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In vivo vectorization and delivery systems for gene therapies and RNA-based therapeutics in oncology†

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Gene and RNA-based therapeutics represent a promising frontier in oncology, enabling targeted modulation of tumor-associated genes and proteins. This review explores the latest advances in payload vectorization and delivery systems developed for *in vivo* cancer treatments. We discuss viral and non-viral organic particles, including lipid based nanoparticles and polymeric structures, for the effective transport of plasmids, siRNA, and self-amplifying RNA therapeutics. Their physicochemical properties, strategies to overcome intracellular barriers, and innovations in cell-based carriers and engineered extracellular vesicles are highlighted. Moreover, we consider oncolytic viruses, novel viral capsid modifications, and approaches that refine tumor targeting and immunomodulation. Ongoing clinical trials and regulatory frameworks guide future directions and emphasize the need for safe, scalable production. The potential convergence of these systems with combination therapies paves the way toward personalized cancer medicine.

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1. Introduction

Nucleic acids (NAs) have emerged as transformative therapeutic molecules, with their conceptual origins dating back to the 1960s. The 1990s marked significant progress, including the first successful demonstration of *in vivo* gene expression *via* mRNA injection and the advent of RNA interference (RNAi). These breakthroughs, coupled with recent innovations

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like mRNA vaccines, have positioned NAs as versatile tools in addressing complex diseases such as cancer. Unlike traditional therapies, which are often limited by toxicity and resistance, NAs offer sequence-specific approaches to target oncogenic drivers, restore cellular functions, and enhance immunotherapy. With cancer representing the focus of over 68% of gene therapy clinical trials,[1] NAs hold immense promise in overcoming tumour heterogeneity and microenvironmental challenges.[2,3] However, hurdles such as optimizing delivery, sustaining gene expression, and mitigating vector toxicity remain critical.[4] Advancements in sequencing and delivery technologies are rapidly transforming NAs into pivotal components of next-generation cancer therapies. In this review, we examine the diverse payloads utilized in gene and RNA-based therapies, recent advancements in vectorization strategies, including viral and organic vectors, as well as ongoing clinical trials and future prospects for cancer treatment.

2. Gene therapy and RNA-based therapeutics in oncology

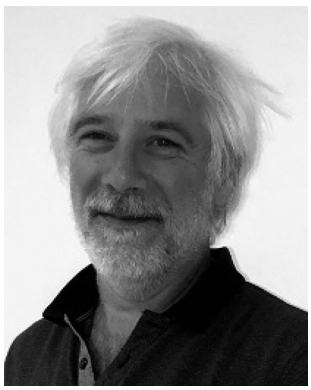
The development of biotechnological therapies has necessitated robust regulatory frameworks to ensure innovation and address evolving healthcare needs.[5] In the EU, regulation No. 1394/2007, along with Directive 2009/120/CE, defines Gene Therapy Medicinal Products (GTMPs) as biological medicines, excluding vaccines such as mRNA COVID-19 vaccines. Historically, vaccines have been confined to immunological drugs for infectious diseases, excluding anti-cancer therapies. For example, Moderna's mRNA-4157/V940, combined with Keytruda to enhance tumour immunity, cannot yet be classified as a vaccine under current EU definitions. In contrast, the FDA does not require a biological origin for gene therapies and categorizes oligonucleotides like siRNA as drugs targeting RNA within cells. The key gene and RNA-based therapies currently under clinical evaluation, as well as those already approved, are presented in ESI 1.† This review distinguishes between gene and RNA-based therapies, considering their unique strategies and regulatory nuances.

2.1. Principles of gene therapy in oncology

Gene therapy in oncology involves introducing DNA to modify cells—cancerous or immune—or modulate immune responses. It targets cancer-related genes or pathways by replacing faulty genes, silencing oncogenes, or enhancing immune responses (immune-gene therapy). Approaches include gene editing, addition, or silencing, delivered *in vivo* or *ex vivo*, with varying tools and vectors depending on tumour characteristics (see Fig. 1). ***Ex vivo* therapy** involves harvesting a patient's cells, modifying them genetically, and reinfusing them. CAR-T (Chimeric Antigen Receptor T-cell) therapy,[6,7] widely used in leukaemia and lymphoma, relies on viral vectors for transduction, which raises production costs and risks such as B-cell depletion, Cytokine Release Syndrome (CRS), and secondary leukaemia due to random integration.[8] Efforts are expanding to include Natural Killer (NK) cells and CAR-based therapies for solid cancers.[9] ***In vivo* therapy** administers therapeutic nucleic acids directly to the patient, eliminating the need for external cell manipulation. This approach is being explored for broader applications, including targeting solid tumours and delivering genetic material efficiently within the patient's body.

2.1.1. Molecular tools for gene therapy. Molecular tools in gene therapy encompass techniques and nucleic elements ("payloads") designed to modify the genetic material of cancerous or healthy cells for therapeutic purposes (Fig. 2).

These nucleic acids possess distinct physicochemical properties, such as a polar sugar-phosphate backbone and hydrophobic nitrogenous bases, which influence solubility and can



Jean-Luc Coll

Dr Jean-Luc Coll is Director of Research at INSERM and Team leader in the Institute for Advanced Biosciences in Grenoble. Dr Coll is a biologist with strong experience in oncology. He is using fluorescence, nanotechnologies, chemistry and large instruments for physics, to develop theranostic nanoparticles for the treatment of tumors and their metastases. His team is in particular focused on the use of near-infrared labeled nanoparticles to target tumors, guide surgery and enhance radiotherapy, phototherapy or innovative therapies. In addition to the developments of nanovectors, he is also involved in the generation of adapted medical devices.

Lucie Sancey

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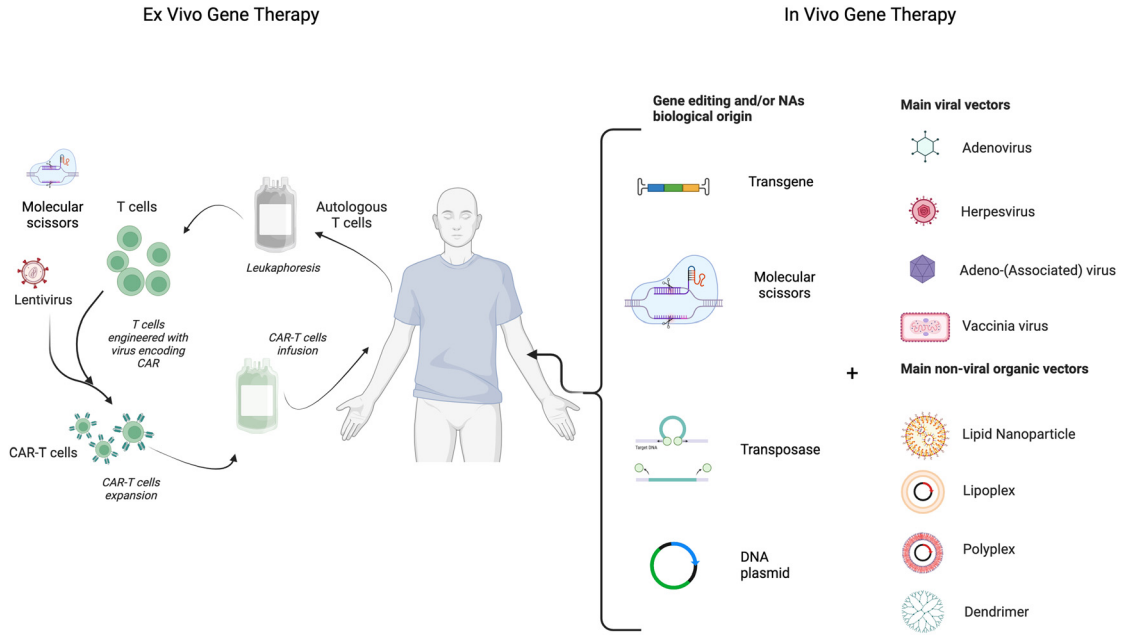

Fig. 1 *Ex vivo versus in vivo* gene therapies.

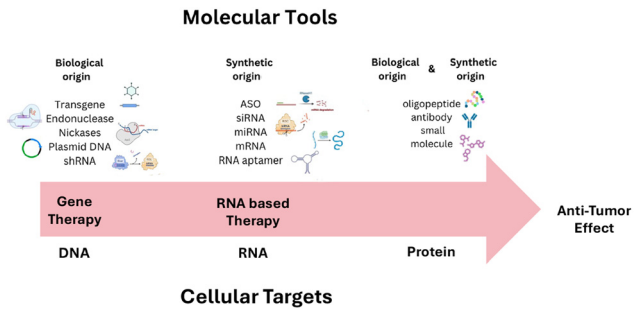

Fig. 2 Molecular tools for gene and RNA-based therapy.

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be enhanced through chemical modifications to resist enzymatic degradation.

2.1.1.1. DNA plasmids carrying a therapeutic gene. Plasmids are small, circular DNA molecules used in gene therapy as delivery vehicles to introduce therapeutic genes into cells, encoding functional RNA. Plasmids can encode various genes, such as cytokines, suppressors, and endonucleases, offering a flexible platform for gene therapy. Episomal plasmids remain extrachromosomal and enable transient gene expression, while integrative plasmids incorporate into the host genome, allowing stable expression regulated by host mechanisms. The choice between these types depends on treatment goals, such as genetic stability and persistence.[10] Integrative plasmids, such as those utilizing transposon systems like Sleeping Beauty (SB)[11] or piggyBac (PB), provide an alternative to viral vectors for stable genome integration. Transposons function *via* a "cut-and-paste" mechanism where transposases facilitate insertion of the gene of interest (GOI) into safe genomic sites. These systems are particularly valuable for cells like NK cells, which are poorly transduced by viral vectors, and have shown promising results in generating CAR immune cells for cancer immunotherapy.[12–14] Clinical applications include a Phase II trial using SB to engineer T cells reactive against cancer neoantigens (NCT04102436).[15] Future advancements, such as combining transposons with CRISPR/Cas9 or functionalized nanoparticles, aim to enhance efficiency and safety in gene delivery.[16–18] Tumour-specific promoters in plasmids enable targeted gene expression by leveraging transcription factors abundant in cancer cells, while tissue-specific or inducible promoters, like the widely used CMV promoter, allow versatile regulation.[19,20]

2.1.1.2. Gene/genome editing with molecular scissors. Gene and genome editing, using programmable nucleases like

CRISPR/Cas9 or transcription activator-like effectors (TALEs) and zinc fingers,[21] has revolutionized therapeutic strategies by enabling precise modifications to DNA through double-strand breaks (DSBs) or more advanced methods like single-strand breaks (SSBs). CRISPR/Cas9 utilizes RNA-guided Cas9 enzymes to target DNA, allowing for gene disruption, insertion, or correction, with applications in both *ex vivo* and *in vivo* therapies.[22] While CRISPR-based therapies, such as CASGEVY, have reached clinical application,[23] challenges like off-target effects, genotoxicity, and risks associated with DSBs in TP53-deficient cancers persist.[24,25] Alternative approaches, including **base editing** (BE) and **prime editing** (PE), enable precise modifications without introducing DSBs, significantly reducing risks.[26] BE alters single nucleotide pairs *via* enzymatic deamination, while PE utilizes a Cas9-reverse transcriptase complex for targeted insertions or corrections guided by pegRNA.[27–29] These advanced tools, particularly in combination with CRISPR systems, hold promise for safer and more effective therapies targeting genes like KRAS in cancer.[27]

2.2. Principles of RNA-based therapy in oncology

RNA-based therapeutics offer innovative strategies for modulating gene expression, with potential to treat various diseases, including cancer. Several RNA therapies, such as RNA interference drugs (*e.g.*, inclisiran, patisiran, givosiran, lumasiran, teprasiran) and antisense oligonucleotides (*e.g.*, nusinersen, eplontersen, tofersen), have received FDA and EMA approval, distinguishing themselves from gene therapies in manufacturing and mechanisms of action. While anti-cancer siRNAs, miRNAs, and antisense nucleotides (ASOs) are not yet commercially available, numerous candidates are in clinical development, highlighting their therapeutic promise (Fig. 2).[30]

2.2.1. Molecular tools for RNA-based therapy

2.2.1.1. Coding RNA. Coding RNAs, such as mRNA, are single-stranded molecules characterized by a 5′ cap, a 3′ polyadenylated tail, and tailored untranslated regions (UTRs) that enhance stability and translation efficiency.[31] Unlike plasmid DNA, mRNA functions without requiring nuclear entry or genome integration, making it a streamlined therapeutic option. Bicistronic mRNAs, capable of encoding two proteins from a single molecule, highlight advanced applications. For instance, OTX-2002, currently under clinical investigation (NCT05497453), targets the MYC oncogene in cancer through epigenetic regulation, encoding two distinct proteins that modulate gene expression pre-transcriptionally. To address the limitations of *ex vivo* CAR-T therapies—such as high costs and prolonged persistence—mRNA-transcribed CARs delivered *via* ionizable lipid nanoparticles (LNPs) present a significant advancement, bypassing the need for viral vectors and enabling transient, safer gene expression with fewer complications.[32–34] Additionally, gene-editing tools like Cas9 can be delivered as mRNA, reducing immunogenicity compared to protein-based systems and facilitating precise genome editing. However, off-target and on-target effects, especially in TP53-mutated cancer cells, require careful monitoring during therapy.[21,31,35,36] Another promising approach is self-amplifying mRNA (samRNA), which combines encoding a target protein with replication genes to amplify RNA messages post-transfection. Platforms such as naked RNA, DNA-launched replicons, and viral replicon particles have demonstrated efficacy in inducing anti-tumour immunity, which can be further enhanced through combination therapies like chemotherapy or immune checkpoint inhibitors. This approach simplifies production and offers a powerful tool for cancer immunotherapy.[37,38]

2.2.1.2. Non-Coding RNA (ncRNAs). ncRNAs play crucial roles in gene regulation and cancer therapy by modulating gene expression without encoding proteins. RNAi therapeutics, including small interfering RNAs (siRNAs)[39] and microRNAs (miRNAs),[40] selectively target mRNA to downregulate protein production. Synthetic miRNA mimics, such as MRX34[41] and miR-193a-3p mimic,[42] and anti-miRNAs, like anti-miR-21[43] and anti-miR-155,[44] have shown preclinical and clinical promise in restoring tumour suppressor functions and inhibiting cancer progression. siRNAs guide the RNA-induced silencing complex (RISC) to complementary mRNA, enabling targeted cleavage but requiring repeated dosing due to their transient effects. Small hairpin RNAs (shRNAs) provide gene silencing by integrating into cellular DNA, producing siRNA continuously.[45] Despite stability challenges, siRNA modifications and delivery innovations, such as lipid encapsulation, have improved therapeutic potential, exemplified by patisiran, the first FDA-approved siRNA drug.[46] Long non-coding RNAs (lncRNAs), including circular RNAs (circRNAs), influence transcription, epigenetic modifications, and mRNA stability through diverse interactions with nucleic acids and proteins. Therapeutics based on lncRNAs and circRNAs are emerging, with applications such as miRNA sponges or oncolytic RNA therapies targeting specific cancer pathways.[47–49]

2.2.1.3. Antisense oligonucleotides (ASOs). ASOs are synthetic single-stranded DNA or RNA analogues, typically 18–30 nucleotides long, designed to bind specific mRNA or non-coding RNA targets.[45] Chemical modifications, such as phosphorothioate backbones and 2′-*O*-methoxyethyl groups, have enhanced their nuclease resistance, potency, and patient tolerance.[50] ASOs have evolved through three generations, with improvements in binding affinity, cell penetration, and reduced off-target effects.[51,52] They function either by recruiting RNase H to degrade mRNA or by steric blocking to alter splicing. In oncology, ASOs like anti-HSP27 (BOREALIS-2 trial)[53] and anti-STAT3[54] show promise in treating metastatic urothelial carcinoma and myelodysplastic syndrome. However, few ASOs have reached clinical use, and none currently utilize nanoparticle-based delivery.

2.3. Strategic objectives of gene and RNA-based therapies

Gene and RNA-based therapies aim to modulate gene expression, restore normal gene functions, enhance immune responses, and induce targeted cancer cell death, offering innovative avenues for effective cancer treatment.

2.3.1. Gene negative regulation. Gene negative regulation involves silencing or reducing the expression of oncogenes or

upregulated genes to counteract cancer progression. **Knockout strategies** completely eliminate gene activity using tools like CRISPR/Cas9, which introduces double-strand breaks repaired by the NHEJ or HDR pathway. For instance, knockout of hTERT, a component of telomerase, disrupts cancer cell immortality,[55] while targeting KRAS mutations, such as c.35G > A, holds promise in resensitising tumours to tyrosine kinase inhibitors (TKIs).[56,57] However, challenges include the multifaceted roles of target genes in cellular homeostasis.[58] **Knock-in approaches** introduce precise genetic modifications, such as stop codons to truncate dysfunctional proteins. In pancreatic cancer, researchers used CRISPR/Cas9 to introduce mutations in BRCA1/2, sensitizing cells to PARP inhibitors like olaparib and enhancing apoptosis.[59] **Knockdown approaches** reduce, rather than eliminate, gene expression using RNAi tools like siRNAs and ASOs. These methods enable multi-gene silencing, an advantage for network-based approaches in cancers such as breast cancer.[60]

2.3.2. Add/restore gene expression. Adding or restoring gene expression can counteract the loss of tumour suppressors or enhance immune responses. Cytokines such as IL-2 stimulate anti-tumour immunity by activating effector lymphocytes like memory T cells and NK cells. High-dose IL-2 (proleukin) therapies have shown efficacy but are limited by significant toxicities. Alternative delivery methods, such as **plasmid DNA encoding IL-2 or IL-2-targeted mRNA**, aim to minimize side effects.[61–63] The SB transposon system, tested in a preclinical glioma model, enhanced IFN-γ immunotherapy by prolonging transgene expression, leading to improved survival and tumour regression compared to episomal plasmid expression.[64] **Emerging neoantigen-based therapies**, such as GRT-C901 and GRT-R902, use viral and mRNA vectors to stimulate T-cell responses against tumour-specific mutations. These strategies, combined with immune checkpoint inhibitors under the GRANITE phase II/III program (NCT05141721), show promise in advanced cancers, improving immune responses and patient survival. **RNA-based antibodies**, like BNT141, offer advantages in production speed and pharmacokinetics. For instance, BNT141 targets claudin-18.2, a protein overexpressed in gastric and pancreatic cancers, showing potential as a targeted therapy.[65] Similarly, plasmid DNA-encoded antibodies have demonstrated efficacy in HER2-positive breast cancer models, providing long-term expression and tumour inhibition.[66] Checkpoint inhibitors, such as anti-PD-L1 and anti-CTLA-4 antibodies, enhance T-cell activity against tumours. RNA-based approaches, including **siRNA targeting PD-L1**, offer alternative methods for delivering immune-modulating therapies, particularly in immune-privileged regions like the brain. Preclinical studies using vectors like HVJ-E demonstrated strong antitumor immunity and prolonged survival in glioblastoma models.[67]

Restoring tumour suppressor genes, such as TP53 and PTEN, offers another avenue.[68] mRNA encoding functional versions of these genes has shown potential in preclinical models,[69,70] particularly when combined with checkpoint inhibitors.[71] CRISPR-based activation of PTEN using dCas9-VPR has also demonstrated success in upregulating tumour suppressor activity in cancer cells.[72] TALEN-mediated targeting of HPV E6 and E7 oncogenes effectively induced apoptosis and inhibited tumorigenicity in HPV-positive cervical cancer models, ultimately restoring tumour suppressors like p53 and RB1.[73]

2.3.3. Gene-directed enzyme prodrug therapy (GDEPT). GDEPT delivers a gene encoding an enzyme that activates a non-toxic prodrug into its cytotoxic form within tumour cells.[74] HSV-thymidine kinase (HSV-TK), delivered *via* viral vectors or transposon systems, converts ganciclovir into a toxic compound, inducing localized tumour cell death with bystander effects enhancing efficacy.[75] Similarly, cytosine deaminase converts 5-fluorocytosine into the cytotoxic 5-fluorouracil, though clinical trials like Toca 5 have faced setbacks in demonstrating significant survival benefits.[76]

3. Strategies in vectorization for gene and RNA-based therapies

The effective delivery of nucleic acids, referred to as "payloads", is a cornerstone of gene and RNA-based therapies, addressing critical challenges in therapeutic development. These payloads, including plasmid DNAs, siRNAs, and mRNAs, face substantial barriers due to their physicochemical properties, such as hydrophilicity, negative charge, and large molecular size. For example, the size of mRNA (~400 kDa) starkly contrasts with small molecules like aspirin (0.18 kDa), creating significant hurdles in crossing cellular membranes. Furthermore, the negative charge of the sugar-phosphate backbone is repelled by the similarly charged cellular membrane, complicating cellular entry. These issues are exacerbated by serum nucleases that rapidly degrade nucleic acids, reducing their stability and therapeutic efficacy. Delivery systems, or vectors, are indispensable for overcoming these barriers, protecting nucleic acids from enzymatic degradation, extending their circulation time, and facilitating cellular uptake. Additionally, vectors can help navigate specific challenges, such as crossing the nuclear membrane or targeting solid tumours *via* abnormal vasculature, which are critical for achieving therapeutic success in gene and RNA therapies (Fig. 3).

The term "vector" refers to the carriers used to deliver nucleic acids to their target sites. No single vector is universally effective for all cancers; their selection must be tailored to the specific phenotypic profile of the cancer type, tumour microenvironment (TME), and the patient's pathophysiological state. This complexity underscores the need for careful vector design to ensure precise targeting and therapeutic efficacy. Strategies for vectorization are broadly categorized into viral and non-viral systems, such as nanoparticles, each with distinct advantages and challenges. The choice between these approaches requires careful consideration of safety, efficiency, and specificity, as well as the unique demands of the therapy and the underlying disease.

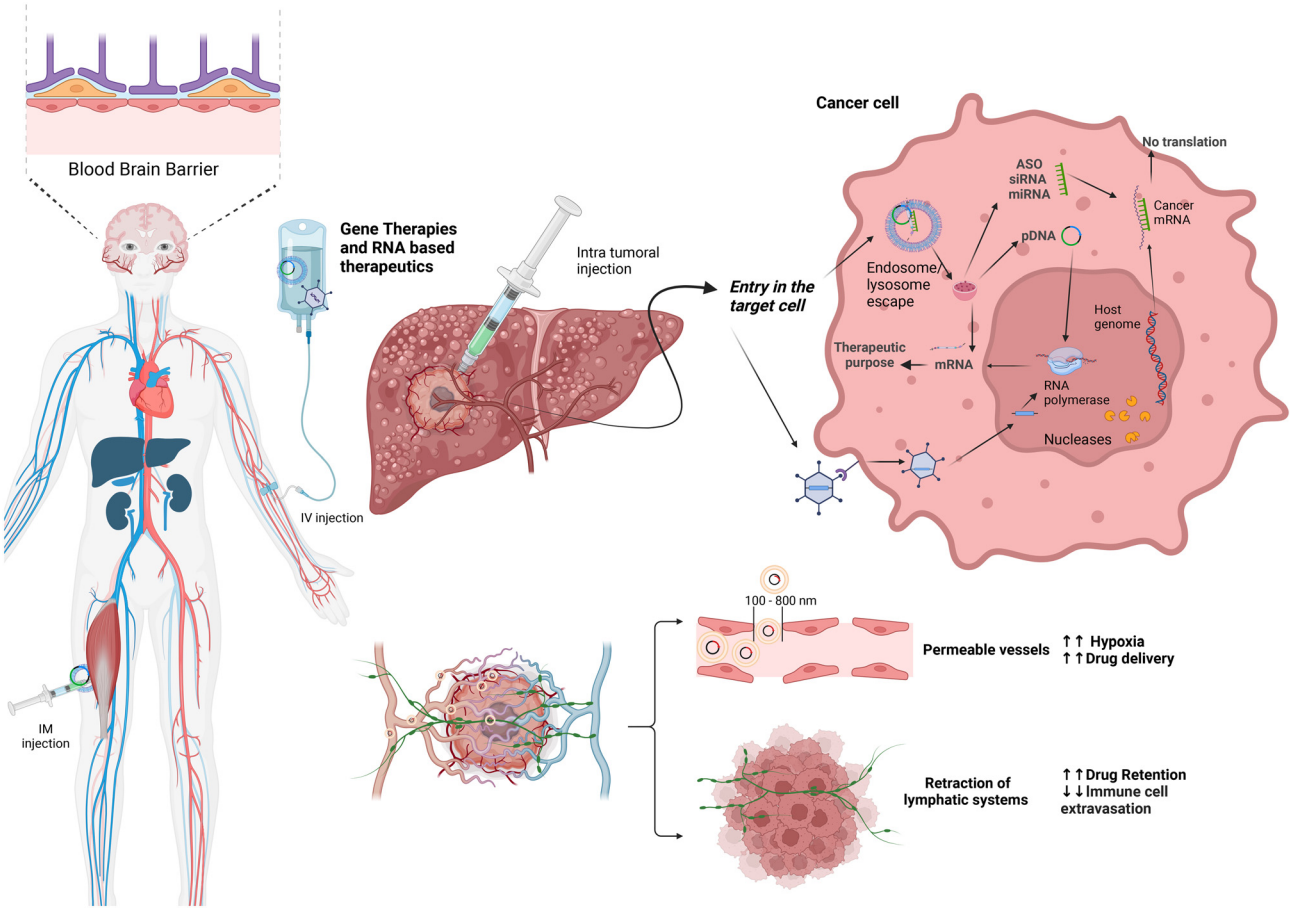


Fig. 3 Patho-physiological barriers to overcome for delivery systems.

3.1. Viral transduction of nucleic acids

Viruses are natural vectors evolved to efficiently deliver their genetic material into host cells, making them ideal tools for gene therapy. Leveraging their ability to enter cells and initiate gene expression, viral vectors have become the most advanced platforms for transferring therapeutic nucleic acids.[1,10] Early efforts in viral gene therapy, beginning in the 1980s with recombinant adeno-associated viruses (AAVs), demonstrated the potential for correcting monogenic diseases. Despite initial success, challenges such as random insertional mutagenesis and adverse immune responses highlighted the need for safer, more precise designs.[77] Modern recombinant viral vectors are stripped of pathogenic elements and engineered to enhance specificity and safety. For cancer therapies, viruses can be tailored to target cancer cells through genetic modifications. This specificity capitalizes on unique characteristics of tumour cells, including defective interferon signalling, frequent division, and immune evasion. Oncolytic viruses, such as poxviruses encoding hNIS, exemplify these modifications, combining tumour cell targeting with imaging and antitumor efficacy.[78,79] Viral vectors can be used for two main purposes: delivering therapeutic genes or as oncolytic agents, with some designs achieving both. Their versatility allows for integration into diverse therapeutic strategies, such as adoptive immune cell therapy using retroviral vectors like lentiviruses, and non-integrative vectors such as adenoviruses and AAVs (Fig. 4).[80]

Complex engineering of capsids or envelopes redirects viral tropism to specific cell types, while chimeric viruses harness features of multiple viral types to optimize delivery.

Despite their advantages, viral vectors pose challenges, including high production costs and potential immunogenicity. Manufacturing under good practice standards involves co-transfection of producer cells and advanced purification methods, which are resource-intensive. Moreover, immune responses triggered by viral vectors require careful management to minimize toxicity.[80,81] This chapter focuses on the role of viral vectors in delivering therapeutic genes, with or without additional oncolytic functions, highlighting their application in cancer gene therapy trials (see Table 1).

3.1.1. Mechanisms and strategies in viral gene delivery. Viral vectors deliver genetic material to target cells either by integrating it into the host genome, enabling stable long-term expression, or maintaining it as episomal DNA for transient expression. This choice depends on the therapeutic objective and the nature of the vector. Oncolytic viruses, for instance, target cancer cells for lysis and stimulate immune responses through tumour antigen release and inflammatory factor

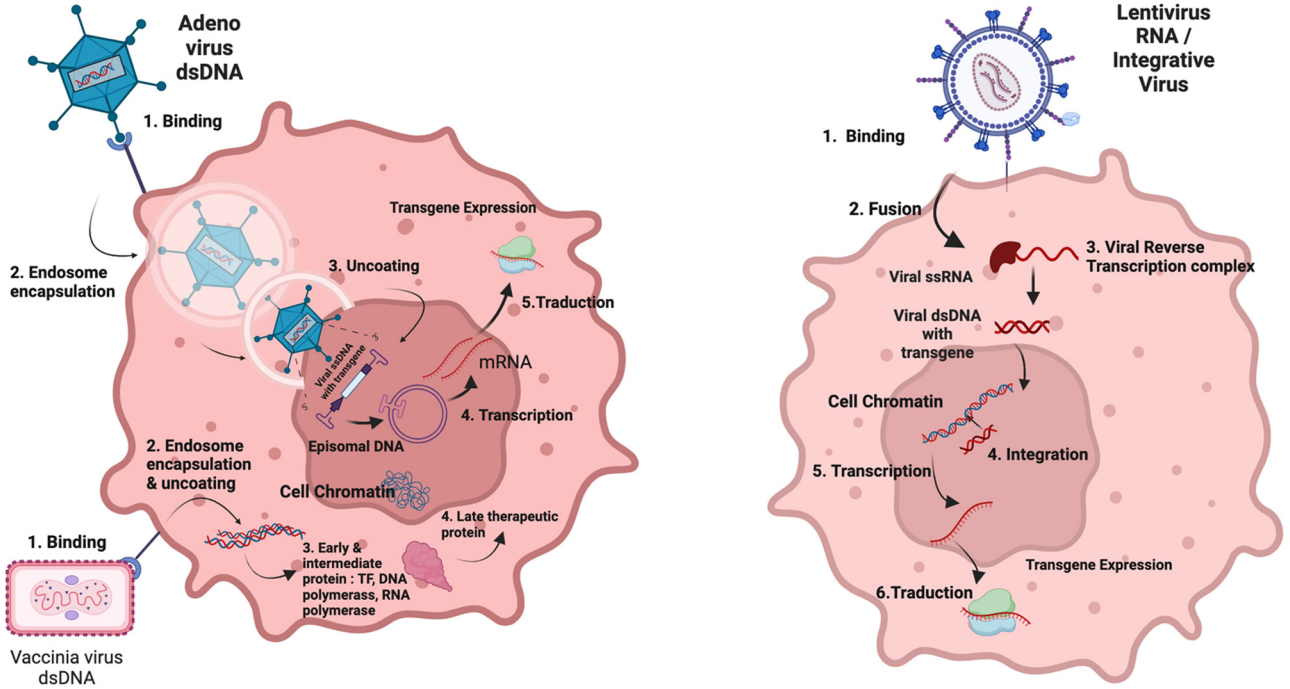


Fig. 4 Viral transduction with integrative or non-integrative viruses.

secretion, while also enabling transgene delivery. Emerging designs combine oncolytic activity with transgene vectorization to enhance therapeutic outcomes.[82]

The specificity of transgene expression in viral vectors relies on promoters. Strong, ubiquitous promoters like CMV and CAG ensure robust gene expression but risk non-specific activity, whereas tumour-specific promoters, such as engineered AFP variants for liver cancer, enhance expression in targeted cells while minimizing off-target effects.[83–85] Inducible systems, like the Ad-RTS-hIL-12 platform, further refine control by activating transgenes only in the presence of exogenous ligands, exemplifying precise regulation.[86]

To improve targeting, viral pseudotyping replaces envelope proteins with those from different viruses, tailoring the vector's tropism. For instance, pseudotyped Sindbis virus lentiviral vectors selectively target T cells, enabling precise CAR-T engineering *in vivo*.[87] Similarly, adenoviral surface protein swapping can improve targeting, though this poses additional manufacturing challenges.

Pharmacokinetics and safety vary among vectors. AAVs are favoured for their low immunogenicity and stable expression but have limited payload capacity, whereas adenoviruses allow larger payloads at the cost of transient expression and stronger immune responses.[88] Retroviral vectors enable long-term expression *via* genome integration but carry oncogenic risks due to random insertional mutagenesis. Advanced manufacturing and dosing strategies, such as particle threshold optimization in clinical trials, continue to improve the scalability, purity, and therapeutic efficacy of viral vector applications.[89,90]

3.1.2. Integrative viruses. Integrative viruses incorporate their genetic material into the host cell genome, enabling stable, long-term transgene expression (Table 1). Retroviruses, particularly lentiviruses, are commonly used in *ex vivo* applications such as CAR-T cell therapy due to their ability to efficiently integrate and express large transgenes.[91,92] However, their use *in vivo* is limited by risks like insertional mutagenesis and uncontrolled gene expression, prompting efforts to redesign lentiviral vectors for safer applications.[92] Foamy viruses (FVs), another retrovirus family, offer safer profiles due to preferential integration into non-coding genomic regions, reducing genotoxic risks.[93–95] Unlike lentiviruses, FVs exhibit stability in quiescent cells, resuming activity upon cell division, and induce less clonal dominance, making them attractive for hematopoietic stem cell therapies and cancer applications.[96] Challenges include the stability of larger transgenes during viral propagation, influencing therapeutic design.[95]

The Moloney murine leukaemia virus (MMLV), a type C retrovirus, has shown potential in clinical applications. Rexin-G, approved for all solid tumours in the Philippines, integrates a dominant-negative mutant of cyclin G1 (dnG1), inducing apoptosis and disrupting tumour vasculature.[97,98] Similarly, Toca 511, encoding a yeast cytosine deaminase, converts the prodrug 5-fluorocytosine into the active chemotherapy agent 5-fluorouracil (5-FU). Although promising in preclinical models, Toca 511 faced challenges in clinical trials for glioblastoma due to inefficient transduction of tumour cells and limited stability *in vivo*.[99,100] Integrative viruses hold significant therapeutic potential but require precise engineering to balance stable expression with safety concerns.

Table 1 Main viruses used in cancer gene therapy

Characteristics	Integrative virus			Non-integrative virus		
Name	Lentivirus	Foamy virus	Moloney Murine Leukaemia Virus	Adenovirus (AdV)	Herpes simplex virus (HSV1, HSV)	Vaccinia
Number of open clinical trials[a]	0 (soon)	NA	NA	179	38	31
Transgene expression	Stable, long-term expression *via* genome integration	Stable, long-term expression *via* genome integration	Stable	Transient, typically non-integrative (rare integration events possible)	Transient	Transient
Genome	ssRNA	dsDNA	RNA with reverse transcriptase	dsDNA	dsDNA	dsDNA
Envelope	YES (glycoprotein envelope)	YES (lipid envelope)	YES	NO	YES	YES
Packaging capacity or cloning capacity	8–10 kb	9–10 kb	8 kb	30 kb	20 kb	25 kb
Size (diameter)	80–100 nm	80–100 nm	100 nm	70–100 nm	150–200 nm	~300 nm
Entry method	Endocytosis + membrane fusion (*via* glycoprotein)	Endocytosis followed by host membrane fusion	Receptor mCAT-1 followed by endocytosis	CD46 and coxsackie/ adenovirus receptor (CAR)	HVEM, Nectin 1; Nectin 2	Receptor-mediated endocytosis
Immunogenicity	Low (envelope incorporates HLA-I from packaging cell membranes during budding)	Low (less than other retroviruses)	Medium	High	High	High
Safety	Risk of insertional mutagenesis; improved safety with self-inactivating (SIN) 3rd generation vectors	Considered safe with modifications; low pathogenic potential; no human disease association from natural infection	Highly mutagenic	Immunogenic, inflammatory	Pathogenic to humans; requires engineering. Neurotropic; complex genome complicates modifications	Immunogenic, inflammatory
Elimination & biodegradability	Viral debris cleared by immune system over time	Degraded intracellularly post-infection.	Likely degraded intracellularly post-infection, similar to other retroviruses.	Not typically specified for adenoviruses, but likely degraded intracellularly post-infection	Degraded intracellularly post-infection; may persist in latent forms in specific cases	Degraded intracellularly post-infection; large genome and cytoplasmic replication may allow persistence depending on immune response
Most advanced clinical stage	Approved for *ex vivo* gene therapy; preclinical stage for *in vivo* applications	Preclinical stage	Phase III (Toca 511)	Approved: *Oncolytic* (oncorine, gendicine) *Vector-based* (adstiladrin with IFN-α2b)	Approved: Imlygic (oncolytic + GM-CSF)	Phase III: *Oncolytic* (Pexa-Vex) *Vector-based* (GDEPT): Oncovirac/oncorine
Advantages	-Stable, long-term transgene expression -Infects both dividing and non-dividing cells -Broad host range with VSV-G pseudotyping	-Long-term stable expression -Low immunogenicity -Integration of transgene without unfavourable recombination	-Stable genome expression -Modifiable for enhanced tropism	-Efficient transduction of dividing and non-dividing cells. -Non-mutagenic and replicative oncolytic properties -Easy to manipulate. -High-level gene expression	-Large genome allows for multiple transgene insertions -Efficient tumour infiltration -Easy to manipulate -Rapid replication in infected cells	-Replicates entirely in the cytoplasm -No risk of genomic integration

Table 1 *(Contd.)*

Characteristics	Integrative virus			Non-integrative virus		
Name	Lentivirus	Foamy virus	Moloney Murine Leukaemia Virus	Adenovirus (AdV)	Herpes simplex virus (HSV1, HSV)	Vaccinia
Disadvantages	-Risk of insertional mutagenesis -Limited packaging capacity -Potential for immune response activation	Potential for random gene insertion, increasing the risk of insertional mutagenesis.	-Possibility of mutagenesis from incorrect insertion -Infects dividing cells only -Low production titres -Requires stringent safety measures for manipulation	-Capsid and transgene can be highly immunogenic -Pre-existing antibodies (anti-AAV, AdV) may lead to rapid vector clearance	-Pathogenic to humans, requiring engineering for safety -High immunogenicity and widespread pre-existing anti-HSV immunity, causing vector clearance -Low production titres hinder large-scale applications	-Immune response to viral proteins -Unknown actions of many genes -Potential cytopathic effects on target cells

[a] Data sourced from ClinicalTrials.gov, last accessed September 2024. NA, not available

3.1.3. Non-integrative viruses. Non-integrative viral vectors are pivotal in gene therapy for delivering genetic material without incorporating it into the host genome, thus minimizing the risk of insertional mutagenesis. These vectors persist as episomes in the nucleus, offering a safer profile for therapeutic interventions (Table 1).

3.1.3.1. Adenoviruses. Adenoviruses (AdVs) are among the most widely utilized viral vectors in gene therapy, particularly in oncology.[1] Engineered through successive generations to enhance safety and efficacy, they offer diverse therapeutic strategies. Wild-type adenoviruses (WTAd) are noted for high cloning capacity and robust immune responses.[101] First-generation AdVs (FGAd) have deletions in essential genes (E1 and E3) to prevent replication, facilitating efficient gene delivery.[102] Second-generation vectors (SGAd) involve further deletions (E2 and E4), reducing viral protein synthesis and immune reactions. Helper-dependent AdVs (HDAd), or "gutless" vectors, have extensive deletions, increasing safety and DNA carrying capacity while enabling long-term gene expression with minimal toxicity.[103]

In cancer therapy, adenoviral vectors are versatile and are therefore currently tested for numerous malignancies (Table S1†). Gendicine, approved in China, delivers wild-type TP53 to induce cell cycle arrest and modulate the tumour TME.[104] Oncorine, another approved therapy, is an oncolytic AdV targeting TP53-deficient cells, selectively replicating in tumour cells while sparing healthy ones.[104] AdVs can also be engineered to secrete immunomodulatory factors, utilizing receptors like the coxsackievirus and adenovirus receptor on epithelial cells to enhance immune responses against tumors.[105]

AdVs have been instrumental in vaccine development, notably in the context of the SARS-CoV-2 pandemic, showcasing their capacity to stimulate immune responses.[106] In oncology, nadofaragene firadenovec (rAd-IFN α2b/Syn3) was approved for non-muscle invasive bladder cancer unresponsive to Bacillus Calmette–Guérin therapy.[107] This therapy employs a recombinant Ad5 vector to deliver the IFN-α2b gene, enhancing immune responses and inducing apoptosis in cancer cells *via* TNF-related apoptosis-inducing ligand (TRAIL)-related pathways.[108] Another adenoviral therapy, aglatimagene besadenovec (CAN-2409), uses a replication-defective AdV encoding herpes simplex virus thymidine kinase (HSV-TK). The clinical developments of aglatimagene besadenovec are presented in Table S2,† this treatment induces local activation of cytotoxic lymphocytes and proinflammatory cytokines, stimulating a robust antitumor immune response.[109] DNX-2440 is an oncolytic AdV engineered to express OX40 ligand (OX40L), selectively replicating in tumour cells deficient in the retinoblastoma gene (Rb) or p16, enhancing T-cell proliferation and cytokine production.[110,111]

A challenge with AdVs is pre-existing immunity in humans, reducing efficacy and posing risks.[112] To address this, non-human AdVs, like simian adenoviruses (*e.g.*, ChAdOx1), are explored for their lower prevalence of pre-existing immunity.[113] GRANITE and SLATE clinical studies utilize chimpanzee AdV

vectors in a prime/boost immunotherapy to deliver personalized or shared neoantigen vaccines, enhancing CD8 T-cell responses in cancer patients.[114]

3.1.3.2. Adeno-associated viruses. Recombinant adeno-associated viruses (rAAVs) are small, non-enveloped single-stranded DNA viruses from the parvovirus family, with a packaging capacity of ~4.7 kb. While this limits their use for complex transgenes in oncology, rAAVs have been approved for other diseases, such as Luxturna for retinal disorders and Zolgensma for spinal muscular atrophy. In cancer, rAAV-mediated interferon β (IFNβ) expression has shown promise in suppressing glioblastoma in mouse models.[115] Capsid engineering enhances tumour specificity and transduction efficiency, exemplified by Her2-modified rAAVs and prostate-targeting rAAV7 and rAAV9.[116,117]

Clinical trials using AAV in oncology are limited. A Phase I trial (NCT02496273) involves *ex vivo* gene therapy for gastric cancer, where patients' T lymphocytes are primed to target carcinoembryonic antigen (CEA)-expressing tumour cells. Another trial (NCT02602249) uses AAV2 to transduce dendritic cells with the MUC1 gene for gastric cancer treatment. AAVs can also deliver RNA interference molecules, such as shRNA, within target cells.[118] However, toxicity from viral vectors and RNAi necessitates careful evaluation, including dose optimization and conditional promoters to minimize immune responses.

A novel approach involves *in vivo* AAV-mediated CAR-T cell therapy. An AAV vector encoding CD4CAR was used to transduce T cells within the patient's body, effectively reducing tumour size in a T-cell leukaemia model.[119] This simplifies CAR-T production, potentially lowering costs and increasing accessibility.

3.1.3.3. Herpes simplex virus. Herpes simplex viruses (HSV-1 and HSV-2) are utilized in oncology as non-replicating vectors and oncolytic agents. The non-replicating HSV-1 vector NP2 was designed to express preproenkephalin (PENK) for pain modulation in cancer patients, showing safety and potential efficacy.[120,121] Oncolytic HSV-1 vectors have also gained attention; talimogene laherparepvec (T-VEC, Imlygic), an HSV-1 vector encoding GM-CSF, was approved for recurrent melanoma.[122] Despite innovative design, challenges like host antiviral responses and limited intratumoral spread affected efficacy. Ongoing trials investigate combinations of T-VEC with immune checkpoint inhibitors to enhance outcomes.[123]

3.1.3.4. Vaccinia virus. Vaccinia virus (VV), an enveloped double-stranded DNA orthopoxvirus, offers advantages like large genome capacity (~190 kb), cytoplasmic replication (avoiding genome integration), and tumour tropism.[124,125] Strains like Western Reserve (WR) and Modified Vaccinia Ankara (MVA) serve different purposes. TG6002, derived from the WR strain, selectively replicates in tumour cells and converts 5-fluorocytosine into 5-fluorouracil, showing efficacy in preclinical studies and ongoing trials for glioblastoma and gastrointestinal tumors.[126] Pexa-Vec (JX-594), another WR-derived oncolytic virus expressing GM-CSF, has been explored in various cancers but did not improve outcomes in a Phase III trial for hepatocellular carcinoma.[127] Research aims to enhance targeting and immune stimulation. MVA is extensively attenuated and used in cancer immunization strategies. A 'prime-boost' approach combining ChAdOx1-MAGE-A3-NY-ESO-1 and MVA vectors is under investigation to enhance immune responses in patients with specific tumour antigens.[128,129]

3.1.3.5. Chimeric orthopox virus. CF33 is a novel chimeric orthopoxvirus created by recombining multiple orthopoxvirus species, showing specificity for breast and pancreatic cancers.[130] CF33-hNIS includes the human sodium iodide symporter for imaging,[78] and CF33-hNIS-antiPDL1 expresses anti-PD-L1 antibodies, enhancing tumour localization and immune activation.[131] Clinical trials, like the OASIS trial, evaluate CF33 variants with anti-CD19 agents for their oncolytic and immunostimulatory properties (Table S3†).[132]

Despite advances with non-integrative viral vectors, concerns over safety, immunogenicity, and production complexity have prompted a shift toward non-viral vectors. Non-viral systems—such as lipid nanoparticles and polymers—offer advantages in safety, ease of production, and lower immunogenicity, making them suitable for repeat administrations and broader therapeutic applications.

3.2. Organic nanoparticles for nucleic acids transfection

3.2.1. Mechanisms and strategies for NP vectorisation. Organic nanoparticles (NPs) have transformed oncology by enabling stabilization and thus precise delivery of NAs for therapeutic purposes. Their unique properties, including small size, high surface area, and functionalisation potential, allow for encapsulating and protecting NAs such as DNA, RNA, siRNA, and mRNA.[3,133] The size and shape, the charge, hydrophobicity, and encapsulation rate of the NP are key (Table S4†). These attributes improve pharmacokinetics, enhance biodistribution, and minimise off-target effects, addressing the inherent challenges of NA therapies, such as enzymatic degradation, low cellular uptake, and rapid systemic clearance.[134] Effective delivery systems must meet specific criteria: shielding NAs from degradation, maintaining stability in circulation, and ensuring efficient release within targeted cells. This is particularly crucial in solid tumours, especially the ones where high interstitial fluid pressure and a dense extracellular matrix pose significant barriers.[135] Two primary mechanisms, the Enhanced Permeability and Retention (EPR) effect and Active Transport Retention (ATR), govern NP distribution. The EPR effect exploits the disorganised vasculature of tumours, which features large pores and poor lymphatic drainage (Fig. 3), enabling NPs of 100–200 nm to accumulate preferentially within tumour tissues.[134,136] However, variability in the EPR effect due to patient-specific tumour biology limits its universal applicability. To enhance this mechanism, strategies such as carbon monoxide-releasing agents (*e.g.*, SMA/CORM2 micelle) improve tumour blood flow and vascular permeability.[137] In contrast, ATR involves active mechanisms like receptor-mediated interactions and the absence of intratumoral lymphatics to enhance NP retention. While ATR comp-

lements EPR, its success depends on understanding tumour-specific biology, requiring tailored approaches.[138] Functionalising NP surfaces with ligands, such as antibodies, aptamers, or peptides, further enhances specificity.[139,140] Tumour-specific antigens (TSAs), derived from genetic mutations or viral infections, and tumour-associated antigens (TAAs), overexpressed on cancer cells, serve as critical targets for ligand conjugation.[141,142] A representative selection of diverse targeting agents is presented in Tables 2 and S5.† These strategies reduce off-target effects and maximise therapeutic efficacy. Additionally, modifying the tumour microenvironment (TME) to deplete the extracellular matrix, inhibit cancer-associated fibroblasts, and reverse immunosuppression facilitates NP penetration and drug delivery.[143,144]

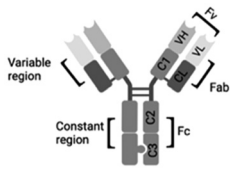
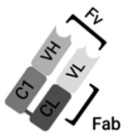
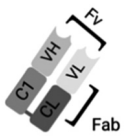
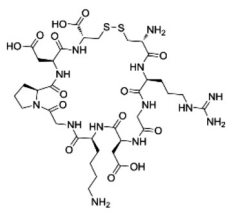
3.2.2. Advanced NP systems for NA delivery. Advancements in NP design have led to the development of systems that address stability, specificity, and controlled release (Fig. 5). Stealth NPs, coated with hydrophilic polymers like polyethylene glycol (PEG), evade immune detection by preventing opsonisation, thereby prolonging circulation time and improving tumour delivery.[135] However, repeated PEGylated NP administration can elicit anti-PEG antibodies, reducing efficacy. Innovative alternatives, such as using different polymers or modifying dosing regimens, aim to mitigate this challenge.[145]

Stimuli-responsive NPs release their payload in response to specific triggers, such as pH, temperature, or redox potential. For example, pH-sensitive liposomes destabilise in the acidic TME, ensuring localised and precise drug activation.[143] These systems enhance therapeutic outcomes while minimising systemic toxicity.

A critical aspect of these applications is overcoming intracellular barriers, particularly endosomal escape. Incorporating polyethylenimine (PEI) into NP formulations induces the proton sponge effect, causing endosomal swelling and rupture, which facilitates NA release into the cytoplasm.[146] This mechanism is essential for achieving high transfection efficiency, particularly in hard-to-target cancers.

Recent advances in NP engineering have focused on improving specificity and therapeutic efficacy. NPs targeting the TME can reshape it by modulating immune responses or

Table 2 Size and molecular characteristics of antibody-derived fragments and peptides for targeted vectors

Protein	IgG(1) 150 kDa (10–15 nm)	Fab' 55 kDa (4–6 nm)	Fab 50 kDa (4–6 nm)	scFv 28 kDa (2–4 nm)	VHH 12 kDa (camelids ab) (2–5 nm)	Peptide 1–4 kDa (0.5 to 2 nm)
Schematic view						 **iRGD:** 9-amino acid cyclic peptide, (sequence: CRGDKGPDC)

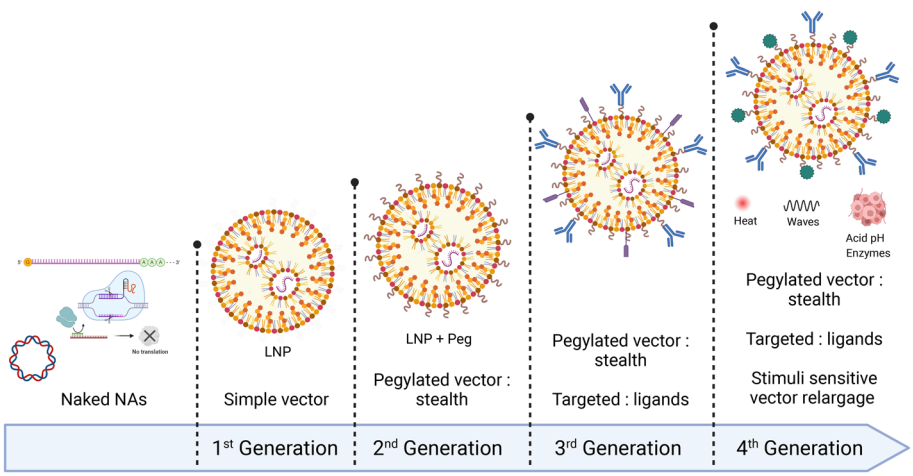


Fig. 5 Evolution of lipid NPs' design.

altering its physical and biochemical properties. For instance, targeting acidic, hypoxic, or fibrotic regions within the TME enhances NP delivery and therapeutic outcomes.[144,147] Aptamers, specific oligonucleotides, can precisely target cell-specific receptors. Fluorescent aptamers now allow real-time monitoring of nucleic acid nanoparticle (NANP) formation and interactions. Advances in customizable NANPs have enabled the design of nanoassemblies that use aptamers to modulate cell signalling or activate therapeutic functions through aptamer–receptor interactions. These innovations pave the way for applications like reshaping the TME with nanoparticle decorations.[148] Such strategies are crucial in overcoming the TME's role as a barrier to treatment and leveraging its unique characteristics for therapy.

3.2.3. Pharmacokinetics, biodistribution, and dose optimisation. Understanding the pharmacokinetics (PK) and biodistribution of NPs is pivotal for optimising their therapeutic potential. Upon administration, NPs interact with plasma proteins to form a "protein corona", which significantly influences their stability, cellular uptake, and clearance. While the corona can enhance targeting by exposing functional ligands, it also marks NPs for immune clearance by macrophages, particularly Kupffer cells in the liver.[89,146] Strategies to address this include engineering NPs with low immunogenicity.

The route of administration, structural properties, and interactions with biological matrices also impact NP performance. Intravenous delivery is most common for systemic cancer therapies, but variability in enzymatic degradation across organs necessitates tailored designs of NPs. For example, PEGylation extends circulation time but can trigger immune responses over time, underscoring the need for balancing stability and adverse immune reactions.[145,149,150]

Recent studies have identified a minimum effective dose threshold for tumour delivery. Administering 1 trillion NPs in mice saturates Kupffer cells, prolonging NP circulation and enhancing tumour accumulation. This approach has improved delivery efficiency in formulations like Caelyx/Doxil and provides a framework for dose optimisation in clinical settings.[89]

Tumour heterogeneity remains a significant challenge. Differences in genetic profiles, TME composition, and therapeutic responses necessitate personalised NP strategies. A complementary approach to reducing tumour progression and dissemination is to deliver NAs with NPs to cancer stem cells, or to target the epithelial–mesenchymal transition, or to block metastatic pathways.[151–153] Similarly, NPs targeting circulating tumour cells (CTCs) offer a novel approach to prevent metastasis. For example, TRAIL-conjugated liposomes induce apoptosis in CTCs while sparing healthy tissues.[154]

Advancing NP-based therapies requires integrating pharmacological, physiological, and pathological considerations into design and application. This includes understanding tumour-specific biodistribution patterns, overcoming immune clearance, and optimising dosing regimens to achieve maximal therapeutic benefit. Standardising manufacturing and purification protocols is also critical for ensuring batch-to-batch consistency, which is essential for regulatory approval and clinical application.[155,156] The DELIVER framework encapsulates the core principles for optimising nanomedicine preclinical development, emphasising design, manufacturing, clinical, and regulatory strategies to accelerate translation and maximise impact.[157]

3.2.4. Lipidic vectors. Lipidic vectors have been transformative in drug delivery, especially in oncology, where they have been applied for over two decades.

These vectors, including liposomes, lipoplexes, and lipid nanoparticles (LNPs) (Fig. 6), offer numerous advantages, such as extended circulation times, avoidance of the reticuloendothelial system (RES), and high biocompatibility. They are known for their ability to encapsulate and protect therapeutic agents, NAs, while facilitating targeted delivery. Among their applications, Doxil, a liposomal formulation of doxorubicin approved in 1995, exemplifies the efficacy of lipidic vectors in utilising the EPR effect for tumour targeting. These systems improve bioavailability, reduce systemic toxicity, and enhance therapeutic outcomes.[158,159]

The roles and characteristics of lipids used in lipidic vectors formulations, such as ionizable, phospholipid, cholesterol, and PEGylated components, are critical for determining stability, delivery efficiency and compatibility with various NAs (Table S6†). While liposomes have been traditionally used for delivering plasmid DNA, the emergence of lipoplexes and LNPs has enabled the delivery of various NAs, including mRNA, siRNA, and ASOs, through electrostatic interactions. Tables S7, S8, and S9† provide an overview of ongoing clinical trials exploring lipid-based vectors, with a focus on liposomes (Table S7†), lipoplexes (Table S8†), and LNPs-based systems (Table S9†), showcasing their distinct roles in advancing nucleic acid delivery technologies. Importantly, only three NA therapies using LNPs—patisiran (siRNA), the Pfizer-BioNTech COVID-19 vaccine, and the Moderna COVID-19 vaccine—have received market approval, highlighting the clinical relevance of lipid-based delivery systems.[160–162]

3.2.4.1. Liposomes. Liposomes are spherical vesicles consisting of one or more phospholipid bilayers encapsulating an aqueous core. Due to their versatile structure, they have been

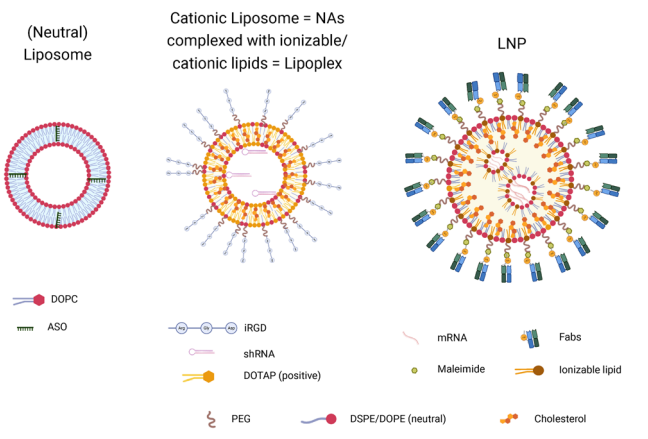


Fig. 6 Representation of selected lipidic vectors.

widely used in drug delivery, including for NAs such as miRNA, siRNA, and ASOs. Their neutral lipid composition, often incorporating phospholipids (Table S6†), enhances their biocompatibility but limits their ability to encapsulate highly charged molecules like DNA and RNA. To overcome this, modified liposomal systems have been developed, targeting smaller or chemically modified NAs with reduced charges.[52]

One significant advancement in liposomal technology is the development of EphA2-targeted siRNA delivery systems. EphA2, a receptor tyrosine kinase overexpressed in multiple cancers, plays a crucial role in tumour progression and metastasis. A liposome composed of DOPC encapsulating EphA2-specific siRNA, termed EPHARNA, demonstrated significant efficacy in preclinical models. For example, in ovarian cancer models, EPHARNA reduced tumour growth and metastasis while exhibiting minimal toxicity. Additionally, its combination with chemotherapy showed synergistic effects, and ongoing clinical trials (NCT01591356) are evaluating its safety and efficacy in human subjects.[163,164]

Another innovative liposomal formulation is BP1001, an antisense oligonucleotide (ASO) targeting Grb2 mRNA. Composed of a P-ethoxy backbone for nuclease resistance, BP1001 is encapsulated in DOPC liposomes, ensuring stability and extended circulation times. This formulation has demonstrated promising results in preclinical models of leukaemia and solid tumours, with clinical trials indicating safety and efficacy in refractory leukaemia (NCT02781883).[165,166]

3.2.4.2. Lipoplexes. Lipoplexes are complexes formed through electrostatic interactions between positively charged lipids and negatively charged NAs. Their simplicity, ease of customisation, and ability to deliver large NAs make them an attractive option for therapeutic applications. However, their reliance on electrostatic interactions necessitates optimisation to ensure stability and minimise aggregation *in vivo*.[136,167]

DOTMA, a cationic lipid widely used in lipoplex formulations, has demonstrated efficacy in gene transfer, particularly when combined with helper lipids like DOPE. The inclusion of DOPE facilitates membrane fusion and endosomal escape, critical steps for effective gene transfection. Additionally, lipoplexes have been modified with tumour-targeting peptides, such as iRGD (a 9-amino acid cyclic peptide, Table 2), to enhance their active targeting capabilities. For example, R-LP, a DOTAP-based lipoplex modified with iRGD, successfully delivered eIF3i-specific shRNA in melanoma models, significantly inhibiting tumour growth and metastasis.[168] A notable clinical example is SGT53, a lipoplex encapsulating wild-type human p53 cDNA. Modified with an anti-transferrin receptor (TfR) scFv for selective targeting, SGT53 has shown efficacy in restoring apoptotic pathways in various cancers. It is currently being evaluated in clinical trials for glioblastoma (NCT02340156) and pancreatic cancer (NCT02340117), where it demonstrated improved progression-free survival in second-line treatments.[169,170]

Despite their potential, lipoplexes face challenges such as detachment of NAs in the bloodstream, leading to rapid aggregation. Innovations in lipid composition and manufacturing processes are addressing these limitations, enhancing the stability and efficacy of lipoplexes for clinical applications.[171]

3.2.4.3. Lipid Nanoparticles. LNPs are among the most advanced lipidic vectors, consisting of a lipid core surrounded by a phospholipid monolayer. Their composition typically includes ionizable or cationic lipids, cholesterol, phospholipids, and PEGylated lipids, allowing for precise tuning of their properties for specific therapeutic applications. Unlike lipoplexes, LNPs leverage pH-sensitive ionizable lipids to stabilise NAs and potentially enhance endosomal escape, making them highly efficient for intracellular delivery.[161,167]

A landmark in LNP technology is patisiran/Onpattro, an siRNA-based therapy encapsulated in LNPs, approved for hereditary transthyretin-mediated amyloidosis. This system exemplifies the clinical success of LNPs in overcoming biological barriers to NA delivery. Similarly, the Pfizer-BioNTech and Moderna COVID-19 vaccines utilised LNPs to deliver mRNA encoding the SARS-CoV-2 spike protein, demonstrating the scalability and efficacy of this platform.[162] Cholesterol plays a pivotal role in stabilising LNP structures, reducing permeability, and enhancing circulation time. However, cholesterol can be rapidly extracted by cell membranes, compromising stability. Modified lipids, such as sphingomyelin derivatives, have been developed to address this issue. For instance, SM-CSS-Chol, a disulfide-bonded cholesterol analogue, improved the stability and delivery efficiency of siRNA-loaded LNPs in preclinical cancer models, highlighting its potential for clinical applications.[172]

Targeted LNPs have also been developed for immune cell transfection. For example, a CD3-targeted LNP encapsulating plasmid DNA for CAR-T therapy demonstrated selective delivery to T cells, enhancing gene expression while minimising off-target effects. This system incorporates PEGylation to improve circulation time and specific lipid combinations to optimise endosomal escape, showcasing the versatility of LNPs in advanced gene therapies.[173]

The toxicity and immunogenicity of LNPs are critical considerations, particularly for repeated dosing. Ionizable lipids, the cornerstone of LNP formulations, exhibit pH-sensitive properties that reduce cytotoxicity compared to permanently charged cationic lipids. Biodegradable linkages, such as ester and amide bonds, further minimise toxicity by facilitating *in vivo* cleavage. Nevertheless, careful evaluation of lipid composition and dosing regimens is necessary to balance efficacy and safety.[174–176]

3.2.5. Polymeric vectors. Polymeric vectors have emerged as a versatile platform in NAs delivery, offering a tunable framework to overcome challenges such as enzymatic degradation, limited stability, and poor cellular uptake of naked NAs. These vectors harness the diversity of polymers (Table S10†), which can be tailored chemically and structurally to meet therapeutic demands, such as protecting NAs during systemic circulation, promoting cellular internalisation, and enabling controlled release at target sites.[177] Among the various systems under development, polymersomes, poly-

plexes, polymeric nanocapsules, and dendrimers stand out for their unique attributes and therapeutic potential (Fig. 7).

Polymers suitable for NA delivery include both natural and synthetic types, such as poly(ethyleneimine) (PEI), poly-L-lysine (PLL), poly(lactic-*co*-glycolic acid) (PLGA), poly(beta-amino ester) (PBAE), and poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) (Table S10†). Each offers specific advantages, including charge density for NA condensation, biodegradability, and biocompatibility. Modifications, such as PEGylation, are often incorporated to enhance systemic stability and minimise immune responses, ensuring the safe and efficient delivery of therapeutic agents.[178–180]

The design of polymeric vectors often involves balancing transfection efficiency and toxicity. Cationic polymers like PEI and PLL effectively bind and condense NAs through electrostatic interactions, forming compact polyplexes that protect NAs from enzymatic degradation. However, their high charge density and lack of biodegradability can lead to cytotoxicity and off-target effects, necessitating structural modifications to enhance safety profiles.[181] PLGA, an FDA-approved polymer, offers an alternative with its excellent biocompatibility and biodegradability, making it a preferred choice for clinical applications, particularly for sustained-release systems.[182]

3.2.5.1. Polymersomes. Polymersomes are vesicular systems formed by the self-assembly of amphiphilic block copolymers into bilayer structures. These vesicles mimic liposomes but possess superior stability due to their thicker membranes, which resist mechanical stress and provide prolonged circulation times *in vivo*. Polymersomes can encapsulate both hydrophilic and hydrophobic NAs, offering a flexible platform for gene delivery.[183] The chemical diversity of block copolymers enables the design of polymersomes with tailored properties for specific applications. For instance, polymersomes made from poly(ethylene glycol)–poly(caprolactone) (PEG–PCL) or poly(ethylene glycol)–poly(lactic acid) (PEG–PLA) demonstrate excellent biocompatibility and efficient encapsulation of plasmids and siRNA. Functionalisation with targeting ligands further enhances cellular uptake, enabling site-specific delivery in tumours.[183,184]

A notable clinical example involves the Local Drug EluteR device, which uses a PLGA-based polymer matrix to deliver siRNA targeting the KRAS G12D mutation in pancreatic cancer. This system has shown promising results in phase I and II trials (NCT01188785, NCT01676259), providing a minimally invasive approach to address one of the most challenging oncogenic mutations.

3.2.5.2. Polyplexes. Polyplexes are complexes formed by the electrostatic interaction between cationic polymers and negatively charged NAs. These compact, stable structures shield NAs from enzymatic degradation, enhance cellular uptake, and promote intracellular delivery. Unlike lipoplexes, polyplexes are composed of hydrophilic polymers, which improve their solubility and adaptability in aqueous environments.[185,186]

PEI, a widely studied polymer, forms polyplexes with high transfection efficiency due to its ability to condense NAs and facilitate endosomal escape *via* the proton sponge effect. However, its high molecular weight variants often exhibit cytotoxicity, prompting the development of biodegradable alternatives, such as poly(beta-amino ester) (PBAE) and PEGylated PEI, which combine high transfection efficiency with reduced toxicity.[187]

Cyclodextrin-based polyplexes, exemplified by CALAA-01, represent a pioneering approach in systemic siRNA delivery. Functionalised with transferrin for tumour targeting, CALAA-01 demonstrated dose-dependent gene silencing in phase I trials but faced challenges related to DLTs, attributed to the polymer components rather than the siRNA.[188]

3.2.5.3. Polymeric nanocapsules. Polymeric nanocapsules feature a core–shell architecture, where therapeutic agents are encapsulated within a polymeric shell, offering protection and controlled release. These structures are versatile platforms for NA delivery, capable of incorporating diverse polymer types, including PLGA, PEG-PBAE, and chitosan. Their rigid structure enhances stability, while their surface can be functionalised to improve targeting and cellular uptake.[182,187]

A key example of polymeric nanocapsule application is STP705, a system that utilises histidine-lysine copolymers to deliver siRNA targeting TGF-β1 and COX-2. This dual-targeted approach addresses both tumour cells and the tumour microenvironment, demonstrating high efficacy in preclinical and early clinical studies for cancers like hepatocellular carcinoma.[189]

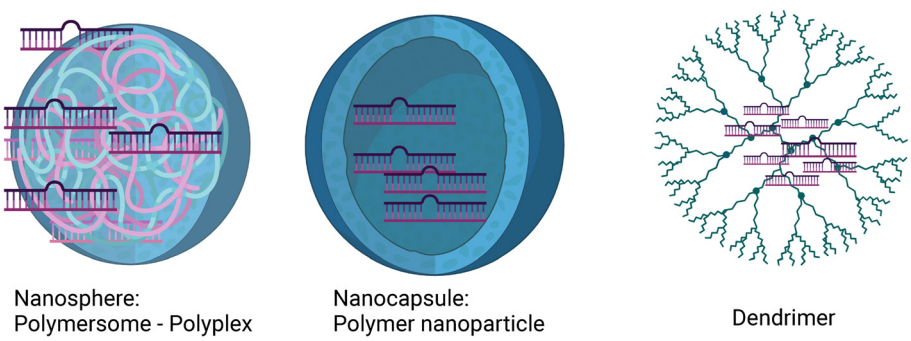


Fig. 7 Representation of selected polymeric vectors.

Another innovative system employs PEG-PBAE nanocapsules co-encapsulating plasmids for glioblastoma treatment. These vectors achieve superior transfection efficiency and tumour penetration compared to non-PEGylated systems, highlighting the role of polymeric nanocapsules in addressing challenges such as endosomal escape and tumour heterogeneity.[187]

3.2.5.4. Dendrimers. Dendrimers are hyperbranched, tree-like macromolecules characterised by a central core and radially symmetric layers of branching units. Their unique architecture allows for precise control over size, shape, and surface functionality, making them highly adaptable for NA delivery. Dendrimers form stable complexes with NAs through electrostatic interactions, protecting the cargo and facilitating intracellular transport.[190]

Poly(amidoamine) (PAMAM) dendrimers are the most extensively studied for gene therapy. Their surface amine groups enable efficient condensation of NAs, while their branched structure allows for multifunctionalisation. Recent advancements include the modification of PAMAM G5 dendrimers with cholesteryl chloroformate and alkyl-PEG for dual delivery of TRAIL and doxorubicin, achieving synergistic anti-tumour effects in preclinical colon cancer models.[191]

Phosphorus dendrimers, incorporating protonated ammonium terminals, have shown efficacy in delivering plasmid DNA encoding tumour suppressor genes like TP53. These systems effectively induce cell cycle arrest and apoptosis in tumour cells, demonstrating their potential in targeted therapies.[192] Carbosilane dendrimers, on the other hand, have been employed to deliver pro-apoptotic siRNA, highlighting their versatility in addressing oncogenic pathways.[193]

A novel application of dendrimers involves the formation of RNA triplex nanoparticles, combining dendritic architecture with RNA molecules for dual-function cancer therapy. These systems enable the simultaneous inhibition of oncogenic microRNAs and replacement of tumour suppressor microRNAs, offering a comprehensive approach to tumour management.[194]

3.2.6. Lipid-polymer hybrid nanoparticles. LPHNs, also named lipopolyplexes, combine lipid structures with cationic or neutral polymers, offering controlled release, stability, and protection for NAs.[195] A PLGA polymer shell enhances sustained delivery of siRNA, enabling long-term gene silencing, reducing administration frequency, and minimising systemic side effects.[195] Cationic polymers like PEI condense NAs into stable complexes, encapsulated by lipid bilayers of DPPC and cholesterol, with PEG and ligands like PR_b for targeted delivery. For glioblastomas, PEI-complexed miR-603 in PR_b-functionalised liposomes sensitises tumour cells to radiation by downregulating IGF1 signalling.[151,196] LPNPs encapsulating CRISPR/Cas9 plasmids targeting MGMT, combined with focused ultrasound and microbubbles, effectively crossed the blood–brain barrier, enhancing glioblastoma sensitivity to temozolomide. Modifications like DSPE-PEG2000 improve tumour targeting and circulation time, enabling advanced therapies for drug-resistant glioblastomas.[197]

3.2.7. Extracellular vesicles (EVs). Extracellular vesicles (EVs), cellular membrane-based nanovesicles ranging from 50 to over 2000 nm, mediate intercellular communication *via* autocrine, paracrine, and endocrine signalling. Subtypes like exosomes, microvesicles, and apoptotic bodies carry diverse cargo, including proteins, NAs, and metabolites, reflecting their cellular origin. EVs' ability to evade immunity and cross barriers like the blood–brain barrier makes them promising vectors for drug and gene therapy delivery.[198] Mesenchymal stem/stromal cell (MSC)-derived EVs offer therapeutic potential for targeting tumours, facilitated by engineering their surfaces with specific ligands for precise delivery of NAs and CRISPR/Cas9 tools.[199] Phase 1 trials, such as KRASG12D-siRNA-loaded exosomes for pancreatic cancer, highlight their clinical potential.[200] Additionally, EVs encapsulating adeno-associated viruses (AAVs) bypass pre-existing immunity, improving vector safety and stability.[201] Novel vexosomes, combining exosomes and viral vectors, enhance gene therapy applications.[202] In hepatocellular carcinoma models, AAV6 vexosomes showed a 2.3-fold increase in tumour regression.[203]

3.3. Cells as vectors

The use of neural stem cells (NSCs) as carriers for oncolytic viruses is a promising strategy for treating malignant gliomas, leveraging NSCs' natural tumour-tropism as biological "Trojan horses". Genetic engineering enhances their specificity, as demonstrated by the FDA-approved NSC line HB1.F3.CD21, which delivers CRAd-S-pk7, a conditionally replicative adenovirus. CRAd-S-pk7 uses a survivin promoter for selective replication in glioma cells and features a polylysine sequence (pK7) to improve viral attachment, enhancing targeting of resistant glioma stem cells. Preclinical and phase I trials (NCT03072134) show improved antitumor activity and survival in glioblastoma models.[204] Similarly, bone marrow-derived mesenchymal stem cells (BM-hMSCs) loaded with DNX-2401, another oncolytic adenovirus, utilize the same principle to precisely target gliomas. This approach demonstrates efficacy in recurrent high-grade gliomas in phase I trials (NCT03896568). These innovations highlight the potential of stem cell-based oncolytic virotherapy to overcome vectorization challenges, offering precise and effective treatment for resistant gliomas.

4. Conclusions

In summary, gene and RNA-based therapies represent an evolving frontier in oncological treatment, offering highly specific mechanisms to modulate gene expression, restore tumour suppressors, or enhance anti-tumour immunity. Advances in molecular tools—from CRISPR-based gene editing to antisense oligonucleotides and self-amplifying mRNAs—have provided unprecedented precision and flexibility. At the same time, the development of robust delivery systems remains central to the successful translation of these approaches. Viral vectors continue to be refined for safety, specificity, and stable integration, while non-viral carriers, such as lipidic or polymeric

nanoparticles and extracellular vesicles, show great promise in overcoming barriers like enzymatic degradation, immunogenicity, and complex intratumoral environments. The integration of cell-based carriers, exemplified by stem cell "Trojan horses" further expands the therapeutic landscape.

Looking ahead, the field is poised to capitalize on emerging technologies for target identification, advanced gene editing methods, improved vector engineering, and more nuanced control over gene expression patterns. Combining these therapies with established treatments—chemotherapy, immunotherapy, radiation—and implementing personalized strategies based on tumour profiling will likely enhance both efficacy and safety. Continued interdisciplinary collaboration, rigorous clinical testing, and regulatory refinement will be pivotal in pushing these next-generation therapies into mainstream cancer care.

Author contributions

JSK screened the literature, wrote the original draft and made illustrations. BB developed the concept, structured the paper and supervised this work. All authors wrote and reviewed the manuscript.

Data availability

This article is a review and does not include original research data. All information discussed is derived from published sources, which are cited in the reference list.

All ESI data supporting this review are included in the manuscript and its ESI.†

Where applicable, data supporting the findings have been obtained from publicly available sources, as detailed in the references.

Conflicts of interest

There are no conflicts to declare.

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