CAR T-CELL THERAPY OF SOLID TUMORS: PROMISING APPROACHES TO MODULATING ANTITUMOR ACTIVITY OF CAR T CELLS

Yana Yu. Kiseleva, AM. M. Shishkin, AV. Ivanov, TM. Kulinich, VK. Bozhenko

Russian Scientific Center of Roentgenoradiology, Moscow, Russia

Adoptive immunotherapy that makes use of genetically modified autologous T cells carrying a chimeric antigen receptor (CAR) with desired specificity is a promising approach to the treatment of advanced or relapsed solid tumors. However, there are a number of challenges facing the CAR T-cell therapy, including the ability of the tumor to silence the expression of target antigens in response to the selective pressure exerted by therapy and the dampening of the functional activity of CAR T cells by the immunosuppressive tumor microenvironment. This review discusses the existing gene-engineering approaches to the modification of CAR T-cell design for 1) creating universal "switchable" synthetic receptors capable of attacking a variety of target antigens; 2) enhancing the functional activity of CAR T cells in the immunosuppressive microenvironment of the tumor by silencing the expression of inhibiting receptors or by stimulating production of cytokines.

Keywords: CAR T-cell therapy, solid tumors, chimeric antigen receptor, CAR T cells, universal CARs, immunosuppressive microenvironment

Author contribution: Yana Yu. Kiseleva analyzed the literature, prepared the draft of the manuscript, created the figures; Shishkin AM analyzed the literature and revised the manuscript; Ivanov AV analyzed the literature and revised the manuscript; Kulinich TM revised the manuscript; Bozhenko VK revised the manuscript.

Correspondence should be addressed: Yana Yu. Kiseleva

Profsoyuznaya, 86, Moscow, 117997; 89036728541 yana.kiseleva@gmail.com

Received: 03.10.2019 Accepted: 17.10.2019 Published online: 18.10.2019

DOI: 10.24075/vrgmu.2019.066

CAR-TЕРАПИЯ СОЛИДНЫХ ОПУХОЛЕЙ: ПЕРСПЕКТИВНЫЕ ПОДХОДЫ К МОДУЛИРОВАНИЮ ПРОТИВООПУХОЛЕВОЙ АКТИВНОСТИ CAR-T-ЛИМФОЦИТОВ

Я. Ю. Киселева, А. М. Шишkin, А. В. Иванов, Т. М. Кулынич, В. К. Боженко

Российский научный центр рентгенорадиологии, Москва, Россия

Адаптивную иммунотерапию, использующую генно-модифицированные аутологичные Т-лимфоциты с искусственным рецептором заданной специфичности (CAR-терапию) рассматривают в качестве перспективного подхода к лечению солидных опухолей, как рецидивирующих, так и на поздних стадиях развития. При использовании этого вида терапии приходится сталкиваться с рядом проблем, таких как способность опухоли к отбору клеток со сниженной экспрессией антигенов-мишеней в ходе терапии и снижение функциональной активности CAR-T-лимфоцитов иммуносупрессивным микросредством опухоли. В обоих обсуждены существующие генно-инженерные подходы к модификации технологии получения CAR-T-лимфоцитов для: 1) создания универсальных искусственных рецепторов, способных в ходе иммунотерапии переключаться и атаковать различные антигены-мишени; 2) повышения функциональной активности CAR-T-лимфоцитов в условиях иммуносупрессивного микросредства путем подавления экспрессии ингибирующих рецепторов или повышения продукции цитокинов.

Ключевые слова: CAR-терапия, солидные опухоли, химерный антигенный рецептор, CAR-T-клетки, универсальные CAR, иммуносупрессивное микросредство

Информация об авторах: Я. Ю. Киселева — анализ литератур, написание рукописи, подготовка рисунков, редактирование; А. М. Шишkin и А. В. Иванов — анализ литературы, редактирование; Т. М. Кулынич и В. К. Боженко — редактирование.

Для корреспонденции: Яна Юрьевна Киселева

ул. Профсоюзная, д. 86, г. Москва, 117997; yana.kiseleva@gmail.com

Статья принята к печати: 17.10.2019 Опубликована онлайн: 18.10.2019

DOI: 10.24075/vrgmu.2019.066

Traditional treatment options for malignancies, such as surgery, radiation therapy and chemotherapy, do not satisfy the criteria for therapeutic efficacy in patients with advanced or relapsed cancer. The need for more effective therapies has driven development of innovative treatment modalities, many of which harness the mechanisms of immune response [1]. One of them is adoptive immunotherapy that makes use of T-cell chimeric antigen receptors (CARs). Eligible patients receive an infusion of autologous T cells genetically modified ex vivo before the procedure to carry a synthetic receptor with a desired specificity on their surface. A CAR is a fusion protein composed of an extracellular single-chain variable immunoglobulin fragment (scFv) and T-cell intracellular signaling domains [2]. Unlike T-cell receptors that recognize antigens processed and embedded within the major histocompatibility complex, chimeric T-cell receptors target native (unprocessed) cell surface antigens associated with malignant cell transformation [3]. Despite the success of CAR T-cell therapy in fighting hematologic malignancies [4], its application to solid tumors has a few limitations related to the presence of tumor-associated antigens on the surface of healthy tissue cells and the ensuing adverse cytotoxicity [5, 6]. Another challenge is that malignant tumors are heterogeneous and can evolve under selective pressure induced by immunotherapy, expressing fewer target antigens on their surface. In addition, once a CAR T cell reaches the tumor, it finds itself in the immunosuppressive microenvironment created by regulatory T cells, tumor-associated macrophages, myeloid-derived suppressor cells, some overexpressed immunosuppressive molecules (PD-L1, PD-L2, CD80, CD86), hypoxia, necrosis, and lack of nutrients [7].

In order to overcome these barriers, a variety of gene engineering approaches have been proposed to the modification of CAR T-cell manufacturing technology [7–9]. The most promising of them include 1) designing universal CARs capable of attacking a wide range of target antigens and 2) enhancing functional activity of CAR T cells in the immunosuppressive microenvironment of the tumor. This review focuses on genetically engineered universal CARs and the possibility of modulating antitumor activity of CAR T cells by downregulating the expression of inhibiting receptors and stimulating production of cytokines.
Universal CARs with an adaptor module

Since classic, i.e. currently used in clinical practice, CARs are monospecific, antigen loss provoked by therapy remains one of the crucial challenges facing CAR therapy. In order to cut down on manufacturing costs and effort involved in creating a CAR construct with new (yet fixed) specificity, as well as to expand the range of simultaneously or consecutively attacked targets, a modular design of the CAR T-cell system has been proposed in which the antigen-recognition and signaling domains are represented by two separate modules. The antigen-binding adaptor is a stand-alone molecule recognized by a CAR ectodomain. Such system can be functional only in the presence of all 3 components, including the target, the adaptor module and the effector CAR T cell. Its design allows controlled activation of CAR T cells and their rapid “switch-off” in case of toxic adverse events, such as the cytokine release syndrome. In addition, a modular CAR T cell can be easily redirected from one target to another, without having to start the engineering process all over in an attempt to obtain a cell with new antigen specificity. This concept lies behind the idea of a universal CAR system (UniCAR). This review looks at the most promising universal CAR systems developed so far.

A modular CAR system equipped with biotin-binding immunoreceptors

In this type of UniCAR T cells, the universal ectodomain is represented by avidin or streptavidin (Fig. 1A) that bind to biotinylated antigen-specific molecules (MAT, scFv and other specific ligands that recognize the target antigen). The first UniCAR system exploited the interaction between biotin and avidin [10]. Its inventors demonstrated that T cells equipped with the biotin-binding immunoreceptor (BBIR), whose ectodomain was represented by an extracellularly modified avidin dimer, could bind to cancer cells pre-incubated with biotinylated antibodies, switch on and lyse the malignant target. The researchers showed that supraphysiological concentrations of avidin, which is present in human blood plasma, did not cause antigen-independent activation of modified CAR T cells and did not inhibit their activity. Biotinylated antibodies were also employed as adaptor modules in another UniCAR system described in [11]. Here, the role of the ectodomain component was played by a high-affinity streptavidin monomer (mSA2). Using biotinylated rituximab (anti-CD20 mAb) as an adaptor module, the researchers demonstrated that modified T cells were capable of switching on and lysing their targets in a dose-dependent manner in vitro. However, the immunogenicity of avidin/streptavidin remains an open question and can limit therapeutic applications of BBIR-based UniCAR T-cell systems [10, 11].

A modular CAR-system containing fluorescein isothiocyanate

In this modular system (Fig. 1B), the universal CAR T-cell ectodomain contains a variable scFv fragment that targets synthetic fluorescein isothiocyanate (FITC), a commonly used fluorescent probe for antibody labeling. Here, FITC is conjugated either to a monoclonal antibody (mAb) or to a receptor ligand that interacts with a target antigen on the surface of the malignant cell. This interaction results in a pseudoimmunological synapse formed between the anti-FITC CAR T cell and the tumor cell expressing the target antigen. Subsequently, the activated CAR T cells lyse the target. CAR T-cell constructs carrying FITC conjugated to mAb (trastuzumab, rituximab and cetuximab) were successfully tested against HER2-expressing cells (breast cancer), CD20 (B-cell lymphoma) and EGFR (pancreatic cancer) in NSG mice [12]. Just like BBIR-based UniCAR T cells, the immunogenicity of FITS is yet to be elucidated [12].

Modular CAR systems with neoepitopes

In this type of CAR systems, the adaptor module contains a neoepitope bound to antigen-specific scFv or Fab, whereas the CAR itself consists of an intracellular domain and an ectodomain (scFv) that recognizes the neoepitope. Neoepitopes are exogenous peptides not found in humans. So far, two modular neoepitope CAR T-cell systems have been developed; they rely on neoepitopes 5B9 and PNE (Fig. 1C).

5B9 is a non-immunogenic peptide 10 amino acids in length. Its sequence is a peptide motif present in the nuclear autoantigen La/SS-B typically found in patients with Sjögren’s syndrome and systemic lupus erythematosus [13]. Initially, the developed 5B9-specific UniCAR system [14] was directed against antigens CD33 and CD123 expressed on acute myeloid leukemia cells. The researchers experimented with both mono- and bispecific antigen-recognition modules conjugated to epitope 5B9. The targeted cells were effectively lysed both in the presence of two independent monomodules (scFv) and the bispecific module (bis-scFv) alone; the latter turned out to be even more effective. It was established that antigen-recognizing modules could effectively induce lysis
even at very low concentrations, regardless of antigen density on the surface of targeted cells [14]. Later, the efficacy of the 5B9-specific UniCAR-system against solid tumors was tested in cell and animal models of prostate cancer [15]. In this case, the neoepitope 5B9 was bound to scFv directed against prostate stem cell antigen (PSCA). The use of the 5B9-specific UniCAR system in NSG mice with high and low tumor burden significantly delayed tumor growth and improved animal survival. Interestingly, after the adaptor module was added to the co-cultured cancer and 5B9-specific UniCAR T cells, the expression of immuno-suppressing PD-L1 and PD-L2 on the cancer cells and their PD-1 receptor on effector T cells significantly increased in comparison with the control culture without the adaptor module [15]. Later, the same research team [16] published preclinical trial data on the successful application of PSCA- and PSMA-specific (prostate specific membrane antigen) 5B9-modules used in combination.

The other peptide known as PNE (peptide neoepitope) and exploited in a neoepitope UniCAR (Fig. 1C) was derived from GCN4, the transcription factor found in yeast. PNE contains 14 amino acid residues, is not found in humans and is expected to have low immunogenicity, according to the in silico analysis. Proposed in [17], the PNE-based adaptor module contained a PNE bound to a Fab fragment of therapeutic antibodies specific either for CD19 or for CD20. The universal CAR ectodomain contained scFv of highly specific 52SR4 mAb that recognize PNE. Using the mouse model of B-cell leukemia xenograft, the researchers demonstrated dose-dependent control over the activity of UniCAR T cells and their localization in tissue in the areas of malignant cell accumulation and cytokine secretion [17]. Interestingly, high doses of adaptor modules caused expansion of CD45RA-CD62L+ (TEMRA, terminal effector phenotype) memory cells (central memory expressing CD45RA+), whereas low adaptor doses led to the prevalence of the CD45RA+CD62L- phenotype (central memory cells) associated with prolonged persistence of CAR T cells and correlated with sustained remission in patients with acute myeloid leukemia or chronic lymphocyte leukemia [18]. The same research team developed a UniCAR-system with PNE targeting the HER2-expressing breast cancer cells [19]. The antigen-recognizing component of the adaptor module was represented by the Fab fragment of trastuzumab (clone PNE targeting the HER2-expressing breast cancer cells [19]. The same research team developed a UniCAR-system with PNE targeting the HER2-expressing breast cancer cells [19].

The antigen-recognizing component of the adaptor module was represented by the Fab fragment of trastuzumab (clone PNE targeting the HER2-expressing breast cancer cells [19]. The same research team developed a UniCAR-system with PNE targeting the HER2-expressing breast cancer cells [19].

Resolution of lesions in NSG mice inoculated subcutaneously with breast cancer cell lines characterized by different levels of HER2 expression.

**SUPRA CAR: a modular CAR-system with a leucine zipper motif component**

One of the most promising universal CARs is known as SUPRA CAR (split, universal and programmable) and was proposed in [20] (Fig. 1D). It is a two-component system that relies on a leucine zipper motif to ensure the interaction between its parts. The zipper is composed of a universal receptor (zipCAR) expressed on the T-cell surface and a scFv adaptor (zipFv). The universal zipCAR receptor arises from the fusion of intracellular signaling domains (CD28, 4-1BB and CD3z) with the ectodomain containing a leucine zipper motif. The adaptor module zipFv consists of an antigen-specific scFv and a leucine zipper motif, which ensures its interaction with zipCAR and subsequent activation of T cells. Unlike “conventional” CARs with fixed specificity, the described construct allows redirecting the system against different antigen targets without preforming any extra manipulations on a patient’s immune cells. Another unique feature of SUPRA CAR is its tunability: it is possible to adjust the wide range of different parameters involved in modulating T-cell response and prevent T-cell overactivation. By varying such parameters as (1) affinity between leucine zipper motifs, (2) affinity between the tumor antigen and scFv, (3) zipFv concentrations, and (4) zipCAR expression, one can modulate the functional activity of T cells, including interferon gamma production [20]. In case of a cytokine storm occurring in response to CAR therapy, the activity of SUPRA CAR T cells can be dampened or completely inhibited by administering a competing low/affinity adaptor zipFv to the patient. The adaptor can dimerize with the leucine zipper domain of specific zipFv introduced into the patient’s organism in the previous step, and thus prevent it from binding to zipCAR. Besides, this system can perform such logic operations as A OR B or A AND NOT B. The former is used when there is a need to attack malignant cells carrying two target antigens, which is achieved by adding two zipFv adaptors specific for the two targets and capable of binding to zipCAR. The second logic operation is performed to mitigate adverse cytotoxic effects on healthy cells expressing the target antigen. The researchers demonstrated...
the feasibility of sparing cells that carry 2 target antigens, one of which was tumor-associated and the other was non-tumor [20]. This effect can be achieved by using the zipFv adaptor that is specific for a non-tumor antigen but competes with zipCAR for binding to the leucine zipper domain of the zipFv adaptor specific for the tumor antigen. When bound to the target, the adaptors form dimers, meaning that zipFv specific for the tumor antigen can no longer interact with zipCAR. As a result, the induced cytokotoxic response of T cells against healthy cells is weak. It was demonstrated that SUPPA CAR T cells can control tumor growth in NGS mice injected with SK-BR-3 breast cancer cells intraperitoneally or with Jurkat cells intravenously [20]. In order to reduce immunoinactivity of the proposed system, the authors humanized leucine zippe using the corresponding sequences of human transcription factors [20].

**Modulation of antitumor activity of CAR T cells in the immunosuppressive tumor microenvironment**

The immunosuppressive microenvironment of solid tumors is one of the major factors preventing the positive outcome of CAR therapy. Coupled with the expression of inhibiting receptors on the surface of CAR T cells, this disrupts the efficacy of the latter [21]. The following 2 inhibiting receptors are worth noting: PD-1 (programmed cell death receptor) and CTLA-4 (cytotoxic T-cell-associated antigen 4); today, they are regarded as leading regulators of the immune system that control the activation of T cells and maintain peripheral tolerance [7, 22]. By interacting with CD80/86, CTLA-4 inhibits potentially autoreactive T cells in the early stage of naive T-cell activation, usually in lymph nodes [23], whereas PD1 bound to PD-L1/2 prevents this activation. In order to reduce immunoinactivity of immune response, exerting its effect in peripheral tissue [24]. Tumor cells expressing the corresponding ligands on their surface can harness those two receptors to inactivate tumor-specific lymphocytes, including CAR T cells, thus acquiring insensitivity to their attacks [22, 25]. Importantly, adoptive immunotherapy makes use of activated T cells. Their activation leads to overexpression of PD-1 and CTLA-4, which makes T cells even more susceptible to the immunosuppressive effect of the tumor [25].

The unwanted interaction between the ligand and the receptor can be blocked by mAb specific for this receptor or ligand [26]. Since 2011, therapeutic regimens for metastatic melanoma adopted in the USA have included ipilimumab, the monoclonal antibody that blocks CTLA-4 (cytotoxic T-cell-associated antigen 4); today, they are regarded as leading regulators of the immune system that control the activation of T cells and maintain peripheral tolerance [7, 22]. By interacting with CD80/86, CTLA-4 inhibits potentially autoreactive T cells in the early stage of naive T-cell activation, usually in lymph nodes [23], whereas PD1 bound to PD-L1/2 prevents this activation. In order to reduce immunoinactivity of immune response, exerting its effect in peripheral tissue [24]. Tumor cells expressing the corresponding ligands on their surface can harness those two receptors to inactivate tumor-specific lymphocytes, including CAR T cells, thus acquiring insensitivity to their attacks [22, 25]. Importantly, adoptive immunotherapy makes use of activated T cells. Their activation leads to overexpression of PD-1 and CTLA-4, which makes T cells even more susceptible to the immunosuppressive effect of the tumor [25].

The unwanted interaction between the ligand and the receptor can be blocked by mAb specific for this receptor or ligand [26]. Since 2011, therapeutic regimens for metastatic melanoma adopted in the USA have included ipilimumab, the monoclonal antibody that blocks CTLA-4 (cytotoxic T-cell-associated antigen 4); today, they are regarded as leading regulators of the immune system that control the activation of T cells and maintain peripheral tolerance [7, 22]. By interacting with CD80/86, CTLA-4 inhibits potentially autoreactive T cells in the early stage of naive T-cell activation, usually in lymph nodes [23], whereas PD1 bound to PD-L1/2 prevents this activation. In order to reduce immunoinactivity of immune response, exerting its effect in peripheral tissue [24]. Tumor cells expressing the corresponding ligands on their surface can harness those two receptors to inactivate tumor-specific lymphocytes, including CAR T cells, thus acquiring insensitivity to their attacks [22, 25]. Importantly, adoptive immunotherapy makes use of activated T cells. Their activation leads to overexpression of PD-1 and CTLA-4, which makes T cells even more susceptible to the immunosuppressive effect of the tumor [25].

Another approach to overcoming the immunosuppressive activity of tumor environment and attacking tumor cells that have stopped expressing the target antigen on their surface lies in modifying T cells to secrete cytokines that can activate tumor microenvironment. Experiments in mouse models have demonstrated that accumulation of IL12 in malignant tissue following adoptive transfer of tumor-specific IL12-secreting cells improves the cytokotic activity of T lymphocytes and stimulates activation and recruitment of innate immunity cells to the tumor site [37]. Secretion of IL12 locally affects myeloid suppressor cells, dysfunctional dendritic cells and alternatively activated macrophages and reprograms them into functional antigen-presenting cells that can present tumor-associated antigens to tumor-infiltrating lymphocytes (TIL), causing regression of large tumor lesions [38]. Clinical trials conducted in patients with metastatic melanoma treated with autologous TIL expressing IL12 under the “supervision” of regulatory NFAT (NFAT stands for nuclear factor of activated T cells) have demonstrated an objective clinical effect in 10 out of 16 patients treated with lower cell doses than recommended in the standard protocol [39]. However, many patients recruited for the trial developed serious side effects, such as severe hepatotoxicity and hemodynamic instability; therefore, the trial was terminated [39]. Pronounced cytotoxicity of TCR T cells with NFAT-regulated expression of IL12 has been detected in vivo in the experiments in mice [40]. Another research group has achieved a positive therapeutic outcome (the absence of toxicity in vivo) by controlling the IL12 expression with the TET-On promoter sensitive to doxycycline [45]. Transient expression of IL12 was enough to inhibit the growth of B16F10 melanoma without provoking systemic cytotoxicity.
There have been studies investigating the effect of the increased IL15 and IL18 expression on the antitumor activity of CAR T cells and TCR T cells. It has been established that IL15 improves survival and promotes proliferation of ex vivo modified CAR T cells redirected against CD19 (in leukemia/lymphoma) [42] and IL13RA2 (in glioblastoma) [43]. Using the melanoma mouse model, the researchers have demonstrated that administration of modified TCR T cells with NFAT-regulated expression of IL18 was safe (no adverse toxicity was observed), resulted in the expansion of CD8+ cells in the lesion and increased antitumor activity [40]. CD4 CAR T cells secreting IL18 have been shown to activate CD8 T-cells that, in turn, proliferate and enhance the antitumor response in mice with induced B16F10 melanoma [44]. CAR T cells secreting IL18 trigger acute Th1 immune response in the tumor, which results in improved survival of mice with pancreatic and lung cancer [45].

CONCLUSION
At present, CAR T-cell therapy is successfully used in clinical practice in patients with hematologic malignancies. However, this positive experience cannot be extrapolated to solid tumors because of the adverse events associated with CAR T-cell toxicity against healthy cells and the inhibiting effect of the immunosuppressive tumor microenvironment on the functional activity of CAR T cells. A few solutions have been proposed, including universal CARs that can be quickly redirected against a new antigen target and CAR T cells that have been genetically modified to resist the immunosuppressive effect of the tumor microenvironment. Results of extensive research in this field instill hope for creating an arsenal of effective and therapeutically safe CAR T cells that can be used to treat solid tumors.

References
1. Palucka AK, Coussens LM. The Basis of Oncoimmunology. Cell. 2016; 164 (6): 1233–47. DOI: 10.1016/j.cell.2016.01.049. PubMed PMID: 26967289; PubMed Central PMCID: PMC4788788.
2. Jena B, Dotti G, Cooper LJ. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. Blood. 2010; 116 (7): 1035–44. DOI: 10.1182/blood-2010-01-043737. PubMed PMID: 20439624; PubMed Central PMCID: PMC2938125.
3. Kielanoff CA, Rosenberg SA, Restifo NP. Prospects for gene-engineered T cell immunotherapy for solid cancers. Nature medicine. 2016; 22 (1): 26–36. DOI: 10.1038/nm.4015. PubMed PMID: 26735408; PubMed Central PMCID: PMC35295670.
4. Park JH, Geyer MB, Brentjens RJ. CD19-targeted CAR T-cell therapeutics for hematologic malignancies: interpreting clinical outcomes to date. Blood. 2016; 127 (26): 3312–20. DOI: 10.1182/blood-2016-02-629063. PubMed PMID: 27207800; PubMed Central PMCID: PMC4299923.
5. Liu B, Yan L, Zhou M. Target selection of CAR T-cell therapy in accordance with the TME for solid tumors. American journal of cancer research. 2019; 9 (2): 228–41. PubMed PMID: 30906625; PubMed Central PMCID: PMC6405971.
6. Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ. Toxicity and management in CAR T-cell therapy. Molecular therapy oncology. 2016; 3 (1): 16011. DOI: 10.1038/mtoso.2016.11. PubMed PMID: 27626862; PubMed Central PMCID: PMC5026256.
7. Tahmassebi S, Elahi R, Esmailzadeh A. CD4 Th1-targeted CAR T-cell therapies for hematologic malignancies: interpreting clinical outcomes to date. Blood. 2016; 127 (26): 3312–20. DOI: 10.1182/blood-2016-02-629063. PubMed PMID: 27207800; PubMed Central PMCID: PMC4299923.
8. Minutolo NG, Hollander EE, Powell DJ, Jr. The Emergence of Universal Immune Receptor T Cell Therapy for Cancer. Frontiers in oncology. 2019; (9): 176. DOI: 10.3389/fonc.2019.00176. PubMed PMID: 30984613; PubMed Central PMCID: PMC6448045.
9. Stoiber S, Cadilha BL, Benmebarek MR, Lesch S, Endres S, Kobold S. Limitations in the Design of Chimeric Antigen Receptors for Cancer Therapy. Cells. 2019; 8 (5). DOI: 10.3390/cells8050472. PubMed PMID: 31108883; PubMed Central PMCID: PMC64562702.
10. Urbanska K, Lanitis E, Poussin M, Lynn RC, Gavin BP, Feldermann A, et al. Universal strategy for adoptive immunotherapy of cancer through use of a novel T-cell antigen receptor. Cancer research. 2012; 72 (7): 1844–52. DOI: 10.1158/0008-5472.CAN-11-3890. PubMed PMID: 22315351; PubMed Central PMCID: PMC319867.
11. Lohmueller JJ, Ham JD, Kvorjak M, Finn OJ, mSAA affinity-enhanced biotin-binding CAR T cells for universal tumor targeting. Oncoimmunology. 2017; 7 (1): e1368604. DOI: 10.1080/21624022.2017.1368604. PubMed PMID: 29296519; PubMed Central PMCID: PMC5739565.
12. Tamada K, Geng D, Sakoda Y, Bansal N, Srivastava R, Li Z, et al. Redirecting gene-modified T cells toward various cancer types using tagged antibodies. Clinical cancer research: an official journal of the American Association for Cancer Research. 2012; 18 (23): 6436–45. DOI: 10.1158/1078-0432.CCR-12-1449. PubMed PMID: 23032741.
13. Koristka S, Cartellieri M, Arndt C, Bippes CC, Feldmann A, Michalki I, et al. Retargeting of regulatory T cells to surface-inducible autoantigen La/SS-B. Journal of autoimmunity. 2013; (42): 105–16. DOI: 10.1016/j.jaut.2013.01.002. PubMed PMID: 23352111.
14. Cartellieri M, Feldmann A, Koristka S, Arndt C, Loff S, Ehninger A, et al. Switching CAR T-cells on and off: a novel modular platform for retargeting of T-cells to AML blasts. Blood cancer journal. 2016; 6 (8): e458. DOI: 10.1038/bcj.2016.61. PubMed PMID: 27518241; PubMed Central PMCID: PMC5022178 directed to CD33, La and the UniCAR platform technology. AE, SL and MC are employed by GEMoAb and CPT, respectively. The other authors declare no conflict of interest.
15. Pshilai Bejestani E, Cartellieri M, Bergmann R, Ehninger A, Loff S, Kramer M, et al. Characterization of a switchable chimeric antigen receptor platform in a pre-clinical solid tumor model. Oncoimmunology. 2017; 6 (10): e1342009. DOI: 10.1080/216240222017.1342009. PubMed PMID: 29123961; PubMed Central PMCID: PMC5665006.
16. Feldmann A, Arndt C, Bergmann R, Loff S, Cartellieri M, Bachmann D, et al. Retargeting of T lymphocytes to PSMA- or PSMA-positive prostate cancer cells using the novel modular chimeric antigen receptor platform ‘UniCAR’. Oncotarget. 2017; 8 (19): 31368–85. DOI: 10.18632/oncotarget.15572. PubMed PMID: 28404896; PubMed Central PMCID: PMC5458214.
17. Rogers DT, Mazagova M, Hampton EN, Cao Y, Ramirezoss NS, Hardy IR, et al. Switch-mediated activation and retargeting of CAR-T cells for B-cell malignancies. Proceedings of the National Academy of Sciences of the United States of America. 2016; 113 (4): E459–68. DOI: 10.1073/pnas.1524155113. PubMed PMID: 26793969; PubMed Central PMCID: PMC4743815.
18. Riddell SR, Sommermeyer D, Berger C, Liu LS, Baikichnann A, Salter A, et al. Adoptive therapy with chimeric antigen receptor-modified T cells of defined subset composition. Cancer composition. 2014; 20 (2): 141–4. DOI: 10.1007/JFFOC-15500000000306. PubMed PMID: 24667960; PubMed Central PMCID: PMC4149222.
19. Cao Y, Rodgers DT, Du J, Ahmad I, Hampton EN, Ma JS, et al. Switching of Chimeric Antigen Receptor T Cells Targeting Breast Cancer. Angewandte Chemie. 2016; 55 (26): 7520–4. DOI: 10.1002/ang.201601902. PubMed PMID: 27145250; PubMed Central PMCID: PMC50207029.
20. Cho JH, Collins JJ, Wong WW. Universal Chimeric Antigen Receptors for Multiplexed and Logical Control of T Cell Responses. Cell. 2018; 173 (6): 1426–38. DOI: 10.1016/j.
Yu Y, Wu H, Tang Z, Zang G. CTLA4 silencing with siRNA
Ren J, Liu X, Fang C, Jiang S, June CH, et al. A versatile system for rapid multiplex genome-edited CAR T cell generation. Oncoarget. 2017; 8 (10): 17002–11. DOI: 10.18632/oncoarget.15218. PubMed PMID: 28199983; PubMed Central PMCID: PMC5357609.

Knochelmann HM, Smith AS, Dwyer CJ, Wyatt MM, Mehrotra S, Paulos CM. CAR T Cells in Solid Tumors: Blueprints for Building Effective Therapies. Frontiers in immunology. 2018; (9): 1740. DOI: 10.3389/fimmu.2018.01740. PubMed PMID: 30140266; PubMed Central PMCID: PMC6094980.

Kerker SP, Muranski P, Kaiser A, Boni A, Sanchez-Perez L, Yu Z, et al. Tumor-specific CD8+ T cells expressing interleukin-12 eradicate established cancers in lymphodepleted hosts. Cancer research. 2010; 70 (17): 6725–34. DOI: 10.1158/0008-5472; CAN-10-0735. PubMed PMID: 20647327; PubMed Central PMCID: PMC2935308.

Pegram HJ, Lee JC, Hayman EG, Imperato GH, Tedder TF, Sadelaar M, et al. Tumor-targeted T cells modified to secrete IL12 eradicate systemic tumors without need for prior conditioning. Blood. 2012; 119 (18): 4313–41. DOI: 10.1182/blood-2011-12-400044. PubMed PMID: 22354001; PubMed Central PMCID: PMC3359735.

Kerker SP, Goldszmid RS, Muranski P, Chinnasamy D, Yu Z, Foger RN, et al. IL12 triggers a programmable change in dysfunctional myeloid-derived cells within mouse tumors. The Journal of clinical investigation. 2011; 121 (12): 4746–57. DOI: 10.1172/JCI58814. PubMed PMID: 22056381; PubMed Central PMCID: PMC3226001.

Zhang L, Morgan RA, Beane JD, Zheng Z, Dudley ME, Kassim SH, et al. Tumor-infiltrating lymphocytes genetically engineered with an inducible gene encoding interleukin-12 for the immunotherapy of metastatic melanoma. Clinical cancer research: an official journal of the American Association for Cancer Research. 2015; 21 (10): 2278–88. DOI: 10.1158/1078-0432.CCR-14-2085. PubMed PMID: 25695689; PubMed Central PMCID: PMC4433819.

Kunert A, Chmielewski M, Wijers R, Berrevoets C, Abken H, Debets R. Intra-tumoral production of IL18, but not IL12, by TCR-engineered T cells is non-toxic and counteracts immune evasion of solid tumors. Oncoimmunology. 2017; 6 (3): e1378842. DOI: 10.1080/2162402X.2017.1378842. PubMed PMID: 29296541; PubMed Central PMCID: PMC5739571.

Alsaieedi A, Holler A, Velica P, Bendle G, Stauss HJ. Safety and efficacy of Tet-regulated IL12 expression in cancer-specific T cells. Oncoimmunology. 2019; 8 (3): 1542917. DOI: 10.1080/2162402X.2018.1542917. PubMed PMID: 30723575; PubMed Central PMCID: PMC6350686.

Hoyos V, Savoldo B, Quintarelli C, Mahendravada A, Zhang M, Vera J, et al. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia. 2010; 24 (6): 1160–70. DOI: 10.1038/leu.2010.75. PubMed PMID: 20428207; PubMed Central PMCID: PMC2888148.

Krenciute G, Pinzling BL, Yi Z, Wu MF, Liu H, Dotti G, et al. Transgenic Expression of IL15 Improves Antiligaemia Activity of IL15Ralph2-CAR T Cells but Results in Antigen Loss Variants. Cancer immunology research. 2017; 5 (7): 571–81. DOI: 10.1158/2326-6066.CIR-16-0376. PubMed PMID: 28550091; PubMed Central PMCID: PMC5746871.

Hu B, Ren J, Luo Y, Keith B, Young RM, Scholler J, et al. Augmentation of Antitumor Immunity by Human and Mouse CAR T Cells Secreting IL18. Cell reports. 2017; 20 (13): 3025–33. DOI: 10.1016/j.celrep.2017.09.002. PubMed PMID: 28954221; PubMed Central PMCID: PMC5602762.

Chmielewski M, Abken H. CAR T Cells Releasing IL18 Convert to T-Bet(high) FoxO1(low) Effectors that Exhibit Augmented Activity against Advanced Solid Tumors. Cell reports. 2017; 21 (11): 3205–19. DOI: 10.1016/j.celrep.2017.11.063. PubMed PMID: 29241547.
1. Palucka AK, Coussens LM. The Basis of Oncoimmunology. Cell. 2016; 164 (6): 1233–47. DOI: 10.1016/j.cell.2016.01.049. PubMed PMID: 26967289; PubMed Central PMCID: PMC4788788.

2. Jena B, Dotti G, Cooper LJ. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. Blood. 2010; 116 (7): 1035–44. DOI: 10.1182/blood-2010-01-043737. PubMed PMID: 20403982; PubMed Central PMCID: PMC2993125.

3. Klebanoff CA, Rosenberg SA, Restifo NP. Prospects for gene-engineered T cell immunotherapy for solid cancers. Nature medicine. 2016; 22 (1): 26–36. DOI: 10.1038/nm.4015. PubMed PMID: 26735408; PubMed Central PMCID:PMC6295670.

4. Park JH, Geyer MB, Brentjens RJ, Curran KJ. Toxicity and pharmacokinetics of CAR T cells: clinical outcomes to date. Blood. 2016; 127 (26): 3512–20. DOI: 10.1182/blood-2016-02-629063. PubMed PMID: 27207800; PubMed Central PMCID:PMC4929923.

5. Liu B, Yan L, Zhou M. Target selection of CAR T cell therapy in solid tumors. American journal of cancer research. 2019; 9 (2): 228–41. PubMed PMID: 30906625; PubMed Central PMCID:PMC6405971.

6. Bonifant CL, Jackson HJ, Brentjens RJ, Curran KJ. Toxicity and management in CAR T-cell therapy. Molecular therapy oncology. 2016; (3): 16011. DOI: 10.1038/mt.2016.11. PubMed PMID: 27626062; PubMed Central PMCID:PMC5008265.

7. Tahmasebi S, Elahi R, Esmaeilzadeh A. Solid Tumors Challenges and New Insights of CAR T Cell Engineering. Stem cell reviews and reports. 2019; 15 (3): 619–36. DOI: 10.1007/s12015-019-09901-7. PubMed PMID: 31161552.

8. Minutolo NG, Hollander EE, Powell DJ, Jr. The Emergence of Universal Immune Receptor T Cell Therapy for Cancer. Frontiers in oncology. 2019; (9): 176. DOI: 10.3389/fonc.2019.00176. PubMed PMID: 30984613; PubMed Central PMCID:PMC6484045.

9. Stoiber S, Cadilha BL, Bennebahre MK, Lesch S, Endres D, Kobold S. Limitations in the Design of Chimeric Antigen Receptors for Cancer Therapy. Cells. 2019; 8 (5). DOI: 10.3390/cells08050472. PubMed PMID: 31108883; PubMed Central PMCID: PMC6562702.

10. Urbanek K, Lantos E, Poussin M, Lynn RC, Gavin BP, Kelderman S, et al. The UniCAR platform technology “UniCAR”. Oncotarget. 2017; 6 (19): 13168–85. DOI: 10.18632/oncotarget.15572. PubMed PMID: 28498437; PubMed Central PMCID:PMC5458214.

11. Riddell SR, Sommerneyer D, Berger C, Liu LS, Balakrishnan A, Saller A, et al. Adoptive therapy with chimeric antigen receptor-modified T cells of defined subset composition. Cancer journal. 2014; 20 (2): 141–4. DOI: 10.1097/PPRO.0000000000000369. PubMed PMID: 24867960; PubMed Central PMCID:PMC4149222.

12. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell. 2016; 165 (1): 35–53. DOI: 10.1016/j.cell.2016.03.038. PubMed PMID: 27076040; PubMed Central PMCID:PMC5984158.

13. Taradà K, Geng D, Sakoda Y, Bansal N, Srivastava R, Li Z, et al. Redirecting gene-modified T cells toward various cancer types using tagged antibodies. Clinical cancer research: an official journal of the American Association for Cancer Research. 2012; 18 (23): 6436–45. DOI: 10.1158/1078-0432.CCR-12-1449. PubMed PMID: 23032741.

14. Koristka S, Cartellieri M, Arndt C, Bippes CC, Feldmann A, Michalk I, et al. Retargeting of regulatory T cells to surface-expressed autoantigen La/SS-B. Journal of autoimmunity. 2013; 42 (4): 105–16. DOI: 10.1016/j.jaut.2013.01.002. PubMed PMID: 23352121.

15. Kobold S. Limitations in the Design of Chimeric Antigen Receptors for Cancer Therapy. Cells. 2019; 8 (5). DOI: 10.3390/cells08050472. PubMed PMID: 31108883; PubMed Central PMCID: PMC6562702.

16. Feldmann A, Arndt C, Bergmann R, Loff S, Cartellieri M, Bachmann D, et al. Retargeting of T lymphocytes to PSMA- or PSMA positive prostate cancer cells using the novel modular chimeric antigen receptor platform technology “UniCAR”. Oncotarget. 2017; 8 (19): 31368–85. DOI: 10.18632/oncotarget.15572. PubMed PMID: 28498437; PubMed Central PMCID:PMC5458214.

17. Rodgers DT, Mazagava M, Hampton EN, Cao Y, Ramadoss NS, Hardy IR, et al. Switch-mediated activation and retargeting of CAR-T cells for B-cell malignancies. Proceedings of the National Academy of Sciences of the United States of America. 2016; 113 (4): 1459–68. DOI: 10.1073/pnas.1524155113. PubMed PMID: 26735969; PubMed Central PMCID:PMC4743815.

18. Liu B, Yan L, Zhou M. Target selection of CAR T cell therapy in solid tumors. American journal of cancer research. 2019; 9 (2): 228–41. PubMed PMID: 30906625; PubMed Central PMCID:PMC6405971.

19. Riddell SR, Sommerneyer D, Berger C, Liu LS, Balakrishnan A, Saller A, et al. Adoptive therapy with chimeric antigen receptor-modified T cells of defined subset composition. Cancer journal. 2014; 20 (2): 141–4. DOI: 10.1097/PPRO.0000000000000369. PubMed PMID: 24867960; PubMed Central PMCID:PMC4149222.

20. Liu B, Yan L, Zhou M. Target selection of CAR T cell therapy in solid tumors. American journal of cancer research. 2019; 9 (2): 228–41. PubMed PMID: 30906625; PubMed Central PMCID:PMC6405971.

21. Riddell SR, Sommerneyer D, Berger C, Liu LS, Balakrishnan A, Saller A, et al. Adoptive therapy with chimeric antigen receptor-modified T cells of defined subset composition. Cancer journal. 2014; 20 (2): 141–4. DOI: 10.1097/PPRO.0000000000000369. PubMed PMID: 24867960; PubMed Central PMCID:PMC4149222.

22. Liu B, Yan L, Zhou M. Target selection of CAR T cell therapy in solid tumors. American journal of cancer research. 2019; 9 (2): 228–41. PubMed PMID: 30906625; PubMed Central PMCID:PMC6405971.

23. Riddell SR, Sommerneyer D, Berger C, Liu LS, Balakrishnan A, Saller A, et al. Adoptive therapy with chimeric antigen receptor-modified T cells of defined subset composition. Cancer journal. 2014; 20 (2): 141–4. DOI: 10.1097/PPRO.0000000000000369. PubMed PMID: 24867960; PubMed Central PMCID:PMC4149222.
in Untreated Melanoma. The New England journal of medicine. 2015; 373 (1): 23–34. DOI: 10.1056/NEJMoa1504030. PubMed PMID: 26027431; PubMed Central PMCID: PMC5698905.

30. Postow MA, Siddow R, Hellmann MD. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. The New England journal of medicine. 2018; 378 (2): 158–68. DOI: 10.1056/NEJMra1703481. PubMed PMID: 29320654.

31. Ren J, Liu X, Fang C, Jiang S, June CH, Zhao Y. Multiplex Genome Editing to Generate Universal CAR T Cells Resistant to PD1 Inhibition. Clinical cancer research: an official journal of the American Association for Cancer Research. 2017; 23 (9): 2255–66. DOI: 10.1158/1078-0432.CCR-16-1300. PubMed PMID: 27815355; PubMed Central PMCID: PMC5413401.

32. Yu Y, Wu H, Tang Z, Zang G. CTLA4 silencing with siRNA promotes deviation of Th1/Th2 in chronic hepatitis B patients. Cellular & molecular immunology. 2009; 6 (2): 123–7. DOI: 10.1038/cmi.2009.17. PubMed PMID: 19403062; PubMed Central PMCID: PMC4002649.

33. Rupp LJ, Schumann K, Roybal KT, Gate RE, Ye CJ, Lim WA, et al. CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells. Scientific reports. 2017; 7 (1): 737. DOI: 10.1038/s41598-017-00462-8. PubMed PMID: 28389661; PubMed Central PMCID: PMC5426439.

34. Ren J, Zhang X, Liu X, Fang C, Jiang S, June CH, et al. A versatile system for rapid multiplex genome-edited CAR T cell generation. Oncotarget. 2017; 8 (10): 17002–11. DOI: 10.18632/oncotarget.15218. PubMed PMID: 28199983; PubMed Central PMCID: PMC5537017.

35. Knochelmann HM, Smith AS, Dwyer CJ, Wyatt MM, Mehrotra S, Paulos CM. CAR T Cells in Solid Tumors: Blueprints for Building Effective Therapies. Frontiers in immunology. 2018; (9): 1740. DOI: 10.3389/fimmu.2018.01740. PubMed PMID: 30140266; PubMed Central PMCID: PMC5694980.

36. Kerker SP, Muranski P, Kaiser A, Boni A, Sanchez-Perez L, Yu Z, et al. Tumor-specific CD8+ T cells expressing interleukin-12 eradicate established cancers in lymphodepleted hosts. Cancer research. 2010; 70 (17): 6725–34. DOI: 10.1158/0008-5472.CAN-10-0735. PubMed PMID: 20647327; PubMed Central PMCID: PMC2935308.

37. Pegram HJ, Lee JC, Hayman EG, Imperato GH, Tedder TF, Sadelain M, et al. Tumor-targeted T cells modified to secrete IL12 eradicate systemic tumors without need for prior conditioning. Blood. 2012; 119 (18): 4133–41. DOI: 10.1182/blood-2011-12-400044. PubMed PMID: 22354001; PubMed Central PMCID: PMC3359735.

38. Kerker SP, Goldszmid RS, Muranski P, Chinnasamy D, Yu Z, Reger RN, et al. IL12 triggers a programmatic change in dysfunctional myeloid-derived cells within mouse tumors. The Journal of clinical investigation. 2011; 121 (12): 4746–57. DOI: 10.1172/JCI58814. PubMed PMID: 22056831; PubMed Central PMCID: PMC3226001.

39. Zhang L, Morgan RA, Beane JD, Zheng Z, Dudley ME, Kassim SH, et al. Tumor-infiltrating lymphocytes genetically engineered with an inducible gene encoding interleukin-12 for the immunotherapy of metastatic melanoma. Clinical cancer research: an official journal of the American Association for Cancer Research. 2015; 21 (10): 2278–88. DOI: 10.1158/1078-0432.CCR-14-2085. PubMed PMID: 25695689; PubMed Central PMCID: PMC4433819.

40. Kunert A, Chmielewski M, Wijers R, Berrevoets C, Abken H, Debets R. Intra-tumoral production of IL18, but not IL12, by TCR-engineered T cells is non-toxic and counteracts immune evasion of solid tumors. Oncoimmunology. 2017; 7 (1): e1378842. DOI: 10.1080/2162402X.2017.1378842. PubMed PMID: 29296541; PubMed Central PMCID: PMC5739571.

41. Alsaieedi A, Holler A, Velica P, Bendle G, Stauss HJ. Safety and efficacy of Tet-regulated IL12 expression in cancer-specific T cells. Oncoimmunology. 2019; 8 (3): 1542917. DOI: 10.1080/2162402X.2018.1542917. PubMed PMID: 30723575; PubMed Central PMCID: PMC6350686.

42. Hoyos V, Savoldo B, Quintarelli C, Mahendraavadha A, Zhang M, Vera J, et al. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. Leukemia. 2010; 24 (6): 1160–70. DOI: 10.1038/leu.2010.75. PubMed PMID: 20428207; PubMed Central PMCID: PMC2888148.

43. Krencicute G, Prinzng BL, Yi Z, Wu MF, Liu H, Dotti G, et al. Transgenic Expression of IL15 Improves Antiglioma Activity of IL13Ralpha2-CAR T Cells but Results in Antigen Loss Variants. Cancer immunology research. 2017; 5 (7): 571–81. DOI: 10.1158/2326-6066.CIR-16-0376. PubMed PMID: 28550091; PubMed Central PMCID: PMC5746871.

44. Hu B, Ren J, Luo Y, Keith B, Young RM, Scholler J, et al. Augmentation of Antitumor Immunity by Human and Mouse CAR T Cells Secreting IL18. Cell reports. 2017; 20 (13): 3025–33. DOI: 10.1016/j.celrep.2017.09.002. PubMed PMID: 28954221; PubMed Central PMCID: PMC6002762.

45. Chmielewski M, Abken H. CAR T Cells Releasing IL18 Conver to T-Bet(high) FoxO1(low) Effectors that Exhibit Augmented Activity against Advanced Solid Tumors. Cell reports. 2017; 21 (11): 3205–19. DOI: 10.1016/j.celrep.2017.11.063. PubMed PMID: 29241547.