CARs once again include the CD3ζ chain in addition to two signaling domains. Examples include the CD3ζ-CD28-4-1BB and CD3ζ-CD28-OX40 [45]. The purpose of this is to enhance the activation signal, upgrade the cytokine production which will lead to potent function and increase the time that the cells proliferate [46]. This is all a result of the third-generation CAR-T cells combining the signaling potential of the two costimulatory domains as mentioned with the second-generation CARs [47]. Separately, one case of the third-generation CARs that included α-CD19-CD3ζ-CD28-4-1BB disclosed of complete remission rates, which was possible by permeation and lysing of the cancer tissue in chronic lymphocyte leukemia patients [38]. Although this success is important since if proves the effectiveness of CAR-T cell therapy, there are severe consequences to the treatment [6]. The cause of this is the CAR-T cells being uncontrollable; this is a double-edged sword because although it eliminates tumors, it potentially causes pulmonary failure with will end in death [48]. Additionally, the sharp increase of production of the pro-inflammatory cytokines is problematic, as well as the possibility of multi-organ dysfunction [49]. One significant phase I trial of BrainChild-01 (NCT0350091) began exploring the maximum capability of the third-generation CAR-T cells that target the tumor cells that express HER2 [50]. This was conducted directly through an inherent CNS (Central Nerve System) catheter [51]. Overall, the third-generation CARs were a success besides the after-effects of treatment [52]. Researchers continue to conduct trials in order to find out how to suppress the T cells after eradication of cancer cells [53].

Finally, the fourth-generation CARs are derived the preceding generations. While its predecessors are contingent on a specific strategy and assisted to arbitrate the antitumor response in the T cells, the fourth-generation CARs consisted of something different [54]. The earlier generations had restrictions like the absence of antineoplastic activity against solid tumors [55]. The cause of this

is the huge phenotypic heterogeneity and the decline allocated to antigen-negative cancer cells [56]. Researchers progressed in study to innovate a novel CAR stratagem. The introduction of the fourth-generation CAR instituted the background of the tumor through the transgenic immune modifiers expression, like interleukin (IL)-12 [57]. The function of the IL-12 is to initiate the activation of innate immune cells, and also to magnify T cell activation, which in turn lowers the antigen-negative cancer cells in the lesion that are marked [58]. The fourth generation CARs' antitumor activity is actually also known as T-cells redirected for universal cytokine-mediated killing, or TRUCKs [59]. These will be further genetically modified and include but are not limited to supplementary transgenes for the purpose of secreting cytokine like IL-12, or perhaps costimulatory ligands [60]. Overall, the CARs have evolved greatly throughout the generations with improvements after each one [61].

Tumor Microenvironment

Problems

In order for CAR-T cells to be effective after being injected into cancer patients, they have to invade the tumor and identify the antigen on the cancer cells to kill them [7]. However, this takes place in the tumor microenvironment, which houses competition and hostile components [62]. Additionally, the TME is hypoxic and lacks many nutrients that T cells need to proliferate [63]. Also, the tumor microenvironment contains tons of metabolic end products that have an immunosuppressive quality [64]. Nevertheless, the TME is essential to the body response to the treatment, along with the capability of decreasing the T cell action, making the therapy less effective [65]. This is shown in the molecular or cellular profiles that specify the T cell dysfunction [66]. Overall, there are many obstacles the T cells will encounter in the TME, but there are also strategies presented that can counter the effects [67].

Along with the solid tumor cells are other populations of cells like myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), and Tregs [68]. As a result, there is intense competition for nutrients, consistent antigenic stimulation, and the immunosuppressive networks can cause the T cell to burn out in the TME [69]. Another disadvantage is that T cell requires amino acids to function properly. Examples of the amino acids include glutamine and arginine, which are typically rare in the TME, meaning T cell abilities are restricted [70]. After T cells activate, there is a surge in glucose uptake and the glycolytic rate increases in order to enhance proliferation so they can function properly and effectively [71]. Additionally, metabolic adaptation in T cells may result in metabolites building up [72]. This can cause change in the epigenetic environment that can impact the fate and the function of the T cells [73]. Finally, the metabolic end products in the TME are immunosuppressive [74]. Once example is when tumorigenic R-2-hydroxyglutarate has isocitrate has isocitrate dehydrogenase ½ mutations and contains electrolyte concentrations that are immunosuppressive [75]. The competition and molecules in the TME could be very dangerous to CAR-T cell therapy.

Solutions

There are some designs for CAR-T cells that are possible through the hypoxia pathway in the TME [76]. However, the option to have the highest costimulatory domains in the CAR could be restricted by the oxygen availability in the TME [77]. Other potential methods include designing CARs that are not active in environments with more oxygen but are active in the TME so that the remote toxicities are reduced. Some innovative approaches to enclose CAR expressions to the TME are presented [78]. One of them is to introduce hypoxia-inducible factors (HRE) regions on the building promoter [79]. Another one is merging the domain of hypoxia-inducible factor (HIF) domains within the cell of CAR to further the degradation and hydroxylation of the CAR when oxygen is present [80]. However, both approaches depend on the mechanism of the endogenous T cell to sense oxygen to control CAR expression [81]. On the other hand, the activity of CAR-T cells can be directed to the antigens that are expected to upregulate during hypoxic conditions in solid tumors like carbonic anhydrase IX [82]. Immunosuppressive pathways in the TME is enhanced by hypoxia, and they provide conjunctive therapeutic strategies [83]. Both HIF and hypoxia increase the expression of programmed cell death ligand 1 (PD-L1) and many other factors that hinder T cell responses [84]. Other immune checkpoints besides PD-L1 includes ligands for TIM-3, LAG-3, and TIGIT [65]. Other immunosuppressive cytokines like IL-10 and transforming growth factor (TGF)-b are secreted by Tregs, MDSCs, and CAFs. The TME contains loads of cytokines that inhibit the function of T cells [85].

As for strategies to better the efficacy of CAR-T in the TME, various preclinical studies concluded that amalgamating PD-1 or PD-L1 blockade with CAR-T cell therapy enhances the function of T cells [86]. In order to decrease consequences from systemic checkpoint blockade, researchers genetically modified CAR-T cells so that they express a PD-1 or CD28 switch; an alternative expression is to truncate PD-1 receptor that works as a dominant negative receptor (DNR) [87]. Additionally, the CRISPR-Cas9 gene editing removed PD-1 from CAR-T cells. These methods improved the function of CAR-T cells in the preclinical models [88]. However, these procedures have been limited in the clinical experience, as only PD-1 knockout abTCR T cells have been considered clinically [89]. There are some other techniques, like prohibiting CTLA-4 or FAS expression on the cell surface of CAR-T or tumor specific cells [90]. Newer research presented that DNR expression FAS receptor also enhanced the T cells and their role in therapy [91].

The other problems that the TME presented were cytokines that inhibited T cell function [92]. However, this can be solved

by DNR expression, or cytokine switch receptors (CSRs) that transform a hindering signal into a signal to promote proliferation [93]. Cytokine DNRs include DNR-TGF-b receptors [94]. The EBV specific T cells that express the DNR-TGF-b for EBV+ lymphoma have been assessed in the early phase of clinical studies to ensure safety measures and correct function in contrast to their unmodified counterparts [95]. The IL-4 that's generated in the TME are used by the CSRs [96]. This includes IL-4/IL-2, IL-4/IL-7, and IL-4/IL-21 CSRs. In fact, the IL-2/IL-4 CSR is being assessed in an early phase clinical study (NCT01818323) [97]. Moreover, colony-stimulating factor-1 (CSF-1) is a cytokine in the TME, but the T cells do not express the cognate receptor [98]. There are studies researching this, like a preclinical study that demonstrated CSF-1R expression in CAR-T cells enhances their function [20].

An additional component in the TME are chemokines, which are necessary in regulating tumor growth and metastasis [99]. If there is a lack of expression of chemokine receptors or mismatches occur between chemokine ligands and receptors, tumors can elude the immune response [100]. Some researchers modified CAR-T cells so that they overexpress chemokine receptors in order to increase CAR-T cell populations in the TME [101]. Overall, the studies conclude that calculating the relevant chemokine-chemokine receptor axes between tumors and the CAR-T cells could potentially allow for better tumor infiltration [102]. There are preclinical, and early-phase clinical trials being held to explore this.

Conclusion

Although CAR-T cell therapy is a new and innovative treatment for malignancies, researchers run into many difficulties that need to be overcome for it to be truly successful [6]. Most importantly, researchers want to maximize survival as well as assure there are durable clinical benefits. In fact, there have already been breakthroughs, but there are many more to come [103]. For now, the TME still presents problems for T cells like competition for resources, physical barriers, and other immunosuppressive cytokines and chemokines [104]. We have yet to have a full understanding of the TME and the therapeutic resistance inherent to it. Nevertheless, CAR-T cell therapy is unquestionably worthwhile to research, and the final perfect model of it has the potential to shape the future of cancer research [105]. According to Nagai, H. and Kim.

References

1. Nagai H, Kim YH (2017) Cancer prevention from the perspective of global cancer burden patterns. J Thorac Dis 9(3): 448-451.
2. Ventola CL (2017) Cancer Immunotherapy, Part 2: Efficacy, Safety, and Other Clinical Considerations. P T 42(7): 452-463.
3. Maus MV, Levine BL (2016) Chimeric Antigen Receptor T-Cell Therapy for the Community Oncologist. Oncologist 21(5): 608-617.
4. Fesnak AD, June CH, Levine BL (2016) Engineered T cells: the promise and challenges of cancer immunotherapy. Nat Rev Cancer 16(3): 566-581.
5. Shah NN, Fry TJ (2019) Mechanisms of resistance to CAR T cell therapy. Nat Rev Clin Oncol 16: 372-385.
6. Srivastava S, Riddell SR (2018) Chimeric Antigen Receptor T Cell Therapy: Challenges to Bench-to-Bedside Efficacy. J Immunol 200(2): 459-468.
7. Zhao L, Cao YJ (2019) Engineered T Cell Therapy for Cancer in the Clinic. Front Immunol 10: 2250.
8. Abou El Enein M, Magdi Elsallab, Steven AF, Andrew DF, Helen EH, et al. (2021) Scalable Manufacturing of CAR T cells for Cancer Immunotherapy. Blood Cancer Discov 2(5): 408-422.
9. Gomes Silva D, Ramos CA (2018) Cancer Immunotherapy Using CAR-T Cells: From the Research Bench to the Assembly Line. Biotechnol J 13(2): 10.
10. Asnani A, Anastasia M, Moussa M, Jeremy R, Ephraim PH, et al. (2017) Management of atrial fibrillation in patients taking targeted cancer therapies. Cardiooncology 3: 2.
11. Schaft N (2020) The Landscape of CAR-T Cell Clinical Trials against Solid Tumors-A Comprehensive Overview. Cancers (Basel) 12(9): 2567.
12. Sidana S, Shah N (2019) CAR T-cell therapy: is it prime time in myeloma? Hematology Am Soc Hematol Educ Program 2019(1): 260-265.
13. Hartmann J, Schussler Lenz M, Bondanza A, Buchholz CJ (2017) Clinical development of CAR T cells-challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med 9(9): 1183-1197.
14. Xu X, Qihang Sun, Xiaoqian Liang, Zitong Chen, Xiaoli Zhang, et al. (2019) Mechanisms of Relapse After CD19 CAR T-Cell Therapy for Acute Lymphoblastic Leukemia and Its Prevention and Treatment Strategies. Front Immunol 10: 2664.
15. Dai H, Wang Y, Lu X, Han W (2016) Chimeric Antigen Receptors Modified T-Cells for Cancer Therapy. J Natl Cancer Inst 108(7): djv439.
16. Haabeth OA, Anders Aune T, Marte Fauskanger, Fredrik Schjesvold, Kristina Berg L, et al. (2014) How Do CD4(+) T Cells Detect and Eliminate Tumor Cells That Either Lack or Express MHC Class II Molecules? Front Immunol 5: 174.
17. Wang L, Zhang S, Wang X (2020) The Metabolic Mechanisms of Breast Cancer Metastasis. Front Oncol 10: 602416.
18. Sentman CL (2013) Challenges of creating effective chimeric antigen receptors for cancer therapy. Immunotherapy 5(8): 783-785.
19. Chakraborty S, Rahman T (2012) The difficulties in cancer treatment. Ecancermedicalscience 6: ed16.
20. Sterner RC, Sterner RM (2021) CAR-T cell therapy: current limitations and potential strategies. Blood Cancer J 11(4): 69.
21. Knochelmann HM, Aubrey S Smith, Connor J Dwyer, Megan M W, Shikhar M, et al. (2018) CAR T Cells in Solid Tumors: Blueprints for Building Effective Therapies. Front Immunol 9: 1740.
22. Jensen MC, Riddell SR (2015) Designing chimeric antigen receptors to effectively and safely target tumors. Curr Opin Immunol 33: 9-15.
23. Greaves M, Maley CC (2012) Clonal evolution in cancer. Nature 481(7381): 306-313.
24. Charrot S, Hallam S (2019) CAR-T Cells: Future Perspectives. Hemasphere 3(2): e188.
25. Guedan S, Calderon H, Posey AD, Maus MV (2019) Engineering and Design of Chimeric Antigen Receptors. Mol Ther Methods Clin Dev 12: 145-156.

26. Bridgeman JS, K Ladell, V E Sheard, K Miners, R E Hawkins, et al. (2014) CD3zeta-based chimeric antigen receptors mediate T cell activation via cis- and trans-signalling mechanisms: implications for optimization of receptor structure for adoptive cell therapy. Clin Exp Immunol 175(2): 258-267.

27. Sadeghi Rad H, James Monkman, Majid EW, Rahul Ladwa, Ken O'Byrne, et al. (2021) Understanding the tumor microenvironment for effective immunotherapy. Med Res Rev 41(3): 1474-1498.

28. Porter DL, Wei Ting H, Noelle VF, Simon FL, Pamela AS, et al. (2015) Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med 7(303): 303ra139.

29. Gacerez AT, Arellano B, Sentman CL (2016) How Chimeric Antigen Receptor Design Affects Adoptive T Cell Therapy. J Cell Physiol 231(12): 2590-2598.

30. Jafarzadeh L, Masoumi E, Fallah Mehrjardi K, Mirzaei HR, Hadjati J (2020) Prolonged Persistence of Chimeric Antigen Receptor (CAR) T Cell in Adoptive Cancer Immunotherapy: Challenges and Ways Forward. Front Immunol 11: 702.

31. Andrea AE, Chiron A, Bessoles S, Hacein Bey AS (2020) Engineering Next-Generation CAR-T Cells for Better Toxicity Management. Int J Mol Sci 21(22): 8620.

32. Mardiana S, Gill S (2020) CAR T Cells for Acute Myeloid Leukemia: State of the Art and Future Directions. Front Oncol 10: 697.

33. Sun, W, Jemily Malvar, Richard Sposto, Anupam Verma, Jennifer J Wilkes, et al. (2018) Outcome of children with multiply relapsed B-cell acute lymphoblastic leukemia: a therapeutic advance in childhood leukemia & lymphoma study. Leukemia 32(11): 2316-2325.

34. Cheng Z, Runhong Wei, Qiuling Ma, Lin Shi, Feng He, et al. (2018) In Vivo Expansion and Antitumor Activity of Coinfused CD28- and 4-1BB-Engineered CAR-T Cells in Patients with B Cell Leukemia. Mol Ther 26(4): 976-985.

35. Sievers NM, Dorrie J, Schaft N (2020) CARs: Beyond T Cells and T Cell-Derived Signaling Domains. Int J Mol Sci 21(10): 3525.

36. Tammana S, Xin Huang, Marianna Wong, Michael C Milone, Linan Ma, et al. (2010) 4-1BB and CD28 signaling plays a synergistic role in redirecting umbilical cord blood T cells against B-cell malignancies. Hum Gene Ther 21(1): 75-86.

37. Subklewe M, von Bergwelt BM, Humpe A (2019) Chimeric Antigen Receptor T Cells: A Race to Revolutionize Cancer Therapy. Transfus Med Hemother 46(1): 15-24.

38. Tang XY, Yao Sun, Ang Zhang, Guo Liang H, Wei Cao, et al. (2016) Third-generation CD28/4-1BB chimeric antigen receptor T cells for chemotherapy relapsed or refractory acute lymphoblastic leukaemia: a non-randomised, open-label phase I trial protocol. BMJ Open 6(12): e013904.

39. Salles G, Martin Barrett, Robin Foà, Joerg Maurer, Susan O'Brien, et al. (2017) Rituximab in B-Cell Hematologic Malignancies: A Review of 20 Years of Clinical Experience. Adv Ther 34(10): 2232-2273.

40. Duong MT, Matthew RCP, Eva Morschl, An Lu, Slawomir PS, et al. (2019) Two-Dimensional Regulation of CAR-T Cell Therapy with Orthogonal Switches. Mol Ther Oncolytics 12: 124-137.

41. Philipson BI, O'Connor RS,May MJ, June CH, Milone MC (2020) 4-1BB costimulation promotes CAR T cell survival through noncanonical NF-kappaB signaling. Sci Signal 13(625): eaay8248.

42. Priceman SJ, Forman SJ, Brown CE (2015) Smart CARs engineered for cancer immunotherapy. Curr Opin Oncol 27(6): 466-474.

43. Han D, Xu Z, Zhuang Y, Ye Z, Qian Q (2021) Current Progress in CAR-T Cell Therapy for Hematological Malignancies. J Cancer 12(2): 326-334.

44. Roselli E, Justin C Boucher,Gongbo Li, Hiroshi Kotani, Kristen Spitler, et al. (2021) 4-1BB and optimized CD28 co-stimulation enhances function of human mono-specific and bi-specific third-generation CAR T cells. J Immunother Cancer 9(10).

45. Weinkove R, George P, Dasyam N, McLellan AD (2019) Selecting costimulatory domains for chimeric antigen receptors: functional and clinical considerations. Clin Transl Immunology 8(5): e1049.

46. Vazquez MI, Catalan Dibene J, Zlotnik A (2015) B cells responses and cytokine production are regulated by their immune microenvironment. Cytokine 74(2): 318-326.

47. Ramos CA, Rayne Rouce, Catherine S R, Amy Reyna, Neeharika N, et al. (2018) In Vivo Fate and Activity of Second- versus Third-Generation CD19-Specific CAR-T Cells in B Cell Non-Hodgkin's Lymphomas. Mol Ther 26(12): 2727-2737.

48. Sun S, Hao H, Yang G, Zhang Y, Fu Y (2018) Immunotherapy with CAR-Modified T Cells: Toxicities and Overcoming Strategies. J Immunol Res 2018: 2386187.

49. Jaffer U, Wade RG, Gourlay T (2010) Cytokines in the systemic inflammatory response syndrome: a review. HSR Proc Intensive Care Cardiovasc Anesth 2(3): 161-175.

50. Li, P, Lingcong Yang, Tong Li, Shufang Bin, Bohao Sun, et al. (2020) The Third Generation Anti-HER2 Chimeric Antigen Receptor Mouse T Cells Alone or Together with Anti-PD1 Antibody Inhibits the Growth of Mouse Breast Tumor Cells Expressing HER2 in vitro and in Immune Competent Mice. Front Oncol 10: 1143.

51. Soderquist RG, Mahoney MJ (2010) Central nervous system delivery of large molecules: challenges and new frontiers for intrathecally administered therapeutics. Expert Opin Drug Deliv 7(3): 285-293.

52. Han X, Wang Y, Han, WD (2018) Chimeric antigen receptor modified T-cells for cancer treatment. Chronic Dis Transl Med 4(4): 225-243.

53. Zamora AE, Crawford JC, Thomas PG (2018) Hitting the Target: How T Cells Detect and Eliminate Tumors. J Immunol 200(2): 392-399.

54. Ma S, Xinchun Li, Xinyue Wang, Liang Cheng, Zhong Li, et al. (2019) Current Progress in CAR-T Cell Therapy for Solid Tumors. Int J Biol Sci 15(12): 2548-2560.

55. Anand P, Ajaikumar BK, Chitra Sundaram, Kuzhuvelil BH, Sheeja TT, et al. (2008) Cancer is a preventable disease that requires major lifestyle changes. Pharm Res 25(9): 2097-2116.

56. Scher HI, Ryon P Graf, Nicole AS, Brigit McLaughlin, Adam Jendrisak, et al. (2017) Phenotypic Heterogeneity of Circulating Tumor Cells Informs Clinical Decisions between AR Signaling Inhibitors and Taxanes in Metastatic Prostate Cancer. Cancer Res 77(20): 5687-5698.

57. Hosseinkhani N, Afshin Derakhshani, Omid Kooshkaki, Mahdi Abdoli S, Khalil H, et al. (2020) Immune Checkpoints and CAR-T Cells: The Pioneers in Future Cancer Therapies? Int J Mol Sci 21(21): 8305.

58. Weiss JM, Subleski JJ, Wigginton JM, Wiltrout RH (2007) Immunotherapy of cancer by IL-12-based cytokine combinations. Expert Opin Biol Ther 7(11): 1705-1721.

59. Schepisi G, Maria Concetta C, Chiara Casadei, Cecilia Menna, Amelia Altavilla, et al. (2019) CAR-T cell therapy: a potential new strategy against prostate cancer. J Immunother Cancer 7: 258.

60. DeRenzo C, Gottschalk S (2019) Genetic Modification Strategies to Enhance CAR T Cell Persistence for Patients with Solid Tumors. Front Immunol 10: 218.

61. Petersen CT, Krenciute G Next (2019) Generation CAR T Cells for the Immunotherapy of High-Grade Glioma. Front Oncol 9: 69.

62. Henke E, Nandigama R, Ergun S (2019) Extracellular Matrix in the Tumor Microenvironment and Its Impact on Cancer Therapy. Front Mol Biosci 6: 160.

63. Lim AR, Rathmell WK, Rathmell JC (2020) The tumor microenvironment as a metabolic barrier to effector T cells and immunotherapy. Elife 9: e55185.

64. Shi R, Tang YQ, Miao H (2020) Metabolism in tumor microenvironment: Implications for cancer immunotherapy. MedComm 1(1): 47-68.

65. Anderson KG, Stromnes IM, Greenberg PD (2017) Obstacles Posed by the Tumor Microenvironment to T cell Activity: A Case for Synergistic Therapies. Cancer Cell 31(3): 311-325.

66. Wherry EJ, Kurachi M (2015) Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol 15(8): 486-499.

67. Safarzadeh Kozani P, Pouya Safarzadeh K, Rahbarizadeh F, Khoshtinat Nikkhoi S (2021) Strategies for Dodging the Obstacles in CAR T Cell Therapy. Front Oncol 11: 627549.

68. Tian X, Shen H, Li Z, Wang T, Wang S (2019) Tumor-derived exosomes, myeloid-derived suppressor cells, and tumor microenvironment. J Hematol Oncol 12: 84.

69. Geltink RIK, Kyle RL, Pearce EL (2018) Unraveling the Complex Interplay Between T Cell Metabolism and Function. Annu Rev Immunol 36: 461-488.

70. Hope HC, Salmond RJ (2021) The Role of Non-essential Amino Acids in T Cell Function and Anti-tumour Immunity. Arch Immunol Ther Exp (Warsz) 69(1): 29.

71. O'Sullivan D, Pearce EL (2015) Targeting T cell metabolism for therapy. Trends Immunol 36(2): 71-80.

72. Rangel Rivera GO, Hannah M K, Connor JD, Aubrey SS, Megan M Wyatt, et al. (2021) Fundamentals of T Cell Metabolism and Strategies to Enhance Cancer Immunotherapy. Front Immunol 12: 645242.

73. Phan AT, Goldrath AW, Glass CK (2017) Metabolic and Epigenetic Coordination of T Cell and Macrophage Immunity. Immunity 46(5): 714-729.

74. Jennings MR, Munn D, Blazeck J (2021) Immunosuppressive metabolites in tumoral immune evasion: redundancies, clinical efforts, and pathways forward. J Immunother Cancer 9(10): e003013.

75. Dang L, Su SM (2017) Isocitrate Dehydrogenase Mutation and (R)-2-Hydroxyglutarate: From Basic Discovery to Therapeutics Development. Annu Rev Biochem 86: 305-331.

76. Berahovich, R, Xianghong Liu, Hua Zhou, Elias Tsadik, Shirley Xu, et al. (2019) Hypoxia Selectively Impairs CAR-T Cells In Vitro. Cancers (Basel) 11(5):602.

77. Poorebrahim M, Jeroen Melief, Yago Pico de C, Stina L Wickström, Angel Cid Arregui, et al. (2021) Counteracting CAR T cell dysfunction. Oncogene 40: 421-435.

78. Miao L, Zhang Z, Ren Z, Tang F, Li Y (2021) Obstacles and Coping Strategies of CAR-T Cell Immunotherapy in Solid Tumors. Front Immunol 12: 687822.

79. Orlando IMC, Véronique NL, Federica Storti, Patrick Spielmann, Lisa Crowther, et al. (2020) Distal and proximal hypoxia response elements cooperate to regulate organ-specific erythropoietin gene expression. Haematologica 105(12): 2774-2784.

80. Juillerat, A, Alan Marechal, Jean Marie F, Yannick Valogne, Julien Valton, et al. (2017) An oxygen sensitive self-decision making engineered CAR T-cell. Sci Rep 7: 39833.

81. Brandt LJB, Barnkob MB, Michaels YS, Heiselberg J, Barington T (2020) Emerging Approaches for Regulation and Control of CAR T Cells: A Mini Review. Front Immunol 11: 326.

82. Martinez M, Moon EK (2019) CAR T Cells for Solid Tumors: New Strategies for Finding, Infiltrating, and Surviving in the Tumor Microenvironment. Front Immunol 10: 128.

83. Lee CT, Mace T, Repasky EA (2010) Hypoxia-driven immunosuppression: a new reason to use thermal therapy in the treatment of cancer? Int J Hyperthermia 26: 232-246.

84. Wen Q, Han T, Wang Z, Jiang S (2020) Role and mechanism of programmed death-ligand 1 in hypoxia-induced liver cancer immune escape. Oncol Lett 19(4): 2595-2601.

85. Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA (2006) Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells. Immunology 117(4): 433-442.

86. Kyte JA (2022) Strategies for Improving the Efficacy of CAR T Cells in Solid Cancers. Cancers (Basel) 14(3): 571.

87. Cherkassky L, Aurore Morello, Jonathan Villena V, Yang Feng, Dimiter S Dimitrov, et al. (2016) Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J Clin Invest 126(8): 3130-3144.

88. Dimitri A, Herbst F, Fraietta JA (2022) Engineering the next-generation of CAR T-cells with CRISPR-Cas9 gene editing. Mol Cancer 21(1): 78.

89. Zolov SN, Rietberg SP, Bonifant CL (2018) Programmed cell death protein 1 activation preferentially inhibits CD28.CAR-T cells. Cytotherapy 20: 1259-1266.

90. Benmebarek MR, Clara Helke K, Bruno Loureiro C, Stefanie L, Stefan Endres, et al. (2019) Killing Mechanisms of Chimeric Antigen Receptor (CAR) T Cells. Int J Mol Sci 20(6):1283.

91. Yamamoto TN, Ping Hsien L, Suman K V, Devikala G, Rigel J Kishton, et al. (2019) T cells genetically engineered to overcome death signaling enhance adoptive cancer immunotherapy. J Clin Invest 129(4): 1551-1565.

92. Dhodapkar MV (2019) Navigating the Fas lane to improved cellular therapy for cancer. J Clin Invest 129(4): 1522-1523.

93. Bell M, Gottschalk S (2021) Engineered Cytokine Signaling to Improve CAR T Cell Effector Function. Front Immunol 12: 684642.

94. Sanjabi S, Flavell RA (2010) Overcoming the hurdles in using mouse genetic models that block TGF-beta signaling. J Immunol Methods 353: 111-114.

95. Foster AE, Gianpietro Dotti, An Lu, Mariam Khalil, Malcolm K B, et al. (2008) Antitumor activity of EBV-specific T lymphocytes transduced with a dominant negative TGF-beta receptor. J Immunother 31(5): 500-505.

96. Wagner J, Wickman E, DeRenzo C, Gottschalk S (2020) CAR T Cell Therapy for Solid Tumors: Bright Future or Dark Reality? Mol Ther 28(11): 2320-2339.

97. Wang Y, Hua Jiang, Hong Luo, Yansha Sun, Bizhi Shi, et al. (2019) An IL-4/21 Inverted Cytokine Receptor Improving CAR-T Cell Potency in Immunosuppressive Solid-Tumor Microenvironment. Front Immunol 10: 1691.

98. Bhattacharya P, Muthusamy T, Hatem A Elshabrawy, Khaled Alharshawi, Prabhakaran Kumar, et al. (2015) GM-CSF: An immune modulatory cytokine that can suppress autoimmunity. Cytokine 75(2): 261-271.

99. Raman D, Baugher PJ, Thu YM, Richmond A (2007) Role of chemokines in tumor growth. Cancer Lett 256(2): 137-165.

100. Idorn M, Thor Straten P (2018) Chemokine Receptors and Exercise to Tackle the Inadequacy of T Cell Homing to the Tumor Site. Cells 7(8): 108.

101. Scarfo I, Maus MV (2017) Current approaches to increase CAR T cell potency in solid tumors: targeting the tumor microenvironment. J Immunother Cancer 5: 28.

102. Foeng J, Comerford I, McColl SR (2022) Harnessing the chemokine system to home CAR-T cells into solid tumors. Cell Rep Med 3(3): 100543.

103. Ayala FJ (2015) Cloning humans? Biological, ethical, and social considerations. Proc Natl Acad Sci U S A 112(29): 8879-8886.

104. Rodriguez GA, Palazon A, Noguera OE, Powell DJ, Guedan S (2020) CAR-T Cells Hit the Tumor Microenvironment: Strategies to Overcome Tumor Escape. Front Immunol 11: 1109.

105. Cheng J, Lei Zhao 1, Yuanyuan Zhang, Yun Qin, Yuqi Guan, et al. (2019) Understanding the Mechanisms of Resistance to CAR T-Cell Therapy in Malignancies. Front Oncol 9: 1237.