Gene Expression **2025** vol. 24(4) | e00042
DOI: 10.14218/GE.2025.00042

Review Article

Targeted Delivery of MicroRNA Sponge Short-hairpin RNA via Vir-inspired Biotechnical Vector: Enhancing Cancer Therapy

Hananeh Rozbahani[1], Alireza Zangooie[2,3], Seyed Mohsen Mirabdolhosseini[4], Nayeralsadat Fatemi[5], Mohsen Norouzinia[6], Amir Sadeghi[6], Zahra Salehi[7,8*] and Ehsan Nazemalhosseini-Mojarad[6*]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran; [2]Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran; [3]Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran; [4]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Doctoral School of Molecular Biology and Biological Chemistry, Warsaw, Poland; [5]Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [6]Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [7]Cell Therapy and Hematopoietic Stem Cell Transplantation Research Center, Research Institute for Oncology, Hematology and Cell Therapy, Tehran University of Medical Sciences, Tehran, Iran; [8]Hematology, Oncology and Stem Cell Transplantation Research Center, Research Institute for Oncology, Hematology and Cell Therapy, Tehran University of Medical Sciences, Tehran, Iran

Received: April 06, 2025 | **Revised:** June 23, 2025 | **Accepted:** July 17, 2025 | **Published online:** August 28, 2025

Abstract

Targeted drug delivery remains a major challenge in cancer therapy, often limiting both efficacy and safety. Although microRNA sponges and short-hairpin RNAs show potential for gene-based cancer treatment, their clinical use is restricted by delivery inefficiency, off-target effects, cytotoxicity, and instability. Viral vectors offer high efficiency but are associated with issues such as immune responses, insertional mutagenesis, and limited cargo capacity. Non-viral carriers are safer and more affordable but suffer from poor transfection efficiency, instability, and inadequate endosomal escape. These limitations hinder the clinical application of RNA therapeutics. The Vir-inspired Biotechnical Vector (VIBV) is a novel hybrid platform that combines viral and non-viral elements with nanotechnology to enable personalized, tumor-specific gene therapy. Engineered with a spindle-shaped nanocore and a polyethylene glycolylated liposomal shell, VIBV ensures immune evasion, prolonged circulation, and controlled therapeutic release triggered by tumor microenvironmental cues such as acidity, hypoxia, and elevated glutathione levels. It delivers oncogenic microRNA sponges, short-hairpin RNAs, tumor-specific antigens, and cyclin-targeting RNAs to enhance gene silencing, immune activation, and tumor suppression. This review examines the limitations of current delivery systems and presents VIBV as a promising next-generation strategy with improved biocompatibility, targeting precision, and potential for cost-effective, personalized cancer therapy, while also addressing its remaining challenges and prospects.

Introduction

Gene therapy holds immense promise as a targeted and potentially curative approach to cancer treatment, offering advantages over traditional methods such as surgery, chemotherapy, and radiation. By precisely targeting disease-causing genes, gene therapy aims to address the underlying molecular mechanisms driving cancer progression, providing the possibility of more effective and durable outcomes with reduced side effects. This is achieved by delivering genetic material, such as DNA, RNA, small interfering RNA (siRNA), short-hairpin RNA (shRNA), or microRNA (miRNA), into target cells using a delivery vehicle known as a vector. These vectors can either replace mutated genes with their functional counterparts or modulate gene expression, for instance, by silencing oncogenes.[1,2]

Gene therapy delivery systems are primarily categorized into viral (e.g., retroviruses) and non-viral (e.g., electroporation) vectors.[3] Viral vectors, derived from viruses, exploit the natural ability of these pathogens to infect cells and deliver genetic cargo. In

Keywords: Drug development; Gene therapy; Targeted drug delivery; Cancer treatment; Vector design; Nanotechnology; Personalized medicine; shRNA; miRNA sponge.
***Correspondence:** Zahra Salehi, Hematology, Oncology and Stem Cell Transplantation Research Center, Research Institute for Oncology, Hematology and Cell Therapy, Tehran University of Medical Sciences, Tehran 14114, Iran. ORCID: https://orcid.org/0000-0002-0839-2729. Tel: +98-21-8490-1000, E-mail: Zahra.salehi6463@yahoo.com; zsalehi@sina.tums.ac.ir; Ehsan Nazemalhosseini Mojarad, Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran 1985717443, Iran. ORCID: https://orcid.org/0000-0001-8914-004X. Tel: +98-21-23871, E-mail: e.nazemalhosseini_mojarad@lumc.nl; ehsanmojarad@gmail.com
How to cite this article: Rozbahani H, Zangooie A, Mirabdolhosseini SM, Fatemi N, Norouzinia M, Sadeghi A, *et al*. Targeted Delivery of MicroRNA Sponge Short-hairpin RNA via Vir-inspired Biotechnical Vector: Enhancing Cancer Therapy. *Gene Expr* 2025;24(4):e00042. doi: 10.14218/GE.2025.00042.

cancer gene therapy, viral vectors are employed both to introduce therapeutic genes that modify cancer cell behavior and as oncolytic viruses that selectively replicate within and lyse tumor cells.[4] Commonly used viral vectors include retroviruses, adenoviruses, and adeno-associated viruses (AAVs). While highly efficient gene delivery vehicles, viral vectors pose potential challenges, including the risk of immunogenicity, production costs, and the possibility of insertional mutagenesis.[5] Conversely, non-viral vectors, constructed from materials such as lipids, polymers, and proteins, offer a safer and more cost-effective alternative.[6,7] These vectors can be engineered for targeted delivery but often exhibit lower transfection efficiency compared to their viral counterparts. Delivering miRNA sponges and shRNA to cancer cells faces significant challenges, including off-target effects, poor cellular uptake, instability in the bloodstream, immune responses, and difficulty in achieving tumor-specific targeting, which limits efficacy and increases toxicity. Hybrid nanocarriers combine viral and non-viral components to leverage the advantages of both systems. They mimic viral entry mechanisms to improve delivery efficiency and tumor targeting while maintaining lower toxicity. These platforms, such as lipid-polymer hybrids and virus-like particles (VLPs), offer enhanced RNA stability and reduced off-target effects. Hybrid vectors show promise in improving therapeutic delivery and specificity in cancer treatment. Their development supports more effective and safer gene and drug delivery strategies.[8]

Nanorobots, or nanobots, are microscopic machines operating at the nanoscale (1–100 nm). Built from materials such as carbon nanotubes or DNA and equipped with nanoscale sensors and actuators, they can perform highly precise tasks. In medicine, they offer significant potential for targeted drug delivery, advanced diagnostics, non-invasive surgeries, and even cellular-level tissue repair.[9] Researchers at the Karolinska Institutet developed DNA origami-based nanorobots that carry a hexagonal pattern of cytotoxic peptides, which remain inactive until exposed to the acidic microenvironment typical of tumors. This pH-triggered "kill switch" ensures that the nanorobot activates only in the vicinity of cancer cells, minimizing damage to normal cells. In mouse models of breast cancer, these nanorobots achieved a significant reduction in tumor growth by about 70%, demonstrating both efficacy and safety with no adverse effects on normal blood coagulation or cell morphology.[10] Nanorobots hold great potential for precise cancer therapy, but clinical translation faces key challenges, including technical control in complex biological environments, safety concerns, regulatory gaps, high development costs, and scalability issues. Overcoming these barriers is crucial for clinical adoption.[11]

Despite decades of progress in gene therapy, current delivery systems for RNA-based cancer therapeutics face major challenges such as immune activation, poor tumor specificity, and inefficient intracellular delivery, all of which have limited their clinical success. Addressing these obstacles, the Vir-inspired Biotechnical Vector (VIBV) represents a novel, next-generation platform that fuses the sophisticated functional elements of viral vectors with the safety and versatility of synthetic, non-viral nanotechnology. VIBV is engineered with a polyethylene glycolylated liposomal coating, a tumor-responsive design, and a spindle-shaped nanostructure, enabling precise targeting, immune evasion, and efficient RNA delivery even in the aggressive tumor microenvironment (TME). By mimicking viral entry mechanisms within a biocompatible and non-immunogenic framework, VIBV achieves both high delivery efficiency and reduced toxicity. This multifunctional system can concurrently deliver miRNA sponges, shRNAs, tumor antigens, and cyclin-targeting RNAs to suppress tumor growth, stimulate anti-tumor immunity, and enhance therapeutic outcomes. Overall, VIBV holds significant potential to overcome the limitations of current vectors, offering improved efficacy, safety, and cost-effectiveness for personalized RNA-based cancer therapies.

Here, we aim to systematically evaluate the major limitations of current viral and non-viral gene delivery systems in RNA-based cancer therapies, specifically off-target effects, low transfection efficiency, instability in systemic circulation, immune activation, and insufficient tumor specificity, and to introduce the VIBV as a novel hybrid platform designed to address these challenges.

shRNA and miRNA sponges

miRNA sponges and shRNAs are two cancer treatment techniques that utilize promising RNA-based therapies. These techniques are similar in that they both control gene expression, but they operate through different mechanisms.[12]

shRNA is an RNA structure that resembles endogenous pre-miRNA and is processed by the RNA interference machinery. In the nucleus, it is transcribed into a piece that Drosha cleaves and exports to the cytoplasm, where Dicer further processes it into siRNA-like molecules. These molecules join the RNA silencing complex (RISC), which results in the degradation and translational repression of target messenger RNA (mRNA) strands.[13] shRNA therapy proves highly effective for silencing oncogenes and other seminal cancer genes, making it crucial in precision medicine. This method has been shown to target Kirsten rat sarcoma viral oncogene homolog, MYC proto-oncogene, and vascular endothelial growth factor, which are essential for tumor growth and angiogenesis.[14] The use of shRNA over siRNA is primarily motivated by the cost-effectiveness of production and its ability to deliver shRNA via lentiviral or retroviral transduction to almost all types of cell lines.[15]

According to the competing endogenous RNA hypothesis, competing endogenous RNAs, including pseudogenes and circular RNAs, can act as miRNA sponges, thereby modulating gene expression. The term 'sponges' refers to artificial RNA strands that bind specific seed regions of target miRNAs and prevent them from functioning. Sponges consist of repeat binding domains that capture miRNAs and stop them from acting directly on their respective mRNAs. This allows the miRNA-regulated mRNA to be translated. miRNA inhibition can be achieved through genetic sequence removal, antisense oligonucleotides, or synthetic miRNA sponges. miRNAs, approximately 23 nucleotides long, bind to the 3′ untranslated regions of mRNAs, silencing their translation.[16] Certain miRNAs, such as miR-1269a and miR-210-5p, contribute to cancer progression by regulating pathways involved in metastasis, apoptosis, and drug resistance.[17] Due to their stability in extracellular fluids, miRNAs serve as potential biomarkers for early cancer diagnosis. As a result, oncogenic miRNAs that downregulate tumor suppressor genes may be overridden. Whenever the miRNA sponge can reverse an oncogenic miRNA's actions, it aids in the expression of genes that have been silenced. This makes sponge miRNAs highly therapeutic. However, they are particularly effective in targeted therapies for dysregulated miRNA arrays in malignant tumors, such as those involving the highly studied oncogenic miRNAs miR-21 and miR-155. These miRNAs are strongly associated with tumor progression and immune escape. These powerful miRNAs are known for accelerating tumor growth, supporting malignant cell indoctrination, and facilitating immune evasion.[18]

Challenges of current delivery methods

Delivering therapeutic genes effectively faces several obstacles, limiting the success of current methods. One major challenge is achieving precise targeting while avoiding unintended effects. Many delivery systems struggle with efficiency, either due to low uptake by cells or instability within the body.[19] Safety concerns also pose significant hurdles. Some methods risk triggering immune responses, while others may interfere with normal cellular functions.[20] Additionally, ensuring long-term effectiveness without unwanted genetic alterations remains difficult.[21] The challenge of reliable system production and scalability adds to the existing problems. Many systems that function well in a controlled laboratory environment do not scale equally well. Resolving these obstacles is essential for enhancing the effectiveness of gene therapy.[22]

Viruses as gene delivery carriers

Viral vectors are widely used in various fields such as gene therapy,[23] oncology (particularly cancer), metabolic diseases,[24] heart defects,[25] and neurological disorders.[26] Furthermore, vaccinology, a field that triggers immune responses in patients via the expression of proteins and stimulates T and B cells, also utilizes viral vectors.[27] Viral impacts on cancer have been known since the early 20th century, exemplified by a 1904 case of leukemia that went into remission after the patient developed an influenza infection, and a rare case of tumor necrosis in a cervical cancer patient attributable to a viral infection following a rabies vaccination in 1912.[28,29] These reports led to speculation about the potential use of viruses to attack tumor cells, and the idea has since evolved into a well-established research field known as oncolytic virus therapy.[30] While the potential of using viruses as delivery vehicles for gene therapy appears promising, concerns surrounding safety, limited cargo capacity, and considerable cost still pose significant challenges that must be overcome.[31]

Viral vectors consist of three key components: a protein shell protecting the genetic cargo and enabling retargeting through structural modifications, a transgene for protein expression or gene regulation, and a regulatory cassette controlling transgene expression.[3,32]

Gene therapy for cancer treatment involves two primary approaches using viral vectors: the transfer of genetic material to modify the behavior of cancer cells and the use of oncolytic viruses.[4] Three classes of viruses are mainly used in the first approach: retroviruses, adenoviruses, and AAVs. Moreover, some researchers are exploring the use of oncolytic viruses in cancer therapy. These viruses specifically target and kill cancer cells, potentially leading to the reduction of cancerous lesions.

Adenoviruses

Adenoviruses are non-enveloped, double-stranded DNA viruses that can cause diseases in humans and animals. The adenovirus type 5 (Ad5) genome is approximately 36 kb and is partitioned into early and late regions.[33] The early region encodes non-structural proteins for replication, while the late region encodes structural proteins for assembly and release.[33] The Ad5 capsid consists of three major capsid proteins and four minor cement proteins. Adenoviruses have unique abilities for packaging and delivering genetic material, making them valuable for research in gene therapy. For delivering vectors, adenoviruses bind to coxsackie and adenovirus receptor and αvβ3/5 integrins, resulting in fast and efficient nuclear transport.[34] These high-capacity adenoviral vectors can hold up to 36 kb of cargo, making them useful for advanced gene expression systems with reduced vector-associated toxicity and immune responses.[35] Their use is diverse and includes gene editing and therapy, vaccination, oncolytic virotherapy, and basic research.[20] In this preclinical glioma study, Lee and colleagues tested whether combining two strategies—an hTERT-targeting ribozyme-controlled HSVtk suicide gene and adenoviral delivery of miR-145—would improve therapy.[36] Individually, HSVtk gene therapy slowed tumor growth, while miR-145 mainly suppressed migration and invasion. When delivered together in a single adenoviral vector (Ad5CMV.Rz.HSVtk.miR145), the treatment significantly enhanced anti-tumor effects and prolonged survival in animal models compared to either approach alone. This demonstrates that combining miR-145 with HSVtk gene therapy yields stronger therapeutic benefit against glioma.[36]

Adenoviral vectors are limited in gene therapy applications primarily due to their high immunogenicity, which can trigger strong cytotoxic T-cell and humoral responses, leading to the rapid elimination of transduced cells and the formation of neutralizing antibodies that hinder repeated administrations.[37–39] Additionally, adenoviruses lack specificity, as they can infect a wide range of cell types due to the widespread presence of their cellular receptor, often necessitating high doses that increase the risk of inflammatory reactions and severe side effects, especially with systemic delivery.[37] Another major drawback is the transient nature of transgene expression, which is insufficient for therapies requiring long-term gene correction. The presence of pre-existing immunity in most individuals further reduces vector efficacy upon re-administration.[37,40] These factors collectively restrict the use of adenoviral vectors for long-term or repeated gene therapy, making them more suitable for applications where only temporary gene expression is needed.

Retroviruses

Retroviruses, members of the RNA virus family known as murine leukemia viruses, utilize reverse transcriptase to translate their genetic information into DNA, which is later integrated into the host genome by the integrase enzyme as a pre-integration complex.[41] The inserted DNA is passed on to all of the cell's descendants, ensuring long-term expression of the introduced genes. Retroviral vectors have been used in gene therapy to deliver target genes into hematopoietic stem cells.[42] human immunodeficiency virus (HIV) is commonly used as a vector, modified to prevent its replication and cause no harm to the patient. New lentiviral vectors allow for HBG derepression through a miRNA targeting the HbF repressor BCL11A.[43] However, retroviral vectors have limited capacity to deliver genetic material, requiring multiple injections to maintain sustained expression. A modified ENV gene allows for targeting specific cell types, promoting entry and delivery of therapeutic genes. Systemic delivery of a lentiviral vector to restore the expression of miR-15a and miR-16 in a de novo mouse model of chronic lymphocytic leukemia has shown significant therapeutic benefits. These microRNAs normally act as tumor suppressors by targeting and downregulating the anti-apoptotic protein Bcl-2, which is commonly overexpressed in chronic lymphocytic leukemia and contributes to the survival and accumulation of malignant cells. The lentiviral system enables efficient, systemic delivery of these miRNAs into the malignant B-1 cells *in vivo*, leading to their increased expression. This miRNA restoration induces apoptosis—the programmed cell death—in the leukemic cells by reducing Bcl-2 levels, thereby decreasing tumor burden in critical organs like the spleen and liver. Importantly, this treatment was well tolerated in the mouse model with minimal systemic toxicity.[44]

Retroviral vectors face significant limitations in gene therapy

applications, primarily due to safety concerns and technical challenges. The most critical risk is insertional mutagenesis, where random integration into the host genome can disrupt tumor suppressor genes or activate oncogenes, potentially triggering malignancies, as evidenced by leukemia cases in X-SCID trials.[45,46] Additionally, these vectors require actively dividing cells for transduction, limiting their utility in non-proliferating tissues, and are susceptible to inactivation by the human complement system, reducing effective viral titers.[47] Immunogenicity poses another hurdle, as immune responses against viral components can neutralize the vector and complicate repeated administrations. While self-inactivating vectors and chromatin insulators mitigate some risks by reducing genotoxicity, challenges persist in achieving targeted integration and preventing replication-competent retrovirus generation. Low production yields further constrain clinical scalability, with current manufacturing processes often insufficient for large-scale treatments.[46]

AAV

AAVs are non-enveloped, non-contagious, and non-pathogenic viruses that hold promise for clinical treatment. The single-stranded SSAAV forms of DNA are inert when they enter the cell nucleus and must undergo second-strand synthesis or annealing of the plus and minus strands to be converted into double-stranded DNA. This conversion is essential to initiate the transcription process.

The single-stranded gene therapy vectors, SCAAVs, are pre-designed to already be double-stranded, allowing them to begin transcription immediately.[48,49] After the virus enters the host nucleus, its capsid undergoes proteolytic processing and releases peptides presented by major histocompatibility complex class I (MHC1) to stimulate T cells.[50] The viral inverted terminal repeats present in the AAV genome can drive inter-molecular or intra-molecular recombination, creating circularized episomal genomes that can persist in the nucleus. Over time, the episome is diluted in dividing cells, but non-dividing cells can maintain the episome for a longer period.[51,52] Researchers are exploring improvements to the properties of AAV9 to increase their ability to penetrate and cross the blood-brain barrier for targeted delivery in rodents and primates.[53,54] Incorporating miR-431 and miR-636 during AAV vector production improved gene transfer potency *in vitro* (3.7-fold increase for AAV2) and enhanced therapeutic efficacy *in vivo*, with AAV6 vectors carrying an inducible caspase 9 gene inducing significant tumor regression (~2.2-fold) in a murine T-cell lymphoma model, demonstrating an effective microRNA-based strategy to optimize AAV gene therapy.[55]

Although AAV vectors have great potential in cancer gene therapy, they come with significant challenges. One of the most concerning issues is insertional mutagenesis, in which viral DNA is integrated within a certain region of the genome, potentially leading to liver cancer. Although studies suggest this is much less probable in adult humans, long-term surveillance is still warranted.[56] The complex dense extracellular matrix, hypoxia, and immune suppression also make treating solid tumors with AAV vectors less effective and pose challenges for repeat treatments.[53] Moreover, AAV vectors have a relatively small packaging capacity (approximately 4.7 kb), which limits the size of therapeutic genes that can be incorporated.[57] These factors are compounded by the high cost and sophistication required to produce AAV vectors, which hinders their more general application in oncology.[58] Despite these drawbacks, research is ongoing to improve targeting efficiency, reduce immune responses, and scale production. Even though AAV vectors remain an important component in the field of gene therapy, their application in cancer treatment is still limited by these challenges.

Bacteriophages

Bacteriophages, or phages, are viruses that specifically infect bacteria and are composed of a protein capsid encapsulating either DNA or RNA genomes of varying sizes and complexities. Structurally, many bacteriophages, such as the well-studied T4 phage, consist of a polyhedral head that houses the genetic material, a short collar, and a helical tail apparatus. The tail includes a hollow tube surrounded by a contractile sheath and ends with a baseplate and tail fibers that recognize and attach to bacterial surface receptors, facilitating genome injection into the host cell. This sophisticated structure enables precise delivery of genetic material into bacteria.[59–61] As vectors in gene therapy, bacteriophages offer several advantages: they are highly stable, non-pathogenic to humans, can be genetically and chemically engineered for targeted delivery, and have a low risk of insertional mutagenesis compared to mammalian viral vectors. Their ability to be modified to display targeting ligands and carry therapeutic genes or RNA molecules makes them versatile tools for selective and efficient gene transfer, particularly in cancer treatment applications.[62] An illustrative example of bacteriophage-mediated miRNA delivery in cancer therapy involves the use of MS2-derived VLPs engineered to carry both a miR-21 sponge and pre-miR-122 to target hepatocellular carcinoma (HCC) cells. The miR-21 sponge sequesters oncogenic miR-21, inhibiting its tumor-promoting effects, while pre-miR-122 restores the tumor-suppressive activity of miR-122, leading to decreased cancer cell proliferation and increased apoptosis. These VLPs were modified with HCC-specific peptides to enhance targeted delivery. Functional assays and reverse transcription quantitative polymerase chain reaction (RT-qPCR) confirmed efficient delivery, with miR-21 target mRNAs derepressed and mature miR-122 successfully expressed. Co-delivery of both RNAs inhibited HCC cell proliferation, migration, and invasion by up to 34%, 63%, and 65%, respectively, and significantly promoted apoptosis. Overall, this dual-miRNA delivery via bacteriophage VLPs represents a stable, targeted, and effective strategy to correct miRNA dysregulation in HCC.[63]

Bacteriophages, while promising as safe and cost-effective vectors for targeted gene delivery in cancer therapy, face significant limitations primarily due to their natural tropism for bacteria rather than mammalian cells, resulting in relatively poor gene transfer efficiency into human cells. Their inability to efficiently express transgenes upon entry into eukaryotic cells restricts their direct application, necessitating the development of hybrid vectors like AAV/adeno-associated phage to improve targeting and transduction. Additionally, the extracellular matrix in tumors acts as a physical barrier, further reducing phage vector penetration and efficacy, although enzymatic degradation of the extracellular matrix can enhance delivery. Another concern is the potential for horizontal gene transfer and contamination with bacterial endotoxins or other pathogenic elements, which pose safety challenges for clinical use. These factors collectively limit the widespread adoption of bacteriophage-based vectors and require ongoing engineering and combinatorial strategies to overcome these hurdles for effective cancer gene therapy.[64,65]

Oncolytic viral vectors

Oncolytic viruses are non-pathogenic viruses being developed for cancer therapy. They exploit compromised immune responses and release tumor-killing proteins, though their exact mechanisms

remain unclear.[66] Nevertheless, issues such as immune NET caspase-cleavage of type I interferon signaling and viral resistance still exist, which undermine their efficacy.[67] It has also been noted that certain tumors become resistant to oncolytic virus infection, as seen with B16F10 mouse melanoma cells, which became refractive to both vesicular stomatitis virus (VSVD51) and the unrelated Sindbis virus.[68] The elimination of oncolytic viruses is driven by tumor cell responses, stromal responses, antiviral immunity, and some peripheral components, with the signaling of interferons identified as associated with resistance.[69] With Talimogene laherparepvec being the most well-known, there are currently five accepted viral therapies.[70] The most frequent oncolytic virus is herpes simplex virus (HSV)-1, though it has low efficiency with systemic injections.[71,72] Physical barriers, as well as the limited capacity of the oncolytic virus genome to encode therapeutic proteins, hinder delivery to solid tumors. Immune responses include antigen-presenting cells, preexisting antibodies, and blood factors like factor IX (FIX), factor X (FX), and C4b-binding protein (C4BP).[73] Oncolytic viruses are known to cause damage to healthy tissues as well, as seen with the HSV-1-based NV1066, which induces apoptosis in about 10% of uninfected gastric cancer cells.[74] Oncolytic VSVd51 viruses engineered to express miR-199a-5p effectively inhibited the epithelial-mesenchymal transition-related transcription factor ZEB1 in human triple-negative breast cancer cells but failed to affect EMT gene expression in mouse triple-negative breast cancer cells. *In vivo*, VSVd51-pre-miR-199 showed no significant impact on tumor growth or survival in either syngeneic or xenograft mouse models. This study highlights the context-dependent challenges of combining miRNA delivery with oncolytic virotherapy and emphasizes the importance of vector and tumor model selection for effective therapeutic outcomes.[75]

Oncolytic virus therapy may exhibit limited selectivity towards target cells, resulting in potential harm to healthy tissue surrounding the affected area. Stanziale *et al*.[74] reported that human gastric cancer cells infected with an HSV-1-based virus (designated NV1066) died via apoptosis, a common form of programmed cell death. The study also found that when both infected and uninfected cells were mixed, approximately 10% of the uninfected cells also underwent apoptosis.[74] Severe side effects have been reported in the oncolytic virus treatment group, including fever, neutropenia, diarrhea, nausea, vomiting, chills, flu-like symptoms, arthralgia, myalgia, extreme pain, and other common symptoms, with an elevated pooled risk ratio and risk difference compared to the control group.[76] Despite this, recent methods to reduce side effects and eliminate oncolytic viruses in the body can be used, such as liquid nitrogen shock and mesenchymal stem cells as vectors to deliver these viruses.[77]

Nonviral vectors

Non-viral vectors for gene therapy include liposomes, exosomes, polymer nanoparticles, hydrogels, gold nanoparticles, dendrimers, and VLPs.[6,78] Limited research has been conducted on their use for gene therapy, primarily due to challenges such as the endosome, a cellular compartment that impedes the delivery and expression of genes, as well as issues like low transfection rates, reduced efficacy over time, and limited infectivity in certain cell types.[79] In a rat hepatoma model, researchers identified seven miRNAs that were downregulated in tumors and used them to design luciferase-expressing gene therapy vectors with improved tumor specificity. These vectors were tested in cell lines, mouse liver, and tumor-bearing rats. Among the miRNAs, miR-26a and miR-122 significantly reduced transgene expression in healthy liver tissue (6.40% and 0.26% of control, respectively; $p < 0.05$), demonstrating effective liver de-targeting. In tumor tissue, miR-122 showed an approximately 50% non-significant reduction, while miR-26a had no significant impact on transgene expression. This study is the first to demonstrate the use of differentially expressed miRNAs, particularly miR-122 and miR-26a, to selectively reduce gene expression in liver tissue while preserving expression in HCC, supporting their potential in developing more targeted and personalized gene therapies for HCC.[80] A vector-based plasmid expressing miR-15a/16-1 was developed to evaluate its anti-tumor effects in colon cancer. miR-15a and miR-16-1 were found to target cyclin B1 (CCNB1), a protein linked to tumor cell survival and proliferation, with their levels inversely correlated to CCNB1 expression in colon cancer cells. Transfection of the miR-15a/16-1 plasmid into colon cancer cell lines reduced cell viability, colony formation, and angiogenesis, while downregulating CCNB1 protein. Systemic delivery of the plasmid encapsulated in cationic liposomes significantly inhibited tumor growth and angiogenesis *in vivo* in colon cancer xenografts, demonstrating that miR-15a/16-1 effectively suppresses colon tumor progression and may represent a promising therapeutic approach for colon cancer treatment.[81]

Non-viral vectors are considered more attractive than viral vectors due to their reduced toxicity, as they target cancer cells without harming healthy ones.[82,83] Viral vector-based gene therapy can be very expensive, whereas non-viral vectors are relatively inexpensive and much easier to produce.[84] Non-viral vectors also have a much lower risk of generating an immune response than viral vectors, which is important when treating patients with compromised immune systems, such as those undergoing chemotherapy.[85] Additionally, non-viral vectors can be designed to be specific to certain tissue types, improving delivery to the desired area, reducing the chances of off-target delivery and potential toxicity. They can also carry larger payloads than viral vectors, allowing for the delivery of larger genes or multiple genes.[86]

Studies show progress in developing lipid nanoparticles (LNPs) and exosomes for the modification of cancer therapies by increasing specificity to tumor cells and reducing off-target effects. Unlike viral vectors, these non-viral alternatives are hypothesized to be cheaper and safer. However, like traditional liposomes and polymer nanoparticles, these lipid vectors suffer from low efficiency due to poor transfection, inadequate endosomal escape, and instability in biological systems. Research is focused on optimizing targeting mechanisms and transfection stability to overcome these issues. LNPs are being designed using various ligands, such as antibodies and peptides, to target cancer cells via receptor-mediated endocytosis. Ionizable cationic lipids, such as DODMA or DLinMC3DMA, known for their better cellular uptake and intracellular release of therapeutic cargo, are used in the composition of the LNPs to improve historically low transfection rates among liposomes, which can be 1/10th to 1/1000th of viral transfection rates.[87] Other modifications, such as coating with distearoyl phosphatidylethanolamine-polyethylene glycol-2000-maleimide, have been reported to improve LNP stability and reduce off-target accumulation.[88]

Considering factors like biocompatibility and active targeting attributes, exosomes (extracellular vesicles) are being studied for use in drug and gene delivery. Researchers are already altering their surfaces with targeting moieties to improve specificity while employing electroporation and sonication, more precise loading techniques that overcome previously encountered difficulties in endosomal escape, to enhance nucleic acid or drug incorporation.[89] Overcoming stability issues for effective *in vivo* deliv-

ery and improved clinical feasibility is being addressed through content modification and genetic engineering. These vectors are likely to be translated into clinical use, as recent trials have demonstrated promising results. Current research is focused on LNPs in mRNA-based cancer vaccines, with exosomes being used to target OX40L T cells in ovarian and solid tumors through Moderna's mid-phase III trials.[90,91] Exosome-based therapies are also advancing in chemotherapy, gene therapy, and immunomodulation, with an emphasis on safety standardization and large-scale production. The application of high-throughput screening techniques, such as DNA/RNA barcoding combined with these technologies, is expected to further enhance the precision medicine scope.[92]

Nanorobotic vectors

Medical nanorobots are tiny, untethered devices capable of converting energy into mechanical force to perform medical tasks. Their small size allows them to interact directly with cells, making them valuable in cancer diagnosis and treatment. These nanoscale machines can deliver drugs or genes, perform therapeutic functions, and target disease sites using active or passive power systems (e.g., light, ultrasound, magnetic fields, or blood flow). Unlike passive nanocarriers, nanorobots possess active propulsion mechanisms. Despite their potential, a major challenge remains: translating nanorobotic technologies into real-world clinical applications.[11] Nanorobots represent a revolutionary advancement in cancer treatment, offering targeted and highly efficient therapeutic solutions at the microscopic level. Unlike traditional macro-scale robots, nanorobots are designed to operate within the unique and complex microenvironment of the human body, executing precise, pre-programmed missions such as drug delivery, tissue repair, or immune modulation. Their construction and control present significant challenges due to the need for biocompatibility, miniaturization, and accurate functionality. Depending on their intended purpose, nanorobots vary in composition and structural design, often incorporating components like molecular sensors, chemotactic systems, and microchips for disease detection and response. Notable examples include Pharmacytes (using chemotactic sensors for targeted drug delivery), Microchips (signaling disease presence electronically), Respirocytes (artificial red blood cells powered by serum glucose), Microbivores (which exhibit rapid phagocytic activity to destroy pathogens), Clottocytes (synthetic platelets capable of instant hemostasis), and Chromallocytes (advanced cellular repair machines that assess and restore cellular functions). While most nanorobotic systems remain in early experimental stages, ongoing innovations are setting the groundwork for their future integration into cancer therapy by emphasizing safety, precision, and scalability.[11]

While nanorobots offer significant potential for precise and effective cancer treatment, several major challenges hinder their clinical application. Technically, the nanoscale design and control of these robots in complex biological environments, like blood or other bodily fluids, are difficult due to low power efficiency, interference from cells, and challenges in movement precision. Safety is also a concern, as malfunctioning nanorobots could cause harm or be ineffective in certain tumor environments, necessitating rigorous testing and quality control. Regulatory gaps present another barrier, as comprehensive guidelines for nanorobot development, testing, and post-market monitoring are still lacking. Additionally, the development of nanorobots is costly and resource-intensive, requiring significant funding and cross-sector collaboration. Scalability is a further hurdle, involving the need for advanced manufacturing techniques, cost reduction strategies, strict quality control systems, and efficient supply chain management. Addressing these multifaceted issues is essential for the successful translation of nanorobotic technologies from the lab to clinical use.[11] Hence, advancements in nanotechnology inform the conceptual foundation of VIBV, which integrates motile elements and environment-sensitive delivery into a single multifunctional platform.

VIBV

This section presents the VIBV, a novel platform applicable to personalized medicine. VIBV is an advanced vector that incorporates features from viral and non-viral gene delivery systems, as well as nanomaterials and nanomotors, for enhanced gene therapy. It uses nanotechnology to maximize therapeutic effectiveness while minimizing side effects and overcoming the traditional delivery challenges associated with viral and non-viral vectors. VIBV has a spindle-shaped nanostructure that allows it to stealthily penetrate tumors, evade the immune system, and prolong circulation due to its polyethylene glycolylated liposomal coat. This coat facilitates direct membrane fusion and efficient cytoplasmic entry at cancer-critical temperatures. VIBV is designed to respond to stimuli and selectively activate in response to tumor acidity, hypoxia, and the high glutathione (GSH) level inside the cytoplasm. Referred to as VIVB, its sperm-like flagellar nanomotor enhances mobility in biological fluids, improving tumor localization.

Once inside, VIBV sequentially releases therapeutic cargo: oncogenic miRNA sponges neutralize malignancy, shRNA silences key oncogenes, and tumor-specific antigen-encoding mRNA triggers immune responses. Cyclin-targeting RNA constructs further inhibit proliferation by halting the cancer cell cycle. These combined mechanisms disrupt tumor growth at both the genetic and immune levels.

Unlike conventional vectors, VIBV effectively navigates the acidic, hypoxic TME while minimizing harm to healthy tissues. Its non-immunogenic liposomal coat reduces immune rejection, and its encapsulation ensures prolonged systemic circulation, surpassing the stability of traditional non-viral vectors. VIBV is likely to enhance tumor-targeting efficiency where conventional primers cannot eliminate the hyperacidity or hypoxia typical of malignant cells. The risk of harming healthy tissues is reduced due to its pH and hypoxia-responsive targeting specificity. The potential for immune rejection is significantly decreased due to the non-immunogenic liposomal coat, offering an advantage over viral vector-based therapies. Unlike conventional non-viral vectors, which degrade quickly, the prolonged systemic circulation of VIBV is ensured by its encapsulation.

Key features and components of the VIBV

We attempt to deliver a single-stranded miRNA sponge via the VIBV vector to eliminate cancer-related miRNAs due to its specific targeting capabilities and the ability to rapidly construct it at a low cost. We will delve into the details of this process below.

Utilizing miRNAs obtained from exosomes for cancer diagnosis shows promise as a non-invasive and rapid approach. This method can be investigated using the cost-effective and accurate bulk RNA barcoding and sequencing technique to sequence these RNAs.[93,94] Comparing miRNAs from healthy cells in the individual's body can help identify specific sequences that can serve as complementary targets for miRNA sponge and mRNA targeting. The minigene cloning method is employed to create the miRNA sponge, which is then inserted into the VIBV vector. A small double-stranded DNA is inserted into the vector, which functions similarly to scAA1V6-

shRNA, commonly used to inhibit proteins like aggrecanase-2 and aggrecanase-1.[95] The vector transcribes the DNA to create the shRNA, which inhibits cancer-related mRNAs and effectively suppresses cancer growth. The choice to utilize shRNA over siRNA is primarily motivated by the cost-effectiveness of production and its capability of delivering shRNA via lentiviral or retroviral transduction to almost all types of cell lines.[96] The vector also contains a third strand that encodes the information necessary to produce a tumor-specific antigen on the surface of cancer cells to stimulate the immune system and promote the destruction of cancerous cells. This process involves using an enzyme to transcribe DNA templates into RNA. The DNA template can be created by PCR using primers that contain the sequence for the tumor-specific antigen. After transcription, the mRNA can be purified and used for gene therapy.[97]

The fourth and final genetic cargo that must be placed inside the vector is RNA that causes the cancer cell cycle and its growth to stop. It has been proven that the replication of vesicular stomatitis virus ΔM51 (but not wild-type vesicular stomatitis virus) and a paramyxovirus (Sendai virus) is greatly enhanced by G2/M cell cycle arrest. This enhanced replication is likely due to the inhibition of antiviral gene expression caused by mitotic suppression of transcription during the G2/M phase. The findings suggest that the G2/M phase represents a vulnerable point in infected cells that can be exploited for virotherapy.[98] Our goal is to ensure that the final strand placed within the vector triggers the expression of the N protein, which will then inhibit the cyclins of the tumor cell and halt its growth in a similar manner to how the N protein of SARS-CoV inhibits S-phase progression in mammalian cells.[99] All the given components have been integrated into the design of the VIBV to ensure an effective and targeted cancer therapy approach.

VIBV design and structure

The VIBV vector has a unique spindle-shaped design. The following are the main reasons behind this design:

1. Length: The vector is small but has enough room to accommodate the genetic material, allowing for compact and efficient cargo delivery.
2. Penetration: Due to its sharp spindle shape, the vector can penetrate deep into the TME and avoid suppression by the harsh extracellular environment, unlike the previously mentioned viral vectors.
3. Increasing the contact surface of the inner layer of the vector with the outer layer of the liposome to facilitate cell entry: The process of viral infection is complex and varies depending on the specific virus. Some viruses, such as vaccinia virus or Newcastle disease virus, lack specific receptors and enter cells through a process called endocytosis, while others, like adenoviruses, use receptors on the cell surface, such as coxsackie and adenovirus receptor or integrins. Measles virus uses CD46 for entry, whereas HSV uses nectin or herpesvirus entry mediator. Although tumor cells are known to upregulate these receptors, they are also present in normal cells.[100] To address this challenge, a liposomal layer will coat the surface of the VIBV vector, which will be discussed further.

Self-assembly is a powerful technique for creating nanostructures. It generates much of the functionality of living cells and can simplify processes, reduce costs, and develop new approaches. It is also highly capable of fabricating nanostructures in the range of 1–100 nm. However, creating complex nanostructures requires careful consideration of critical parameters such as well-defined geometry and specific interactions between basic units. Self-assembly is relatively underused in microfabrication but holds potential for using components too small to be manipulated robotically, integrating components made using incompatible technologies, and generating structures in three dimensions and on curved surfaces.[101,102]

Viral capsids are resilient and easy to produce in large quantities. They self-assemble into symmetric and highly uniform structures, are programmable through genetic and chemical engineering, and can form arrays. Their ability to protect cargo while allowing for controlled uncoating is key in capsid design.[103,104] Using IP6 to stabilize the capsid can prevent the collapse of the VIBV vector during the process of entering cells to detect abnormal mRNA and miRNA.

The lipo-VIBV delivery system is a novel approach that utilizes liposomes to protect the vector and enhance its effectiveness for targeting cancerous cells. Polyethylene glycolylation, a process of attaching polyethylene glycol molecules to the surface of the liposomes, creates a stealth outer layer around the vectors. This reduces aggregation and nonspecific interactions, extends the blood circulation time of the vectors, and decreases rapid clearance by the reticuloendothelial system.[105] Unlike exosomes, liposomes are more uniform in structure with a more controllable surface charge as well as physical and chemical properties. These factors, along with the increased blood circulation and reduced clearance rate of pegylated liposomes, make them an appealing candidate for a delivery system.[106,107]

While most delivery systems rely on lipofection, a process that induces immune responses and cytotoxic effects, ibidi GmbH has developed Fuse-It liposomal reagents that increase the efficiency of fusion with the cell membrane by 80–100%. This molecule transfer does not rely on biological processes such as endocytosis, pinocytosis, or specific receptor binding.[108] Such commercial fusogenic nano-carriers containing RNA molecules were recently investigated by Hoffmann *et al*.[109] The results indicated that the RNAs were successfully transferred to the cytoplasm, and cytokine expression decreased. Moreover, immune responses were minimal in zebrafish embryos, zebrafish brains and cortical tissue, and the brains of mammals.[109]

After the successful transfer of the vector to the cells in the tumor area, the liposomal layer of the lipo-VIBV delivery system fuses with the membrane of the cells concentrated in the tumor area, causing the vector to enter and initiate the treatment process. The fabrication of a responsive nanocarrier, DANPCT, is an example of a hypoxia- and acidity-sensitive nanoparticle designed for the specific treatment of tumor cells. The nanocarrier exhibited improved stability at neutral pH and displayed a rapid response to pH-activated transcriptional trans-activator (TAT) function, accelerating internalization by tumor cells. This example contributes to the rational design of TME-responsive nanocarriers for precise and effective cancer treatment.[110] Incorporating pH, oxidative stress, or hypoxia-sensitive substances into the vector can increase its ability to target and enter cancerous cells.

GSH-responsive nanocarriers are a promising approach in cancer therapy, designed to target cancer cells and release drugs effectively by exploiting the high GSH levels found inside these cells. These carriers remain stable in the bloodstream but break down in the presence of elevated GSH concentrations (2–10 mM in cancer cells compared to 2–20 μM outside), ensuring precise drug release within tumors.[111,112] Some tumor tissues have a phenomenon known as leaky blood vessels, which causes certain particles to accumulate in tumor tissues—this is referred to as the enhanced permeability and retention effect.[113] Nanoparticles have

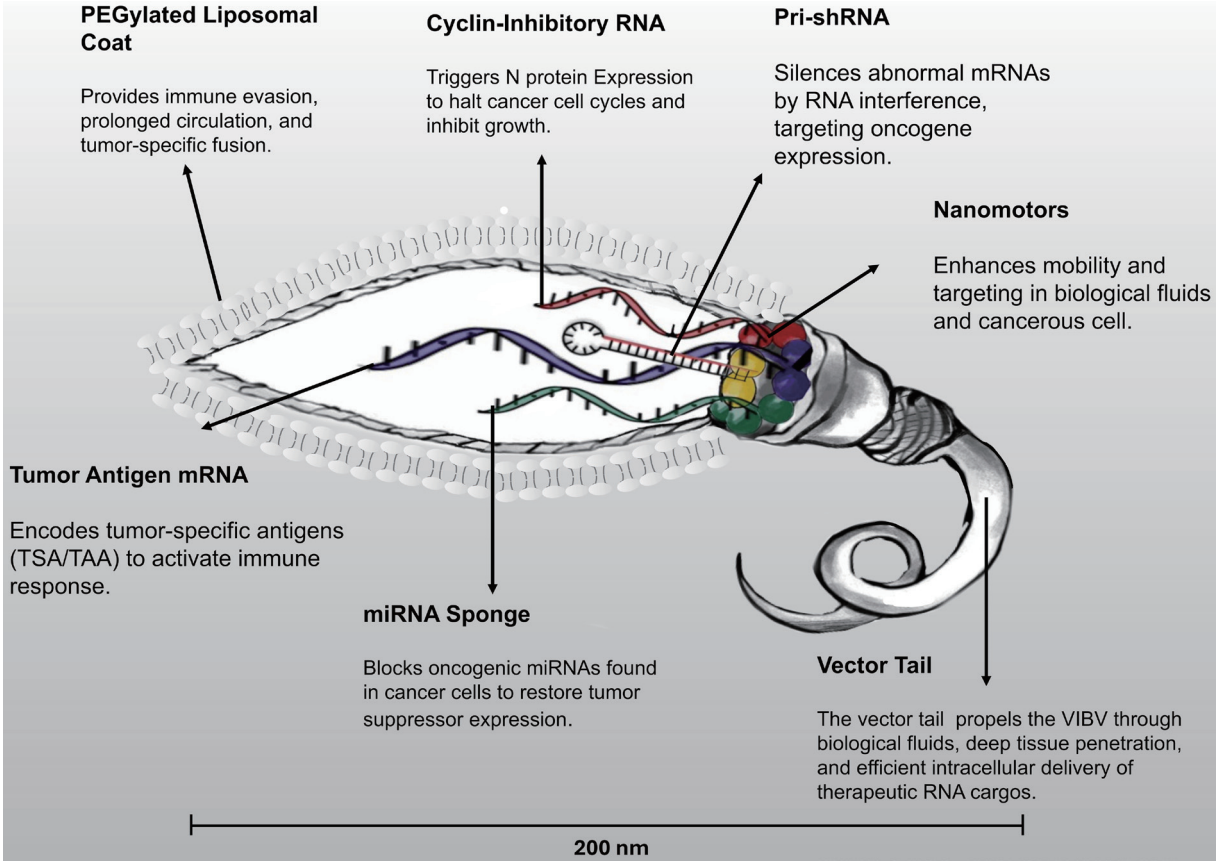

Fig. 1. Schematic representation of the VIBV. The vector consists of a polyethylene glycolylated liposomal outer coat (providing immune evasion and prolonged circulation), a spindle-shaped nanomotor (resembling a flagellum, facilitating targeted movement), and four distinct internal cargo compartments: (1) miRNA sponge, (2) shRNA, (3) tumor-specific antigen mRNA, and (4) cyclin-inhibitory RNA. These compartments enable multi-step cancer-targeting actions. The approximate length of the spindle-shaped structure is approximately 200 nm. The structure shown is conceptual and illustrates the design framework of the VIBV. miRNA, microRNA; mRNA, messenger RNA; PEG, polyethylene glycol; shRNA, short hairpin RNA; TAA, tumor associated antigen; TSA, tumor specific antigen; VIBV, Vir-inspired Biotechnological Vector.

been shown to deliver platinum drugs, resulting in rapid cancer cell death via GSH-triggered drug release. For instance, Ling *et al*.[114] demonstrated how nanoparticles transport platinum drugs with rapid GSH-induced release, killing cancer cells in the process. These carriers help overcome drug resistance and improve therapy outcomes.[115] One of the most promising aspects is the ability to image and track drug carriers using fluorescence and PET imaging, which allows for real-time monitoring of drug delivery and can assist in tailoring treatments.[116] Upon entering the cell, the vector ensures it has reached a cancerous environment by detecting elevated GSH levels, which are significantly higher in tumor cells than in normal tissues. This biochemical cue triggers the controlled opening of the vector, releasing its therapeutic RNA cargo. These RNA molecules then utilize the cell's microtubules as transport highways to reach their designated intracellular targets, facilitating precise gene silencing and therapeutic action. This targeted approach enhances treatment specificity, minimizing harm to healthy cells while maximizing therapeutic efficacy within the tumor.

In the design of sperm-based nanorobots, one goal is to utilize the intrinsic driving force of motile sperm to power the machines. The VIBV vector has the ability to move through a sperm-inspired tail, which resembles biological fluids. An additional aspect of the flagellum is its ability to penetrate deep into tissue with the help of its propulsive force, accelerating the treatment process. In 2021, Celi *et al*.[117] successfully self-assembled superparamagnetic heads and flexible Au/PPy flagella nanorobots. Under magnetic fields, the heads rotated the tails and generated undulatory waves for bi-directional locomotion. Locomotive properties varied with field strength, frequency, direction, and tail length.[117] By using self-assembly, artificial molecular motors are placed inside a vector, and up to four nucleic acid strands are connected to them. These strands include a miRNA sponge, double-stranded DNA for shRNA, messenger RNA for expressing tumor-associated antigens on the surface of the cancerous cell, and RNA that causes the cell cycle to stop and inhibit cyclins. Inside the cancer cell, these molecular motors move these strands with linear movements on the microtubules of the host cell. When these motors are inside the vector, they cause the vector's flagellum to move like that of sperm. The basic structure of the VIBV vector is presented in Figure 1.

VIBV vector mechanism of action

VIBV is injected systemically into the body to treat cancer. This modified vector carries designed strands that specifically target and eliminate cancer cells, demonstrating promising theoretical potential for significant tumor reduction. The vectors, using their flagella to swim through the blood, search for tumors guided by

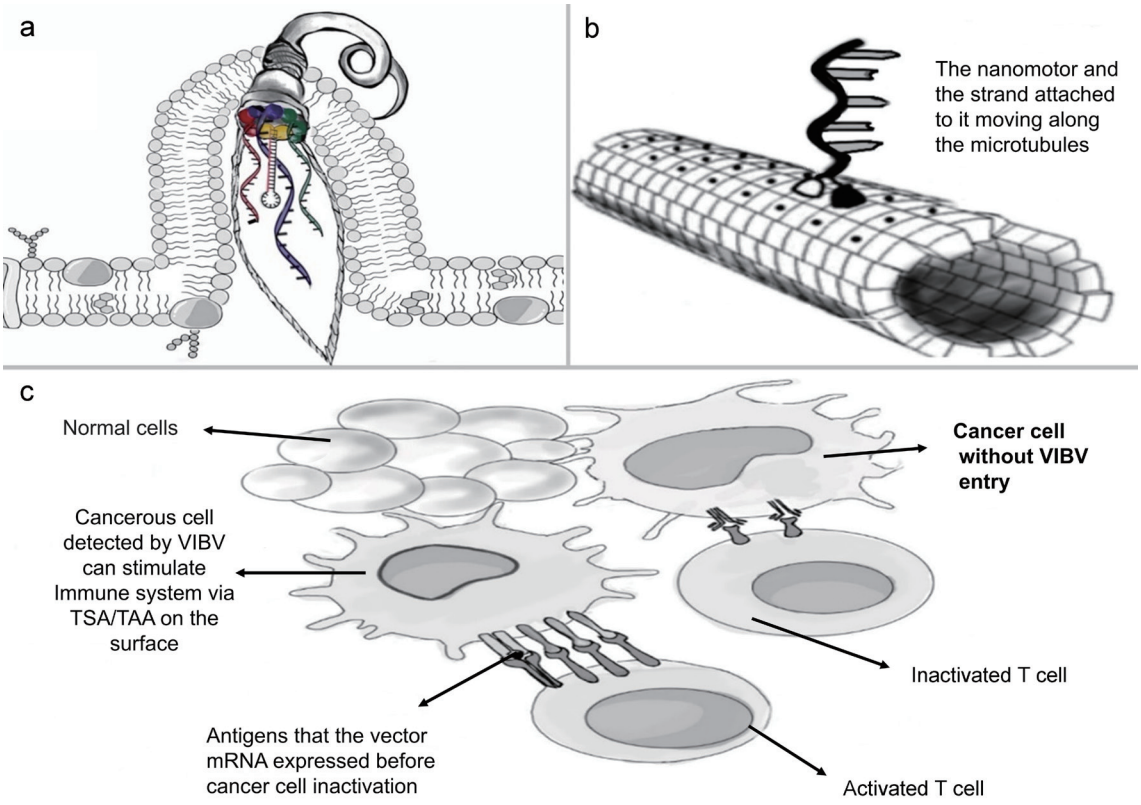

Fig. 2. Conceptual mechanism of action of the VIBV. (a) The VIBV's polyethylene glycolylated liposomal coat fuses with the tumor cell membrane, allowing vector entry. (b) Once inside, nanomotors and RNA cargo interact with the microtubules for intracellular transport. (c) Released RNAs sequentially perform therapeutic tasks: miRNA sponges neutralize oncogenic miRNAs, shRNA silences tumor genes, tumor antigen mRNA activates immune responses, and cyclin-inhibitory RNA arrests cancer cell division. The mechanisms illustrated are based on theoretical design and currently reflect preclinical hypotheses rather than fully validated *in vivo* pathways. GSH, glutathione; miRNA, microRNA; mRNA, messenger RNA; RISC, RNA-induced silencing complex; shRNA, short hairpin RNA; TAA, tumor associated antigen; TME, tumor microenvironment; TSA, tumor specific antigen; VIBV, Vir-Inspired Biotechnological Vector.

acidity and hypoxia. The presence of nanoparticles further enhances the vector's attraction to tumors, increasing the chances of making contact. Once the vector has entered the TME, it passes through the cell membrane of the tumor cell, allowing it to access the interior while shedding the outer liposomal layer during fusion (Fig. 2a).

Upon entry into the cancerous cell, the GSH nanosensor will facilitate the release of the vector's cargo into the cytoplasmic environment. The negative charge on the microtubules within the tumor cell then facilitates the connection of the vector to the microtubules, enabling the treatment process to begin. After connecting to the microtubule, the vector compartment opens, exposing its cargo to the alkaline cytoplasm of the cancer cell. Four pairs of nanomotors, as mentioned in this article, are activated in this environment. By halting their spinning movements, they detach from the vector and move along the microtubule to reach their designated destination (Fig. 2b).

These four pairs of molecular motors are labeled based on their sensitivity to the alkaline environment. The first nanomotor, which is the most sensitive, begins to move linearly and guides the attached miRNA sponge to its target. If the cell is cancerous and the miRNA sponge sequence matches the miRNA found in the exosomes of the patient, the process of eliminating the cell continues. However, if there is no match between the miRNA sponge and the RISC, it indicates that the cell is not cancerous, and the miRNA target of the miRNA sponge is not present. In this case, the activity of the miRNA sponge will not lead to the cleavage of any miRNA. Ultimately, the healthy cell's lysosomes will eliminate the vector. If the miRNA sponge is complementary to the targeted miRNA, the process of gradual destruction of defective miRNAs begins in the cell. Simultaneously, the stimulus that triggers the second nanomotor is generated, initiating the movement of the second strand, which is a double-stranded polynucleotide chain, along the cancerous cell's microtubule.

In this scenario, the polynucleotide double strand is constructed with promoter sequences suitable for the polymerization of either RNA polymerase III or II for shRNA. The molecular motor then directs this strand to the cell nucleus for transcription by the RNA polymerase enzyme to produce multiple copies. A series of enzymes, including DGCR8 and Drosha, will convert the shRNA into its precursor form, Pri-shRNA. This molecule is subsequently exported from the cell nucleus by exportin 5. The final step involves processing by the enzyme Dicer, which adds it to the RISC. At this point, the passenger strand of the double strand is discarded. The guide strand then directs RISC to bind its mRNA target that possesses a complementary sequence, ultimately leading to mRNA degradation.

After stimulation, the third nanomotor moves toward the ribosomes, allowing the mRNA strand attached to the nanomotor to initiate the production of tumor-specific and tumor-associated antigens on the surface of the cancer cell. This action triggers the immune system to respond and directs the body's immune cells to recognize and destroy the cancer cell, which contributes to tumor suppression.

Table 1. Overview of recent RNA-targeting strategies for cancer treatment, focusing on circRNAs, miRNAs, and siRNA-based therapies in preclinical studies

Cancer type	Therapeutic approach	Mechanism of action	Preclinical findings	Reference
PDAC	miR-506-3p delivered via PEI F25-LMW nanoparticles	Induces apoptosis, necrosis, autophagy, and ROS production	High biocompatibility in mice, no significant side effects, but no complete tumor regression	118
TNBC	Nanodrug targeting miR-10b (antisense oligonucleotides + magnetic nanoparticles)	Prevents metastasis and inhibits the growth of pre-existing metastases	Effective in TNBC models	119
HCC	shRNA targeting KIF23	Regulates miR-107/KIF23 axis, reducing hepatoma cell proliferation	Prevented oncogene-induced liver cancer in mouse models	120
CRC	RNAi or shRNA targeting circRNAs	Inhibits circRNAs that act as miRNA sponges	Efficacy demonstrated in preclinical *in vivo* models	121
cRCC	Fusogenic liposome encapsulating siRNA against C14orf142	Targets functional genes required for metastasis	Designed to interfere with metastatic dissemination	122
TNBC	Nanodrug targeting miR-10b	Prevents metastasis and inhibits cancer stem cell survival	Demonstrated efficacy in TNBC metastasis models	119
NSCLC	siRNA or shRNA targeting up-regulated circRNAs	Inhibits circRNAs that sponge miRNAs, preventing mRNA target cleavage	Identified circRNAs promoting NSCLC growth in preclinical models	123
CRC	RNAi or shRNA targeting chemoresistance-associated circRNAs	Suppresses circRNAs mediating drug resistance and upregulating key cellular components	Effective in preclinical models against drug-resistant CRC	121
PDAC	miR-506-3p replacement therapy	Induces autophagy, apoptosis, senescence, and mitochondrial alterations	Modulates oncogene and suppressor gene expression	118
RCC	siRNA via fusogenic liposomes and lncRNA blockade	Targets genes required for metastasis and blocks lncRNA pathways to overcome drug resistance	Potential synergy with TKIs	122
PCa	Targeting circFKBP5 and circRNA-ARC1	Reduces miRNA sponging to regulate genes involved in cancer progression	Potential suppression of metastasis and improved therapeutic outcomes	124

Studies included in this table were selected based on their relevance to RNA-based cancer therapies involving miRNA, siRNA, or circRNA delivery, with a focus on preclinical models published in peer-reviewed journals between 2018 and 2024. Only studies providing mechanistic insight and measurable therapeutic outcomes were included. C14orf142, chromosome 14 open reading frame 142; circFKBP5, circular RNA FKBP prolyl isomerase 5; circRNA-ARC1, circular RNA- activity-regulated cytoskeleton-associated protein 1; CRC, colorectal cancer; cRCC, clear cell renal cell carcinoma; HCC, hepatocellular carcinoma; KIF23, Kinesin family member 23; NSCLC, non-small cell lung cancer; PCa, prostate cancer; PDAC, pancreatic ductal adenocarcinoma; PEI F25-LMW, polyethylenimine F25-low molecular weight; RNAi, RNA interference; ROS, reactive oxygen species; shRNA, short-hairpin RNA; siRNA, small interfering RNA; TKIs, tyrosine kinase inhibitors; TNBC, triple-negative breast cancer.

The final nanomotor contains RNA that triggers the production of the N protein, which blocks the cyclins in the cell cycle of the cancer cell. This action leads to the termination of cell division and the arrest of the growth of cancer cells, preventing the spread of cancer (Fig. 2c).

Preclinical evidence and supporting data for the VIBV vector

The progress in the development of the VIBV stems from the recent integration of viral gene delivery systems with non-viral-based systems. The great efficiency of transfection is only one component to consider. As the constraints of conventional delivery systems, including, but not limited to, immunogenicity, lack of specificity, and low intracellular uptake, continue to increase, new bioengineered approaches for RNA-based cancer therapy are desperately needed.

In recent years, preclinical and clinical studies have demonstrated the potential of miRNA-based interventions in cancer cell therapy. Various delivery strategies, including nanoparticles, viral vectors, and exosomes, have been explored to enhance the stability and efficacy of miRNA therapeutics. A brief summary has been provided in Table 1.[118–124] Despite these advancements, challenges such as immune responses, off-target effects, and efficient tumor targeting remain significant hurdles. Below, we explore some of the most relevant research studies in this area.

Hybrid vector systems and enhanced delivery efficiency

The use of some virus-derived components in fully artificial nanocarrier systems has been shown to improve their transfection efficiency and retention. Research within the scope of VLPs and lipid-polymer hybrid nanospheres suggests that there are many benefits to be gained from imitating viral cell entry mechanisms, while non-viral vectors remain less toxic. VIBV has been designed to provide higher stability of RNA, better tumor delivery, and lower non-specific systemic exposure. A number of VLP-enabled

Table 2. Recent advances in the applications of nanomotors for different fields of therapy

Name	Model	Target	Results	Efficiency	Reference
CMPBC	*In vitro*: Intestinal epithelial and mucus-secreting cells, colon cancer cells. *In vivo*: Various colorectal cancer mouse models, including orthotopic and advanced tumor models	CRC	3.5× higher drug delivery, 8.3× improved mucus penetration, 98.6% tumor inhibition, 60% survival at day 60, no significant toxicity	Mucus penetration: 8.3× Drug delivery: 3.5× Therapeutic: 98.6%	132
Gd-MCNs/ Pt-RAPA-AC	ApoE−/− mice, high-fat diet	Atherosclerosis	Reduced plaque ratio to 8.03%, decreased inflammation	Plaque ratio 8.03%	129
Helical magnetic	Mice	Safety assessment	No toxicity up to 55 mg/kg	Safe up to 55 mg/kg	130
MSNPs	Tumor-bearing mice	Bladder cancer	Promising drug delivery nano system	At high radiochemical yields (73 ± 10%) and excellent radiochemical purity (≥99%). The labelled nanobots showed good stability at 37 °C in both water and 300 mM urea	133
AG-DMSNs	Mouse wound model	MRSA infection	99% anti-biofilm efficiency, bacterial burden reduced	99% anti-biofilm	131
Urea-fueled, AMPs	Murine infection model	Acinetobacter baumannii	Reduced infections by 3 orders of magnitude	3 orders reduction	131
RBC-HNTM-Pt@Au	MRSA-infected osteomyelitis	MRSA	99.9% antibacterial after 15 m US, eradication in 4 weeks	99.9% antibacterial	131

AMPs, antimicrobial peptides; CMPBC, CP@MSN/PB@CWL; CRC, colorectal cancer; MRSA, methicillin-resistant Staphylococcus aureus; MSNPs, mesoporous silica nanoparticles; US, ultrasound.

prophylactic vaccines are in development or already available. However, only those directed against Hepatitis B Virus (HBV) and Human papillomavirus (HPV) are licensed for clinical application in humans. The rest remain in the phase of preliminary tests.[8]

Research indicates that VLPs could be valuable for enhancing preclinical tumor models since they can improve drug delivery and have shown promise as nanocarriers in targeted therapy for cancer. For example, potato virus X modified with doxorubicin (DOX) demonstrates greater efficacy than free DOX, significantly shrinking tumors in different models of MDA-MB-231 breast cancer xenografts.[125] Tobacco mosaic virus (TMV)-based platforms have also been examined for glioblastoma and triple-negative breast cancer (TNBC).[126] DOX-loaded TMV increased survival in intracranial glioblastoma models, while mitoxantrone-loaded TMV was found to be more useful in TNBC xenografts.[126,127] Additionally, TRAIL-potato virus X completely regressed tumors in TNBC models 30 days post-administration. These studies suggest the need for developing VLPs for cancer nanomedicine, emerging as a new platform that warrants further exploration with respect to their stability, targeting, and therapeutic delivery efficacy.[128]

Nanomotors

In 2025, a study developed a dual-mode-driven nanomotor capable of targeting inflammatory macrophages for magnetic resonance imaging and atherosclerosis therapy. These nanomotors were Gd-doped mesoporous carbon nanoparticles/Pt with rapamycin AntiCD36 antibody modification, which, besides using hydrogen peroxide, were also propelled by near-infrared laser. In ApoE−/− mouse models, self-motion and photothermal ablation were enhanced, and MR imaging showed a peak signal at 6 h post-injection, with the most intense signals in the Gd-doped mesoporous carbon nanoparticles/Pt-rapamycin AntiCD36 antibody modification + near-infrared group. Aortic oil red O staining showed a plaque burden of 8.03% in the experimental group compared to 34.41% in the controls, alongside lower inflammatory markers (interferon-γ, interleukin-6, tumor necrosis factor-α) and total cholesterol, suggesting anti-inflammation.[129] In a 2022 study on magnetic nanomotors powered by external fields, complete biocompatibility was observed, with no toxicity found in mice up to 55 mg/kg. These nanomotors had a circulation phase in the blood before they were observed to adhere to the walls of blood vessels, and perfusion was shown to reduce adhesion, supporting their use for safe drug delivery.[130] In the area of nanomotors, there is great potential for their use as antimicrobial agents. Silver-gated dendritic mesoporous silica nanoparticles were shown to penetrate MRSA-infected wound biofilms in mice, resulting in 99% effectiveness and a 10,000-fold decrease in bacteria. In other studies, urea-fueled enzymatic nanomotors with antifouling peptides improved Acinetobacter baumannii infection rates in mouse models by over 1,000 times. These results highlight the greater potential of nanomotors beyond drug delivery, extending to imaging and multitargeted therapies.[131] See Table 2 for more details.[129-133]

Future directions

While progress has been made in preclinical research on miRNA-based therapeutics, they are still in their infancy, with very few advancing to phase III clinical trials or receiving the approval of the U.S. Food and Drug Administration. Other challenges include the need to improve target specificity, reduce immunogenicity, enhance delivery methods, and optimize dosing for efficacy and safety.[134]

The integration of viral and non-viral vector systems, alongside modern techniques in nanotechnology, in the VIBV formulation marks an important milestone in precision medicine for cancer therapy. VIBV is designed to increase specificity and stability while achieving greater delivery efficiency and reduced off-target effects. The tumor cells are locally targeted by VIBV through pH and hypoxia sensitivity, enabling gene silencing and tumor suppression. In other words, the combination of hypoxia sensitivity, high GSH levels, and the precise matching of engineered RNAs within the vector ensures effective treatment inside cancer cells while minimizing damage to healthy tissues. This targeted approach leverages the unique TME, where hypoxia and elevated GSH levels act as triggers for the vector to release its therapeutic cargo. The engineered RNAs, such as miRNA sponges and shRNA, are designed to match specific cancer-related targets, ensuring accurate gene silencing and immune activation within cancer cells. By responding to these tumor-specific conditions, the vector maximizes therapeutic efficacy while reducing off-target effects. As a result, patients are likely to experience fewer side effects during treatment, making this approach a promising advancement in cancer therapy. Its fusogenic liposomal coat lowers immunogenicity and enhances uptake, and the multi-modal approach of VIBV, encompassing oncogenic miRNA suppression, oncogene silencing, tumor antigen expression, and disruption of the cell cycle, offers a comprehensive anti-cancer strategy. Due to the spindle-like form and the movement provided by the nanomotors, VIBV is expected to be more penetrative toward tumors in proportion to its systemic circulation throughout the body. The toxicity of VIBV is also lower than that of other viral vectors, as its non-immunogenic coating renders it non-immunogenic. These features are important because such a self-assembling structure increases the reproducibility and scalability of VIBV. Optimizing large-scale production and further refining specificity toward tumors to evade off-target interactions remain priorities. Maximizing controlled release of the cargo and minimizing off-target effects are also key to achieving the desired impact. Extensive preclinical and clinical testing will be needed to confirm safety and therapeutic efficacy. Improvement of tracking imaging modalities, changes in targeting ligands, and potential integration for other genetic disorders could broaden the scope of applications for VIBV. The use of clustered regularly interspaced short palindromic repeats for gene editing may further improve its application in the precision medicine field. While the reviewed VIBV platform demonstrates an innovative integration of viral and non-viral elements for targeted cancer therapy, several limitations warrant careful consideration to assess its translational viability. Notably, the self-assembled architecture of VIBV may face batch-to-batch reproducibility issues, which could hinder large-scale production and regulatory approval. Additionally, although polyethylene glycolylation is employed to minimize immune detection, the use of sperm-like nanomotors could still provoke unforeseen immunogenic responses *in vivo*. Another critical limitation is the absence of comparative *in vivo* efficacy data against well-established delivery systems, such as AAVs or LNPs. Addressing these challenges through rigorous preclinical testing and direct benchmarking will be essential before clinical translation can be realistically envisioned.

Conclusions

Due to the heterogeneity of cancers across individuals, the use of personalized treatments is becoming increasingly important. It is no longer feasible to apply a one-size-fits-all approach to cancer treatment, as each case requires a tailored therapeutic strategy for optimal efficacy. VIBV presents an innovative and exciting solution for gene therapy and personalized medicine across different cancer types, addressing major problems in delivery, treatment accuracy, and efficiency simultaneously by combining novel and promising therapies. Innovations like VIBV could dramatically alter cancer treatment, but further development and proof of concept will be necessary for clinical use. Rigorous preclinical studies, optimization of large-scale production, and thorough safety evaluations will be critical for translating VIBV into a viable therapeutic platform. Addressing challenges such as immune compatibility, regulatory approval, and precise tumor targeting will define its future clinical success. As advancements in gene delivery and nanotechnology continue to evolve, VIBV offers a promising foundation for the next generation of precision oncology treatments.

Acknowledgments

The authors would like to thank all the staff of the Cancer Department at the Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Funding

None.

Conflict of interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Conceptualization (HR), data extraction (HR, SMM, NF, MN), resources (ENM, AS), validation (SMM, NF, ENM), writing – original draft preparation (HR, AZ), writing – review & editing (ZS, ENM), and supervision (ZS, ENM). All authors approved the final version to be published, and they all agreed to be accountable for all aspects of the work.

References

[1] Cesur-Ergün B, Demir-Dora D. Gene therapy in cancer. J Gene Med 2023;25(11):e3550. doi:10.1002/jgm.3550, PMID:37354071.
[2] Schott JW, Morgan M, Galla M, Schambach A. Viral and Synthetic RNA Vector Technologies and Applications. Mol Ther 2016;24(9):1513–1527. doi:10.1038/mt.2016.143, PMID:27377044.
[3] Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. Signal Transduct Target Ther 2021;6(1):53. doi:10.1038/s41392-021-00487-6, PMID:33558455.
[4] Bezeljak U. Cancer gene therapy goes viral: viral vector platforms come of age. Radiol Oncol 2022;56(1):1–13. doi:10.2478/raon-2022-

0002, PMID:35148469.
[5] Lundstrom K. Viral Vectors in Gene Therapy: Where Do We Stand in 2023? Viruses 2023;15(3):698. doi:10.3390/v15030698, PMID: 36992407.
[6] Wang C, Pan C, Yong H, Wang F, Bo T, Zhao Y, *et al*. Emerging non-viral vectors for gene delivery. J Nanobiotechnology 2023;21(1):272. doi:10.1186/s12951-023-02044-5, PMID:37592351.
[7] Zu H, Gao D. Non-viral Vectors in Gene Therapy: Recent Development, Challenges, and Prospects. AAPS J 2021;23(4):78. doi:10.1208/s12248-021-00608-7, PMID:34076797.
[8] Tornesello AL, Tagliamonte M, Buonaguro FM, Tornesello ML, Buonaguro L. Virus-like Particles as Preventive and Therapeutic Cancer Vaccines. Vaccines (Basel) 2022;10(2):227. doi:10.3390/vaccines10020227, PMID:35214685.
[9] Antil M, Gupta V. Nanorobots in Medicine: Advancing Healthcare through Molecular Engineering: A Comprehensive Review. IgMin Res 2024;2(11):938–949. doi:10.61927/igmin271.
[10] Wang Y, Baars I, Berzina I, Rocamonde-Lago I, Shen B, Yang Y, *et al*. A DNA robotic switch with regulated autonomous display of cytotoxic ligand nanopatterns. Nat Nanotechnol 2024;19(9):1366–1374. doi:10.1038/s41565-024-01676-4, PMID:38951595.
[11] Kong X, Gao P, Wang J, Fang Y, Hwang KC. Advances of medical nanorobots for future cancer treatments. J Hematol Oncol 2023;16(1):74. doi:10.1186/s13045-023-01463-z, PMID:37452423.
[12] Chakrabortty A, Patton DJ, Smith BF, Agarwal P. miRNAs: Potential as Biomarkers and Therapeutic Targets for Cancer. Genes (Basel) 2023;14(7):1375. doi:10.3390/genes14071375, PMID:37510280.
[13] Huang L, Xia L, Nie T, Cui B, Lu J, Lu F, *et al*. Maintaining Drosha expression with Cdk5 inhibitors as a potential therapeutic strategy for early intervention after TBI. Exp Mol Med 2024;56(1):210–219. doi:10.1038/s12276-023-01152-4, PMID:38200156.
[14] Seres M, Spacayova K, Sulova Z, Spaldova J, Breier A, Pavlikova L. Dynamic Multilevel Regulation of EGFR, KRAS, and MYC Oncogenes: Driving Cancer Cell Proliferation Through (Epi)Genetic and Post-Transcriptional/Translational Pathways. Cancers (Basel) 2025;17(2):248. doi:10.3390/cancers17020248, PMID:39858030.
[15] Shirsath NR, More MP, Deshmukh PK, Bagul VS, Patel JK, Bhavsar BV, *et al*. Novel Drug Delivery Systems in the Treatment of Cancer: A Better Future Hope. In: Keservani RJ, Laddha UD, Ahire ED, Kshirsagar SJ (eds). Novel Drug Delivery Systems in the Management of Chronic Diseases. 1st ed. New York: Apple Academic Press; 2024:199–254. doi:10.1201/9781032654881-10.
[16] Singh S, Sinha T, Panda AC. Regulation of microRNA by circular RNA. Wiley Interdiscip Rev RNA 2023;15(1):e1820. doi:10.1002/wrna.1820, PMID:37783567.
[17] Niveditha D, Jasoria M, Narayan J, Majumder S, Mukherjee S, Chowdhury R, *et al*. Common and Unique microRNAs in Multiple Carcinomas Regulate Similar Network of Pathways to Mediate Cancer Progression. Sci Rep 2020;10(1):2331. doi:10.1038/s41598-020-59142-9, PMID:32047181.
[18] Zhang Z, Huang Q, Yu L, Zhu D, Li Y, Xue Z, *et al*. The Role of miRNA in Tumor Immune Escape and miRNA-Based Therapeutic Strategies. Front Immunol 2021;12:807895. doi:10.3389/fimmu.2021.807895, PMID:35116035.
[19] Yao R, Xie C, Xia X. Recent progress in mRNA cancer vaccines. Hum Vaccin Immunother 2024;20(1):2307187. doi:10.1080/21645515.2024.2307187, PMID:38282471.
[20] Araújo NM, Rubio IGS, Toneto NPA, Morale MG, Tamura RE. The use of adenoviral vectors in gene therapy and vaccine approaches. Genet Mol Biol 2022;45(3 Suppl 1):e20220079. doi:10.1590/1678-4685-GMB-2022-0079, PMID:36206378.
[21] Braun CJ, Boztug K, Paruzynski A, Witzel M, Schwarzer A, Rothe M, *et al*. Gene therapy for Wiskott-Aldrich syndrome—long-term efficacy and genotoxicity. Sci Transl Med 2014;6(227):227ra33. doi:10.1126/scitranslmed.3007280, PMID:24622513.
[22] Rossi M, Breman E. Engineering strategies to safely drive CAR T-cells into the future. Front Immunol 2024;15:1411393. doi:10.3389/fimmu.2024.1411393, PMID:38962002.
[23] Balakrishnan B, David E. Biopolymers augment viral vectors based gene delivery. J Biosci 2019;44(4):84. PMID:31502562.
[24] Chuecos MA, Lagor WR. Liver directed adeno-associated viral vectors to treat metabolic disease. J Inherit Metab Dis 2024;47(1):22–40. doi:10.1002/jimd.12637, PMID:37254440.
[25] Zacchigna S, Zentilin L, Giacca M. Adeno-associated virus vectors as therapeutic and investigational tools in the cardiovascular system. Circ Res 2014;114(11):1827–1846. doi:10.1161/CIRCRESAHA.114.302331, PMID:24855205.
[26] Okada M, Ortiz E. Therapeutic potential of viral vectors that express venom peptides for neurological diseases. Med Hypotheses 2022;161:110809. doi:10.1016/j.mehy.2022.110809.
[27] Travieso T, Li J, Mahesh S, Mello JDFRE, Blasi M. The use of viral vectors in vaccine development. NPJ Vaccines 2022;7(1):75. doi:10.1038/s41541-022-00503-y, PMID:35787629.
[28] Dock G. The influence of complicating diseases upon leukaemia. The American Journal of the Medical Sciences 1904;127(4):563–592. doi:10.1097/00000441-190404000-00001.
[29] Kelly E, Russell SJ. History of oncolytic viruses: genesis to genetic engineering. Mol Ther 2007;15(4):651–659. doi:10.1038/sj.mt.6300108, PMID:17299401.
[30] Larson C, Oronsky B, Scicinski J, Fanger GR, Stirn M, Oronsky A, *et al*. Going viral: a review of replication-selective oncolytic adenoviruses. Oncotarget 2015;6(24):19976–19989. doi:10.18632/oncotarget.5116, PMID:26280277.
[31] Kay MA, He CY, Chen ZY. A robust system for production of minicircle DNA vectors. Nat Biotechnol 2010;28(12):1287–1289. doi:10.1038/nbt.1708, PMID:21102455.
[32] Bouard D, Alazard-Dany D, Cosset FL. Viral vectors: from virology to transgene expression. Br J Pharmacol 2009;157(2):153–165. doi:10.1038/bjp.2008.349, PMID:18776913.
[33] Lee CS, Bishop ES, Zhang R, Yu X, Farina EM, Yan S, *et al*. Adenovirus-Mediated Gene Delivery: Potential Applications for Gene and Cell-Based Therapies in the New Era of Personalized Medicine. Genes Dis 2017;4(2):43–63. doi:10.1016/j.gendis.2017.04.001, PMID:28944281.
[34] Greber UF, Suomalainen M. Adenovirus entry: Stability, uncoating, and nuclear import. Mol Microbiol 2022;118(4):309–320. doi:10.1111/mmi.14909, PMID:35434852.
[35] Lewis TB. Development of an adenoviral vector platform for selective gene delivery to neurons [Dissertation]. Birmingham: The University of Alabama at Birmingham; 2012.
[36] Lee SJ, Kim SJ, Seo HH, Shin SP, Kim D, Park CS, *et al*. Over-expression of miR-145 enhances the effectiveness of HSVtk gene therapy for malignant glioma. Cancer Lett 2012;320(1):72–80. doi:10.1016/j.canlet.2012.01.029, PMID:22269208.
[37] Muravyeva A, Smirnikhina S. Adenoviral Vectors for Gene Therapy of Hereditary Diseases. Biology (Basel) 2024;13(12):1052. doi:10.3390/biology13121052, PMID:39765719.
[38] Wold WS, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. Curr Gene Ther 2013;13(6):421–433. doi:10.2174/15665232133666131125095046, PMID:24279313.
[39] Christ M, Lusky M, Stoeckel F, Dreyer D, Dieterlé A, Michou AI, *et al*. Gene therapy with recombinant adenovirus vectors: evaluation of the host immune response. Immunol Lett 1997;57(1-3):19–25. doi:10.1016/s0165-2478(97)00049-7, PMID:9232420.
[40] Nász I, Adám E. Recombinant adenovirus vectors for gene therapy and clinical trials. Acta Microbiol Immunol Hung 2001;48(3-4):323–348. doi:10.1556/AMicr.48.2001.3-4.6, PMID:11791337.
[41] Alan E, Peter C. Retroviral integrase structure and DNA recombination mechanism. In: Chandler M, Gellert M, Lambowitz AM, Rice PA, Sandmeyer SB (eds). Mobile DNA III. Washington, DC: ASM Press; 2015:1011–1033. doi:10.1128/9781555819217.ch44.
[42] Dunbar CE, High KA, Joung JK, Kohn DB, Ozawa K, Sadelain M. Gene therapy comes of age. Science 2018;359(6372):eaan4672. doi:10.1126/science.aan4672, PMID:29326244.
[43] Brusson M, Chalumeau A, Martinucci P, Romano O, Felix T, Poletti V, *et al*. Novel lentiviral vectors for gene therapy of sickle cell disease combining gene addition and gene silencing strategies. Mol Ther Nucleic Acids 2023;32:229–246. doi:10.1016/j.omtn.2023.03.012, PMID:37090420.
[44] Kasar S, Salerno E, Yuan Y, Underbayev C, Vollenweider D, Laurindo MF, *et al*. Systemic in vivo lentiviral delivery of miR-15a/16 reduces malignancy in the NZB de novo mouse model of chronic lympho-

cytic leukemia. Genes Immun 2012;13(2):109–119. doi:10.1038/gene.2011.58, PMID:21881595.
[45] Anson DS. The use of retroviral vectors for gene therapy-what are the risks? A review of retroviral pathogenesis and its relevance to retroviral vector-mediated gene delivery. Genet Vaccines Ther 2004;2(1):9. doi:10.1186/1479-0556-2-9, PMID:15310406.
[46] Yi Y, Hahm SH, Lee KH. Retroviral gene therapy: safety issues and possible solutions. Curr Gene Ther 2005;5(1):25–35. doi:10.2174/1566523052997514, PMID:15638709.
[47] Kurian KM, Watson CJ, Wyllie AH. Retroviral vectors. Mol Pathol 2000;53(4):173–176. doi:10.1136/mp.53.4.173, PMID:11040938.
[48] Mnyandu N, Limani SW, Arbuthnot P, Maepa MB. Advances in designing Adeno-associated viral vectors for development of anti-HBV gene therapeutics. Virol J 2021;18(1):247. doi:10.1186/s12985-021-01715-9, PMID:34903258.
[49] Domenger C, Grimm D. Next-generation AAV vectors-do not judge a virus (only) by its cover. Hum Mol Genet 2019;28(R1):R3–R14. doi:10.1093/hmg/ddz148, PMID:31261383.
[50] Barnes C, Scheideler O, Schaffer D. Engineering the AAV capsid to evade immune responses. Curr Opin Biotechnol 2019;60:99–103. doi:10.1016/j.copbio.2019.01.002, PMID:30807882.
[51] Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov 2019;18(5):358–378. doi:10.1038/s41573-019-0012-9, PMID:30710128.
[52] Naso MF, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. BioDrugs 2017;31(4):317–334. doi:10.1007/s40259-017-0234-5, PMID:28669112.
[53] Xu X, Chen W, Zhu W, Chen J, Ma B, Ding J, *et al*. Adeno-associated virus (AAV)-based gene therapy for glioblastoma. Cancer Cell Int 2021;21(1):76. doi:10.1186/s12935-021-01776-4, PMID:33499886.
[54] Yao Y, Wang J, Liu Y, Qu Y, Wang K, Zhang Y, *et al*. Variants of the adeno-associated virus serotype 9 with enhanced penetration of the blood-brain barrier in rodents and primates. Nat Biomed Eng 2022;6(11):1257–1271. doi:10.1038/s41551-022-00938-7, PMID:36217021.
[55] Khan N, Cheemadan S, Saxena H, Bammidi S, Jayandharan GR. MicroRNA-based recombinant AAV vector assembly improves efficiency of suicide gene transfer in a murine model of lymphoma. Cancer Med 2020;9(9):3188–3201. doi:10.1002/cam4.2935, PMID:32108448.
[56] Kasimsetty A, Sabatino DE. Integration and the risk of liver cancer-Is there a real risk? J Viral Hepat 2024;31(Suppl 1):26–34. doi:10.1111/jvh.13915, PMID:38606944.
[57] Hadi M, Qutaiba B, Allela O, Jabari M, Jasoor AM, Naderloo O, *et al*. Recent advances in various adeno-associated viruses (AAVs) as gene therapy agents in hepatocellular carcinoma. Virol J 2024;21(1):17. doi:10.1186/s12985-024-02286-1, PMID:38216938.
[58] El Andari J, Grimm D. Production, Processing, and Characterization of Synthetic AAV Gene Therapy Vectors. Biotechnol J 2021;16(1):e2000025. doi:10.1002/biot.202000025, PMID:32975881.
[59] Aoki K, Shimada S, Simantini DS, Tun MM, Buerano CC, Morita K, *et al*. Type-I interferon response affects an inoculation dose-independent mortality in mice following Japanese encephalitis virus infection. Virol J 2014;11:105. doi:10.1186/1743-422X-11-105, PMID:24903089.
[60] Leiman PG, Kanamaru S, Mesyanzhinov VV, Arisaka F, Rossmann MG. Structure and morphogenesis of bacteriophage T4. Cell Mol Life Sci 2003;60(11):2356–2370. doi:10.1007/s00018-003-3072-1, PMID:14625682.
[61] Molineux IJ. No syringes please, ejection of phage T7 DNA from the virion is enzyme driven. Mol Microbiol 2001;40(1):1–8. doi:10.1046/j.1365-2958.2001.02357.x, PMID:11298271.
[62] Szymanski CM. Bacteriophages and their unique components provide limitless resources for exploitation. Front Microbiol 2024;15:1342544. doi:10.3389/fmicb.2024.1342544, PMID:38380101.
[63] Zhang J, Li D, Zhang R, Peng R, Li J. Delivery of microRNA-21-sponge and pre-microRNA-122 by MS2 virus-like particles to therapeutically target hepatocellular carcinoma cells. Exp Biol Med (Maywood) 2021;246(23):2463–2472. doi:10.1177/15353702211035689, PMID:34644206.
[64] Pranjol MZ, Hajitou A. Bacteriophage-derived vectors for targeted cancer gene therapy. Viruses 2015;7(1):268–284. doi:10.3390/v7010268, PMID:25606974.
[65] Doub JB. Risk of Bacteriophage Therapeutics to Transfer Genetic Material and Contain Contaminants Beyond Endotoxins with Clinically Relevant Mitigation Strategies. Infect Drug Resist 2021;14:5629–5637. doi:10.2147/IDR.S341265, PMID:34992389.
[66] Rahman MM, McFadden G. Oncolytic Viruses: Newest Frontier for Cancer Immunotherapy. Cancers (Basel) 2021;13(21):5452. doi:10.3390/cancers13215452, PMID:34771615.
[67] Stetson DB, Medzhitov R. Type I interferons in host defense. Immunity 2006;25(3):373–381. doi:10.1016/j.immuni.2006.08.007, PMID:16979569.
[68] Larrieux A, Sanjuán R. Cellular resistance to an oncolytic virus is driven by chronic activation of innate immunity. iScience 2023;26(1):105749. doi:10.1016/j.isci.2022.105749, PMID:36590165.
[69] Bhatt DK, Chammas R, Daemen T. Resistance Mechanisms Influencing Oncolytic Virotherapy, a Systematic Analysis. Vaccines (Basel) 2021;9(10):1166. doi:10.3390/vaccines9101166, PMID:34696274.
[70] Shalhout SZ, Miller DM, Emerick KS, Kaufman HL. Therapy with oncolytic viruses: progress and challenges. Nat Rev Clin Oncol 2023;20(3):160–177. doi:10.1038/s41571-022-00719-w, PMID:36631681.
[71] Zhang N, Li J, Yu J, Wan Y, Zhang C, Zhang H, *et al*. Construction of an IL12 and CXCL11 armed oncolytic herpes simplex virus using the CRISPR/Cas9 system for colon cancer treatment. Virus Res 2023;323:198979. doi:10.1016/j.virusres.2022.198979, PMID:36283533.
[72] Tang G, Wang D, Zhao X, Feng Z, Chen Q, Shen Y. The Dilemma of HSV-1 Oncolytic Virus Delivery: The Method Choice and Hurdles. Int J Mol Sci 2023;24(4):3681. doi:10.3390/ijms24043681, PMID:36835091.
[73] Zheng M, Huang J, Tong A, Yang H. Oncolytic Viruses for Cancer Therapy: Barriers and Recent Advances. Mol Ther Oncolytics 2019;15:234–247. doi:10.1016/j.omto.2019.10.007, PMID:31872046.
[74] Stanziale SF, Petrowsky H, Adusumilli PS, Ben-Porat L, Gonen M, Fong Y. Infection with oncolytic herpes simplex virus-1 induces apoptosis in neighboring human cancer cells: a potential target to increase anticancer activity. Clin Cancer Res 2004;10(9):3225–3232. doi:10.1158/1078-0432.ccr-1083-3, PMID:15131064.
[75] St-Cyr G, Daniel L, Giguère H, Birtch R, Ilkow CS, Tai LH. Therapeutic limitations of oncolytic VSVd51-mediated miR-199a-5p delivery in triple negative breast cancer models. Sci Rep 2025;15(1):16634. doi:10.1038/s41598-025-01584-0, PMID:40360587.
[76] Li Z, Jiang Z, Zhang Y, Huang X, Liu Q. Efficacy and Safety of Oncolytic Viruses in Randomized Controlled Trials: A Systematic Review and Meta-Analysis. Cancers (Basel) 2020;12(6):1416. doi:10.3390/cancers12061416, PMID:32486227.
[77] Zhu X, Fan C, Xiong Z, Chen M, Li Z, Tao T, *et al*. Development and application of oncolytic viruses as the nemesis of tumor cells. Front Microbiol 2023;14:1188526. doi:10.3389/fmicb.2023.1188526, PMID:37440883.
[78] Ren S, Wang M, Wang C, Wang Y, Sun C, Zeng Z, *et al*. Application of Non-Viral Vectors in Drug Delivery and Gene Therapy. Polymers (Basel) 2021;13(19):3307. doi:10.3390/polym13193307, PMID:34641123.
[79] Hosseinkhani H. Viral/Non-Viral Vectors in DNA/RNA Delivery Technology. Recent Progress in Materials 2024;6(4):027. doi:10.21926/rpm.2404027.
[80] Ronald JA, Katzenberg R, Nielsen CH, Jae HJ, Hofmann LV, Gambhir SS. MicroRNA-regulated non-viral vectors with improved tumor specificity in an orthotopic rat model of hepatocellular carcinoma. Gene Ther 2013;20(10):1006–1013. doi:10.1038/gt.2013.24, PMID:23719066.
[81] Dai L, Wang W, Zhang S, Jiang Q, Wang R, Dai L, *et al*. Vector-based miR-15a/16-1 plasmid inhibits colon cancer growth in vivo. Cell Biol Int 2012;36(8):765–770. doi:10.1042/CBI20110404, PMID:22574716.
[82] Lee AY, Cho MH, Kim S. Recent advances in aerosol gene delivery systems using non-viral vectors for lung cancer therapy. Expert Opin Drug Deliv 2019;16(7):757–772. doi:10.1080/17425247.2019.1641083, PMID:31282221.
[83] Hajebi S, Yousefiasl S, Rahimmanesh I, Dahim A, Ahmadi S, Kadumudi FB, *et al*. Genetically Engineered Viral Vectors and Organic-Based Non-Viral Nanocarriers for Drug Delivery Applications. Adv Healthc Mater 2022;11(20):e2201583. doi:10.1002/adhm.202201583, PMID:35916145.
[84] Picanço-Castro V, Pereira CG, Covas DT, Porto GS, Athanassiadou A, Figueiredo ML. Emerging patent landscape for non-viral vectors used

for gene therapy. Nat Biotechnol 2020;38(2):151–157. doi:10.1038/s41587-019-0402-x, PMID:32034383.
[85] Amer MH. Gene therapy for cancer: present status and future perspective. Mol Cell Ther 2014;2:27. doi:10.1186/2052-8426-2-27, PMID:26056594.
[86] Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nat Rev Genet 2014;15(8):541–555. doi:10.1038/nrg3763, PMID:25022906.
[87] Hidai C, Kitano H. Nonviral Gene Therapy for Cancer: A Review. Diseases 2018;6(3):57. doi:10.3390/diseases6030057, PMID:29970866.
[88] Khabazian E, Vakhshiteh F, Norouzi P, Fatahi Y, Dinarvand R, Atyabi F. Cationic liposome decorated with cyclic RGD peptide for targeted delivery of anti-STAT3 siRNA to melanoma cancer cells. J Drug Target 2022;30(5):522–533. doi:10.1080/1061186X.2021.1973481, PMID:34482780.
[89] Yang Q, Li S, Ou H, Zhang Y, Zhu G, Li S, *et al*. Exosome-based delivery strategies for tumor therapy: an update on modification, loading, and clinical application. J Nanobiotechnology 2024;22(1):41. doi:10.1186/s12951-024-02298-7, PMID:38281957.
[90] Li X, Qi J, Wang J, Hu W, Zhou W, Wang Y, *et al*. Nanoparticle technology for mRNA: Delivery strategy, clinical application and developmental landscape. Theranostics 2024;14(2):738–760. doi:10.7150/thno.84291, PMID:38169577.
[91] Kanvinde S, Kulkarni T, Deodhar S, Bhattacharya D, Dasgupta A. Non-Viral Vectors for Delivery of Nucleic Acid Therapies for Cancer. BioTech (Basel) 2022;11(1):6. doi:10.3390/biotech11010006, PMID:35822814.
[92] Wei PS, Thota N, John G, Chang E, Lee S, Wang Y, *et al*. Enhancing RNA-lipid nanoparticle delivery: Organ- and cell-specificity and barcoding strategies. J Control Release 2024;375:366–388. doi:10.1016/j.jconrel.2024.08.030, PMID:39179112.
[93] Janjic A, Wange LE, Bagnoli JW, Geuder J, Nguyen P, Richter D, *et al*. Prime-seq, efficient and powerful bulk RNA sequencing. Genome Biol 2022;23(1):88. doi:10.1186/s13059-022-02660-8, PMID:35361256.
[94] Alpern D, Gardeux V, Russeil J, Mangeat B, Meireles-Filho ACA, Breysse R, *et al*. BRB-seq: ultra-affordable high-throughput transcriptomics enabled by bulk RNA barcoding and sequencing. Genome Biol 2019;20(1):71. doi:10.1186/s13059-019-1671-x, PMID:30999927.
[95] Mern DS, Thomé C. Synergetic enrichment of aggrecan in nucleus pulposus cells by scAAV6-shRNA-mediated knockdown of aggrecanase-1 and aggrecanase-2. Exp Biol Med (Maywood) 2023;248(13):1134–1144. doi:10.1177/15353702231171905, PMID:37354087.
[96] Park SK, Kee Y, Ryu T, Kim H, Hwang BJ. Enzymatic construction of shRNA library from oligonucleotide library. Genes Genomics 2019;41(5):573–581. doi:10.1007/s13258-019-00800-2, PMID:30830681.
[97] Pardi N, Muramatsu H, Weissman D, Karikó K. In vitro transcription of long RNA containing modified nucleosides. Methods Mol Biol 2013;969:29–42. doi:10.1007/978-1-62703-260-5_2, PMID:23296925.
[98] Bressy C, Droby GN, Maldonado BD, Steuerwald N, Grdzelishvili VZ. Cell Cycle Arrest in G(2)/M Phase Enhances Replication of Interferon-Sensitive Cytoplasmic RNA Viruses via Inhibition of Antiviral Gene Expression. J Virol 2019;93(4):e01885–18. doi:10.1128/JVI.01885-18, PMID:30487274.
[99] Surjit M, Liu B, Chow VT, Lal SK. The nucleocapsid protein of severe acute respiratory syndrome-coronavirus inhibits the activity of cyclin-cyclin-dependent kinase complex and blocks S phase progression in mammalian cells. J Biol Chem 2006;281(16):10669–10681. doi:10.1074/jbc.M509233200, PMID:16431923.
[100] Howells A, Marelli G, Lemoine NR, Wang Y. Oncolytic Viruses-Interaction of Virus and Tumor Cells in the Battle to Eliminate Cancer. Front Oncol 2017;7:195. doi:10.3389/fonc.2017.00195, PMID:28944214.
[101] Boncheva M, Whitesides GM. Making things by self-assembly. MRS Bulletin 2005;30(10):736–742. doi:10.1557/mrs2005.208.
[102] Rothemund PWK. ICCAD-2005. IEEE/ACM International Conference on Computer-Aided Design, 2005. Design of DNA origami. Piscataway, NJ: IEEE; 2005:471–478. doi:10.1109/ICCAD.2005.1560114.
[103] Mallery DL, Márquez CL, McEwan WA, Dickson CF, Jacques DA, Anandapadamanaban M, *et al*. IP6 is an HIV pocket factor that prevents capsid collapse and promotes DNA synthesis. Elife 2018;7:e35335. doi:10.7554/eLife.35335, PMID:29848441.
[104] Schulz O, Diebold SS, Chen M, Näslund TI, Nolte MA, Alexopoulou L, *et al*. Toll-like receptor 3 promotes cross-priming to virus-infected cells. Nature 2005;433(7028):887–892. doi:10.1038/nature03326, PMID:15711573.
[105] Yang J, Luo GF. Peptide-Based Vectors for Gene Delivery. Chemistry 2023;5(3):1696–1718. doi:10.3390/chemistry5030116.
[106] Ren H, He Y, Liang J, Cheng Z, Zhang M, Zhu Y, *et al*. Role of Liposome Size, Surface Charge, and PEGylation on Rheumatoid Arthritis Targeting Therapy. ACS Appl Mater Interfaces 2019;11(22):20304–20315. doi:10.1021/acsami.8b22693, PMID:31056910.
[107] Xu X, Xu L, Wen C, Xia J, Zhang Y, Liang Y. Programming assembly of biomimetic exosomes: An emerging theranostic nanomedicine platform. Mater Today Bio 2023;22:100760. doi:10.1016/j.mtbio.2023.100760, PMID:37636982.
[108] Fuse-It, ibidi's Novel, Membrane Fusion Technology, is a Highly Synergistic Alternative to Gene Transfection. BioSpace; 2014. Available from: https://www.biospace.com/fuse-it-b-ibidi-b-s-novel-membrane-fusion-technology-is-a-highly-synergistic-alternative-to-gene-transfection. Accessed November 8 20.
[109] Hoffmann M, Gerlach S, Takamiya M, Tarazi S, Hersch N, Csiszár A, *et al*. Smuggling on the Nanoscale-Fusogenic Liposomes Enable Efficient RNA-Transfer with Negligible Immune Response In Vitro and In Vivo. Pharmaceutics 2023;15(4):1210. doi:10.3390/pharmaceutics15041210, PMID:37111695.
[110] Zhang Z, Feng J, Zhang T, Gao A, Sun C. Application of tumor pH/hypoxia-responsive nanoparticles for combined photodynamic therapy and hypoxia-activated chemotherapy. Front Bioeng Biotechnol 2023;11:1197404. doi:10.3389/fbioe.2023.1197404, PMID:37362218.
[111] Gaucher C, Boudier A, Bonetti J, Clarot I, Leroy P, Parent M. Glutathione: Antioxidant Properties Dedicated to Nanotechnologies. Antioxidants (Basel) 2018;7(5):62. doi:10.3390/antiox7050062, PMID:29702624.
[112] Liu J, Li M, Luo Z, Dai L, Guo X, Cai K. Design of nanocarriers based on complex biological barriers in vivo for tumor therapy. Nano Today 2017;15:56–90. doi:10.1016/j.nantod.2017.06.010.
[113] Lahooti B, Akwii RG, Zahra FT, Sajib MS, Lamprou M, Alobaida A, *et al*. Targeting endothelial permeability in the EPR effect. J Control Release 2023;361:212–235. doi:10.1016/j.jconrel.2023.07.039, PMID:37517543.
[114] Ling X, Tu J, Wang J, Shajii A, Kong N, Feng C, *et al*. Glutathione-Responsive Prodrug Nanoparticles for Effective Drug Delivery and Cancer Therapy. ACS Nano 2019;13(1):357–370. doi:10.1021/acsnano.8b06400, PMID:30485068.
[115] Kashani GK, Naghib SM, Soleymani S, Mozafari MR. A review of DNA nanoparticles-encapsulated drug/gene/protein for advanced controlled drug release: Current status and future perspective over emerging therapy approaches. Int J Biol Macromol 2024;268(Pt 1):131694. doi:10.1016/j.ijbiomac.2024.131694, PMID:38642693.
[116] Ding Y, Dai Y, Wu M, Li L. Glutathione-mediated nanomedicines for cancer diagnosis and therapy. Chemical Engineering Journal 2021;426:128880. doi:10.1016/j.cej.2021.128880.
[117] Celi N, Gong D, Cai J. Artificial flexible sperm-like nanorobot based on self-assembly and its bidirectional propulsion in precessing magnetic fields. Sci Rep 2021;11(1):21728. doi:10.1038/s41598-021-00902-6, PMID:34741063.
[118] Borchardt H, Kogel A, Kalwa H, Weirauch U, Aigner A. Therapeutic miR-506-3p Replacement in Pancreatic Carcinoma Leads to Multiple Effects including Autophagy, Apoptosis, Senescence, and Mitochondrial Alterations In Vitro and In Vivo. Biomedicines 2022;10(7):1692. doi:10.3390/biomedicines10071692, PMID:35884996.
[119] Halim A, Al-Qadi N, Mondal SK, Kenyon E, Savan NA, Mackie H, *et al*. Identification of efficacy biomarkers for therapies targeting miR-10b. Cancer Res 2024;84(6_Suppl):4726–4726. doi:10.1158/1538-7445.AM2024-4726.
[120] Castoldi M, Roy S, Angendohr C, Pellegrino R, Vucur M, Singer MT, *et al*. Regulation of KIF23 by miR-107 controls replicative tumor cell fitness in mouse and human hepatocellular carcinoma. J Hepatol 2025;82(3):499–511. doi:10.1016/j.jhep.2024.08.025, PMID:40235270.
[121] Weidle UH, Nopora A. Up-regulated Circular RNAs in Colorectal Cancer: New Entities for Therapy and Tools for Identification of Therapeutic Targets. Cancer Genomics Proteomics 2023;20(2):132–153. doi:10.21873/cgp.20369, PMID:36870691.

[122] Hong SH, Hwang HJ, Ha NG, Park SY, Moon HS, Yoon YE. PD43-06 EZH2 INHIBITION AS THERAPEUTIC STRATEGY TO DYSREGULATE LATS1 IN RCC. J Urol 2022;207(Suppl 5):e703. doi:10.1097/JU.0000000000002604.06.
[123] Weidle UH, Birzele F. Circular RNA in Non-small Cell Lung Carcinoma: Identification of Targets and New Treatment Modalities. Cancer Genomics Proteomics 2023;20(6suppl):646–668. doi:10.21873/cgp.20413, PMID:38035705.
[124] Xu P, Yang JC, Chen B, Nip C, Van Dyke JE, Evans CP, *et al*. Abstract 5151: Enzalutamide resistance results in immunosuppressive alterations in prostate tumor immune microenvironment. Cancer Res 2023;83(7_Suppl):5151. doi:10.1158/1538-7445.AM2023-5151.
[125] Le DH, Lee KL, Shukla S, Commandeur U, Steinmetz NF. Potato virus X, a filamentous plant viral nanoparticle for doxorubicin delivery in cancer therapy. Nanoscale 2017;9(6):2348–2357. doi:10.1039/c6nr09099k, PMID:28144662.
[126] Finbloom JA, Aanei IL, Bernard JM, Klass SH, Elledge SK, Han K, *et al*. Evaluation of Three Morphologically Distinct Virus-Like Particles as Nanocarriers for Convection-Enhanced Drug Delivery to Glioblastoma. Nanomaterials (Basel) 2018;8(12):1007. doi:10.3390/nano8121007, PMID:30563038.
[127] Lin RD, Steinmetz NF. Tobacco mosaic virus delivery of mitoxantrone for cancer therapy. Nanoscale 2018;10(34):16307–16313. doi:10.1039/c8nr04142c, PMID:30129956.
[128] Mummidivarapu VVS, Rennie ML, Doolan AM, Crowley PB. Non-covalent PEGylation via Sulfonatocalix[4]arene-A Crystallographic Proof. Bioconjug Chem 2018;29(12):3999–4003. doi:10.1021/acs.bioconjchem.8b00769, PMID:30445810.
[129] Wei M, Jiang Q, Bian S, Chang P, Li B, Shi C, *et al*. Dual-mode-driven nanomotors targeting inflammatory macrophages for the MRI and synergistic treatment of atherosclerosis. J Nanobiotechnology 2025;23(1):54. doi:10.1186/s12951-025-03136-0, PMID:39881324.
[130] Ramachandran RV, Barman A, Modak P, Bhat R, Ghosh A, Saini DK. How safe are magnetic nanomotors: From cells to animals. Biomater Adv 2022;140:213048. doi:10.1016/j.bioadv.2022.213048, PMID:35939957.
[131] Jiang Z, Fu L, Wei C, Fu Q, Pan S. Antibacterial micro/nanomotors: advancing biofilm research to support medical applications. J Nanobiotechnology 2023;21(1):388. doi:10.1186/s12951-023-02162-0, PMID:37875896.
[132] Wang ZH, Zeng X, Huang W, Yang Y, Zhang S, Yang M, *et al*. Bioactive nanomotor enabling efficient intestinal barrier penetration for colorectal cancer therapy. Nat Commun 2025;16(1):1678. doi:10.1038/s41467-025-57045-9, PMID:39956840.
[133] Simó C, Serra-Casablancas M, Hortelao AC, Di Carlo V, Guallar-Garrido S, Plaza-García S, *et al*. Urease-powered nanobots for radionuclide bladder cancer therapy. Nat Nanotechnol 2024;19(4):554–564. doi:10.1038/s41565-023-01577-y, PMID:38225356.
[134] Seyhan AA. Trials and Tribulations of MicroRNA Therapeutics. Int J Mol Sci 2024;25(3):1469. doi:10.3390/ijms25031469, PMID:38338746.