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Engineering Strategies And Therapeutic Applications Of In Vivo Anti-Tumor Gene Delivery Platforms

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Abstract. Gene therapy for cancer has received extensive attention in recent years. Adeno-associated virus (AAV), messenger RNA delivered by lipid nanoparticles (mRNA-LNP), and oncolytic viruses (OVs) are the three most actively studied delivery platforms. The three have different advantages in anti-tumor factor expression, immune activation, and targeting ability. Current research has made positive progress in animal models and preclinical experiments, but there are still obvious challenges in delivery efficiency, targeting control, and safety. This article systematically analyzes the engineering optimization strategies of the three types of gene therapy, including capsid modification and promoter regulation of AAV, lipid screening and surface engineering of mRNA-LNP, and targeted diffusion mechanism and immune enhancement design of OVs. The results show that these delivery platforms can achieve more efficient tumor targeting and therapeutic gene expression through engineering means. The study provides comprehensive theoretical support and technical reference for future cancer gene therapy, but problems such as off-target effects, immunogenicity, and clinical transformation efficiency still need to be solved. Future work can focus on the construction of individualized delivery platforms and multi-therapy synergistic strategies to achieve more precise, safe, and broad-spectrum tumor treatment effects.

Keywords: Gene Therapy Vectors; AAV (Adeno-associated Virus); mRNA-Lipid Nanoparticles (LNPs); Oncolytic Viruses (OVs); Cancer Immunotherapy.

1. Introduction

Cancer has always been an important topic that biological researchers around the world have been committed to tackling. At present, research in related fields has been committed to finding chemotherapy drugs that are less harmful to the body but more effective. With the increasing maturity of in vivo delivery vectors and therapeutic protein technologies, researchers have targeted the delivery of highly specific drugs designed to cancer cells to ensure that the drugs only kill cancer cells and minimize the negative effects of treatment on the body.

Currently, there are three common vectors used for in vivo anti-tumor treatment: adeno-associated virus (AAV), lipid nanoparticles (LNP)-mRNA and oncolytic viruses (OVs) [1-3]. Gene therapy using AAV as a vector has been widely used in the clinical stage to treat some diseases caused by single gene mutations [1]. In the experiments that have been carried out so far, the use of AAV to deliver anti-angiogenic factors (sFlt1, 3TSR), suicide genes (HSV-TK), immune factors (IL-12), etc. has significantly inhibited tumor growth in various tumor models (liver cancer, glioma, ovarian cancer, etc.) [4-7].

mRNA cancer immunotherapy can reshape the tumor microenvironment (TME) through mRNA expression and programmed cell death protein-1/ligand-1 (PD-1/PD-L1) antibodies or cytokines (such as IL-12) [2]. Compared with AAV, the carrying capacity of mRNA-LNP can meet more anti-tumor factor delivery conditions. However, the types of cells or organs that can be targeted are relatively small, because the biotechnology required to develop a specific targeted mRNA-LNP shell is difficult and the screening conditions are strict [2]. OVs can directly achieve specific replication of viruses in tumor cells through genetic modification, and ultimately activate the immune system to kill tumors. Among the current OVs, the most successful HSV-1 drug T-VEC has shown significant effects in the treatment of melanoma and has been approved for marketing by the US Food and Drug

Administration (FDA). It is the first approved OVs therapeutic drug [3]. Compared with AAV and mRNA-LNPs, OVs can achieve specific replication in tumor cells. This feature allows OVs to act only in tumor cells, making them highly targeted and more controllable in off-target effects.

This review focuses on the advantages and disadvantages of three in vivo anti-tumor therapeutic vectors, as well as the corresponding vector engineering strategies required to achieve their functions. Then, this review describes the current strategies for anti-tumor protein expression mediated by in vivo delivery vectors. This article aims to provide a comprehensive reference for the optimization and future development direction of in vivo anti-tumor vectors.

2. Engineering Design of Anti-tumor Gene Therapy Vectors

2.1. AAV

As an anti-tumor factor delivery vector, AAV needs to target tumor cells and avoid being cleared by the immune system before entering tumor cells. At the same time, it also needs to ensure long-term stable expression in tumor cells. To achieve these, AAV vectors need to be engineered.

2.1.1. Rational Design of Tumor-specific Transduction

Although AAV has high in vivo stability and sustained expression, the tissue distribution of natural serotypes is limited, which makes it difficult to meet the needs of tumor-specific delivery [1]. Therefore, researchers have improved its tumor targeting through rational design. Certain proteins in tumor tissues are highly expressed in the TME. AAV can target these proteins through rational design to achieve the purpose of targeting tumor cells. For example, aminopeptidase N (or CD13) is a membrane-bound enzyme that is highly expressed in cancer tissues and blood vessels. Grifman et al. modified AAV2 with NGR peptide to target CD13-expressing tumors, with an efficiency increase of 10-20 times [8]. Tumor progression and metastasis require a protein called integrin, so integrin is also highly expressed in tumor cells and blood vessels [1]. By adding RGD peptide modification to the surface of AAV capsid, it can bind to integrins αvβ3 and αvβ5, thereby targeting AAV to tumor tissue [1]. In TME, the expression of matrix metalloproteinases (MMPs) is significantly higher than that of other normal tissues [9]. Therefore, a negatively charged short peptide can be added to the surface of AAV capsid to block the binding of AAV to heparin on the surface of normal cells, and the negatively charged short peptide can be added with an MMP protease cleavage site. The AAV designed in this way is not easy to bind to normal cell surface receptors, but in the TME where MMP is highly expressed, the negatively charged short peptide will be destroyed by MMP, restoring the binding of AAV to cell surface heparin [1].

2.1.2. AAV Directed Evolution

AAV has widespread problems with existing neutralizing antibodies and insufficient tumor specificity in clinical applications [1], which may limit its distribution in the body and the effect of repeated administration. To meet this challenge, researchers use directed evolution of capsids to screen for more efficient AAV vectors.

Directed evolution of AAV includes three screening methods: random peptide insertion library screening, in vivo library screening, and serotype DNA shuffling library screening (chimeric capsid) [1]. Random peptide insertion library screening is to insert random peptide sequences at specific sites of the AAV capsid protein to construct a diverse AAV library (library), and then screen for mutants that can better infect target cells. Michelfelder et al. screened out AAVs with high infection efficiency for breast cancer cells (PymT) from a random peptide insertion library based on AAV2 [10]. The screened AAV variants shared the RGDXXXX motif (RGD can bind to integrins), and the transduction efficiency of tumor cells was increased by more than 15 times. In vivo library screening is to directly inject the AAV library into tumor-bearing mice, then recover the successfully infected virus particles from the tumor tissue, and then analyze their peptide sequences. Serotype DNA shuffling library screening (chimeric capsid) is to recombinant DNA fragments of cap genes between

different AAV serotypes (such as 1, 2, 5, 9, rh8, rh10) to construct a large library of chimeric AAV capsids, and then screen tumor-specific AAV capsids from this library.

2.1.3. Optimization of Tumor-specific Transduction Expression Regulatory Elements

Given that some therapeutic proteins may be toxic to non-target tissues, the long-term expression characteristics of AAV also bring challenges to expression control. In order to achieve precise spatiotemporal regulation of expression, AAV engineering usually integrates tumor-selective promoters to limit its expression activity in non-tumor tissues [1]. By selecting appropriate promoters and regulatory elements, the gene expression specificity of AAV vectors in tumors or specific cell types can be significantly enhanced, thereby improving the safety and accuracy of treatment. For example, the promoter of C-X-C chemokine receptor type 4 (CXCR4) is overexpressed in many tumor tissues, and experiments have shown that CXCR4-controlled AAV vectors are more strongly expressed in tumor tissues, while off-target expression in muscle tissue is significantly lower (only 10%–50%) [1].

2.2. RNA-LNP

As the main platform for mRNA delivery, LNP has shown great potential in tumor immunotherapy due to its highly modular and non-integrated genome. However, its in vivo application still faces several key challenges. First, mRNA is easily retained in endosomes after cell uptake, resulting in inefficient cytoplasmic release, thereby limiting its translation expression [2]. Moreover, since mRNA itself has a short expression cycle, how to prolong its stable expression time is also key to achieving sustained anti-tumor effects. In response to the above challenges, LNP carriers need to be engineered through multiple aspects, including lipid design and screening, design of LNP component ratio and structure and targeting to enhance its efficacy in tumor treatment.

2.2.1. Design and Screening of Lipid Monomers

Ionic lipids are the key to LNP delivery systems. They can bind to mRNA to protect it from degradation and improve translation efficiency [2]. They also promote LNP cellular uptake, endosomal escape and mRNA release [2]. Ionic lipids are composed of a head group, a hydrophobic tail and a linker. The head group affects the charge state, binding force and toxicity, and is often designed based on a polyamine structure [2]. The hydrophobic tail affects membrane fusion and stability, and asymmetric or branched designs help escape from the endosomal structure [2]. The linker determines the stability and degradability of the lipid. Stable bonds enhance the structure, and degradable bonds enhance biocompatibility [2]. Responsive linkers can be used to specifically release drugs. At the tumor level, the level of intracellular reactive oxygen species (ROS) is high, and linkers that can be degraded by ROS can promote selective mRNA delivery to tumor cells [11]. The Structure–Activity Relationship (SAR) model is used to quickly screen high-performance ionic lipids [2].

2.2.2. Optimization of Other Components of LNP

LNP not only relies on the performance of a single lipid molecule, but also needs to optimize the overall performance by rationally matching four core components - ionic lipids, phospholipids, cholesterol and PEG lipids. Ionic lipids are mainly responsible for the packaging and delivery of mRNA. Phospholipids enhance membrane fusion and endosomal escape. 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), which has strong membrane fusion properties, can promote LNP to release mRNA into cells [2]. Cholesterol improves membrane stability and structural compactness. Increasing the cholesterol ratio can stabilize the particle structure [2]. PEG lipids regulate particle size and prolong circulation time, but may also inhibit cellular uptake and cause LNP to escape after endocytosis. Its density and chain length need to be precisely matched to avoid the "PEG dilemma" [2].

2.2.3. LNP Surface Structure Engineering

Through the engineering design of the LNP surface, it's in vivo distribution, targeting efficiency and immunogenicity can be precisely adjusted to improve the safety and therapeutic effect of the mRNA delivery system. Cleavable PEG, which can automatically break under the acidic or specific enzyme conditions of the TME, has been developed to solve the "PEG dilemma". The modified LNP can dePEG at the target site and release mRNA [2]. To achieve active targeted delivery, specific ligands can be coupled to the LNP surface. The commonly used RGD peptide can target the integrins αvβ3/αvβ5 that are highly expressed in the TME [1]. Aptamers are a class of precisely designed oligonucleotides that can bind to specific proteins with high affinity and have been used to target tumor cells that highly express specific proteins with LNPs [2]. In addition to targeting tumor cells, LNPs modified with CD3, CD4, or CD5 antibodies can target immune cells and deliver mRNA to T lymphocytes to generate chimeric antigen receptor T cells (CAR-T) cells or immune factors in vivo [2].

2.3. OVs

Currently, OVs face a series of technical difficulties in their in vivo application. Their systemic delivery efficiency is strongly interfered by the host immune system, especially neutralizing antibodies and complement systems, which can quickly clear viral particles and limit their distribution in distant tumors [12]. The dense extracellular matrix unique to solid tumors also hinders the spread of viruses within the tumor, further reducing their therapeutic range [12]. In addition, the toxicity of oncolytic viruses is also a problem that should be solved. To overcome these obstacles, the engineering design of OVs needs to be systematically engineered through genetic engineering, delivery platform optimization, and immune regulation strategies.

2.3.1. Optimization of Viral Spread Ability

When the appropriate virus is selected to target tumor cells, subsequent spread and proliferation become important factors in the anti-tumor effect of oncolytic viruses.

Viral spread is mainly divided into local spread and systemic spread. Local spread may occur through cell-to-cell fusion, direct transfer from infected cells to neighboring cells, and release and local migration of viral progeny particles through the interstitial matrix [12]. Fusogenic viruses, such as measles virus, are naturally capable of cell fusion and can spread covertly through cell-to-cell fusion, causing tumor cells to form multinucleated cells that are inviable and contain a large number of tumor antigen fragments. These large multinucleated cells can release these tumor antigen fragments after apoptosis, and the released antigens are phagocytosed by antigen-presenting cells (APCs); these APCs then activate T cells, especially cytotoxic T cells (CTLs), to initiate or enhance immune attacks against tumors. Non-fusogenic viruses can achieve the same effect by genetically modifying them to carry fusion membrane glycoprotein (FMG) [12]. In most clinical cases, tumor metastasis is common, and even micrometastatic foci and inaccessible tumor foci may occur. Therefore, systemic spread is necessary to treat tumors in multiple locations. However, for free virus particles, the tumor matrix may limit the spread of the virus within the tumor and even prevent systemic release [12]. The extracellular matrix (ECM) is a component of the tumor matrix and is a mesh-like structure with pores similar to or slightly smaller than those of the virus body [12]. Disruption of the ECM components can promote the spread of the virus within the tumor and also facilitate the release of the virus "breaking through the tumor boundary". Conventional chemoradiotherapy or delivery of matrix-degrading enzymes can achieve the destruction of ECM structure [12].

2.3.2. Expression Regulation

Although oncolytic viruses are used clinically to fight cancer, they are still viruses in nature and may cause harm to the human body. Therefore, it is crucial to regulate the expression of OVs and control their large-scale expression only in target cells. Deleting the virulence-related genes of OVs can control the toxicity of OVs. Among them, the TK gene encoding Thymidine Kinase (TK) carried by

HSV is a typical example. The TK gene in HSV-1 encodes a thymidine kinase that can phosphorylate thymidine for DNA synthesis. In non-proliferating cells (such as nerve cells), the supply of thymidine is low, and the virus relies on its own TK to synthesize DNA. The TK gene can help wild-type HSV replicate in non-dividing cells. Therefore, HSV that retains TK may infect normal tissues and produce toxicity. After deleting the TK gene, HSV cannot replicate efficiently in normal cells because it lacks the ability to synthesize DNA. However, tumor cells are in a state of rapid proliferation and have sufficient thymidine, so HSV with the TK gene deleted can still replicate in tumor cells [12]. In addition, genes that affect the host's antiviral mechanism (such as HSV γ34.5) can also be deleted. The modified virus can be eliminated in normal cells, but can still replicate efficiently in tumor cells with defects in the interferon pathway [3]. Some viruses are also designed to integrate and express only in tumor cells with active cell cycles, such as Toca-511, which relies on the host DNA replication mechanism to replicate, thereby avoiding infection of quiescent cells [12].

3. Vector-mediated Anti-tumor Gene Therapy Strategies

AAV, LNP and OVs are three representative gene delivery vectors, each of which has developed different anti-tumor strategies based on its virological or nanotechnology characteristics: AAV relies on continuous expression of anti-tumor factors, LNP expresses proteins through short-term and efficient delivery of mRNA, and OVs uses viral lysis and immune activation as the core mechanism, and partially assists in expression to enhance immunity. The three respectively embody the technical characteristics of "fine expression control", "flexible and agile delivery", and "strong aggressivity".

3.1. AAV-mediated Anti-tumor Strategy

Vascular endothelial growth factor (VEGF) is a potent angiogenesis factor that is particularly active in tumor cells. Various methods of inhibiting VEGF using AAV delivery have achieved results in animal experiments. For example, in one experiment, after the modified AAV2 was injected into mice with subcutaneous human ovarian cancer cell line (SKOV3.ip1) xenograft tumors, a significant reduction in tumor volume was observed, and the survival rate of mice was increased to 83%; in contrast, all mice that did not receive treatment died within six weeks after tumor implantation [1]. Injection of other vectors with similar functions into the quadriceps of mice can significantly inhibit lymph node metastasis in renal cell carcinoma (Caki-2) and prostate cancer (PC-3) models, with inhibition rates of 70% and 75%, respectively [1]. In addition to VEGF receptor-related strategies, researchers have also tried to use other naturally occurring angiogenesis inhibitors for anti-angiogenic gene therapy. Pigment epithelium-derived factor (PEDF) is a potent endogenous inhibitor that inhibits the formation of new blood vessels in endothelial cells. In the Lewis lung cancer (LCC) mouse model, the AAV2-PEDF vector was directly injected into the tumor. The results showed that compared with the control group, the tumor volume was reduced by 58%, and the median survival time of the mice was extended by 75% [1]. In addition, a variety of anti-angiogenic genes have been successfully delivered via AAV vectors. For example, AAV2 has been used to deliver tissue factor pathway inhibitor (TFPI-2) to a glioblastoma (SNB19) model; and in an ovarian cancer mouse model (MA148), AAV vectors have also been used to deliver the plasminogen kringle 5 fragments, which has potent anti-angiogenic properties.

Delivery of cytokines such as immunostimulatory factors can effectively enhance the host immune response to tumors. Among them, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a molecule widely used in anticancer therapy [1]. In an orthotopic lymphoma mouse model (EL-4), portal vein injection of AAV2-TRAIL significantly inhibited tumor growth and prolonged median survival by 92% [1]. In another study, TRAIL packaged with AAV2 inhibited the growth of liver cancer xenografts by approximately 70% and significantly prolonged survival [1]. Interferon (IFN) has the effect of inhibiting cancer cell division and preventing tumor progression. Using AA8 to deliver hIFN-β inhibited the growth of human neuroblastoma by 90% [1]. AAV2-IFN-β has also been used in colorectal cancer (SW620) and lung cancer (A549) models, inhibiting tumor growth by more than 90% and significantly improving long-term survival rates to 87% and 83%, respectively [1].

Another immune activation strategy is to enhance the cytotoxic T cell (CTL) response against tumors by inhibiting negative immune regulators such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and PD-1 [1]. Currently, blocking monoclonal antibodies (mAbs) against CTLA-4, PD-1 and their ligand PD-L1 have achieved remarkable results in the treatment of various cancers, including melanoma, renal cell carcinoma and non-small cell lung cancer [1]. AAV has been used in preclinical studies to deliver anti-angiogenic mAbs (such as bevacizumab) to tumor models and has shown good therapeutic potential. Therefore, the use of AAV-mediated local expression of blocking antibodies (such as anti-CTLA-4 or anti-PD-1) is expected to continuously activate CTL responses in the TME [1].

3.2. LNP-mediated Anti-tumor Strategies

3.2.1. Cancer Vaccines

Since mRNA vaccines have made significant breakthroughs in responding to infectious diseases, they are now also widely used in cancer treatment, mainly through encoding and expressing tumor-associated antigens (TAAs). The key to achieving anti-tumor immune responses is to effectively deliver mRNA to APCs. Within APCs, TAAs or tumor neoantigens are translated and displayed on the cell surface through the major histocompatibility complex (MHC), thereby activating CD4+ and CD8+ T cells and initiating specific immune killing responses against tumor cells [2]. This type of vaccine strategy has shown good prospects in multiple clinical studies, and some studies have entered the Phase II clinical trial stage [2]. Among them, a study developed a lymph node-targeted lipid nanoparticle (LNP) platform 113-O12B to deliver mRNA encoding melanoma-specific TAA-tyrosinase-related protein 2 (TRP-2) peptide. [2] The nanovaccine showed significant tumor growth inhibition in the B16F10 mouse melanoma model, verifying its anti-tumor immune potential.

3.2.2. Therapeutic Antibodies

In the past few decades, antibody-mediated cancer therapy has made significant progress and has been widely used in clinical practice. With the development of sequence design technology, mRNA has been shown to have the potential to encode therapeutic antibodies. For example, rituximab is a mAb targeting CD20 and is commonly used to treat non-Hodgkin's lymphoma. Researchers used LNPs to deliver mRNA to express rituximab in vivo and verified its anti-tumor effect in vitro and in vivo [2]. The results showed that in the Raji lymphoma model, mRNA-LNPs showed superior tumor inhibition ability to recombinant rituximab protein [2]. Similarly, another study developed mRNA-LNPs encoding anti-HER2 mAbs (trastuzumab) and confirmed that they had significant anti-tumor activity in HER2-positive tumor models [2]. The signaling pathway formed by PD-1/PD-L1 has become an important target for cancer immunotherapy because of its key role in inhibiting T cell activity. Currently, a variety of mAbs that block this pathway have been approved for cancer treatment. On this basis, researchers have developed mRNA molecules encoding PD-1/PD-L1 antibodies and achieved in vivo expression through LNP delivery. Among them, one study used LNP to deliver mRNA encoding Pembrolizumab (an anti-PD-1 antibody) and successfully induced the synthesis of functional antibodies in vivo. Compared with direct injection of Pembrolizumab protein, this mRNA-LNP platform showed superior anti-tumor effects in the MC38 mouse tumor model with human PD-1 gene knock-in [2].

3.2.3. Immunomodulatory Factors

Cytokines are a class of secreted proteins that can regulate a variety of cell types and their functions, especially playing a key role in the immune system. They can mediate pro-inflammatory or anti-inflammatory responses, and therefore are of great significance in regulating the immune environment and disease progression [2]. In recent years, some cytokines have been widely used in cancer treatment research due to their potential in inhibiting tumor occurrence and progression. IL-12 is one of the more typical candidate molecules. IL-12 can activate T lymphocytes and induce the secretion of a large amount of interferon-β (IFN-β), thereby achieving tumor cell clearance and further enhancing anti-tumor immune response by promoting immune cell recruitment [2]. In one study,

researchers developed liver-targeted IL-12 mRNA-LNP and verified its anti-tumor effect in a hepatocellular carcinoma (HCC) mouse model. [2] The results showed that the system could induce significant infiltration of CD44+ CD3+ CD4+ T helper cells into tumor tissues and upregulate the expression level of IFNα, thereby significantly inhibiting tumor growth.

3.3. OVs-mediated Anti-tumor Strategy

As an anti-tumor therapeutic carrier, OVs have the unique advantage of having a dual mechanism of directly killing tumor cells and stimulating host immune response. They can specifically infect tumor cells and replicate in them, eventually triggering cell lysis and releasing TAA and damage-related signaling molecules. These "danger signals" can significantly activate the body's immune system, especially APCs such as dendritic cells (DCs), thereby inducing tumor-specific T cell-mediated immune responses [3]. On this basis, OVs can also be "armed" through engineering methods to express immune regulatory factors, thereby further enhancing their anti-tumor ability. For example, the T-VEC virus constructed based on HSV-1 is a classic type of armed OVs, which improves safety by deleting neurotoxicity-related genes and inserting GM-CSF to enhance immune response. It directly induces tumor lysis to release antigens, and promotes DCs to migrate to tumors and activate CD8+ and CD4+ T cells, thereby inducing systemic immunity [3]. Clinical data also support this mechanism: T-VEC had an objective response rate of 26% and a complete remission rate of 11% in patients with unresectable melanoma in a phase III clinical trial [3]. In addition, OVs can also enhance their ability to spread horizontally within tumor tissues by expressing fusion membrane glycoproteins (FMGs) associated with intercellular transmission. Such fusion proteins can induce infected cells to form syncytia with neighboring cells, allowing the virus to spread between cells without being released as free virus particles, helping to evade recognition by neutralizing antibodies. It can also induce the synchronous death of a large number of tumor cells and further release antigens [12]. This strategy not only improves the infection efficiency of the virus, but also forms highly immunogenic cell fragments, accelerating the intervention of the immune system [12].

4. Conclusion

As cancer treatment gradually shifts from traditional chemotherapy and radiotherapy to precision and individualization, the application of in vivo delivery systems in the delivery of anti-tumor factors is receiving more and more attention. AAV, mRNA-LNP and OVs are the three most widely studied in vivo delivery platforms, representing three different directions of viruses, nanoparticles and replicable vectors, respectively. They show significant differences in treatment strategies, engineering optimization ideas and application adaptability, which also reflects the diversity and complexity of current gene therapy technologies. These three types of in vivo delivery platforms each have unique advantages in cancer treatment and face different challenges. AAV is more suitable for gene delivery that requires long-term expression, LNP is suitable for rapid and short-term immune activation therapy, and OVs form a persistent and systemic anti-tumor effect through self-replication and immune amplification mechanism. AAV, mRNA-LNP and OVs, as emerging gene therapies, show broad prospects in future cancer treatment. AAV is expected to improve targeting and expression efficiency through directed evolution and capsid engineering, and expand its application in combination with traditional therapies. mRNA-LNP has potential in in vivo CAR-T therapy due to its programmability. OVs have tumor lysis and immune activation effects, and are developing towards combined treatment with chemotherapy, immune checkpoint inhibitors (ICIs) and bispecific T cell engagers (BiTEs). The three enter from different paths and constitute the technical panorama of contemporary in vivo delivery systems. At present, research around these three types of platforms has gradually shifted from single delivery to multifunctional integration and intelligent regulation.

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