CAR-based Therapies: A Pathway Leading to Promising Therapy against B Cell Malignancy

Jinlu Liu[1, *, †], Taiyi Wu[2, *, †], Minghao Zhang[3, *, †]

[1]McMaster University, Hamilton, Canada, L8S 4L8

[2]St. Michael's College, University of Toronto, Toronto, Canada, M5S 1J4

[3]New college, University of Toronto, Toronto, Canada, M5S 1C7

*Corresponding author: minghao.zhang@mail.utoronto.ca, liuj241@mcmaster.ca, apollo.wu@mail.utoronto.ca

[†]These authors contributed equally

Keywords: CAR-based therapies, Cancer immunology, Immunotherapy, B cell malignancy.

Abstract: Cancer has always been a medical challenge that scientists have been trying to resolve for centuries. After the successful bone marrow transplant which aims to deal with leukemia was developed in 1956, numerous measures including chemotherapy, physical therapy and surgical intervention have taken turns to be tumor fighters. During recent decades, immunotherapy is a hot academic area to step on. Among all promising techniques that has been developed, CAR-T has shown impressive results in treating hematological malignancies and progress in conquering solid tumors clinically. However, clinical trials also showed some drawbacks such as severe side effects (neurotoxicity, cytokine release syndrome, etc.), high cost and resistance in making progress in solid tumors. Here, this review will introduce three emergent CAR-based therapies: CAR-NK, CAR-NKT and CAR-macrophage, as these extensions of CAR-based techniques shed light on further clinical development of CAR strategies. Respectively, the mechanism, evolution, application, and challenges of these three therapies will also be discussed.

1. Introduction

CAR-T therapy is a promising technique that treats different cancers by affecting the immune system and could be personalized within individuals. As known, T cells could recognize the diseased cells and destroy them, however, cancer cells have the ability of immune escape which means they could hide from the T cells inspection. The way CAR-T therapy works is that T cells are extracted from patients through apheresis, the cells could therefore be genetically engineered to have chimeric antigen receptors (CARs) to target the specific antigen on the target cells so that they could be recognized and destroyed, the tumor-specific T cells is then let grown and harvest to transfer back to the patient. The simplest form of CAR is composed of a single-chain variable fragment (scFv) recognition domain, a cluster of differentiation (CD)-3zeta chain, extracellular hinge domain, and a transmembrane domain. scFv could recognize the target cell surface antigens, while the CD-3zeta chain could activate T cell binding to the antigens. Once the target cells are recognized and bind to, CAR T kills the target cells through the perforin and granzyme axis, the Fas and Fas ligand axis, and the releasing of cytokines [1].

As shown in Table 1 are several applications of CAR-T and their structures as well as the target antigens, CAR-T therapy is now used predominantly in the treatment of hematological malignancies such as ALL (Acute Lymphoblastic Leukemia), CLL (Chronic Lymphocytic Leukemia) and lymphoma. Clinical trials have shown less successful in treating solid tumors, the major obstruction is due to the immunosuppressive microenvironment of tumors, such as inhibitory metabolic factors and immunosuppressive checkpoints [2]. Therefore, in treating solid tumors, it is found more effective with the combination of other therapy strategies such as chemotherapy.

In order to overcome the resistance to CAR-T, CAR therapy is explored utilizing different types of lymphocytes such as NK cells, NKT cells, and macrophages. Take an example of CAR-NK, natural killer cells are innate immune cells defending virally infected cells, named for their ability to kill tumor cells without prior activation, and are able to kill target cells through the cytotoxic mechanism. Comparing to conventional CAR-T, CAR-NK has advantages such as better safety, multiple mechanisms of activation and better infiltrating into solid tumor [3]. Even though these newly modified CAR based techniques enhanced the development of CAR therapy, there are challenges such as difficulties in lacking a universal protocol to make new CAR-modified cells. Here, we discussed the most recent discoveries in both experimental research and clinical studies to provide an overview of these advanced CAR technologies and highlight their advantages versus CAR-T.

Table.1. Selected application of CAR-T therapy in treating cancers along with CAR structure and their target [4]

Type of Cancer	CAR Structure	Target Antigen
Acute Lymphoblastic Leukemia (ALL)	CD3zeta and 41BB	CD33
	CD3zeta and CD28	CD123 or LeY
Chronic Lymphocytic Leukemia (CLL)	CD3zeta and 41BB	Igk
	CD3zeta and CD28	ROR1
Lymphoma	CD3zeta and CD28	CD19
	CD3zeta and 41BB	CD19 CD20
Multiple Myeloma (MM)	CD3zeta and 41BB	BCMA CD138
Small Lymphocytic Lymphoma (SLL)	CD3zeta and 41BB	ROR1
Neuroblastoma	CD3zeta and 41BB	CD171
	CD3zeta, CD28 and 41BB	CD171
Breast Cancer	CD3zeta and CD28	HER MUC1
	CD3zeta and CD27	FR
Lung Cancer	CD3zeta and CD28	FAP
	KIR2DS2 and DAP12	FAP
Prostate Cancer	CD3zeta and CD28	PSMA

2. B cell malignancy and Current Therapy Against B cell Malignancy

B cells, or also referred to as B lymphocytes, play an important role in our immune system, they are responsible for making antibodies that protect us from invading pathogens. However, B cells sometimes could be affected by lymphoma, leading to the accumulation of abnormal B cells in our body. They could include slow-growing Hodgkin's lymphomas and non-Hodgkin lymphomas which could be aggressive, one of the most common BCL (B cell lymphoma) is Diffuse large B-cell lymphoma (DLBCL). Traditionally, BCL can be treated with chemotherapy using drugs CHOP (Cyclophosphamide or Cytoxan, Hydroxydaunorubicin or Doxorubicin hydrochloride, Oncovin or Vincristine and Prednisone), which usually comes with the combination of an immunotherapy drug Rituximab (Rituxan). For early-stage BCL, radiation therapy could be the main treatment with a possible combination of chemotherapy or other therapies recommended by doctors. If patients show no response to the treatments mentioned above, this innovative technique -- CAR-T therapy might be suggested by the doctor.

3. Side effects of CAR-T against B cell malignancy

The advent of CAR-T has raised enormous attention across the clinical industry due to its high efficiency to treat cancer cells. However, this therapeutic approach has also demonstrated side effects and even severe toxicities. Specifically, cytokine release syndrome (CRS) and neurotoxicity are the two most noticeable negative outcomes that have been characterized recently [5]. In October 2017, following the FDA approval of Yescarta (an anti-CD19 CAR-T product named axicabtagene ciloleucel), a phase II clinical trial was conducted, and it was observed that grade 3 or higher CRS occurred in 13% of the patients who received the treatment to handle refractory B cell lymphoma, whereas the occurrence rate of neurological disorder is 28% [6].

Similarly, Kymriah, a drug which has its approval in August 2017 against acute lymphoblastic leukemia (ALL), also showed grade 3 or 4 adverse events in 73% of patients suspected of association with Kymriah in a 2018 clinical trial to treat CD19+ relapsed or refractory ALL [7]. Therefore, CAR-T, praised for its panacea-like effects and regarded as a promising treating strategy to end cancer in the future by the public media, has also raised significant concerns in this field. Several reviews that examined CAR-T critically have pointed out that more trials are required to investigate its safety, and some late toxicities like cytopenia are also worth special consideration during the administration, so a standard grading system upon the toxicities is urgent to be widely employed in the increasing clinical trials worldwide [6, 8,10]. Fortunately, the American Society for Transplantation and Cellular Therapy (ASTCT) supported a meeting three years ago and experts actively involved in this field have established a well-recognized consensus about the evaluation of CRS and neurotoxicity [11] and the grading system has been applied to a few studies, showing its decent degree of adaptability [12, 13].

On the other hand, this personalized medicine also poses a heavy burden in terms of treatment expense upon patients that yearn for lifesaving chances. In a comparative study, although Yescarta was shown to extend approximately more than 3-fold of estimating years and quality-adjusted life years compared with traditional chemotherapy, its cost ($552f,921) is approximately 3-fold than the conventional treating strategy ($172,737) to deal with refractory large B cell lymphoma [14]. In this way, most patients are unable to afford such a huge amount of spending, which further exacerbates the widespread application of CAR-T to more patients.

Considering the obstacle CAR-T encountered with respect to both its issue of safety and luxury, scientists have continued to find new adjustments using CAR so that more off-the-shelf products with lower toxicity can be developed. In recent years, three types of immune cells have their own supporters to combine with CAR, and the newly emerging stars are NK cells, NKT cells, and macrophages.

4. CAR-NK Cells: Mechanism, Evolution, and Application

The integration of both activating and inhibitory receptors expressed on NK cells governs the final effector function of these cells. Due to the variation of the receptors, different CAR-NK products may employ a variety of mechanistic pathways leading to cytotoxic outcomes [3]. Normally, interferon γ is produced by NK cells to promote downstream immune response, while the production of this cytokine was shown to increase in recent CAR-NK studies [15] with enhanced targeted killing abilities through degranulation or selective cytotoxicity [16, 17, 18]. Therefore, the incorporation of CAR further boosts the function of NK cells by making them more specific to recognize stress molecules presented by tumor cells, acting as a group of well-trained soldiers to deal with tumor growth.

One of the earliest attempts which employed the idea of CAR-NKs was performed by a group of scientists who used cord blood (CB) to derive NK cells for engineering. Since allogeneic T cells usually give rise to graft-versus-host disease (GVHD) that requires careful consideration before administration, NK cells, with their similar functional mechanism as CD8+ T cells but without causing GVHD, have been identified to be a potential CAR-based candidate. Therefore, the

CB-derived NK cells were transduced with a retroviral vector to incorporate genes for CAR-CD19. In the same logic as CAR-T therapy, the gene for CAR-CD19 ensures the specificity of the NK cells to target CD19 positive tumor cells, which are mostly derived from B cell malignancy [16]. Around the same time, researchers also modified a specific NK cell line- NK-92 cells with their design of CAR to observe their therapeutic effect in a Raji B cell lymphoma model within mice. As expected, variation arose from the difference of components of CARs, while the major CAR-NK92 cells have demonstrated better killing activity than their parental cells [17], suggesting the great potential to harvest these immune cells. Not just focusing on CD19 as a canonical target, the combination of different CAR-NK cells is also under careful scrutiny. Aiming to generate treatment with higher quality, the mixture of CD19-NK-92 and CD138-NK-92 cells resulted in a better performance against leukemia, lymphoma, and multiple myeloma cell lines [19]. In this manner, multitargeting strategy, as well as advanced optimization and development of new CAR display, should catch more attention for further investigation.

Expanding more on their CB-derived NK cells, the original group put these cells into clinical practice. After administering into 11 patients with either non-Hodgkin's lymphoma or chronic lymphocytic leukemia, it was astonishing that 8 complete remissions from CD-19 positive cancers with 12-month long lasting effects and lower toxicity exemplified by the serum cytokines were observed, suggesting CAR-NK to be a safer alternative than CAR-T [21]. NK92 cells, after being modified with various CAR formations, exhibited promising results against tumors like acute myeloid leukemia with few adverse effects [20, 21].

5. CAR-NKT Cells: Mechanism, Evolution, and Application

Vα24-invariant natural killer T (NKT) cells are a group of innate lymphocytes that plays a role in innate immunity [22]. However, they are less studied and explored for their therapeutic possibility due to their low prevalence in the human body [22, 23]. Still, figuring out how they perform their effector responses in the tumor environment, a few studies have highlighted their functions against cancer and also marked the chance of NKT cells being a platform for CAR-based immunotherapy.

As mentioned by Rotolo et al., in 2018, anti CD19 CAR-T cells have been noticed to be a powerful way to fight B cell malignancy [24]. Nonetheless, the relapse rate within patients is also very significant, yearning for better and stronger immunotherapy in place to solve the issue. Consequently, they equipped NKT cells with the CAR19 to achieve a dual antitumor effect through CD19 and CD1d, as glycolipid antigens are primarily presented by CD1d to these cells to activate their robust responses against cancer cells normally. Through their experiments, not only indicating that the two pathways with CD19 and CD1d involved may work cooperatively targeting B cell malignancy, but they also found that short- as well as long-term reactivity towards cancer has been enhanced significantly with the employment of CAR-NKT cells relative to the CAR-T Cells, demonstrating the potency of such strategy. Again, by examining the possibility of GVHD, the use of CAR-NKT cells seem not to incite this side effect, which may make it more advanced than CAR-T [20].

The focus of CAR-NKT cells to control cancer growth was not limited to only B cell malignancy. Instead, more cancer types are being tested using this new tool in recent years. In 2019, Xu et al. engineered NKT cells to co-express a GD2-specific CAR together with IL15 and investigated the efficacy of the modified cell products in controlling neuroblastoma [25], a pediatric tumor that affects the development of nerve cells in early childhood [26]. After treating the mice that were injected with three lines of neuroblastoma cells with the CAR-NKT cells they produced, the tumor size shrank dramatically and the mice received the treatment survived longer compared with those who did not in the absence of severe toxicity and GVHD, further proving that CAR-NKTs are worthy of more research for their use in clinical trials and containment of more cancer types. As shown by an interim study later, in 2020, Heczey et al. conducted CAR-NKT cell infusion into three patients and objective response presented by regression of metastatic lesions has been obtained, and

this first-in-human phase I clinical trial signified the subsequent application of CAR-NKTs in a larger clinical scale upon their encouraging results [23].

6. CAR-Macrophages: Mechanism, Evolution, and Application

Macrophages, often nicknamed “big eaters”, is a type of innate immune cells that engulf pathogens and produce cytokines to mediate and coordinate the immune response [27].

Lately, its antitumor activity has also been gradually characterized and reviewed [28, 29]. As a result, research focusing on the manipulation of macrophages using CAR has also been proposed, and this idea was recently first realized by Zhang et al. in 2020, with the employment of induced pluripotent stem cells (iPSCs) to produce ample CAR-macrophages for experimental use. In their study, they reprogrammed the peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor into iPSCs. Then, after selecting the most suitable CD19 CAR, they used a lentiviral transduction method to integrate the CAR gene into the iPSCs and established their protocol to drive the differentiation of the iPSCs into macrophages, which eventually lead to the production of CAR-expressing iPSC-induced macrophage (CAR-iMac). Similar to both CAR-NK and CAR-NKT cells, the primary origin of CAR-iMac, PBMCs, does not need to come from an autologous source but from an externally allogeneic healthy donor without causing GVHD, which additionally add to the list of CAR-based therapies that could generate “off-the-shelf” products for prevalent use in clinical trials [30].

It was also reported that the CAR-iMacs generated from iPSCs performed well in the murine model. After injecting ovarian cancer cells into NSG mice, the mice were subsequently either treated or not treated with the CAR-iMac cells. Compared to the control group, CAR-iMac treated mice demonstrated lower tumor burden in all selected days post-treatment [30], suggesting the underlining capacity of such a new emerging technique for restricting tumor progression even though this area is still underdeveloped and requires more creative modification and further exploration.

7. Challenges on CAR-based therapies

Due to the powerful function, the side-effect of CAR NK cells should not be ignored and should be managed. Therefore, CARs are engineered in the short-lived form, which can result in potential disadvantage of short-lived CAR-NK-92 cells in terms of efficiency and persistency; to recover this, more frequent infusions of CAR-NK-92 cells are required, and this may lead to higher complexity and cost of funds [31]. Similar to CAR-NK cells, CAR-NKT cells have strong potency while performing their anti-tumor function. Thus, even if these CAR-NKT cells have shown the properties that may prove the promising antitumor ability [32], the amount of control in a patient’s body has become one of the main challenges of this technology, and this challenge might become the barrier for further clinical application of CAR-NKT. Moreover, in terms of CAR-Macrophages, overcoming the factors that could influence the persistence of CAR-Macrophages in the solid tumor is a challenge that needs to be considered. To surmount this challenge, enhancing the expression level of migratory molecules on CAR-Macrophages has become the focus. Several candidates to be targeted include receptor-like protein tyrosine phosphatase, metalloproteinase 10, CDF-1 receptor tyrosine kinase, the integrin co-activator Kindlin-3, and alpha M beta 2 as well as alpha D beta 2 integrins [33]. By targeting these potential factors may help to find the breakthrough of solving these challenges, but more studies are required to identify the optimal target.

8. Comparison of different CAR-based therapy

In terms of fighting against B cell malignancy, CAR-T technology has shown unexpected success. However, multiple factors can still affect its efficiency. For example, the lymphomatous or leukemia cell surface expression can be potentially influenced by any antigen-targeted therapy, which may reduce the CAR T cell efficiency. Meanwhile, as mentioned above, CAR19iNKT plays the

anti-tumor role sufficiently, and it was observed that this CAR19iNKT is even more efficient than the 19 cells applied CAR-T technology while against CD19+CD1d+B cell malignancies. In addition, in an NSG xenograft model of lymphoma, the survival rate of T cells and iNKT cells are equal. Thus, both CAR-T and CAR- iNKT technologies can be sufficiently applied, but it is worth saying that the latter showed better disease control and higher tumor-free survival. Moreover, for CAR-NK technology, the CAR-NK product, termed FT596, showed the same efficiency in tumor cell clearance as primary CD19-targeted CAR-T cells while against the target cells of CD19+ CD20+ B lymphoblast. Other than B cell malignancy, FT596 also played an equivalent role in tumor cell elimination as primary CD19-targeted CAR-T cells while targeting cells of CD19+ acute lymphoblastic leukemia.

9. Conclusions

As discussed above, recent studies have progressed substantially in expanding the field of employing CAR into different cell types for B cell malignancy. Cancer treatment, regarded as one of the most significant medical problems in the 21st century, has attracted enormous research groups to conduct experiments for discovering new mechanisms, new strategies, and new therapeutic trials. As expected, these new data and clinical results raise further questions about the prospective development of CAR-based therapies.

The potential neurotoxicity and cytokine release syndrome of CAR-T, as also recently investigated by newly derived CAR-based methods, demonstrated alleviated or even completely devoid of these side effects [20, 24]. However, it is always unavoidable that concerns are present for these novel tools as a medication because of the lack of clinical studies and trials to examine their other potential side effects after treatment. Therefore, instead of rapidly equipping cells with CAR to boost their original response against stress-induced molecules, the accurate manipulation of these cells for avoiding exceedingly strong inflammatory responses should also be prioritized and supplemented by strict criteria to monitor their toxicity. In this case, a standard like ASTCT is urgently required.

Additionally, previous studies have also regarded CAR-NK, CAR-NKT, and CAR-Macrophage as possible “off-the-shelf” candidates. These important insights not only shed light on the great possibility of reducing the heavy economic burden associated with these cellular therapies, but also may be applicable to more cancer cell types, awaiting widespread application in the coming years.

References

[1] Benmebarek M, Karches C, Cadilha B, et al. Killing mechanisms of chimeric antigen receptor (CAR) T Cells. International Journal of Molecular Sciences, 2019, 20 (6), 1283.

[2] Zhao Z, Xiao X, Saw P, et al. Chimeric antigen receptor T cells in solid tumors: A war against the tumor microenvironment. Science China Life Sciences, 2020, 63 (2), 180–205.

[3] Xie G, Dong H, Liang Y, et al. CAR-NK cells: A promising cellular immunotherapy for cancer. EBioMedicine, 2020, 59, 102975.

[4] Xu D, Jin G, Chai D, et al. The development of CAR design for tumor CAR-T cell therapy. Oncotarget, 2018, 9 (17), 13991–14004.

[5] Chou C, Turtle C. Insight into mechanisms associated with cytokine release syndrome and neurotoxicity after CD19 CAR-T cell immunotherapy. Bone Marrow Transplantation, 2019, 54 (Suppl 2), 780–784.

[6] Neelapu S, Locke F, Bartlett N, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-Cell lymphoma. The New England Journal of Medicine, 2017, 377 (26), 2531–2544.

[7] Maude S, Laetsch T, Buechner J, et al. Tisagenlecleucel in children and young adults with B-Cell lymphoblastic leukemia. The New England Journal of Medicine, 2018, 378 (5), 439–448.

[8] Gust J, Taraseviciute A, Turtle C. Neurotoxicity associated with CD19-targeted CAR-T cell therapies. CNS Drugs, 2018, 32 (12), 1091–1101.

[9] Neelapu S. Managing the toxicities of CAR T-cell therapy. Hematological Oncology, 2019, 37 (Suppl 1), 48–52.

[10] Brudno J, Kochenderfer J. Recent advances in CAR T-cell toxicity: Mechanisms, manifestations and management. Blood Reviews, 2019, 34, 45–55.

[11] Lee D, Santomasso B, Locke F, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with Immune effector cells. Biology of Blood and Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation, 2019, 25 (4), 625–638.

[12] Pennisi M, Jain T, Santomasso B, et al. Comparing CAR T-cell toxicity grading systems: application of the ASTCT grading system and implications for management. Blood Advances, 2020, 4 (4), 676–686.

[13] Gutgarts V, Jain T, Zheng J, et al. Acute kidney injury after CAR-T Cell Therapy: Low incidence and rapid recovery. Biology of Blood and Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation, 2020, 26 (6), 1071–1076.

[14] Roth J, Sullivan S, Lin V, et al. Cost-effectiveness of axicabtagene ciloleucel for adult patients with relapsed or refractory large B-cell lymphoma in the United States. Journal of Medical Economics, 2018, 21 (12), 1238–1245.

[15] Gang M, Marin N, Wong P, et al. CAR-modified memory-like NK cells exhibit potent responses to NK-resistant lymphomas. Blood, 2020, 136 (20), 2308–2318.

[16] Liu E, Tong Y, Dotti G, et al. Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. Leukemia, 2018, 32 (2), 520-531.

[17] Oelsner S, Friede M, Zhang C, et al. Continuously expanding CAR NK-92 cells display selective cytotoxicity against B-cell leukemia and lymphoma. Cytotherapy, 2017, 19 (2), 235-249.

[18] Oelsner S, Waldmann A, Billmeier A, et al. Genetically engineered CAR NK cells display selective cytotoxicity against FLT3-positive B-ALL and inhibit in vivo leukemia growth. International Journal of Cancer, 2019, 145 (7), 1935-1945.

[19] Luanpitpong S, Poohadsuan J, Klaihmon P, et al. Selective cytotoxicity of single and dual anti-CD19 and anti-CD138 chimeric antigen receptor-natural killer cells against hematologic malignancies. Journal of Immunology Research, 2021, 2021, 5562630.

[20] Liu E, Marin D, Banerjee P, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. The New England Journal of Medicine, 2020, 382 (6), 545-553.

[21] Tang X, Yang L, Li Z, et al. First-in-man clinical trial of CAR NK-92 cells: Safety test of CD33-CAR NK-92 cells in patients with relapsed and refractory acute myeloid leukemia. American Journal of Cancer Research, 2018, 8 (6), 1083–1089.

[22] Kriegsmann K, Kriegsmann M, von Bergwelt-Baildon M, et al. NKT cells - new players in CAR cell immunotherapy? European Journal of Haematology, 2018, 101 (6), 750–757.

[23] Heczey A, Courtney A, Montalbano A, et al. Anti-GD2 CAR-NKT cells in patients with relapsed or refractory neuroblastoma: an interim analysis. Natural Medicine, 2020, 26 (11), 1686-1690.

[24] Rotolo A, Caputo V, Holubova M, et al. Enhanced anti-lymphoma activity of CAR19-iNKT cells underpinned by dual CD19 and CD1d targeting. Cancer Cell, 2018, 34 (4), 596-610.

[25] Xu X, Huang W, Heczey A, et al. NKT cells coexpressing a GD2-Specific chimeric antigen receptor and IL15 show enhanced in vivo persistence and antitumor activity against neuroblastoma. Clin Cancer Res, 2019, 25 (23), 7126-7138.

[26] Tsubota S, Kadomatsu K. Origin and initiation mechanisms of neuroblastoma. Cell Tissue Res, 2018, 372 (2), 211-221.

[27] Shapouri-Moghaddam A, Mohammadian S, Vazini H, et al. Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol, 2018, 233 (9), 6425-6440.

[28] Mills C, Lenz L, Harris R. A breakthrough: Macrophage-directed cancer immunotherapy. Cancer Res, 2016, 76 (3), 513-516.

[29] Anderson N, Minutolo N, Gill S, et al. Macrophage-based approaches for cancer immunotherapy. Cancer Res, 2021, 81 (5), 1201-1208.

[30] Zhang L, Tian L, Dai X, et al. Pluripotent stem cell-derived CAR-macrophage cells with antigen-dependent anti-cancer cell functions. J Hematol Oncol, 2020, 13 (1), 153-155.

[31] Romanski A, Uherek C, Bug G, et al. CD19-CAR engineered NK-92 cells are sufficient to overcome NK cell resistance in B-cell malignancies. Journal of Cellular and Molecular Medicine, 2015, 20 (7), 1287-1294.

[32] Rotolo A, Dubois O, Baxan N, et al. Invariant NKT are a more effective and versatile platform than T cells for CAR immunotherapy of CD1d-expressing B lineage malignancies: Cellular and molecular mechanisms. Blood, 2017, 130, 4613-4616.

[33] Santoni M, Massari F, Montironi R, et al. Manipulating macrophage polarization in cancer patients: From nanoparticles to human chimeric antigen receptor macrophages. BBA - Reviews on Cancer, 2021, 1876 (1), 188547-188552.