Application of Nano-Delivery Technology in Tumor Immunotherapy

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Abstract. Immunotherapy has made great progress in cancer treatment, showing amazing results in clinical applications. However, a number of factors contribute to a lower response to immunotherapy in some cancer patients. T cells play an important role in tumor immune response. However, it is difficult to achieve the directed infiltration of T cells by current methods. In addition, the expression of non-specific drugs in normal tissues may cause serious adverse reactions. Now a new strategy is needed to increase the infiltration of T cells, increase the specificity of drug expression, improve the therapeutic effect and reduce the risk of adverse reactions. Nanocarriers can be enriched in tumor tissues through a series of effects, and tumor-specific genes can ensure that drugs will not work in normal tissues, so nanoparticles can be used to encapsulate tumor-specific gene nanocarriers to achieve precise treatment. In this paper, the types of nanocarriers and gene drugs and the application of this technology in practical research are introduced in detail. Finally, the prospect and challenge of future development are expressed.

Keywords: Cancer immunotherapy; Nano-carrier; Gene nanomedicine; PD-1/PD-L1; T cell infiltration.

1. Introduction

Cancer remains the leading cause of death worldwide. Traditional treatment methods such as surgery, chemotherapy, and radiotherapy often have significant limitations and side effects. However, in recent years, cancer immunotherapy has emerged as a breakthrough approach, which mobilizes the body's own immune system to fight tumors, completely transforming the landscape of cancer treatment. This method has demonstrated remarkable efficacy in clinical applications, bringing new hope to cancer patients.

Despite the progress made in cancer immunotherapy, it still faces many challenges. Although current antibody drugs have made key advancements in immunotherapy, some patients do not respond well to this approach. The main reason is that T cells cannot effectively infiltrate cancer cells, resulting in a weak immune response. Additionally, the non-specific expression of drugs in normal tissues may cause severe adverse reactions, which could affect treatment efficacy and patient safety.

To address these challenges, drug delivery systems based on nanotechnology have received extensive attention. Nanocarriers can be designed to accumulate in tumor tissues through various effects, such as the enhanced permeability and retention (EPR) effect. Moreover, by coupling drug expression genes with tumor-specific promoters, drug expression can be restricted to tumor tissues, minimizing side effects. Encapsulating tumor-specific gene nanocarriers in nanoparticles enables precise treatment, enhances therapeutic efficacy, and reduces the risk of adverse events.

This article delves into various types of nanocarriers and gene drugs, exploring their applications in cancer immunotherapy research. It highlights how nanotechnology promotes the targeted delivery of gene drugs and the recruitment and activation of immune cells in the tumor microenvironment. Additionally, the article discusses the broad applicability of this strategy in various cancer models, demonstrating its potential to overcome the limitations of traditional immunotherapy. Finally, it provides insights into the future prospects and challenges of nanotechnology in cancer immunotherapy, aiming to offer researchers a comprehensive perspective and contribute to the development of this promising field.

2. Research Methods and Research Objectives

This review will systematically search for relevant literature published in the past five years, including high-impact journals such as Nature, Science, and Cell, as well as databases like PubMed and Web of Science. It will focus on the current status, challenges, and future directions of nanodelivery technology in cancer immunotherapy. Specifically, it will cover the following aspects: The basic principles of nanodelivery technology and its application in cancer immunotherapy. The mechanism of action of nanoparticles in the tumor microenvironment, including the regulation of T-cell infiltration and activation.The progress of nanodelivery technology in preclinical and clinical studies.

Current challenges, such as the biodistribution of nanoparticles, tumor-specific targeting, and the regulation of immune responses.Future development directions, including the research and development of novel nanomaterials, exploration of combination therapy strategies, and the realization of personalized treatment.

The objective of this review is to summarize the research progress of nanodelivery technology in cancer immunotherapy, analyze the current challenges, and explore future development directions. By systematically reviewing relevant literature, it aims to provide researchers with a comprehensive perspective to promote the further development of nanodelivery technology in cancer immunotherapy.

3. Organization of the Text

3.1. Cancer Immunotherapy and Immune Checkpoint Inhibiors (ICIs)

Tumor immunotherapy is a major breakthrough in the field of cancer therapy in recent years. It harnesses the body's own immune system to fight cancer, changing the landscape of traditional cancer treatments. Beginning in the 1980s, scientists began exploring ways to harness the immune system to treat cancer. In recent years, with the in-depth understanding of the function of immune cells (such as T cells, B cells, etc.), tumor immunotherapy has gradually become a research hotspot. The 2018 Nobel Prize in Physiology or Medicine awarded to James Allison and Koshi Tsuda for their pioneering work in the field of immunotherapy gave a further boost to the field[1].

Checkpoint inhibitors are a type of treatment designed to unblock the suppressive effect of tumor cells on the immune system. Cancer cells often evade attack by the immune system by expressing certain "checkpoint" molecules. Checkpoint inhibitors restore the immune system's ability to attack cancer cells by blocking these molecules. The main checkpoint molecules include PD-1, PD-L1[2,3].

During immunomodulation, antigenic presenting cells (APCs) interact with CD28 and CTLA-4 on T cells through B7-1 (CD80) and B7-2 (CD86) molecules on their surface, thereby affecting T cell activation and function. MHC Class I molecules on the APC surface bind to T cell receptors (TCR) to provide specific antigen recognition signals, while B7 molecules bind to CD28 or CTLA-4 to provide co-stimulatory or co-inhibitory signals. After binding with B7, CD28 transmits stimulus signals through CD3 and CD8 molecules, promoting the activation and proliferation of T cells and the secretion of IL-2 and other cytokines, and enhancing immune response. In contrast, CTLA-4 binds to the B7 molecule and transmits inhibitory signals, leading to T cell impotence or apoptosis, reducing IL-2 production, and thus suppressing the immune response. Clinically, CTLA-4 blocking antibodies (such as ipilimumab) can block the binding of CTLA-4 and B7 molecules, enhance the activation and proliferation of T cells, improve the anti-tumor immunity of the body, and provide a theoretical basis for immune checkpoint inhibitor therapy.

In the tumor immune escape mechanism, tumor cells bind to PD-1 on the surface of cytotoxic T cells by expressing PD-L1 or PD-L2, transmitting inhibitory signals, leading to T cell depletion and reduced cytotoxicity. By blocking this interaction, PD-1-blocking antibodies (such as nabuliumab, Pembrolizumab) and PD-L1 blocking antibodies (such as attezumab) unblock the inhibitory signal and restore the killing ability and cytokine secretion function of T cells. Specifically, the PD-1 antibody prevents its interaction with PD-L1/L2 by binding to PD-1, while the PD-L1 antibody blocks

its interaction with PD-1 by binding to PD-L1 on the surface of tumor cells. This intervention can significantly enhance the cytotoxicity of T cells and increase the production of cytokines, so as to effectively inhibit tumor growth, providing an important basis for the application of immune checkpoint inhibitors in tumor therapy[4].

3.1.1 Adoptive Cell Therapy(ACT)

Adoptive cell transfer therapy (ACT) is a treatment in which T cells from a patient are extracted, expanded and activated in vitro, and then injected back into the patient. The function of T cells in patients can be rebuilt, and the specific recognition and immune response of T cells to tumor cells can be enhanced, so as to fight against primary and metastatic tumors, which is a promising immunotherapy[5]. Among them, CAR-T is the most popular way in clinical treatment[6]. The theory of CAR-T is using genetic engineering method that modifies CAR molecules onto T cells, then they can specifically recognize tumor cells and play an anti-tumor role[7]. First, blood is drawn from the patient and T cells are isolated. These T cells are a type of white blood cell in the immune system that has the ability to recognize and attack abnormal cells, including cancer cells. Next, a gene encoding a chimeric antigen receptor (CAR) is inserted into the T cell's genome using an engineered virus (usually a lentivirus or a retrovirus) as a carrier. This process allows T cells to acquire the ability to recognize antigens on the surface of specific cancer cells. The surface of T cells is genetically modified to express cars that can specifically recognize specific antigens on the surface of cancer cells. At this point, the T cells have transformed into CAR-T cells with anti-cancer capabilities. In the laboratory, the modified CAR-T cells will be massively expanded to reach the number of cells needed for treatment. These amplified CAR-T cells were then re-injected into the patient. The CAR-T cells injected back into the patient will circulate in the body, and once they encounter cancer cells expressing a specific antigen, the CAR will bind to them, activate the T cells, cause them to proliferate and release cytotoxic substances, thus killing the cancer cells[8].

3.2. Gene Medicine and its combination with nano technology

Gene-based medicine is The target gene with a specific sequence is delivered to a specific target cell through a carrier, and is properly expressed to produce a drug that interferes with or regulates the function of the gene, so as to treat the disease[9]. Usually, it includes plasma, RNAi, CRISPR Cas9 and so on. In the field of gene editing, CRISPR-CAS9 technology occupies an important position. This technique utilizes plasmid DNA, which contains the Cas9 protein and sgRNA encoding, to be transcribed into Cas9 mRNA and sgRNA within the cell. These molecules then enter the nucleus, where the Cas9 protein binds to the sgRNA to form a complex with gene-editing capabilities. The complex is able to accurately identify specific PAM sequences on genomic DNA and guide the Cas9 protein to cut double-stranded DNA there. Cleaved DNA double-strand breaks can be repaired by two main pathways: nonhomologous end joining (NHEJ) and homologous directed repair (HDR). The NHEJ pathway usually occurs at any stage of the cell cycle and is repaired by directly connecting broken DNA ends, but this process is prone to introducing base insertion or deletion, resulting in gene inactivation. The HDR pathway relies on the presence of homologous sequences and is usually active in the S and G2 phases of the cell cycle, enabling precise repair of DNA double-strand breaks and precise editing of genes, such as gene knock-in or correction of mutated genes.

Gene addition strategies are mainly implemented by introducing therapeutic genes into cells in the form of mRNA or plasmid DNA. In the case of mRNA, it is translated directly into proteins in the cytoplasm without entering the nucleus, a process that is fast and efficient and suitable for rapid replenishment of missing proteins in the cell. Plasmid DNA must first enter the nucleus, where it is transcribed into mRNA, and then the mRNA is transported to the cytoplasm for translation. This pathway, although relatively slow, is capable of sustained expression of therapeutic proteins and is suitable for situations where long-term protein supplementation is required. Both methods have their own advantages, and the appropriate form of gene addition is selected according to the specific disease and treatment needs.

Gene suppression mainly relies on small RNA molecules such as shRNA and siRNA. Shrnas are usually expressed by plasmid DNA, while siRNA can be introduced directly into cells. These small RNA molecules are processed by Dicer enzymes in the cytoplasm to cut into siRNA with a specific structure, which is subsequently integrated into the RNA-induced silencing complex (RISC). siRNA in RISC can specifically recognize and bind to complementary sequences of target MRnas, and then guide RISC to degrade target mrnas, thereby inhibiting the expression of specific genes. This mechanism has important application value in regulating gene expression and treating diseases caused by gene over expression[10].

3.2.1 Tumor-specific Gene-based medicine

Tumor immunotherapy drugs have non-specific distribution in normal tissues, which carries certain risks. On the one hand, it may hinder the directional infiltration of T cells into the tumor site; on the other hand, it may activate T cells that were originally in a resting state in normal tissues, thereby causing adverse reactions. To solve this problem, we can insert tumor-specific promotors in the gene sequence.

Tumor-specific promoters are active only in specific types of cancer cells and their activity varies greatly in different tumors. Their key feature is that they have little or no activity in normal cells, which ensures their specificity for malignant tumor processes, but they are not specific for specific tissues because they are activated by the tumor microenvironment[11].

3.3. Nano Drug Delivery System

3.3.1 The Classification of Nanoparticle

With the rapid development of the nano technology, there are several mature nanoparticles now. Lipid-based nanoparticle, include Liposome、 Lipid nanoparticle(LNP) and Nanoemulsion（NE）,etc. It can contain water-soluble drugs. Inorganic Nanoparticles, like Metal nanoparticles, silicon nanoparticles, etc., usually have excellent physical and chemical properties. Polymeric Nanoparticles, Polymer nanoparticles are nanocarriers made of synthetic or natural polymers with high tunability and diverse functionalities. More details can be found in Table 1.

Table 1 Classification of nanoparticles

Category	Polymer Nanoparticle	Lipid nanoparticles	Inorganic nanoparticle
Materials	● Polyethylene glycol (PEG) ● polylactic acid (PLA) ● polylactic acid-glycolic acid copolymer (PLGA)	● Ionizable lipids ● Cholesterol ● Auxiliary lipids polyethylene glycol (PEG) lipids	● Silica ● Gold ● magnetic nanoparticles
Particle Size	50-300 nm	20-200 nm	10-500 nm
Advantage	● Good biocompatibility ● High drug load ● sustained release	● Simple formula ● Large drug load ● Low drug degradation	● High stability ● Low production cost ● Efficient delivery of mRNA
Targeting	● Cell targeting through surface modification targeting molecules (such as RGD sequence) ● Tissue targeting can be achieved through particle size and surface charge optimization	● Cell targeting through surface modification targeting molecules; ● Tissue targeting can be achieved by optimizing the size and surface charge of liposomes	● Cell targeting through surface modification targeting molecules ● Magnetic targeted therapy can be achieved through magnetic nanoparticles

3.3.2 Cationic lipid-assisted PEG-b-PLA nanoparticles

Our lab team designed a nanoparticle calles Cationic Lipid Assisted PEG-b-PLA Nanoparticles (CLANs). CLANs are a nanoparticle carrier with nanoparticles used in nucleic acid therapy. It consists of cationic lipid and PEG-b-PLA polymer, prepared by double emulsification method, which can efficiently wrap and protect nucleic acid molecules, such as siRNA, miRNA and CRISPR-Cas9, avoid their degradation in vivo, and promote their release and expression in target cells[12] (Fig 1). CLANs granules have good biocompatibility and scalability, and can be used in the treatment of a variety of diseases, including cancer, cardiovascular disease, immune-related diseases and gene editing therapy.

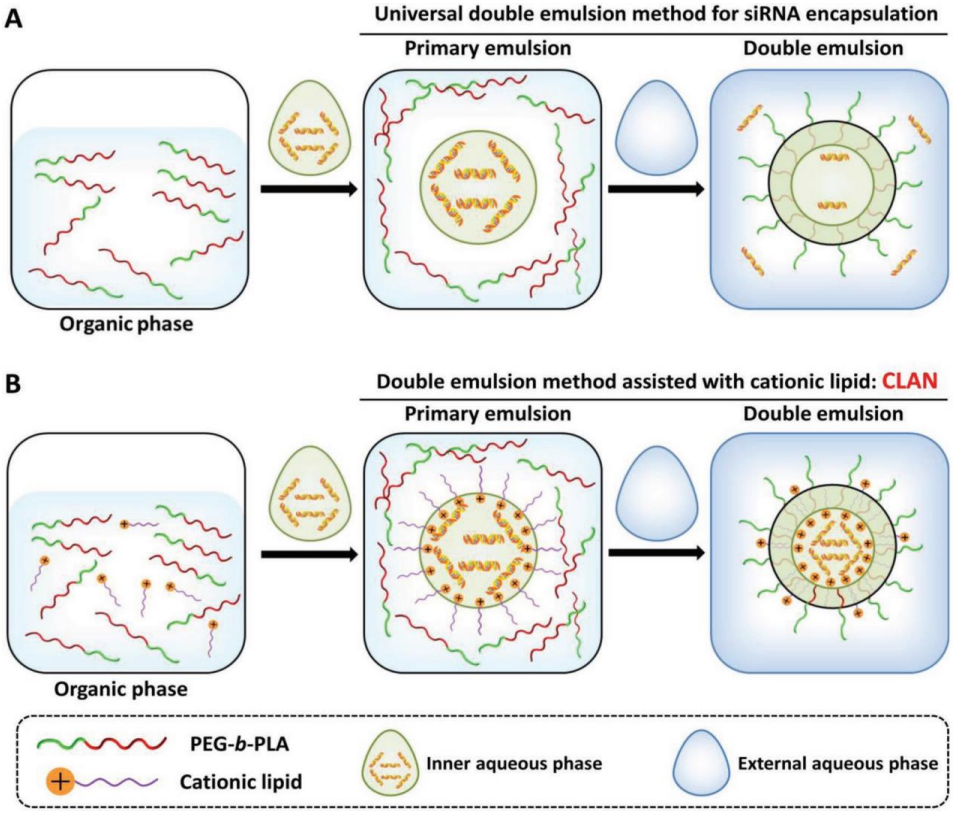

Figure 1 A The Universal double emulsion method and B CLAN wraps nucleic acid molecules[12]

3.4. Nanocarriers for delivery of nucleic acid drugs

Nanocarriers can deliver gene editing tools such as the CRISPR-Cas9 system to edit and regulate specific genes for the treatment of genetic diseases and cancer. Nanocarriers can deliver mRNA vaccines, such as the COVID-19 mRNA vaccine, to enhance the immunogenicity of the vaccine b.0y improving the stability of mRNA and the efficiency of cellular uptake. Nanocarriers can deliver nucleic acid drugs such as siRNA and miRNA to treat cancer by inhibiting the expression of tumor-related genes and inducing apoptosis of tumor cells [10].

3.5. Nanotechnology and cancer immunotherapy combine to improve tumor immune efficacy

Wang Y. et al express the usage of nano technology and how it improves the effort of cancer immunotherapy in article “Engineering tumor-specific gene nanomedicine to recruit and activate T cells for enhanced immunotherapy”

3.5.1 Nanotechnology delivers targeted gene drugs to enhance recruitment and activation of immune cells

The research team designed a tumor-specific gene-nanodrug (NPTyr−C9AP) to encapsulate a plasmid that co-expresses CXCL9 (T cell chemokine) and αPD-L1 (PD-L1 blocker) via pegyl-polylactic glycolic acid (PEG-PLGA) and cationic liposome (DOTAP)(Fig.2). The nanoparticle

utilizes tyrosinase (Tyr) or survivin (Sur) promoters to achieve specific expression of the gene in melanoma or multiple tumor cells. The experiment shows that:

Targeting and safety: Nanoparticles are enriched in tumor tissues through enhanced osmotic retention (EPR) effect, and the promoter-driven expression system is activated only within tumor cells, significantly reducing non-specific expression in normal tissues.

Synergistic effect: CXCL9 formed a chemotactic gradient and recruited a large number of CD8+ T cells to infiltrate the tumor. At the same time, αPD-L1 blocks the PD-L1/PD-1 pathway and unactivates T cell inhibition signals. Together, the number of CD8+ T cells in the melanoma model increased to 5.1 times that of the control group, and the expression of cytotoxic markers (granase B and perforin) was significantly enhanced.

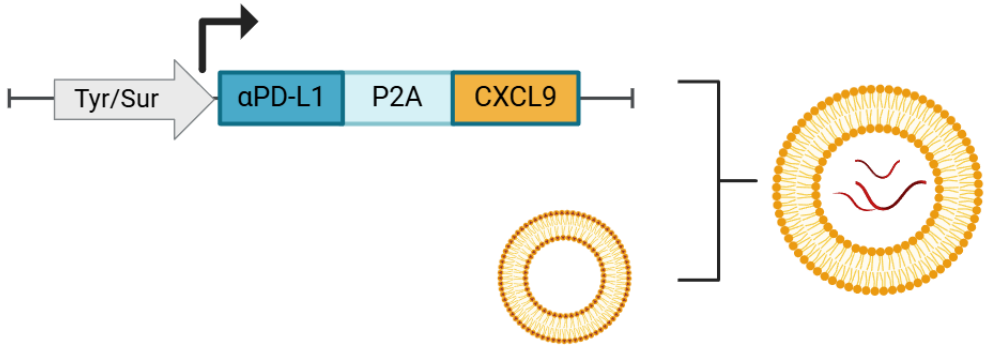

Figure 2 The process of making the nanomedicine.

Broad-spectrum applications: By replacing promoters such as Sur, the strategy was equally effective in colorectal cancer (CT26), pancreatic cancer (Panc02), and breast cancer (4T1) models, demonstrating the scalability of nanotechnology.

3.5.2 Overcoming the bottleneck of traditional immunotherapy

Nanotechnology can significantly enhance the anti-tumor function by regulating the dynamic balance of immune cells in the tumor microenvironment. In this paper, the nanocarrier-mediated CXCL9 secretion can efficiently recruit T cells to the tumor site, and αPD-L1 can release T cell inhibition by blocking the PD-L1/PD-1 pathway. Experimental data showed that after NPTyr−C9AP treatment, the amount of CD8+ T cell infiltration in melanoma increased to 5.1 times that of the control group, the expression of activation marker CD69 was significantly upregulated, and the secretion of cytotoxic molecules (granase B, perforin) and effector cytokines (IFN-γ) was enhanced. In addition, nanotherapy reshaped the immunosuppressive microenvironment: the proportion of regulatory T cells (Tregs) and myeloid derived suppressor cells (MDSCs) decreased, and the proportion of M1-type macrophages increased. This pleiotropic regulation suggests that nanotechnology can not only enhance the function of immune cells, but also synergically reverse the immune escape mechanism of tumors.

4. Conclusion

Cancer immunotherapy has revolutionized oncology by harnessing the body's immune system to combat malignancies, yet challenges such as insufficient T-cell infiltration, off-target toxicity, and immunosuppressive microenvironments persist. This review highlights how nanotechnology-driven gene delivery systems offer a transformative strategy to address these limitations. By encapsulating tumor-specific gene drugs within nanocarriers, researchers can achieve precise, localized drug expression in tumor tissues while minimizing systemic side effects. Key innovations, such as cationic lipid-assisted PEG-PLGA nanoparticles (CLANs) and promoter-driven nanomedicines (e.g., $NP_{Tyr–C9AP}$), demonstrate enhanced T-cell recruitment, immune checkpoint blockade, and remodeling of the tumor microenvironment. These advancements not only amplify therapeutic efficacy but also exhibit broad applicability across diverse cancer models.

The integration of nanotechnology with immunotherapy presents a dual advantage: spatial-temporal control over drug release and synergistic modulation of immune cell dynamics. However, challenges remain in optimizing nanoparticle biodistribution, scaling up production, and ensuring long-term safety. Future efforts should focus on developing multifunctional nanomaterials, refining tumor-specific targeting mechanisms, and advancing combinatorial strategies (e.g., coupling gene editing with immune modulation). Clinical translation will require rigorous validation of biocompatibility and efficacy in human trials, alongside efforts to standardize regulatory frameworks for nanomedicine.

In conclusion, nanotechnology holds immense potential to overcome the bottlenecks of traditional immunotherapy, paving the way for next-generation precision therapies. By bridging the gap between genetic engineering and immune modulation, this interdisciplinary approach promises to redefine cancer treatment paradigms, ultimately improving patient outcomes and quality of life.

References

[1] Dobosz P, Dzieciatkowski T. The Intriguing History of Cancer Immunotherapy. Front Immunol, 2019, 10: 2965.

[2] Manso T, Kushwaha A, Abdoliahi N, et al. Mechanisms of action of monoclonal antibodies in oncology integrated in IMGT/mAb-DB. Front Immunol, 2023, 14: 1129323.

[3] Shuptrine C W, Surana R, Weiner L M. Monoclonal antibodies for the treatment of cancer. Semin Cancer Biol, 2012, 22(1): 3-13.

[4] Goodman A, Patel S P, Kurzrock R. PD-1-PD-L1 immune-checkpoint blockade in B-cell lymphomas. Nat Rev Clin Oncol, 2017, 14(4): 203-20.

[5] Bashor C J, Hilton I B, Bandukwala H, et al. Engineering the next generation of cell-based therapeutics. Nat Rev Drug Discov, 2022, 21(9): 655-75.

[6] Pan K, Farrukh H, Chittepu V, et al. CAR race to cancer immunotherapy: from CAR T, CAR NK to CAR macrophage therapy. J Exp Clin Cancer Res, 2022, 41(1): 119.

[7] June C H, O'connor R S, Kawalekar O U, et al. CAR T cell immunotherapy for human cancer. Science, 2018, 359(6382): 1361-5.

[8] Choudhery M S, Arif T, Mahmood R, et al. CAR-T-Cell-Based Cancer Immunotherapies: Potentials, Limitations, and Future Prospects. J Clin Med, 2024, 13(11).

[9] Dunbar C E, High K A, Joung J K, et al. Gene therapy comes of age. Science, 2018, 359(6372).

[10] Mendes B B, Conniot J, Avital A, et al. Nanodelivery of nucleic acids. Nat Rev Methods Primers, 2022,2.

[11] Montano-Samaniego M, Bravo-Estupinan D M, Mendez-Guerrero O, et al. Strategies for Targeting Gene Therapy in Cancer Cells with Tumor-Specific Promoters. Front Oncol, 2020, 10: 605380.

[12] Xu C F, Iqbal S, Shen S, et al. Development of "CLAN" Nanomedicine for Nucleic Acid Therapeutics. Small, 2019, 15(16): e1900055.