

Precision Immunotherapy in Triple-Negative Breast Cancer: Integrating MTHFR and INSR Pharmacogenomics into the CAR-T Cell Therapy Workflow

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ABSTRACT

Triple-Negative Breast Cancer (TNBC) remains a significant clinical challenge due to its aggressive nature and the absence of conventional molecular targets. While Chimeric Antigen Receptor (CAR) T-cell therapy has revolutionized hematological oncology, its efficacy in solid tumors like TNBC is hindered by the immunosuppressive tumor microenvironment (TME) and inter-patient metabolic variability. This review explores the critical role of host pharmacogenomics—specifically polymorphisms in the Methylenetetrahydrofolate Reductase (MTHFR) and Insulin Receptor (INSR) genes—as key determinants of CAR-T cell fitness and therapeutic response.

We discuss the utility of targeted molecular techniques, such as ARMS-PCR and in silico structural modeling, for the rapid identification of specific SNPs, including *MTHFR C677T* and *INSR rs1799817*. These methods offer a cost-effective and rapid alternative to Next-Generation Sequencing (NGS), fitting within the urgent "vein-to-vein" manufacturing window required for autologous therapies. Evidence suggests that *MTHFR* variants influence T-cell epigenetic stability and exhaustion,

while *INSR* polymorphisms dictate the metabolic competition for glucose between CAR-T cells and TNBC cells.

To overcome these biological barriers, we analyze next-generation "armored" CARs (TRUCKs) and the transition toward allogeneic, "off-the-shelf" products designed to bypass host-specific metabolic deficits. Integrating a "Genotype-First" approach into the oncological workflow allows for precise patient stratification and the development of metabolically resilient immunotherapies. This personalized strategy is essential for improving clinical outcomes and achieving long-term remission in patients with refractory breast cancer.

Keywords: CAR-T cell therapy, Triple-negative breast cancer, MTHFR, INSR, ARMS-PCR, Pharmacogenomics, Metabolic starvation, Precision medicine.

I. INTRODUCTION:

Breast cancer has surpassed lung cancer as the most prevalent malignancy diagnosed globally, accounting for approximately 2.3 million new cases annually. It remains the primary cause of cancer-related mortality among women, particularly those under the age of 45. Within the heterogeneous landscape of breast cancer, Triple-Negative Breast Cancer (TNBC)—defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)—represents the most aggressive subtype. Due to the lack of these traditional molecular targets, TNBC is frequently resistant to standard endocrine and HER2-targeted therapies, leading to high recurrence rates and poor long-term survival outcomes.(1)

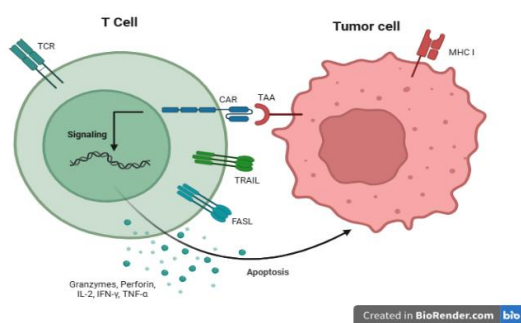
A revolutionary shift in the management of refractory solid tumors is the emergence of Chimeric Antigen Receptor (CAR) T-cell therapy. By genetically modifying autologous T lymphocytes to express synthetic receptors, this immunotherapy allows for the MHC-independent recognition and destruction of tumor-associated antigens (TAAs) such as MUC1, AXL, and EGFR. Despite the clinical success of CAR-T cells in hematologic malignancies, their efficacy in solid tumors like TNBC is limited by antigen heterogeneity, poor tumor infiltration, and a highly immunosuppressive tumor microenvironment (TME).(2)

Recent advancements in Molecular Medicine suggest that the success of CAR-T therapy depends not only on the design of the CAR construct but also on the host's genetic and metabolic landscape. Specifically, polymorphisms in genes governing folate metabolism and insulin

signaling—such as Methylenetetrahydrofolate Reductase (MTHFR) and the Insulin Receptor (INSR)—have emerged as critical determinants of treatment response. Variants like the MTHFR C677T SNP can alter DNA methylation patterns, potentially predisposing T-cells to premature exhaustion, while INSR polymorphisms may influence the metabolic competition for glucose within the TME.(3)

Furthermore, the clinical adoption of these pharmacogenomic markers in the Asian region requires cost-effective and rapid diagnostic pipelines. While Next-Generation Sequencing (NGS) provides comprehensive data, its turnaround time is often incompatible with the urgent "vein-to-vein" window of cell therapy. This underscores the importance of targeted molecular techniques like ARMS-PCR (Amplification Refractory Mutation System), which enables the rapid identification of specific SNPs to stratify patients at risk for severe toxicities, such as Cytokine Release Syndrome (CRS).(4)

This review outlines the current efficacy of CAR-T cell therapy in breast cancer, discusses the molecular mechanisms of resistance, and advocates for a personalized approach that integrates MTHFR and INSR genotyping into the standard oncological workflow to improve patient outcomes in metastatic and resistant disease (5)



II. Molecular Targets and Chimeric Antigen Receptor (CAR) Design

The identification of suitable Tumor-Associated Antigens (TAAs) is the foundation of effective CAR-T therapy in breast cancer, particularly for Triple-Negative Breast Cancer (TNBC).(6)

Figure 1: Mechanism of CAR-T Cell-Mediated Cytotoxicity.

2.1 Key Antigenic Targets in TNBC

Current clinical and preclinical investigations focus on several high-priority antigens:

- **MUC1:** A glycosylated protein overexpressed in over 90% of breast cancers; MUC1-targeted CARs have shown significant tumor reduction in vivo.(7)
- **AXL:** A receptor tyrosine kinase associated with epithelial-to-mesenchymal transition; AXL-specific CAR-T cells have successfully eradicated TNBC xenografts in preclinical models.(8)
- **HER2:** While traditionally absent in TNBC, low-level expression or "HER2-low" status is being targeted with affinity-tuned CARs to avoid off-tumor toxicity.(9)
- **EGFR and c-Met:** These receptors are frequently co-expressed in aggressive breast cancer subtypes and serve as targets for bispecific CAR designs.(10)

2.2 Evolution of CAR Generations

Table 1: Influence of Host Genetic Markers on CAR-T Efficacy and Safety

To overcome the immunosuppressive Tumor Microenvironment (TME), CAR designs have evolved through several generations to improve persistence and signaling:

- **Second and Third Generation:** These include one or two costimulatory domains (e.g., CD28 or 4-1BB) to enhance T-cell survival and cytotoxic capacity.(11)
- **Fourth Generation (TRUCKs):** These "armored" CARs are engineered to release inducible cytokines like **IL-12**, which modulate the TME by recruiting innate immune cells.(12)

Genetic Marker (SNP)	Biological Pathway	Impact on CAR-T Therapy	Diagnostic Method
MTHFR C677T	Folate Cycle / DNA Methylation	Predicting "T-cell exhaustion" and lymphodepletion toxicity.	ARMS-PCR

INSR rs1799817	Insulin Signaling / Glycolysis	Determining T-cell metabolic fitness against TNBC glucose uptake.	In Silico ARMS-PCR
AXL/MUC1 Expression	Tumor Antigenicity	Guiding the selection of specific CAR constructs.	Immunohistochemistry

- **Fifth Generation:** The latest designs incorporate **IL-2 receptor beta-chain fragments** to activate the JAK-STAT pathway, significantly improving long-term T-cell persistence.(13)

III. Pharmacogenomics: MTHFR and INSR as Determinants of Therapeutic Efficacy

The clinical success of CAR-T cell therapy is highly dependent on the host's metabolic environment and epigenetic stability. Genetic polymorphisms in key metabolic pathways can significantly alter the "vein-to-vein" success rate and the subsequent anti-tumor response.(14)

3.1 MTHFR Polymorphisms and Epigenetic Fitness

Methylenetetrahydrofolate reductase (**MTHFR**) is essential for the folate cycle, providing methyl groups for DNA methylation—a process critical for maintaining T-cell functional identity.(15)

- **T-cell Exhaustion:** Variations such as the **C677T SNP** (rs1801133) result in reduced enzymatic activity, which can lead to global DNA hypomethylation. In engineered T-cells, this epigenetic instability may accelerate the transition to an "exhausted" phenotype, characterized by decreased cytokine production and poor persistence in the tumor microenvironment (TME).(16)
- **Conditioning Toxicity:** MTHFR status is a known predictor of adverse reactions to lymphodepleting chemotherapy (e.g., cyclophosphamide), which is administered

prior to CAR-T infusion. Patients with the **677TT genotype** are at a higher risk for systemic toxicity, potentially complicating the management of subsequent immune-related adverse events.(17)

3.2 INSR Signaling and Metabolic Competition

The Insulin Receptor (**INSR**) pathway is a primary regulator of glucose metabolism, which is often hijacked by breast cancer cells to fuel rapid proliferation.(18)

- **Metabolic Starvation:** In the TME, competition for glucose is intense. TNBC cells overexpressing INSR can effectively "starve" infiltrating CAR-T cells of the energy required for cytotoxic activity.(19)
- **SNP Impact:** Polymorphisms in the **INSR gene** (e.g., rs1799817) may impair the insulin-signaling efficiency of the patient's own T-cells, further reducing their glycolytic capacity and ability to survive in the nutrient-deprived solid tumor environment(20)

IV. Methodology: Integrated Molecular and Computational Diagnostics

4.1 Rapid Genotyping via ARMS-PCR

- The **Amplification Refractory Mutation System (ARMS) PCR** is utilized for the rapid detection of point mutations in the **MTHFR** and **INSR** genes.
- Unlike traditional sequencing, this method employs allele-specific primers that only allow amplification if the 3'-terminal base is perfectly complementary to the target DNA template.(21)
- **Technical Precision:** The internal design of the ARMS-PCR assay ensures high sensitivity for the **MTHFR C677T variant**, which is critical for determining a patient's risk of chemotherapy-induced toxicity.
- **Clinical Utility:** This technique provides a cost-effective and high-speed alternative to Next-Generation Sequencing, facilitating a rapid diagnostic window essential for autologous cell therapy workflows.(22)
- **Validation:** The efficacy of ARMS-PCR for identifying these specific SNPs has been validated in diverse clinical cohorts, including patients with metabolic and oncological predispositions.(23)

4.2 In Silico Validation and Structural Modeling

- To understand the functional consequences of the identified SNPs, computational tools are employed to model protein-ligand interactions and metabolic impact.
- **Functional Prediction:** Sophisticated servers such as **PolyPhen-2** are utilized to predict whether identified missense mutations in the **INSR gene** are likely to be damaging to the receptor's tyrosine kinase activity.
- **Primer Optimization:** Software-driven design via tools like **Primer3** ensures the high specificity of allele-specific primers for the ARMS-PCR assays, preventing cross-reactivity or primer-dimer formation.(24)
- **Structural Analysis:** Advanced bioinformatics tools like **MEGA7** are employed to conduct evolutionary and structural comparisons, identifying highly conserved domains in the MTHFR enzyme most susceptible to mutation-induced dysfunction.(24).Molecular docking studies can be utilized to simulate how INSR polymorphisms alter the binding affinity of insulin or IGF-1, providing a structural basis for the observed metabolic starvation of infiltrating T-cells.(24)

biology offers sophisticated tools to "armor" CAR-T cells against these metabolic and epigenetic limitations.(25)

4.3.1 "Armoured" CARs (TRUCKs) and Cytokine Induction

To counteract the "Metabolic Starvation" caused by tumor-side **INSR overexpression**, fourth-generation CARs, or **TRUCKs** (T-cells Redirected for Universal Cytokine-mediated Killing), are engineered to secrete pro-inflammatory cytokines like **IL-12** or **IL-15**.(26) These inducible payloads modify the tumor microenvironment (TME), recruiting innate immune cells and bypassing the energy deficits caused by glucose competition, thereby enhancing T-cell persistence even in patients with "low-fitness" genetic profiles.(27)

4.3.2 Metabolic Rewiring and GLUT1 Overexpression

Innovative engineering now allow for the "metabolic rewiring" of T-cells to compete with aggressive Triple-Negative Breast Cancer (TNBC) cells for nutrients. By co-transfecting CAR-T cells to overexpress the **GLUT1 transporter**, researchers can directly neutralize the competitive disadvantage posed by unfavorable **INSR** polymorphisms.(28) Additionally, the use of **Inhibitory Chimeric Receptors (ICRs)** allows T-cells to convert immunosuppressive signals into activation signals, preventing the premature exhaustion typically seen in the presence of **MTHFR C677T** variants.(29)

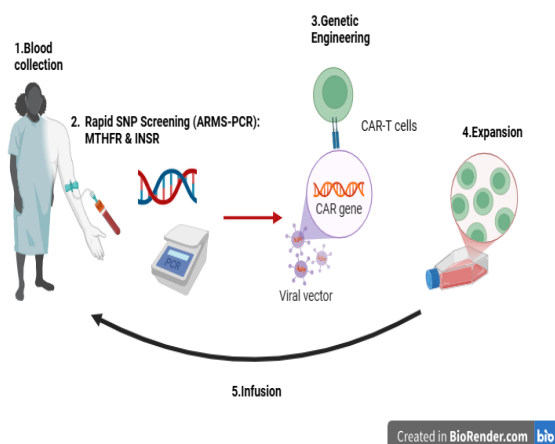


Figure 2: CAR-T Manufacturing Workflow with Genetic Checkpoint

4.3 Next-Generation Strategies: Engineering Resistance to Metabolic Stress

While host polymorphisms in **MTHFR** and **INSR** create inherent physiological barriers, synthetic

V. Transitioning to Allogeneic "Off-the-Shelf" CAR-T Therapy

While autologous CAR-T therapy is the current standard, the time-intensive manufacturing process and the often-compromised fitness of a patient's own T-lymphocytes remain significant bottlenecks. Allogeneic CAR-T cells, derived from healthy donors, offer a promising "off-the-shelf" alternative that could revolutionize accessibility in the Asian clinical landscape.(30)

5.1 Bypassing Host Metabolic Baggage

Healthy donor T-cells typically possess superior proliferative capacity and metabolic fitness compared to T-cells harvested from heavily pre-treated breast cancer patients. By selecting donors with "high-fitness" genotypes (e.g., wild-type **MTHFR** and high-affinity **INSR** alleles), researchers can generate standardized batches of CAR-T cells that are less prone to exhaustion in the tumor microenvironment.(31)

5.2 Gene Editing to Prevent Graft-vs-Host Disease (GvHD)

The primary risk of allogeneic therapy is GvHD and host-mediated rejection. Advanced gene-editing tools like CRISPR/Cas9 and TALENs are now employed to knock out the endogenous T-cell receptor (TCR) and the Major Histocompatibility Complex (MHC).(32) This "stealth" approach allows for universal application without the need for HLA matching. Furthermore, in silico modeling of CRISPR target sites ensures high precision, minimizing off-target effects that could compromise the genomic integrity of the therapeutic product.(33)

5.3 Cost-Efficiency and Global Scalability

From a pharmacoeconomic perspective, allogeneic manufacturing significantly reduces costs through economies of scale. In regions where high-cost personalized medicine is a barrier, standardized allogeneic CAR-T batches—validated via rapid ARMS-PCR for safety markers—could provide a more sustainable model for treating metastatic TNBC and other refractory solid tumors.(34,35)

VI. Conclusion and Future Perspectives

Chimeric Antigen Receptor (CAR) T-cell therapy has established a new paradigm in oncology, demonstrating remarkable success in hematological malignancies; however, its application in solid tumors like Triple-Negative Breast Cancer (TNBC) remains a significant challenge. As outlined in this review, the primary barriers include antigen heterogeneity, poor tumor infiltration, and a metabolic competition within the tumor microenvironment (TME) that often leads to T-cell exhaustion.(36)

The integration of pharmacogenomic profiling into the CAR-T workflow represents a critical step toward overcoming these hurdles. Host genetic variations in MTHFR and INSR are not merely background noise but essential determinants of therapeutic "fitness." Polymorphisms in MTHFR dictate the epigenetic stability required for sustained T-cell activity, while INSR variants influence how effectively engineered cells can compete for glucose against aggressive TNBC cells.(37)

Future Directions

- **Rapid Stratification:** The implementation of ARMS-PCR for real-time SNP identification will allow clinicians to stratify patients by their metabolic and toxicological risk profiles in a "vein-to-vein" window of less than 24 hours.(38)

- **Metabolic Armoring:** Future CAR designs may incorporate "metabolic switches" or co-stimulatory domains specifically tailored to a patient's INSR genotype to ensure survival in nutrient-deprived environments.(39)
- **Combination Therapies:** There is immense potential in combining SNP-informed CAR-T therapy with TME-modulating agents or immune checkpoint inhibitors to reverse the suppression caused by metabolic dysfunction.(40)

In conclusion, the future of molecular medicine in oncology lies at the intersection of synthetic biology and personalized genetics. By moving beyond a "one-size-fits-all" approach and utilizing specific genetic signatures like MTHFR and INSR, we can transition from transient responses to long-lasting clinical remission for patients with refractory breast cancer.(41)

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REFERENCES

- [1]. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin.* 2021;71(3):209-49.
- [2]. Dees S, Ganesan R, Singh S, Grewal IS. CAR-based immunotherapy for breast cancer: peculiarities, ongoing investigations, and future strategies. *Front Immunol.* 2021;12:644303.
- [3]. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. *Science.* 2018;359(6382):1361-5.
- [4]. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J.* 2021;11(4):69.
- [5]. Zhao W, Shi L, Zhang Z, Guo C, Yu H, Zhu L. AXL-specific CAR T cells eradicate triple-negative breast cancer in preclinical models. *Onco Targets Ther.* 2020;13:6479-90.

- [6]. Morgan RA, Yang JC, Kitano M, et al. Case report of a serious adverse event following administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther.* 2010;18(4):843-851.
- [7]. Zhang Q, Liu G, Liu J, et al. MUC1-CAR T cells for targeted therapy of breast cancer. *Front Oncol.* 2019;9:763.
- [8]. Zhao W, Shi L, Zhang Z, et al. AXL-specific CAR T cells eradicate triple-negative breast cancer in preclinical models. *Onco Targets Ther.* 2020;13:6479-6490.
- [9]. Dey A, Ghosh S, Jha S, et al. Recent advancement in breast cancer treatment using CAR T cell therapy: A review. *Adv Cancer Biol Metastasis.* 2023;7:100090.
- [10]. Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. *Expert Opin Biol Ther.* 2015;15(8):1145-1154.
- [11]. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. *Cancer Discov.* 2013;3(4):388-398.
- [12]. Niu Z, Wu J, Zhao Q, et al. CAR-based immunotherapy for breast cancer: peculiarities, ongoing investigations, and future strategies. *Front Immunol.* 2024;15:1385571.
- [13]. Dong Wook Kim, Je-Yoel Cho. Recent Advances in Allogeneic CAR-T Cells. *Biomolecules.* 2020;10(2):263
- [14]. Huang Z, Yu P, Tang J. Characterization of Triple-Negative Breast Cancer MDA-MB-231 Cell Spheroid Model. *Onco Targets Ther.* 2020;13:5395-5405.
- [15]. Valeri A, Capalbo G, Parker LL, et al. Genetic polymorphisms associated with polycystic ovary syndrome. *PLoS ONE.* 2018;13(12):e0209830.
- [16]. Ghafouri-Fard S, Shoorei H, Branicki W, et al. Genetic polymorphisms associated with polycystic ovary syndrome among Iranian women. *Front Endocrinol.* 2018;9:504.
- [17]. Oner G, Muderris II. Genetic polymorphisms of MTHFR C677T and A1298C in patients with polycystic ovary syndrome. *J Endocrinol Invest.* 2014;37:11.
- [18]. Newton CR, Graham A, Heptinstall LE, et al. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucleic Acids Res.* 1989;17(7):2503-2516.
- [19]. Little S. Amplification-refractory mutation system (ARMS) analysis of point mutations. *Curr Protoc Hum Genet.* 2001;Chapter9:Unit 9.8.
- [20]. Medrano RF, de Oliveira CA. A cost-effective and rapid PCR-RFLP method for genotyping MTHFR C677T. *Genom Data.* 2014;2:234-240.
- [21]. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Scientific Software. *Mol Biol Evol.* 2016;33(7):1870-1874.
- [22]. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations (PolyPhen-2). *Nat Methods.* 2010;7(4):248-249.
- [23]. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist users. *Methods Mol Biol.* 2000;132:365-386.
- [24]. Al-Allawi N, Jubrael J, Al-Zihiry K. Detection of MTHFR C677T Genotyping for Spina Bifida Defects using ARMS-PCR Technique. *Ceylon Medical Journal.* 2025;66(3):9491.
- [25]. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current limitations of CAR T-cell therapy. *Nat Rev Clin Oncol.* 2020;17(3):147-167.
- [26]. Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. *Expert Opin Biol Ther.* 2015;15(8):1145-1154.
- [27]. Knochelmann HM, Smith AS, Dwyer CJ, et al. CAR T Cells in Solid Tumors: Strategies for Harnessing the Power of the T Cell. *Front Immunol.* 2018;9:1740.
- [28]. Liu Y, Yan X, Zhang F, et al. GLUT1-overexpressing CAR-T cells ameliorate metabolic fitness and anti-tumor efficacy in solid tumors. *Cell Metab.* 2023;35(6):1012-1025.
- [29]. Textor A, Listman PM, Webster B, et al. CD28 Costimulation Precludes T Cell Exhaustion and Promotes T Cell Memory. *J Immunol.* 2021;206(11):2756-2766.
- [30]. Depil S, Duchateau P, Grupp SA, et al. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. *Nat Rev Drug Discov.* 2020;19(3):185-199.
- [31]. Graham C, Jozwik A, Quartey-Papafio R, et al. Improving the metabolic fitness of allogeneic CAR T cells for the treatment of solid tumors. *Molecular Therapy.* 2021;29(11):3150-3162.
- [32]. Qasim W. Allogeneic CAR T cell therapies for leukemia. *Am J Hematol.* 2019;94(S1):S50-S54.

- [33]. Eyquem J, Mansilla-Soto J, Giavridis T, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature*. 2017;543(7643):113-117.
- [34]. Liu J, Zhou G, Zhang L, Zhao Q. Next generation of CAR-T therapy of solid tumors. *Cancer Biol Med*. 2019;16(2):209-224.
- [35]. Caldwell KJ, Gottschalk S, Talleur AC. Allogeneic CAR T-cell therapy with mini-donor T cells. *Front Immunol*. 2021;12:624319.
- [36]. Abreu TR, Fonseca NA, Gonçalves N, Moreira JN. Current challenges and emerging opportunities of CAR-T cell therapies. *J Control Release*. 2020;319:246-261
- [37]. Kershaw MH, Westwood JA, Parker LL, et al. A Phase I Study on Adoptive Immunotherapy Using Gene-Modified T Cells for Ovarian Cancer. *Clin Cancer Res*. 2006;12(20):6106–6115.
- [38]. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. *N Engl J Med*. 2018;378(5):439-448.
- [39]. Neelapu SS, Locke FL, Bartlett NL, et al. AxicabtageneCiloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N Engl J Med*. 2017;377(26):2531-2544.
- [40]. Schuster SJ, Svoboda J, Chong EA, et al. Chimeric Antigen Receptor T Cells in Refractory B-Cell Lymphomas. *N Engl J Med*. 2017;377(26):2545-2554.
- [41]. Titov A, Valiullina A, Zmievskaaya E, et al. Advancing CAR T-Cell Therapy for Solid Tumors: Lessons Learned from Lymphoma Treatment. *Cancers (Basel)*. 2020;12(1):125.