

NOVEL STRATEGIES TO IMPROVE CAR-T CELLS IN SOLID TUMORS: A MINI REVIEW

Manal M. E. Ahmed

Assistant Professor, Pharmacology Department, National Research Centre, Egypt

ARTICLE INFO

Received: 27 July 2022

Revised: 25 Sept 2022

Accepted: 28 Sept 2022

Keywords:

CAR-T Cells, Solid Tumors, Cell Therapy

Corresponding Author:

Manal M E Ahmed

Email:

thinktankteam2014@gmail.com

Copyright © 2022 by author(s)

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
<http://creativecommons.org/licenses/by/4.0/>



ABSTRACT

Purpose: The paper reviews the obstacles that face CAR-T cells in solid tumors and the suggested novel strategies to overcome these obstacles in association with the efficacy as well as the safety profile of CAR-T cell therapy.

Subjects and Methods: This is a systematic review exploring the novel strategies to improve CAR-T cells in solid tumors.

Results: Several strategies have been developed, including: 1) targeting multiple antigens; 2) improving trafficking of CAR-T cells into tumor tissues; 3) Preventing the immunosuppression of TME on CAR-T cells by different approaches such as blocking the recruitment of MDSCs or TAM in TME, saving CAR-T cells viability from exhaustion by blocking negative signals, and enhancement of CAR-T cells persistence activity by blocking PD-1/PDL-1 pathway in TME, 4) designing novel CAR-T cells to prevent the exhaustion; and 5) inducing a self T cells response in the tumor-bearing host to prevent tumor escape and enhance CAR-T cells activity.

Conclusions: Recent researches about CAR-T cells therapy carries new hope for targeting solid tumors.

INTRODUCTION

One of interesting cellular immunotherapy that used for cancer treatment is chimeric antigen receptor (CAR) T cell therapy. CAR-T cell is a genetically modified T cell that to express chimeric antigen receptor (CAR) for specifically identify and destroy the cancerous cells in an MHC-independent manner, and prevent them from escaping (Jensen & Riddell, 2014; Labanieh et al., 2018).

Thirty years since Gross et al, first innovated CAR-T cells. Basically, CARs composed of three components, extracellular antigen binding region, transmembrane region and intracellular signal transduction region. Each part has a specific function. The extracellular antigen binding region target antigen is composed of the single-chain variable fragment (scFv) of an antibody and identifies the target antigen. The transmembrane region connects extracellular region with intracellular region. The intracellular signal transduction region is

the activation domain with one or more co-stimulatory domains that responsible for the activation of CAR-T cell(Gross et al., 1989).

Based on the structure of intracellular signals transduction region, there are four generations of CAR-T cells. The first generation CAR-T cells have only one signal domain (CD3 ζ). The first generation CARs clinically failed in the majority of patients as CAR-T cells did not persist and expand in vivo for more than a few days. Therefore, additional costimulatory molecule used in the second generation such as CD28, OX40 or 4-1BB etc., to prolong the survival time and significantly increase the anti-tumor efficacy of CAR-T cells. The third-generation CARs contain more than one costimulatory molecules that stimulate cell proliferation and cytokine release according to the preclinical trials. The fourth-generation CAR-T cells have extra powerful tools including ascytokines, receptors for chemokines suicide gene and controlled suicide gene that provide not only rapid expansion and high tumor killing activity, but also superior advantages in safety and persistence(Ahmed, 2021).

The optimized CAR-T cells demonstrated obvious success in CD19+ B cell line hematological malignancies. For solid tumors, there are many obstacles and challenges, such as interfering with CAR-T cells identification of tumor antigens, hindering the infiltration of CAR-T cells and no persistence of CAR-T cell activity(Ahmed, 2021). In this review, we discussed the obstacles that face CAR-T cells in solid tumors and the suggested novel strategies to overcome these obstacles in association with the efficacy as well as the safety profile of CAR-T cell therapy.

METHODOLOGY

This is a systematic literature review drawing upon previous studies. Google scholar, Scopus, PubMed were searched for relevant studies. There is no time frame binding the study.

RESULTS AND DISCUSSION

Improving trafficking of CAR-T cells into tumor tissues:

The main cause of poor efficacy of CAR-T cell therapy is insufficient number of CAR-T cells to enter the solid tumor. Therefore, the enhancement of CAR-T cell penetration into tumor microenvironment (TME) is important to improve its efficacy in solid tumors. Obstacles that hinder CAR-T cells infiltration into solid tumors are including: (1) fibrosis of tumor-associated fibroblasts (CAFs) and abnormal tumor blood vessels represent a physical barrier, (2) the chemokines of solid tumors is not matched with the chemokine receptor of CAR-T cells(Jo et al., 2020).

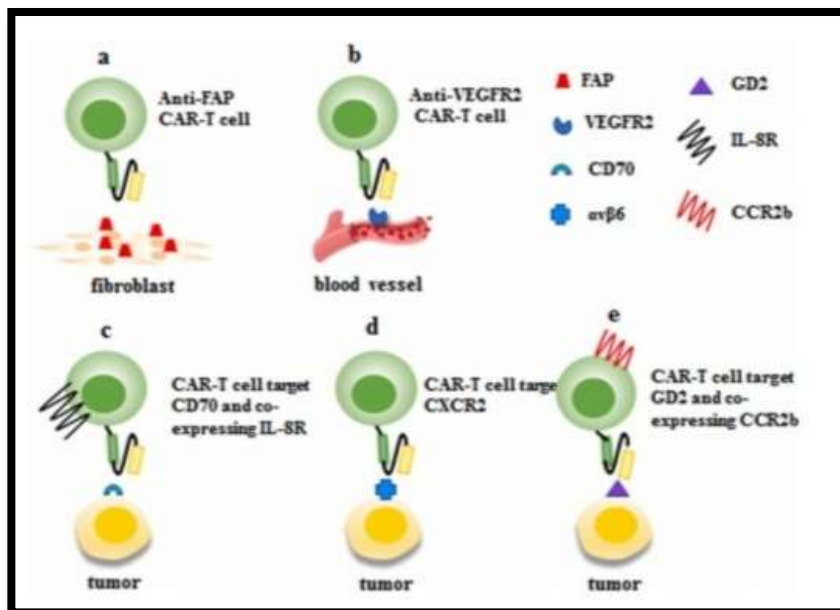
Overexpression of fibroblast activation protein (FAP) in CAFs activates CAFs by FAP-STAT3 axis to drive CCL2 expression and promote bone marrow-derived inhibitory cells (MDSCs) recruitment in TME resulting in increasing tumor growth. For modulation of immune microenvironment and overcoming CAFs barrier, FAP-redirectioned CAR-T cells are used. Preclinical trial in tumor-bearing mouse model of malignant pleural mesothelioma, CAR-T cells targeting FAP stopped tumor development significantly and prolonged the survival time(Ahmed, 2021).

Formation of abnormal blood vessels by tumors to gain more nutrition for growth and metastasis leads to the spatio-temporal heterogeneity of tumor blood flow leading to abnormal TME including interstitial hypertension, hypoxia and acidosis. This abnormal

vascular tissue negatively impacts on CAR-T cells infiltration. Therefore, targeting tumor blood vessels is essential to improve CAR-T cells infiltration. The vital signal in angiogenesis process is VEGF/VEGFR axis that can enhance endothelial cells proliferation, improve the expression of adhesion molecules, and increases vascular permeability. Using anti-angiogenic therapy against VEGF/VEGFR can normalize tumor vessels. It has been found that anti-VEGFR2 CAR-T cells can extend the survival time of B16 tumor-bearing mice when used in combination with T cells specific for the melanoma tumor antigens (gp100 or TRP-1). The synergistic therapy could promote vascular normalization. The anti-VEGFR-2 CAR-T cells may reduce the populations of immunosuppressive cells such as Tregs and MDSCs which express VEGFR-2(Ahmed, 2021). Also, both of VEGFR1-targeted CAR-T cells and VEGFR-2/3 CAR-T suppress tumor growth by the same mechanism(Schuberth et al., 2013).

Mismatched chemokines/chemokine receptors is also considered one of physical barriers that hinders CAR-T cells infiltration into solid tumors. The development of CAR-T cells expressing chemokine receptors can improve CAR-T cells entry into tumors through binding to their corresponding ligands. For example, CD70+ primary gliomas can secrete many chemokines, particularly IL-8. So, the CD70 specific CAR-T cell co-expressing IL-8R could be recruited to tumor tissue to do its effect. Additionally, CXCR2-expressing CAR-T-cell that targets the tumor-associated $\alpha\beta6$ integrin, could recruit to tumor site and perform its anti-tumor activity against $\alpha\beta6$ -expressing ovarian or pancreatic tumor xenografts. [14] When CCR2b expressing CAR-T cells that target GD2 are injected into tumor-bearing mice with CCL2-secreting neuroblastoma, the homing ability of CAR-T cells enhanced and the antitumor activity increased (Figure 2)(Jin et al., 2019).

Figure (2): Enhancement of CAR-T cells infiltrating into solid tumors. (a), (b), overcoming CAFs barrier or



Destroying abnormal tumor blood vessels. (c), (d), (e), Improving the entry of CAR-T cells expressing chemokine receptors into tumor tissue (Zhang et al.,2021)

Preventing the immunosuppression of TME on CAR-T cells

Potent immunosuppressive and immunomodulatory signals against CAR-T-cells are provided by TME via cell-cell interactions and secreted factors. The main factors in TME affecting the CAR-T cells efficacy are many types of immune cells, hypoxia, upregulated immune

checkpoint ligands and the immunosuppressive mediators. Cell-cell interactions are occurred by immune cells in TME, including, myeloid derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and regulatory T cells (Tregs) resulting in a production of different mediators, such as, reactive oxygen species (ROS), lactic acid, arginase, indoleamine 2, 3- dioxygenase (IDO), prostaglandin E2(PGE2), adenosine and TGF- β . Therefore, improving of CAR-T cells anti-tumor activity requires blocking of immune cells or molecular signals included in TME immunosuppression(Fultang et al., 2019; Parihar et al., 2019).

Blocking the recruitment of MDSCs or TAM in TME

Myeloid-derived suppressor cells (MDSCs) recruitment can be suppressed by Olaparib or eliminated by Gemtuzumab Ozogamicin (against CD33) or NK cells expressing a chimeric activating receptor NKG2D. ζ to restore CAR-T cells proliferation and subsequently death of tumor cells . Furthermore, the ligand of NKG2D (NKG2DL) is overexpressed on MDSCs and in some solid tumors not in healthy tissues. Administration of NKG2D-Fc fusion protein by tumor-bearing mice eliminates MDSCs through NKG2D/NKG2DL axis and improve the anti-tumor activity of CAR-T cells(Feng et al., 2020). For TAMs, Glycophosphatidylinositol-anchored receptor (Folate receptor β “FR β ”) is over-expressed on TAMs. The presence of FR β + TAMs in tumor tissues always has a bad prognosis. Therefore, using folate receptor β (FR β) redirected-CAR-T cells that eliminates FR β + TAMs from the TME leads to improve endogenous tumor-specific CD8+ T cells infiltration in the TME and inhibit tumor growth(Kurahara et al., 2012).

Saving CAR-T cells viability from exhaustion by blocking negative signals

One of important soluble regulators in TME is TGF- β . it suppresses CD8+ T cells cytotoxic function by downregulation of granzymes expression. By inhibiting the endogenous TGF- β receptor (TGFBR2) signal in CAR-T cells, the anti-tumor activity of ROR1-specific CAR-T cells enhanced for triple-negative breast cancer via inhibiting of Treg conversion and preventing CAR-T cells exhaustion. [20] The novel CD19 CAR-T cell that secrete bispecific trap protein co-targeting PD-1 (α PD-1 ScFv) and TGF- β (TGF β R II Domain) blocked endogenous TGF- β signaling, reduced the Treg proportion and improved CAR-T cells therapeutic effect in PC3-CD19 prostate tumor xenograft model(Lind et al., 2020).

One of the most important characteristics of the TME is hypoxia that can enhance tumor progression by different mechanisms, such as adenosine receptor expression upregulation in immuno-suppressive cells. Therefore, Improving the anti-tumor activity and saving CAR-T cells from exhaustion could be achieved by blocking the adenosine signal of CAR-T cells with CRISPR/Cas 9 system, shRNA or overexpressing denosine Deaminase 1(Giuffrida et al., 2021).

Redox micromilieu and lactate of the TME are apart from the cytokines and adenosine signals. They cause damage to CAR-T cells. In order to save CAR-T cells from damaging by ROS in TME, CAR-T cells co-expressing catalase could prevent the loss of CAR-T cell functions caused by oxidative stress and promote T cells antioxidant capacity by metabolizing H₂O₂ subsequently save anti-tumor function(Ligtenberg et al., 2016). Also, high amount of lactate in TME inhibits T cell functions via suppressing IL-2 production. Improving anti-PD-1 efficacy (Daneshmandi et al., 2019) and CAR-T activity (Mane et al., 2021)could be achieved by blocking lactate dehydrogenase A (LDH-A) in tumor cells.

Enhancement of CAR-T cells persistence activity by Blocking PD-1/PDL-1 pathway in TME

One of the potential tumor markers is PD-1/PD-L1 axis. Suppression of anti-tumor activity of T cells and interface of CAR-T cells infiltration into TME occur by the Interaction between PD-1 on T cells and PD-L1 on cancerous cells. IFN- γ , TGF- β and TNF- α induce the expression of PD-1 on tumor tissues via activation of Ras, ERK/MAPK, PTEN/PI3K, and Akt/mTOR signaling pathways. Enhancement of CAR-T cells anti-tumor activity is induced by knocking down or knocking out of the PD-1 gene in CAR-T cells or using immune-checkpoint blockades in combination with CAR-T cells. this modified CAR-T cells are safer because of the scFv secreted by CAR-T cells is restricted to tumor tissues. For knocking out PD-1 gene in CART cells, the CRISPR/Cas9 gene editing system has been designed (Figure 3). In human, using CRISPR/Cas9 ribonucleoprotein-mediated editing to disrupted PD-1 in CAR-T cells enables from good tumor control and prevents the relapse triple-negative breast cancer cells xenograft PDXs models(Hu et al., 2019).

Despite of EGFRvIII CAR-T cells administration (iv) is effective in destroying intracerebral tumors, the increase in PD-L1 expression on tumor tissue hinders CAR-T cells functions leading to tumor progression. While generating EGFRvIII Δ PD-1 CAR-T cells using CRISPR/Cas9 gene-editing of the PDCD1 locus in CAR-T cells specific for EGFRvIII was effective in NSG mice against EGFRvIII expressing glioma. Also, a third-generation PD-1-disrupted EvCAR-T cells targeting EGFRvIII could improve the efficacy of CAR-T cells and inhibit the growth of GBM cells. On the other hand, PD-L1-targeted CAR-T or CAR-T cells expressing a dominant-negative form of PD-1 significantly enhanced the activity of mesothelin-targeted CAR-T cells with xenograft PDXs models. Interestingly, blocking PD-1/PD-L1 signal not only decrease CAR-T cells exhaustion, and improve the persistence but also increase the ratio of CD8⁺/CD4⁺ T cells in the TME(Choi et al., 2019).

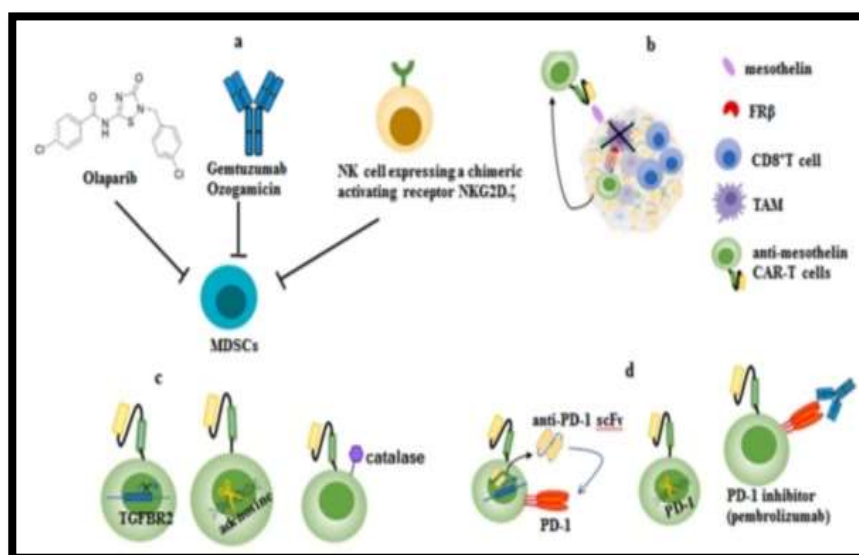


Figure (3): Overcoming the suppression of CAR-T cells activity in TME by new approaches. (A) hindering the infiltration of MDSCs in TME by Olaparib, CD33-targeting antibodies or NK cells expressing a chimeric activating receptor NKG2D. ζ . (B) FR β -targeting CAR-T cells deleted TAM for activation of mesothelin-redirected CAR-T cells and endogenous tumor-specific CD8⁺ T. (C) relieving exhaustion signals or oxidative stress by deleting TGF- β or adenosine signal or overexpressing catalase. (D) inhibiting PD-1 signal by

Preventing the exhaustion of CAR-T cells by engineering novel strategies

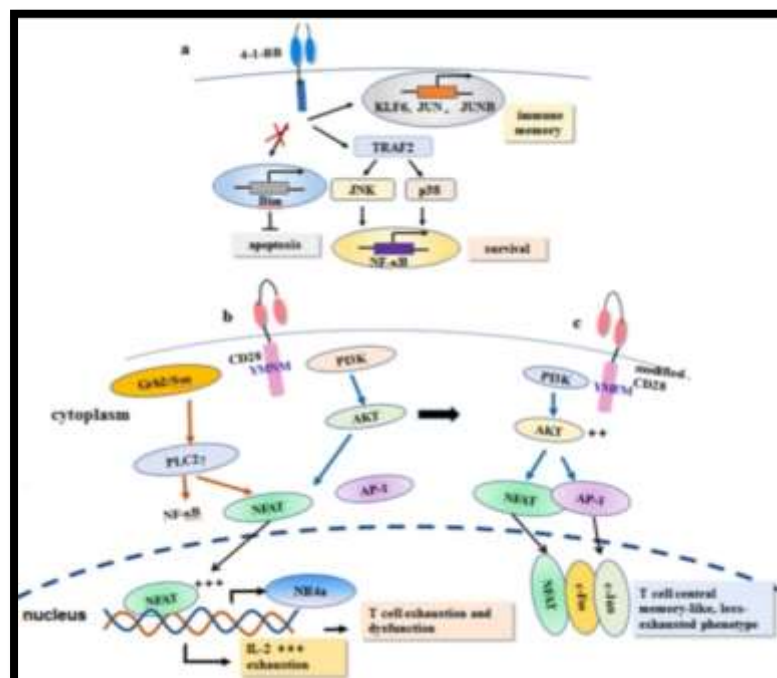
The exact mechanism mediating CAR-T cell exhaustion is not yet clear. The available results may open up new approaches for solving the problem and providing potential novel targets for improving CAR-T cell therapies. Dysfunction of CAR-T cells may be happened due to TME suppression, antigen density and T cell exhaustion. The main characteristic of exhausted CAR-T cell is marked decrease in the production of IL-2. It is caused by continuous antigen stimulation and inflammatory signals in chronic infections as well as tumors. This exhausted T cells are not only inactive to eradicate tumors, but also express a wide range of inhibitory receptors including PD-1, TIM-3 and LAG-3. Although, till now, the mechanisms by which exhaustion of CAR-T cells occurs are not completely understood, various engineering strategies have been developed recently in order to prevent exhaustion and improve the persistence of CAR-T cells (Wherry & Kurachi, 2015).

Persistence of CAR-T cells is affected by various co-stimulatory molecules. For example, continuous proliferation, persistent and activity of CD19-based CAR-T cells in clinical trials influenced by the tandem signaling endo-domains of co-stimulatory molecules including CD28, ICOS, OX40, and 4-1BB as well as cytokine signals. Each of them has a specific role for CAR-T cells persistence (Ghorashian et al., 2019). Also, 4-1BB-based CAR-T cells demonstrated clinical durable persistence in comparison to CD28-based CAR-T cells. This may be regarded to CD28-based CAR-T cells expressed genes encoding for inhibitory receptors, including LAG3, TIM-3, CTLA4, BTLA, and CD244(2B4), and exhaustion-related transcription factors such as T-bet, EOMES, Blimp-1, and Helios while 4-1BB-based CAR-T cells expressed transcription factors related to immune memory, including KLF6, JUN and JUNB. Additionally, 4-1BB-based CAR-T cells persistence is because of potent activation of CAR engineered T cells to $\text{NF-}\kappa\text{B}$ signaling by 4-1BB cytoplasmic domain signals that promotes CAR-T cells survival via repressing the pro-apoptotic protein Bim expression. It is suggested that post antigen identification, CD28-based CAR-T cells can be activated and expressed effector molecules and immunosuppressive molecules more rapidly than 4-1BB-based CAR-T cells. The relatively slower activation process of 4-1BB-based CAR-T cells has a beneficial effect on effector T cells to differentiate into memory T cells. Based on the mechanism, both CD28 and 4-1BB redirected CAR-T cells have a particular glycolytic and fatty acid metabolism. CD28-based CAR-T cells are more dependent on a glycolytic-based metabolism which is characteristics of effector T cells while 4-1BB-based CAR-T cells used fatty acids as the essential source of energy, which is characteristics of memory T cells(Philipson et al., 2020; Kawalekar et al., 2016).

In addition to, inducible costimulator (ICOS) is a member of the CD28 family and has an YMFM signal motif in the intracellular domain. ICOS signaling is essential for optimal proliferation and activation of human Th17 cells. ICOS-based CD4⁺ CAR-T cells showed a Th17 core molecular signature with a Th1 bias, including overexpression of IFN- γ , TNF- α and T-bet. Also, the Th17 cells that were redirected with ICOS-based CARs mediated efficient antitumor responses. Thus, the selection of appropriate costimulatory signals molecule is a critical part for enhancing the persistence of CAR-T cells(Paulos et al., 2010; Guedan et al., 2014).

Enhancement of CAR-T cells persistence can be achieved by designing CD28 motifs. CD28 includes YMNM, PRRP, and PYAP as intracellular subdomains. Each sub domains has an important role in inducing T cell activation and differentiation. YMNM signal motif, starting at Tyrosine 170/173 (mouse/human), has a specific function to recruit important Src-homology 2(SH2)-domain proteins, such as PI3K and Grb2/Sos adapters, leading to activation of of NF- κ B, AP-1, and NATF and subsequently expression of IL-2 and differentiation of T-cell to short-lived effectors (Pagán et al., 2012). Previous experience elicited that replacement of YMNM and PYAP subdomains with FMNM and AYAA, in the CD28 costimulatory domain that retained only one functional CD28 motif PRRP, could develop durable antitumor control in xenograft models (Boucher et al., 2021). Similar studies have demonstrated that by replacing N (asparagine) with F (phenylalanine) in the continuation of YMNM in CD28 will decrease CD28-CAR-T cells exhaustion and increase their persistence. Therefore, designing CD28 motif subdomain to modulate CAR-T cells persistence has also significant impact on the therapeutic efficacy and safety of CAR-T cells (Guedan et al., 2020). It is suggested that CD28 signal modification may provide a better suitable combination of downstream transcription factors and is more conducive for gene transcription that inhibits the exhaustion of CAR-T cells (Figure 4).

Figure (4): The persistence of CAR-T cells viability is affected by co-stimulatory signals of CARs . (A) CAR based on 4-1BB induced the persistence of CAR-T cells viability by reducing apoptotic protein expression, and enhancing immune memory related transcription factors expression. (B) CD28 with YMNM motif is conducive to the production of high levels of NFAT and NR4a, leading to production of large amounts of IL-2 and developing of the exhaustion state. (C) CD28 with YMFM motifs enhances the production of transcription factor complexes, including NFAT/c-Fos/c-Jun that maintains the persistence of CAR-T cells by increasing the activity of AKT(Zhang et al.,202).



Another approach for improving of CAR-T cells persistent vitality can be achieved by CD3 ϵ and ζ ITAMs. CD3 modification has a beneficial effect on CAR-T cells persistence. Three intracellular ITAM motifs are contributed in CD3 ζ providing signal 1 for activating T cell, promoting cytolysis, regulating IL-2 secretion and enhancing anti-tumor activity. Studies demonstrated that a single functional ITAM has an effective antitumor activity leading to a more memory/naïve phenotype. It was superior to the triple-ITAM-containing wild-type CD3 ζ chain. [38] Another different study showed that using CD3 ϵ cytoplasmic domain in a second-generation CAR enhanced CAR-T cells antitumor activity(Wu et al., 2020). This is regarded to CD3 ϵ ITAMs has Lck kinase that recruited the inhibitory Csk kinase to balance the activating and inhibitory motifs of TCR signals, and subsequently improve antitumor activity of CAR-T cells.

Additionally to designing the CAR costimulatory signals, novel approaches to develop specific cytokines for full activation of CAR-T cells were used. It was approved that the expression of cytokines IL-7 (Ma et al., 2020), IL-15 and IL-23 improved the activation and expansion of CAR-T cells. In tumor-bearing mice, IL-7 is found to be promoted CAR-T cells proliferation, prolonged the survival of CAR-T cells and prevented the exhaustion by downregulation of PD-1 expression. Also, T cells redirected with an optimized GD2-specific CAR and interleukin-15 is found to be reduced PD-1 expression and enhanced CAR-T cells proliferative ability. There is a particular integration between both cytokines IL-7 and IL-15 to generate CD45RA⁺ CCR7⁺ early memory populations and favor the emergence of an intratumoral CD8⁺CD62L⁺TCF7⁺IRF4⁻ population that was highly responsive to anti-PD-1 therapy. As shown in figure (5), mbIL15-CAR-T cells (coexpressing CAR with a membrane-bound chimeric IL-15) resulted in high persistent CAR-T cells by stopping the downstream effects of CD28 that cause CAR-T cells exhaustion.

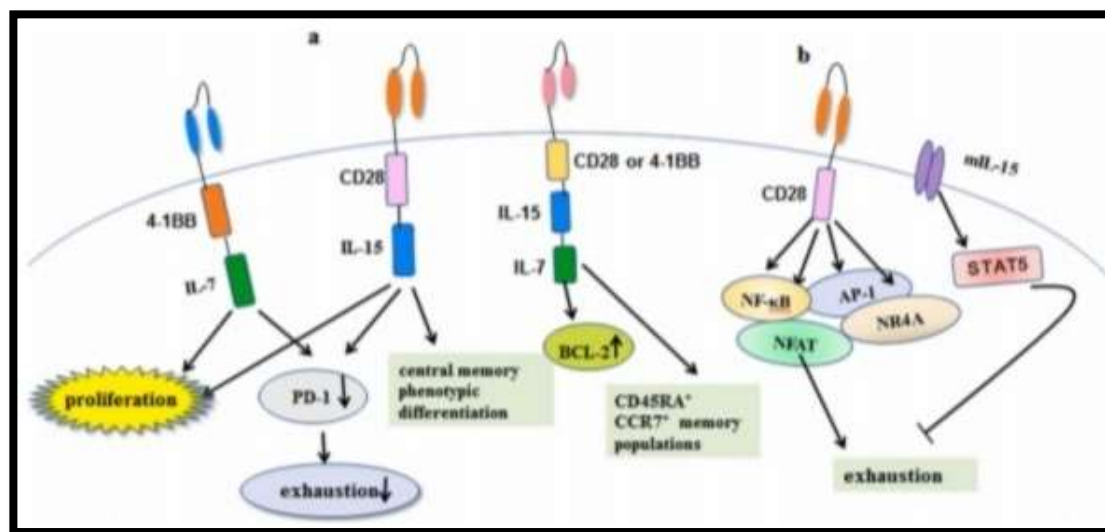


Figure (5): Cytokines improved CAR-T cells activity and persistence. (A):The expression of cytokines IL-7, IL-15 enhanced cytokine-driven CAR-T cell ctivation and expansion. (B): mbIL15-CAR-T cells demonstrated durable activation through STAT5 activation to stop the downstream effects of CD28 that cause CAR-T cell exhaustion(Ma et al., 2020)

Other strategy for extending long-lasting CAR-T cell activity is achieved by blocking the inhibitory receptors expression such as PD-1, TIM-3 and LAG-3 in CAR-T cells (Zou et al., 2019) or combining CAR-T cells with antibody therapy of inhibitory receptors (Figure 3) (John et al., 2019) to prevent T cell failure produced by inhibitory signals or reversed CAR-T

cells with an exhausted phenotype. Also, genetically engineering T cells for over expressing mutated Fas variants in order to prevent FasL-mediated apoptosis, demonstrate better persistence, and have more antitumor efficacy against both of hematological and solid tumors (Yamamoto et al., 2019). Furthermore, REGNASE-1 gene has an excellent capability to affect the persistence and efficacy of CD8+T cells. CAR-T cells lacking REGNASE-1 gene demonstrated enhanced cytotoxicity against a wide range of tumors and promoted differentiation of T cells into memory T cells(Wei et al., 2019).

Inducing a self T cells response in the tumor-bearing host to prevent tumor escape and enhance CAR-T cells activity

Targeting mouse EGFRvIII (an antigen specifically expressing on glioma and completely absent from any normal tissues) by third-generation CAR-T cells (mCAR-T) destroyed mouse gliomas, and demonstrated significant antitumor activity even when antigenic peptides derived from EGFRvIII completely blocked mCAR-T cells (Sampson et al., 2014). Furthermore, the cured mice became resistant for rechallenging with EGFRvIII^{NEG} tumors. Assessment of mCAR-T cells mechanism of action revealed that lysing of cancerous cells by mCAR-T cells resulted in activation of dendritic cells (DCs) and macrophages of tumor-bearing mice followed by killing of the EGFRvIII^{NEG} tumor by tumor-specific T cells of mice because of T cell clones of the tumor-bearing host were activated by epitope spreading and kill cancerous cells with altered tumor antigens (Sampson et al., 2014).

It has been reported that CAR-T cells expressing IL-7 and CCL19 (7 ×19 CAR-T cells) could improve migration of DCs and T cells into TME for complete control of the pre-established solid tumors growth and prolonged the survival [48]. Moreover, muFAP-CAR-T cells (expressing the specific CARs for the mouse fibroblast activation protein “FAP”) found to have no antitumor effects on tumors in the immunodeficient NSG mice while significantly suppressing the tumor growth in the wild-type mice by augment of the endogenous CD8+ T-cell (Wang et al., 2014).

CONCLUSION

For improving CAR-T cell therapy in the management of solid tumors, several strategies have been developed, including: 1) targeting multiple antigens; 2) improving trafficking of CAR-T cells into tumor tissues; 3) Preventing the immunosuppression of TME on CAR-T cells by different approaches such as blocking the recruitment of MDSCs or TAM in TME, saving CAR-T cells viability from exhaustion by blocking negative signals, and enhancement of CAR-T cells persistence activity by blocking PD-1/PDL-1 pathway in TME, 4) designing novel CAR-T cells to prevent the exhaustion; and 5) inducing a self T cells response in the tumor-bearing host to prevent tumor escape and enhance CAR-T cells activity. Recent researches about CAR-T cells therapy carries new hope for targeting solid tumors. The final task in this field is to carry out clinical application.

CONFLICT OF INTEREST: No conflict of interest was declared by the authors.

FINANCIAL DISCLOSURE: The authors declared that this study received no financial support.

REFERENCES

- Ahmed, M. M. E. (2021). CAR-T cell therapy: current advances and future research possibilities. *Journal of Scientific Research in Medical and Biological Sciences*, 2(2), 86-116. <https://doi.org/10.47631/jsrmb.v2i2.234>
- Boucher, J. C., Li, G., Kotani, H., Cabral, M. L., Morrissey, D., Lee, S. B., ... & Davila, M. L. (2021). CD28 Costimulatory Domain–Targeted Mutations Enhance Chimeric Antigen Receptor T-cell Function. *Cancer immunology research*, 9(1), 62-74. <https://doi.org/10.1158/2326-6066>
- Choi, B. D., Yu, X., Castano, A. P., Darr, H., Henderson, D. B., Bouffard, A. A., ... & Maus, M. V. (2019). CRISPR–Cas9 disruption of PD-1 enhances activity of universal EGFRvIII CAR T cells in a preclinical model of human glioblastoma. *Journal for immunotherapy of cancer*, 7(1), 1-8. <https://doi.org/10.1186/s40425-019-0806-7>
- Daneshmandi, S., Wegiel, B., & Seth, P. (2019). Blockade of lactate dehydrogenase-A (LDH-A) improves efficacy of anti-programmed cell death-1 (PD-1) therapy in melanoma. *Cancers*, 11(4), 450. <https://doi.org/10.3390/cancers11040450>
- Feng, P. H., Lam, B., Tseng, S. H., Kung, Y. J., Farmer, E., Cheng, M. A., & Hung, C. F. (2020). NKG2D-Fc fusion protein promotes antitumor immunity through the depletion of immunosuppressive cells. *Cancer Immunology, Immunotherapy*, 69(10), 2147-2155.
- Fultang, L., Panetti, S., Ng, M., Collins, P., Graef, S., Rizkalla, N., ... & De Santo, C. (2019). MDSC targeting with Gemtuzumab ozogamicin restores T cell immunity and immunotherapy against cancers. *EBioMedicine*, 47, 235-246. <https://doi.org/10.1016/j.ebiom.2019.08.025>
- Ghorashian, S., Kramer, A. M., Onuoha, S., Wright, G., Bartram, J., Richardson, R., ... & Amrolia, P. J. (2019). Enhanced CAR T cell expansion and prolonged persistence in pediatric patients with ALL treated with a low-affinity CD19 CAR. *Nature medicine*, 25(9), 1408-1414. <https://doi.org/10.1038/s41591-019-0549-5>
- Giuffrida, L., Sek, K., Henderson, M. A., Lai, J., Chen, A. X., Meyran, D., ... & Beavis, P. A. (2021). CRISPR/Cas9 mediated deletion of the adenosine A2A receptor enhances CAR T cell efficacy. *Nature communications*, 12(1), 1-18. <https://doi.org/10.1038/s41467-021-23331-5>
- Gross, G., Waks, T., & Eshhar, Z. (1989). Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proceedings of the National Academy of Sciences*, 86(24), 10024-10028. <https://doi.org/10.1073/Pnas.86.24.10024>
- Guedan, S., Chen, X., Madar, A., Carpenito, C., McGettigan, S. E., Frigault, M. J., ... & June, C. H. (2014). ICOS-based chimeric antigen receptors program bipolar TH17/TH1 cells. *Blood, The Journal of the American Society of Hematology*, 124(7), 1070-1080. <https://doi.org/10.1182/blood-2013-10-535245>
- Guedan, S., Madar, A., Casado-Medrano, V., Shaw, C., Wing, A., Liu, F., ... & Posey, A. D. (2020). Single residue in CD28-costimulated CAR-T cells limits long-term persistence and antitumor durability. *The Journal of clinical investigation*, 130(6), 3087-3097. <https://doi.org/10.1172/JCI133215>
- Hu, W., Zi, Z., Jin, Y., Li, G., Shao, K., Cai, Q., ... & Wei, F. (2019). CRISPR/Cas9-mediated PD-1 disruption enhances human mesothelin-targeted CAR T cell effector functions. *Cancer Immunology, Immunotherapy*, 68(3), 365-377. <https://doi.org/10.1007/s00262-018-2281-2>
- Kawalekar, O. U., O'Connor, R. S., Fraietta, J. A., Guo, L., McGettigan, S. E., Posey Jr, A. D., ... & June, C. H. (2016). Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. *Immunity*, 44(2), 380-390. <https://doi.org/10.1016/j.immuni.2016.01.021>
- Kurahara, H., Takao, S., Kuwahata, T., Nagai, T., Ding, Q., Maeda, K., ... & Natsugoe, S. (2012). Clinical significance of folate receptor β -expressing tumor-associated macrophages in pancreatic cancer. *Annals of surgical oncology*, 19(7), 2264-2271. <https://doi.org/10.1245/s10434-012-2263-0>
- Jensen, M. C., & Riddell, S. R. (2014). Design and implementation of adoptive therapy with chimeric antigen receptor-modified T cells. *Immunological reviews*, 257(1), 127-144. <https://doi.org/10.3390/cancers12082087>
- Jin, L., Tao, H., Karachi, A., Long, Y., Hou, A. Y., Na, M., ... & Huang, J. (2019). CXCR1-or

- CXCR2-modified CAR T cells co-opt IL-8 for maximal antitumor efficacy in solid tumors. *Nature communications*, 10(1), 1-13. <https://doi.org/10.1038/s41467-019-11869-4>
- Jo, Y., Ali, L. A., Shim, J. A., Lee, B. H., & Hong, C. (2020). Innovative CAR-T cell therapy for solid tumor; current duel between CAR-T spear and tumor shield. *Cancers*, 12(8), 2087. <https://doi.org/10.1111/imr.12139>
- John, L. B., Devaud, C., Duong, C. P., Yong, C. S., Beavis, P. A., Haynes, N. M., ... & Darcy, P. K. (2013). Anti-PD-1 Antibody Therapy Potently Enhances the Eradication of Established Tumors By Gene-Modified T Cells. *Clinical cancer research*, 19(20), 5636-5646. <https://doi.org/10.1158/1078-0432>
- Labanieh, L., Majzner, R. G., & Mackall, C. L. (2018). Programming CAR-T cells to kill cancer. *Nature biomedical engineering*, 2(6), 377-391. <https://doi.org/10.1038/s41551-018-0235-9>
- Lind, H., Gameiro, S. R., Jochems, C., Donahue, R. N., Strauss, J., Gulley, J. L., ... & Schlom, J. (2020). Dual targeting of TGF- β and PD-L1 via a bifunctional anti-PD-L1/TGF- β RII agent: status of preclinical and clinical advances. *Journal for immunotherapy of cancer*, 8(1). <https://doi.org/10.1136/jitc-2019-000433>
- Ligtenberg, M. A., Mougiakakos, D., Mukhopadhyay, M., Witt, K., Lladser, A., Chmielewski, M., ... & Kiessling, R. (2016). Coexpressed catalase protects chimeric antigen receptor–redirected T cells as well as bystander cells from oxidative stress–induced loss of antitumor activity. *The Journal of Immunology*, 196(2), 759-766. <https://doi.org/10.4049/jimmunol.1401710>
- Ma, X., Shou, P., Smith, C., Chen, Y., Du, H., Sun, C., ... & Xu, Y. (2020). Interleukin-23 engineering improves CAR T cell function in solid tumors. *Nature biotechnology*, 38(4), 448-459. <https://doi.org/10.1038/s41587-019-0398-2>
- Mane, M. M., Cohen, I. J., Ackerstaff, E., Shalaby, K., Ijoma, J. N., Ko, M., ... & Blasberg, R. G. (2020). Lactate dehydrogenase a depletion alters MyC-CaP tumor metabolism, microenvironment, and CAR T cell therapy. *Molecular Therapy-Oncolytics*, 18, 382-395. <https://doi.org/10.1016/j.omto.2020.07.006>
- Pagán, A. J., Pepper, M., Chu, H. H., Green, J. M., & Jenkins, M. K. (2012). CD28 promotes CD4+ T cell clonal expansion during infection independently of its YNM and PYAP motifs. *The Journal of immunology*, 189(6), 2909-2917. <https://doi.org/10.4049/Jimmunol.1103231>
- Parihar, R., Rivas, C., Huynh, M., Omer, B., Lapteva, N., Metelitsa, L. S., ... & Rooney, C. M. (2019). NK Cells Expressing a Chimeric Activating Receptor Eliminate MDSCs and Rescue Impaired CAR-T Cell Activity against Solid Tumors. *Cancer immunology research*, 7(3), 363-375. <https://doi.org/10.1158/2326-6066.CIR-18-0572>
- Paulos, C. M., Carpenito, C., Plesa, G., Suhoski, M. M., Varela-Rohena, A., Golovina, T. N., ... & June, C. H. (2010). The inducible costimulator (ICOS) is critical for the development of human TH17 cells. *Science translational medicine*, 2(55), 55ra78-55ra78. <https://doi.org/10.1126/scitranslmed.3000448>
- Philipson, B. I., O'Connor, R. S., May, M. J., June, C. H., Albelda, S. M., & Milone, M. C. (2020). 4-1BB costimulation promotes CAR T cell survival through noncanonical NF- κ B signaling. *Science signaling*, 13(625), eaay8248. <https://doi.org/10.1126/scisignal.aay8248>
- Sampson, J. H., Choi, B. D., Sanchez-Perez, L., Suryadevara, C. M., Snyder, D. J., Flores, C. T., ... & Johnson, L. A. (2014). EGFRvIII mCAR-Modified T-Cell Therapy Cures Mice with Established Intracerebral Glioma and Generates Host Immunity against Tumor-Antigen Loss. *Clinical Cancer Research*, 20(4), 972-984. <https://doi.org/10.1158/1078-0432>
- Schuberth, P. C., Hagedorn, C., Jensen, S. M., Gulati, P., van den Broek, M., Mischo, A., ... & Petrusch, U. (2013). Treatment of malignant pleural mesothelioma by fibroblast activation protein-specific re-directed T cells. *Journal of translational medicine*, 11(1), 1-11. <https://doi.org/10.1186/1479-5876-11-187>
- Wherry, E. J., & Kurachi, M. (2015). Molecular and cellular insights into T cell exhaustion. *Nature Reviews Immunology*, 15(8), 486-499. <https://doi.org/10.1038/nri3862>
- Wu, W., Zhou, Q., Masubuchi, T., Shi, X., Li, H., Xu, X., ... & Xu, C. (2020). Multiple signaling roles

- of CD3 ϵ and its application in CAR-T cell therapy. *Cell*, 182(4), 855-871. <https://doi.org/10.1016/j.cell.2020.07.018>
- Wei, J., Long, L., Zheng, W., Dhungana, Y., Lim, S. A., Guy, C., ... & Chi, H. (2019). Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy. *Nature*, 576(7787), 471-476. <https://doi.org/10.1038/s41586-019-1821-z>
- Wang, L. C. S., Lo, A., Scholler, J., Sun, J., Majumdar, R. S., Kapoor, V., ... & Albelda, S. M. (2014). Targeting Fibroblast Activation Protein in Tumor Stroma with Chimeric Antigen Receptor T Cells Can Inhibit Tumor Growth and Augment Host Immunity without Severe Toxicity FAP-Redirected CAR T Cells. *Cancer immunology research*, 2(2), 154-166. <https://doi.org/10.1158/2326-6066>
- Yamamoto, T. N., Lee, P. H., Vodnala, S. K., Gurusamy, D., Kishton, R. J., Yu, Z., ... & Klebanoff, C. A. (2019). T cells genetically engineered to overcome death signaling enhance adoptive cancer immunotherapy. *The Journal of clinical investigation*, 129(4), 1551-1565. <https://doi.org/10.1172/JCI121491>
- Zhang, Z. Z., Tian, W. A. N. G., Wang, X. F., Zhang, Y. Q., & Song, S. X. (2021). Improving the ability of CAR-T cells to hit solid tumors: Challenges and strategies. *Pharmacological Research*, 106036. <https://doi.org/10.1016/j.phrs.2021.106036>
- Zou, F., Lu, L., Liu, J., Xia, B., Zhang, W., Hu, Q., ... & Zhang, H. (2019). Engineered triple inhibitory receptor resistance improves anti-tumor CAR-T cell performance via CD56. *Nature communications*, 10(1), 1-14. <https://doi.org/10.1038/s41467-019-11893-4>