





# Advancing Cancer Immunotherapy Using Lipid Nanoparticle-Based Approaches

Pedro Henrique Dias Moura Prazeres <sup>1,\*</sup>, Gabriel Henrique Costa da Silva <sup>1,\*</sup>,  
Gabriel Vieira Azevedo <sup>1</sup>, Natalia Jordana Alves da Silva <sup>1</sup>, Pedro Augusto Carvalho Costa <sup>1</sup>,  
Walison Nunes Da Silva <sup>1</sup>, Anderson Oliveira Lobo <sup>2</sup>, Pedro Pires Goulart Guimaraes <sup>1</sup>

<sup>1</sup>Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil;

<sup>2</sup>Department of Materials Engineering, Federal University of Piauí, Teresina, PI, 64049-550, Brazil

\*These authors contributed equally to this work

Correspondence: Pedro Pires Goulart Guimaraes, Department of Physiology and Biophysics, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, Email [ppiresgo@reitoria.ufmg.br](mailto:ppiresgo@reitoria.ufmg.br)

**Abstract:** Cancer immunotherapy, including adoptive cell therapies, cancer vaccines, and cytokine-based therapies, have revolutionized targeted approaches in the treatment of different tumors. However, the broader application of immunotherapies, such as for engineered T cells expressing a chimeric antigen receptor (CAR-T cells), remains limited by challenges in production, systemic toxicity, and inefficient delivery, especially in solid tumors. Recent advances in nucleic acid delivery technologies, notably ionizable lipid nanoparticles (LNP), offer promising solutions to overcome these barriers. LNPs have shown potential in delivering messenger RNA (mRNA), and DNA for the generation of CAR-T cells, cancer vaccines, bispecific antibodies, and cytokine-based immunotherapies. The clinical success of LNP-based platforms in mRNA COVID-19 vaccines and interference RNA therapies for genetic disorders further validates their effectiveness in gene delivery, highlighting LNPs as versatile carriers for therapeutic nucleic acids. Furthermore, LNPs can be optimized for off-the-shelf formulations, enabling personalized treatments targeting specific patient needs. In this review, we highlight the role of LNP platforms in advancing mRNA and DNA delivery for cancer immunotherapy. We explore their potential to improve CAR-T cell production, advance cancer vaccines, and support the development of bispecific antibody- and cytokine-based therapies, ultimately paving the way for more effective, scalable, and accessible immunotherapeutic strategies.

**Keywords:** cancer immunotherapy, adoptive cell therapy, CAR-T cells, cancer vaccines, lipid nanoparticles, gene delivery

## Introduction

Cancer in its various forms remains the second major cause of mortality, also representing a great challenge to the public healthcare systems worldwide.<sup>1-3</sup> The increase in incidence and mortality of this disease relates to contemporary diets and lifestyle as well as environmental changes.<sup>1</sup> Taken together, this outlines the need for investment in basic and clinical research that focus on developing preventive and therapeutic approaches against this disease.

Different immunotherapies have emerged as effective alternatives to conventional treatments like chemotherapy, offering more targeted and enhanced responses across various cancers.<sup>4,5</sup> Among these, chimeric antigen receptor (CAR)-T cell therapy has revolutionized the treatment of hematological tumors by harnessing engineered T cells to selectively target tumor antigens.<sup>6</sup> However, the broader application of CAR-T cells, particularly in solid tumors, is limited by challenges in manufacturing, antigen selection, and the immunosuppressive tumor microenvironment.<sup>7</sup> Clinical efforts are ongoing to extend CAR-T therapy to solid tumors such as hepatocarcinoma, pediatric tumors, and neuroblastoma.<sup>6,8</sup>

In parallel, other immunotherapy strategies, such as cytokine therapies, cancer vaccines, and bispecific antibodies (BsAbs), seek to activate the patient's immune system without the need for cell transfer. These approaches aim to stimulate immune effector cells, reverse T cell exhaustion, and promote tumor cell killing. While promising, their clinical

efficacy is often hindered by systemic toxicity, short half-life, and poor biodistribution, which limit their ability to effectively reach and act within the tumor microenvironment. Overcoming these delivery barriers remains critical to unlocking the full potential of these immunotherapeutic modalities.

To overcome the limitations faced by current immunotherapeutic strategies, lipid nanoparticles (LNP) can be applied as versatile gene delivery systems in multiple contexts.<sup>9,10</sup> These platforms enable a fast and effective delivery of nucleic acids to cells and tissues, both in vivo and ex vivo without affecting cell viability. Compared to viral vectors currently used in CAR-T cell production, LNPs do not integrate the genetic material into the host genome, thereby reducing safety concerns and significantly shortening the engineering timeline. Moreover, LNPs can be tailored to deliver different nucleic acid cargos, offering alternatives to protein-based delivery of agents like cytokines, and enabling the generation of potent cancer vaccines and bsAbs directly in the body. These features make LNP platforms attractive for both off-the-shelf therapies targeting different tumor antigens and personalized approaches based on individual patient profiles.<sup>11–13</sup> In this review, we explore the expanding role of LNP platforms in cancer immunotherapy, with a focus on their application in messenger RNA (mRNA), and DNA-based delivery strategies to enhance their efficacy, specificity, and accessibility of treatments such as CAR-T cells, cancer vaccines, cytokines, and bsAbs.

## Overview of Lipid Nanoparticles

### Lipid Nanoparticles

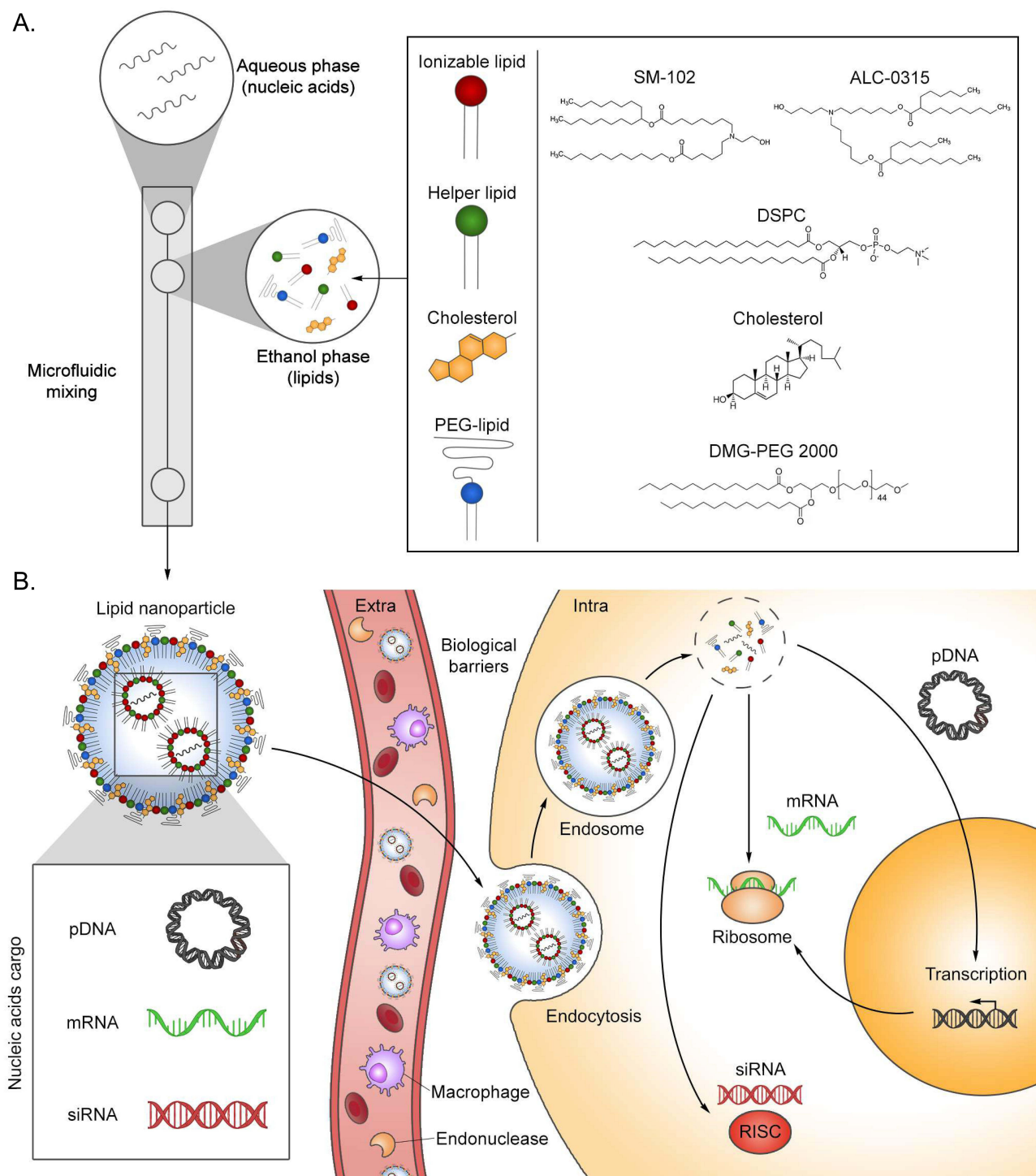
Nanoparticles or nanomaterials are defined as materials with external dimensions ranging from 1 to 200nm and have been employed in antitumor therapy for several years.<sup>14</sup> These materials can serve as drug delivery systems, increasing permeability in the tumor microenvironment and circulation time.<sup>15</sup> The importance of these nanoparticles is substantial, attracting investments and indicating a significant advancement in the treatment of various diseases.<sup>16</sup>

LNPs are included in the class of nanomaterials, among other types of nanoparticles, which are characterized by a spherical shape containing at least one lipid bilayer surrounding an internal aqueous compartment.<sup>16</sup> These LNPs are widely used as delivery systems because of their low-complexity production process, are biocompatible, have good biodistribution<sup>17,18</sup> and are easy to optimize depending on the load to be delivered.<sup>19</sup> Additionally, LNPs have the capacity to accommodate large payloads within them, justifying their use in therapies already approved by the US regulatory agency (FDA).<sup>20</sup> Recently, in response to the COVID-19 pandemic, LNPs platforms have been extensively employed by companies such as Moderna and Pfizer in the production of mRNA vaccines.<sup>17,21</sup>

LNPs are formed from a multicomponent lipid system, composed of an ionizable lipid, a phospholipid, cholesterol, and a polyethylene glycol (PEG)-lipid conjugate<sup>13</sup> (Figure 1A). Ionizable cationic lipids are typically defined by the presence of a tertiary amine that remains unprotonated under neutral conditions and becomes positively charged in acidic pH environments.<sup>22</sup> These lipids are primarily responsible for the encapsulation of nucleic acids within LNPs. During LNP formation, ionizable lipids are positively charged, enabling electrostatic interactions with the negatively charged phosphate groups of nucleic acids, which facilitates their complexation within the nanoparticle.<sup>23</sup> These lipids also mediate endosomal escape and the subsequent release of the nucleic acid cargo into the cell. Endosomal acidification leads to lipid protonation, promoting interaction with endogenous lipids and destabilization of the endosomal membrane, thereby releasing the genetic material into the cytoplasm.<sup>22</sup> Although the exact mechanism has not been fully elucidated, this remains a widely accepted hypothesis.

Phospholipids are helper lipids that enhance the stability of the system by contributing to the structure of the lipid bilayer. They assist in the efficient encapsulation of nucleic acids and some of those possess fusogenic properties, which improve the transfection efficiency of LNPs.<sup>24</sup> Phospholipids such as 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) have been widely used in lipid formulations for years. DSPC, in particular, has been clinically used for siRNA delivery in the drug Onpattro®,<sup>25</sup> as well as in mRNA vaccines against SARS-CoV-2 (Moderna mRNA-1273 and Pfizer/BioNTech BNT162b).<sup>26</sup>

Cholesterol is another helper lipid, primarily responsible for providing structural integrity and directly influencing the membrane fluidity of LNPs.<sup>27</sup> It stabilizes the LNP structure by filling the gaps between phospholipids, which also



**Figure 1** Development and use of LNPs. **(A)** LNPs are prepared using a microfluidic mixing method that combines an aqueous phase containing the nucleic acid and an ethanolic phase containing the lipid mix. **(B)** LNPs loaded with one of various types of nucleic acids protect their cargo from nuclease degradation, evade immune detection and efficiently deliver it to the cells. LNPs are endocytosed by the cells, leading to the formation of an endosome containing the LNP within the cytoplasm. Following endosomal escape and LNP destabilization, the nucleic acid is released into the cytoplasm. Once released, it may be directly translated (mRNA), processed for gene silencing (siRNA), or transported to the nucleus for transcription (DNA).

contributes to enhanced stability in the presence of serum proteins.<sup>28</sup> This stabilization is critical to ensuring nanoparticle integrity during systemic circulation.

The final component of lipid nanoparticles is PEG-lipids, which influence several characteristics of LNPs, including particle size and population homogeneity.<sup>29</sup> They prevent particle aggregation, impact circulation and biodistribution profiles *in vivo*,<sup>30,31</sup> and also play an important role in modulating the immune response.<sup>32,33</sup> These lipids create a hydrophilic steric barrier on the nanoparticle surface, contributing to the stability.<sup>34</sup> It has been demonstrated that nanoparticles lacking PEG-lipids are unstable and polydisperse.

Therefore, each component plays a critical role in the architecture and performance of LNPs, making them a versatile platform with a high potential for modulation. This versatility helps explain their success in both clinical and preclinical settings, particularly in gene therapy, vaccines, and cancer immunotherapy. Given their complexity, detailed characterization of LNPs is essential to ensure reproducibility and safety.

## Characterization and Manufacturing

Lipid nanoparticles can be produced through various methods, among which microfluidic mixing stands out as the most commonly employed approach.<sup>35</sup> This method has gained prominence due to its scalability, reproducibility, and precise control over operational parameters.<sup>36</sup> Microfluidic mixing is based on the simultaneous injection of an ethanolic phase containing the lipids and an aqueous buffered phase containing the nucleic acids (Figure 1A). These phases meet within microchannels where chaotic mixing occurs, promoting the self-assembly and complexation of components to form LNPs.<sup>37</sup> Several factors influence the characteristics of LNPs, including the buffer pH, lipid-to-nucleic acid ratio, and flow rate parameters during mixing.<sup>36–38</sup>

The characterization of LNPs is essential to ensure reproducibility and to predict their performance.<sup>39</sup> Key parameters include particle size and polydispersity index (PDI), typically measured by dynamic light scattering (DLS) or nanoparticle tracking analysis (NTA).<sup>40,41</sup> Zeta potential is used to estimate the colloidal stability of the formulations.<sup>42</sup> Encapsulation efficiency and nucleic acid content are usually assessed through fluorescence-based assays, such as RiboGreen (for RNA) and PicoGreen (for DNA).<sup>43,44</sup> Meanwhile, transmission electron microscopy (TEM) or cryo-TEM are employed to analyze the morphology and internal structure of LNPs.<sup>13,45</sup> An important aspect of LNP quality control is their stability under different storage conditions, which can affect particle size, structural integrity, nucleic acid retention, and transfection capability.<sup>46</sup> Sterility control and endotoxin levels are also critical to prevent adverse immune responses.<sup>47,48</sup> These parameters are crucial for ensuring the clinical translation and therapeutic application of LNP formulations.

Recent advances in LNP manufacturing have increasingly leveraged systematic optimization strategies, notably Design of Experiments (DOE). DOE enables the simultaneous evaluation of multiple variables, avoiding the inefficiency of one-factor-at-a-time approaches.<sup>49</sup> This framework has been applied to modulate lipid molar ratios for high-throughput LNP library generation,<sup>50</sup> to investigate structural variants of specific components—such as PEG-lipids—and assess their *in vivo* performance,<sup>51</sup> and to fine-tune process parameters, including flow rate and pH, for control over key physico-chemical attributes.<sup>49</sup> The resulting statistical models facilitate the concurrent optimization of hydrodynamic diameter, polydispersity index, and encapsulation efficiency.<sup>39</sup> By reducing experimental burden while maximizing information yield, DOE expedites formulation development and enhances predictive control over LNP performance.<sup>52</sup>

In parallel, machine learning (ML) is emerging as a powerful tool for LNP design, enabling the extraction of predictive relationships from large datasets.<sup>53</sup> ML approaches have been used to forecast mRNA vaccine efficacy from compositional data, revealing critical substructural features of ionizable lipids that influence immunogenicity.<sup>54,55</sup> The integration of DOE with ML holds particular promise, coupling statistically rigorous experimentation with adaptive predictive modeling. Together, these computational and statistical tools are poised to become foundational to the next generation of rational, application-tailored LNP development, reducing both cost and development timelines.<sup>56</sup>

Overall, stringent quality parameters and accelerated development timelines are essential for advancing LNP technologies, particularly in the context of nucleic acid delivery for next-generation immunotherapies.

## LNP Pharmacokinetics and Pharmacodynamics

Understanding the *in vivo* biodistribution and metabolism of LNPs is determinant for the development of optimized and safe therapies. In cancer, a major challenge is to engineer delivery systems that can efficiently reach the tumor microenvironment (TME) and perform their therapeutic function.<sup>57</sup> Additionally, reducing toxicity to healthy tissues and adverse effects caused by immunotherapies is a central motivation to employ LNPs,<sup>10</sup> as they can be finely tuned to accumulate in tumor sites, while reducing off-target delivery to the organs.<sup>16</sup> To achieve these goals, some strategies can be explored, including rigorous studies of LNP pharmacokinetics, through variations in administration routes and tailoring lipid composition and molar ratios.

In the context of cancer vaccines, intramuscular and subcutaneous administration routes are the most commonly used.<sup>58</sup> These routes aim to deliver LNPs to the lymph nodes and spleen and, consequently, increase the transfection in antigen-presenting cells (APCs).<sup>59,60</sup> This is critical, as antigen presentation by APCs promotes strong induced anti-tumor immunity.<sup>61</sup> On the other hand, intravenous injection is commonly used for systemic drug delivery, although this approach promotes a great accumulation of LNPs in the liver.<sup>62</sup> This occurs mainly due to the interaction of LNPs with plasma proteins, such as apolipoprotein E (ApoE), which directs them to hepatocytes expressing low-density lipoprotein receptors and facilitates their internalization.<sup>63</sup> Notably, tailoring LNP composition modifies the protein corona, changing the biodistribution of formulations.<sup>64</sup> To overcome hepatic tropism, high-throughput screening methods have been used to identify LNPs with enhanced extrahepatic delivery.<sup>65</sup> For example, DNA barcode technology has been applied to quantify the nucleic acid delivery of large LNP libraries and find those with differential biodistribution.<sup>66</sup> Most recently, this method was used to screen 94 unique LNPs, enabling the identification of formulations that preferentially deliver mRNA to head and neck squamous cell carcinoma, by systematically varying the lipidic composition and molar ratios.<sup>67</sup>

For many years, passive accumulation of nanoparticles in tumors was attributed to the enhanced permeability and retention (EPR) effect, caused by abnormal growth of endothelial tissue, containing intercellular gaps in blood vessels, and reduced lymphatic drainage.<sup>16,57</sup> However, new discoveries demonstrate that just a small fraction of nanoparticles reach the TME through passive transport. Instead, active trans-endothelial transport is the main mechanism for their accumulation.<sup>68</sup> Understanding these mechanisms provides valuable insights for rational development of effective nanosystems. In this context, active targeting approaches, such as LNP surface functionalization with antibodies<sup>69,70</sup> or peptides,<sup>71</sup> have gained significant attention and will be further discussed in subsequent sections.

Beyond active and passive targeting, alternative administration routes have also been investigated. Intratumoral injection provides localized therapy that can reduce systemic toxicity, while triggering systemic anti-tumor immune responses capable of reducing tumor burden at distant sites.<sup>72–74</sup> Similarly, intranasal and inhalable administration have emerged as promising strategies for targeting primary or metastatic tumors in the lungs.<sup>75</sup> Early pre-clinical studies using nebulized nanoparticles for intranasal delivery demonstrated regression of lung metastases in an MC38 cell model.<sup>23</sup>

Once absorbed and distributed, LNPs enter cells through endocytosis. This process can involve different pathways, including protein-mediated mechanisms, such as clathrin and caveolin-dependent endocytosis, as well as independent pathways, like macropinocytosis.<sup>76</sup> Initially, LNPs are maintained in early endosomes, which subsequently mature and acidify.<sup>77</sup> The decrease of endosomal pH protonates the ionizable cationic lipids which interact with negatively charged lipids in bilayer, destabilizing the membrane and promoting nucleic acid release into the cytoplasm.<sup>78</sup> This process, known as endosomal escape, must occur before endosomes mature into lysosomes, where both LNPs and their cargo would be degraded. Importantly, the apparent pKa of the LNP is a key physicochemical parameter for this process, with several studies reporting that values between 6.0 and 6.5 are optimal for efficient endosomal escape and enhanced transfection.<sup>79</sup>

After overcoming all barriers to efficient cargo delivery, metabolism and excretion are the last pharmacokinetic processes that influence LNP function. Metabolism plays a fundamental role to guarantee the safe elimination of LNP-based therapies. However, if it occurs too fast, therapeutic efficacy may be compromised.<sup>80</sup> Thus, developing LNPs with an optimal balance between safe metabolism and efficiency is essential. One factor that strongly influences this balance is the presence of PEG-lipids. While non-PEGylated LNPs are rapidly cleared from circulation, PEGylated LNPs exhibit reduced clearance, resulting in longer circulation times, extended half-life and improved chances of reaching target organs or TME.<sup>31,81</sup> It is important to emphasize that most lipids used in clinical formulations are designed to be

biodegradable. For example, the ionizable lipids used in the Pfizer and Moderna vaccines (ALC-0315 and SM-102, respectively) contain ester groups that undergo hydrolysis, allowing efficient elimination.<sup>82</sup>

In conclusion, the efficacy of LNP-based immunotherapies is multifactorial and demands robust basic research. Advances in the understanding of cell biology and the mechanisms of LNP interactions with biological systems are critical for the design of optimized formulations. Moreover, the ability to modulate the biodistribution of LNPs has revolutionized the field of drug delivery and continues to open new opportunities for safer and more effective therapies.

## mRNA vs DNA Delivery

The choice of the genetic cargo to be encapsulated within LNPs should take into account the specific properties and limitations of each type of molecule, depending on the intended therapeutic application.<sup>83</sup> For immunotherapies to be effective, nucleic acids must overcome multiple biological barriers, both extracellular and intracellular.<sup>16</sup> When administered in vivo, nucleic acids (specially mRNA) must be protected from degradation by endonucleases present in physiological fluids.<sup>26</sup> Then, LNPs must reach the target tissue, be internalized by the target cells, and subsequently escape from the endosome.<sup>84</sup> Once in the cytoplasm, mRNA is immediately available for translation, whereas plasmid DNA (pDNA) must first overcome an additional barrier by crossing the nuclear membrane<sup>85</sup> (Figure 1B).

mRNA offers rapid and direct expression kinetics, as translation begins as soon as the molecule is delivered into the cytoplasm, typically peaking between 6 to 24 hours.<sup>86</sup> This expression is short-lived and transient, decreasing progressively as the mRNA is degraded,<sup>87</sup> which is desirable to control the effects of cellular therapies such as CAR-T, and limit systemic effects of cytokines.<sup>70</sup>

pDNA, on the other hand, has slower expression kinetics—it must enter the nucleus to be transcribed into mRNA before translation can occur in the cytoplasm, with peak expression generally occurring 24 to 72 hours after delivery.<sup>86</sup> pDNA enables prolonged antigen expression, making it an excellent option for vaccines, as extended exposure can promote a stronger and more durable immune response.<sup>88</sup>

In terms of stability, pDNA exhibits greater chemical and structural stability compared to mRNA. This allows for longer-term storage at milder temperatures, or even at room temperature.<sup>29,46</sup> mRNA is more susceptible to hydrolysis and requires stricter storage conditions to preserve its integrity, typically at  $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$ .<sup>89</sup> This difference significantly impacts the logistics of distribution, storage, and the overall cost of nucleic acid-based therapies, directly affecting their accessibility and global reach. Insights gained from comparing mRNA and pDNA delivery serve as a foundation for evaluating the broader landscape of gene delivery, particularly the advantages and limitations of LNPs relative to other platforms (Table 1).<sup>90–96</sup>

## LNPs Vs Other Gene Delivery Systems

Viral vectors, electroporation, and LNPs are the most commonly used approaches to gene delivery. Viral vectors, such as lentiviruses and adenoviruses, are widely employed due to their high transduction efficiency and potential for long-term expression.<sup>59</sup> However, viral vectors provide high transduction efficiency and prolonged expression—permanent in the case of lentiviruses due to genomic integration, and as long-lasting episomes with adenoviruses—but carry risks of insertional mutagenesis, increased immunogenicity, limitations on repeat dosing, and high production costs.<sup>97–100</sup> In contrast, LNPs do not pose a risk of genomic integration, have a lower probability of triggering undesired immune responses, offer greater scalability, and involve lower production costs,<sup>26,101</sup> however, LNP-mediated transgene expression is inherently transient—reaching maximal protein output 24 hours post-administration and declining to subtherapeutic levels within one week—thus necessitating repeated dosing or sustained-release formulations to sustain therapeutic efficacy.<sup>87,102,103</sup>

Electroporation is a frequently used method for ex vivo cell engineering, such as the generation of CAR-T cells.<sup>104,105</sup> Although it demonstrates high efficacy, it is often accompanied by significant cytotoxicity and reduced cell viability,<sup>106</sup> which limits its broader clinical application. In comparison, LNPs exhibit very low toxicity, making them suitable for both ex vivo and in vivo strategies.<sup>70,106–108</sup>

Cationic polymers, such as polyethyleneimine (PEI), are also used as non-viral vectors,<sup>108</sup> offering advantages like low production costs and ease of optimization.<sup>109</sup> However, they are often associated with high toxicity and only

**Table I** RNA vs DNA Delivery

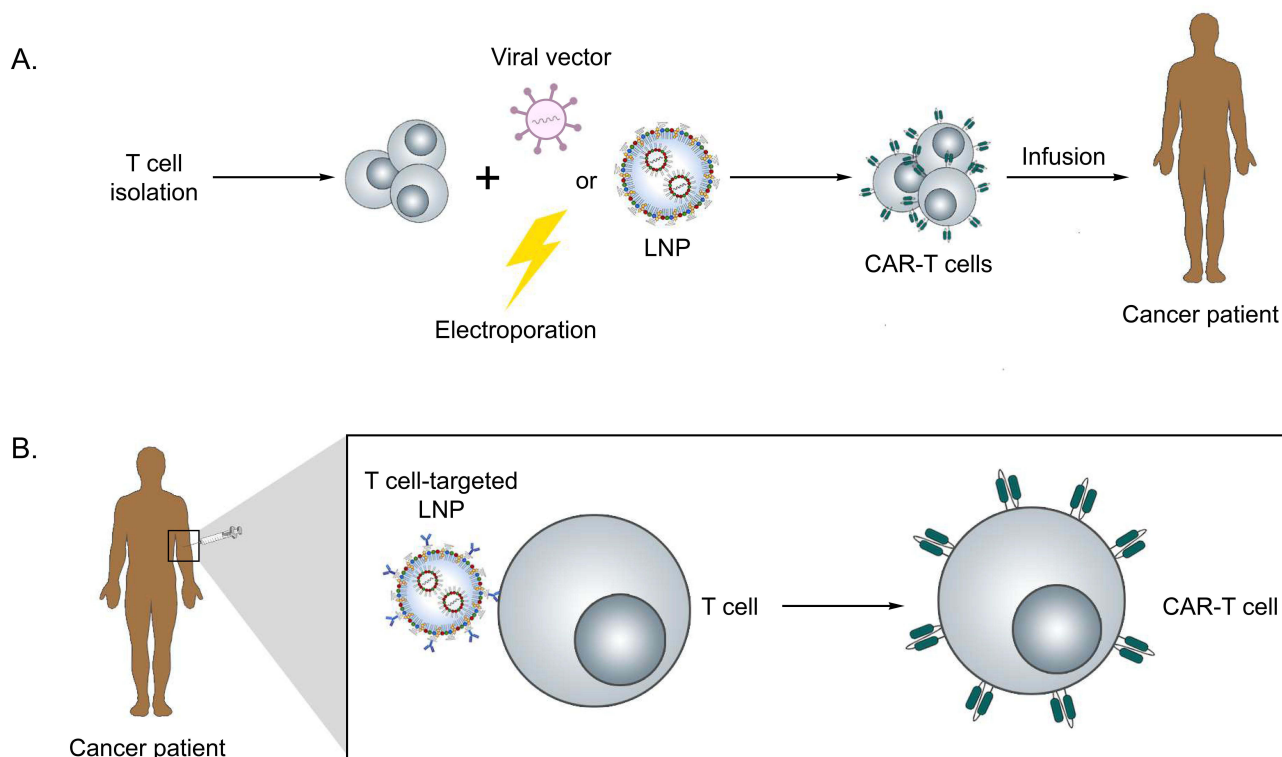
| Features                                | RNA   | DNA   | References                                |
|---|---|---|---|
| <b>Mechanism</b>                        | Direct cytoplasmic translation (mRNA). Also includes RNAi agents (siRNA/miRNA) for oncogene silencing.  | Must enter nucleus → transcription to mRNA → translation to protein.  | [26]                                      |
| <b>Nanoparticle platforms</b>           | LNPs, liposomes, polymeric NPs (PLGA, PEI), hybrid lipid–polymer systems.   | Cationic liposomes, polymeric NPs (PLGA, PEI), hybrid systems; some approaches combine with electroporation.  | [90]                                      |
| <b>Cancer applications</b>              | • mRNA vaccines encoding tumor antigens (neoantigens, WTI, MUC1, NY-ESO-1)  | • DNA vaccines encoding tumor antigens (eg, HPV E6/E7, HER2, PSA)   | [91–93]                                   |
|   | • mRNA encoding cytokines (eg, IL-12, GM-CSF)   | • Plasmid DNA encoding cytokines (eg, IL-12)  | NCT01304524                               |
|   | • siRNA/miRNA for oncogene/pathway silencing (eg, PKN3, RRM2)   | -   |   |
| <b>Representative clinical examples</b> | • Personalized neoantigen vaccine mRNA-4157 (V940) + pembrolizumab in resected melanoma – improved RFS (Phase 2; NCT03897881). Weber et al, 2024.                           | • VGX-3100 (DNA vaccine against HPV16/18 E6/E7) for cervical HSIL/cancer (multiple trials; eg, NCT01304524) often delivered with electroporation; some NP formulations under study. | [94]                                      |
|   | • BioNTech BNT111 (RNA-LPX, FixVac) in melanoma – Lipo-MERIT (Phase I; NCT02410733) and Phase 2 combo with cemiplimab (NCT04526899).  | • Intratumoral IL-12 plasmid (tavokinogene telseplasmid) approaches – melanoma (eg, NCT01502293).   | NCT03739931<br>NCT00689065<br>NCT00938574 |
|   | • Intratumoral cytokine LNP mRNA-2752 (IL-12/IL-23/TSLP) ± durvalumab – solid tumors/lymphoma (Phase I; NCT03739931).   | -   | NCT03739931                               |
|   | • siRNA NPs: CALAA-01 (cyclodextrin NP targeting RRM2; NCT00689065);  | -   | NCT00689065                               |
|   | • Atu027 (liposomal siRNA targeting PKN3; NCT00938574).   | -   | NCT00938574                               |
| <b>Clinical status (high-level)</b>     | Multiple Phase 1–3 programs: mRNA-4157 in Phase 3 for melanoma adjuvant; BNT111 reported positive Phase 2 readout; several siRNA oncology NPs completed early-phase trials. | DNA vaccines show activity in HPV-related disease (VGX-3100 advanced); plasmid cytokines explored mainly in early-phase or with electroporation adjuncts.                           | [95, 96]                                  |
|   |   |   | NCT03897881<br>NCT04526899                |

moderate in vivo performance,<sup>109,110</sup> limitations that are effectively addressed by LNPs. While polymeric nanoparticles offer advantages in formulation stability and customizability, they generally show lower gene delivery efficiency and more variable in vivo outcomes. In terms of immunogenicity, LNPs elicit strong innate immune responses that can be beneficial in vaccine settings, whereas polymeric systems may provoke unpredictable immune activation depending on their charge and composition.<sup>111,112</sup>

In summary, when comparing different platforms for genetic material delivery, LNPs emerge as a highly promising solution, combining safety, adaptability, and efficiency. This positions them as one of the main alternatives to viral vectors, even overcoming other non-viral methods such as electroporation and polymer-based platforms, especially for emerging applications in cancer immunotherapy. The use of nanoparticles has increasingly stood out as an effective and safe approach. Moreover, this methodology offers remarkable flexibility, allowing for application in both in vivo and ex vivo delivery strategies. This enables greater personalization of treatments and broadens the potential uses of the technique.

## Advantages of in vivo Delivery Over ex vivo Manipulation

The use of lipid nanoparticles for in vivo delivery of nucleic acids offers a novel perspective compared to traditional ex vivo cell manipulation, which is used in the production of CAR-T cells.<sup>104,105,107</sup> In this approach, mononuclear cells must be harvested from the peripheral blood of a patient or a donor, followed by T cell isolation, commonly using anti CD3 microbeads, and activation. After that, cells are genetically modified through viral (lentivirus or retrovirus) or non-viral (vectors, such as electroporation or LNPs) systems. The engineered T cells are then expanded in vitro using media supplemented with cytokines such as IL-2, IL-7 and IL-15 to promote clonal proliferation and maturation. Throughout this workflow, rigorous quality-control measures (including flow cytometric assessment of CAR expression, cell viability assays, sterility testing, and functional cytotoxicity assays) are performed to ensure both potency and safety.<sup>107,113</sup>



**Figure 2** CAR-T cell therapy production cycle. **(A)** Ex vivo CAR-T cell engineering: The patient's peripheral blood is collected and lymphocytes are isolated. T cells are genetically modified ex vivo using various methods, such as LNPs, viral vectors, or electroporation. Following genetic modification, CAR-T cells are expanded in vitro, and finally infused intravenously into the patient. **(B)** In vivo CAR-T engineering: LNPs are administered intravenously, enabling the transfection of T cells to induce CAR expression in vivo.

Finally, the modified cells are reinfused into the patient (Figure 2A). Although effective, this process is costly, labor-intensive, time-consuming, and requires sophisticated infrastructure and strict closed-system protocols under Good Manufacturing Practices (GMP) conditions.<sup>114</sup>

In contrast, *in vivo* delivery bypasses the need for cell isolation and manipulation (Figure 2B). Lipid nanoparticles carrying mRNA or pDNA can be directly injected into the patient, overcoming biological barriers and allowing the genetic engineering process to occur within the body.<sup>70,115</sup> These LNPs can be surface-functionalized with anti-CD3 antibodies or T cell-specific ligands, thereby enabling preferential targeting of circulating T lymphocytes following systemic administration.<sup>116</sup> Upon receptor-mediated endocytosis, the particles deliver mRNA into the cytosol, leading to CAR expression directly in endogenous T cells.<sup>117,118</sup> Current efforts are increasingly directed toward refining targeting ligands, optimizing lipid composition, and incorporating controlled mRNA release systems, all of which represent active avenues of investigation aimed at enhancing therapeutic efficacy and prolonging the durability of responses.<sup>119–121</sup> This approach drastically reduces processing time, overall cost, and the need for large-scale infrastructure, enabling more affordable and scalable therapies and expanding access across different socioeconomic groups. Finally, it is important to emphasize that *ex vivo* culture conditions differ significantly from the homeostatic *in vivo* environment. Therefore, it is crucial to investigate how each step of the manufacturing process impacts T cell quality, including potential induction of cellular exhaustion.<sup>122,123</sup>

As previously mentioned, the use of lipid nanoparticles has profoundly transformed the field of molecular delivery, providing innovative solutions across a wide range of therapeutic applications. LNPs constitute a highly versatile and clinically validated platform capable of efficiently encapsulating and delivering diverse nucleic acids, with substantial translational potential. Beyond serving as general delivery vehicles, they act as key enablers of next-generation therapies by directly addressing major challenges in immune modulation, targeted gene transfer, and *in vivo* cell engineering. Collectively, these properties position LNPs as a pivotal link between nucleic acid delivery technologies and the rapidly evolving field of immunotherapy, where their ability to reprogram immune responses can be harnessed to advance cancer treatment and expand therapeutic accessibility. In the following sections, we will discuss recent advances in immunotherapies and highlight the applications of LNPs in these strategies.

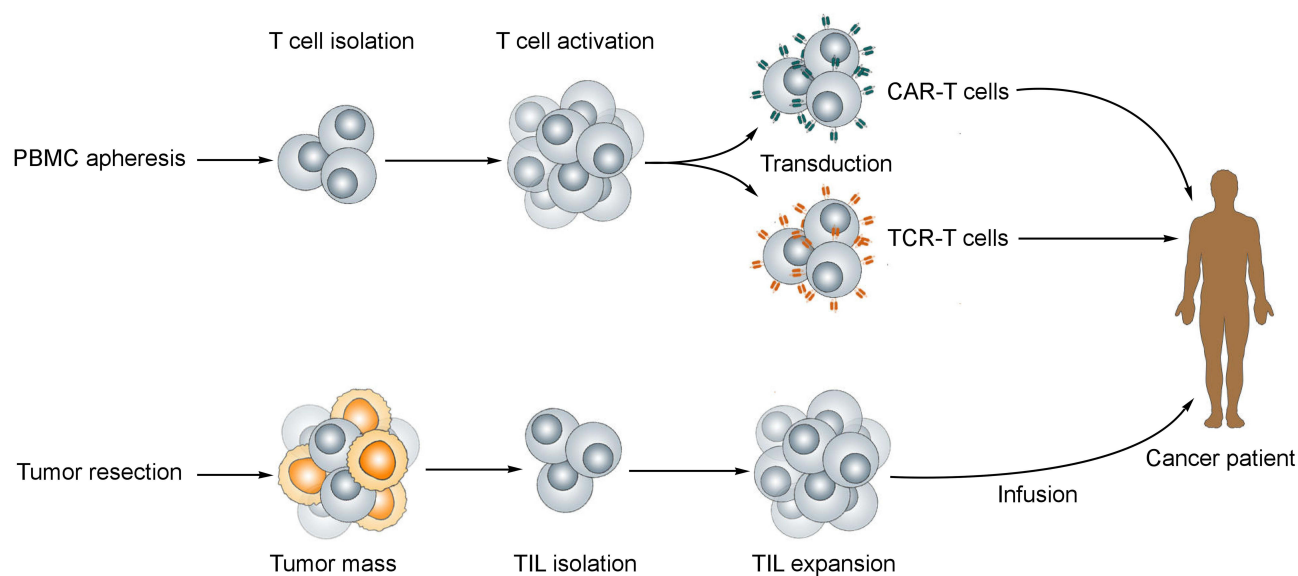
## Cancer Immunotherapies Overview

Immunotherapies against cancer have become a hallmark in the treatment of solid and hematological tumors. These therapies aim at inducing or improving immune cell responses against tumor cells. The first line of immunotherapy was based on cytokines, such as interferon alpha (IFN- $\alpha$ ) and interleukin 2 (IL-2), which resulted in severe adverse effects, despite their success against leukemia and melanoma.<sup>124–126</sup> In the late 1990s, monoclonal antibodies emerged as a new line of treatment, promising a more targeted approach against lymphoma (Rituximab) and lymphocytic leukemia (Ibrutinib).<sup>35,127</sup> These advances led to the development of immune checkpoint blockers (ICBs), which revolutionized the treatment of metastatic melanoma and other solid tumors by targeting CTLA-4 (Ipilimumab) and PD-1 (Pembrolizumab).<sup>128–130</sup> The use of ICBs facilitates the effective recognition and elimination of tumors, and promotes the activation of immune cells, especially T cells.<sup>129,130</sup>

Over time, targeted approaches have been developed to render immunotherapies more effective. In this context, an “acquired immunity” approach known as adoptive cell therapy (ACT) was developed.<sup>131</sup> ACT comprises three forms of adoptive transfer of T cells, which include tumor infiltrating lymphocytes (TILs), T cells engineered to express a T cell receptor (TCR) or a CAR<sup>6</sup> (Figure 3). These different types of cellular immunotherapy have shown significant results in the clinic against leukemias and other neoplasms, and their applications keep increasing with new technologies.<sup>132,133</sup>

Another approach to activate the immune cells against specific tumor targets is the use of therapeutic vaccines. This category of immunotherapy overcomes the necessity of *ex vivo* manipulation of T cells, as well as their transplantation,<sup>134</sup> which opens the way for faster and more personalized treatments. These vaccines rely on the presentation of tumor antigens to T cells by APCs, and may be one of the most personalized forms of immunotherapy when allied with targeted delivery by LNPs.

In summary, the field of immunotherapy has thrived in recent years. However, hurdles in the production process, biodistribution and adverse effects that arise due to autoimmunity still prevent a broader use of these therapies. In this



**Figure 3** Adoptive T cell therapy. For the production of CAR-T and TCR-T cells (top), T lymphocytes are collected from the patient's peripheral blood and transduced to express the desired receptor. TILs (bottom) are isolated from a resected tumor mass, and infused back to the patients following ex vivo activation and expansion.

context, LNPs provide a means of facilitating the production process of engineered T cells, and rapid application of gene delivery technologies that would favor therapeutic vaccines and other necessities for targeted delivery of genetic cargo.<sup>10</sup> CAR-T cell therapy is a significant methodology that has been applied in immunotherapy and it means a strong candidate to revolutionize immunotherapy, and even autoimmune diseases.

## CAR-T Cell Therapy

The concept of chimeric receptors was elucidated in the late 1980s as a tool to induce the recognition of peptides independently of the interaction of T-cell receptor (TCR) and major histocompatibility complex (MHC).<sup>135</sup> This marked a significant improvement to TILs or TCR T cells, as tumor cells commonly employ MHC masking as an immune evasion mechanism.<sup>136</sup> The CAR is a membrane-bound protein composed by extracellular, transmembrane and intracellular domains.<sup>137</sup> These domains have been optimized for clinical use against different tumors, and, in summary, lead to the activation of the engineered T cell upon antigen engagement.<sup>138</sup> The development of new generations of CARs led to better survival and function of the T cells, in addition to a reduction in exhaustion and improved tumor infiltration, which are important features in the context of solid tumors.<sup>139</sup> Approved products against hematological tumors commonly target CD19 and B cell maturation antigen (BCMA), although clinical studies have investigated alternative tumor associated markers for hematological tumors and for solid tumors.<sup>140,141</sup> Novel CAR constructs have been developed over the years, with additional features that allow the control of antigen engagement as well as the control of cytokine release.<sup>142,143</sup> However, T cells engineered with new designs as well as clinically approved ones rely on ex vivo manipulation, and the introduction of exogenous genetic material in these cells.<sup>7</sup> Alternatively, non-viral vectors have been applied in the manufacture of CAR-T cells. Some investigations have explored electroporation using transposon-based systems, which require less time for ex vivo manipulation than viral transduction.<sup>144,145</sup>

Integrative viral vectors increase both the price and manufacture time of CAR-T cells products. Combined, these factors become impeditive to a broader application of this therapy, while also affecting the function of the final product.<sup>146</sup> A common issue of using integrative approaches, either viral or non-viral, is the constitutive expression of the receptor in the engineered cell. This has been associated with the immunogenicity provoked by the therapy.<sup>147</sup> These long-term adverse effects, such as B cell aplasia and immune effector cell-associated neurotoxicity syndrome (ICANS) arise from CAR-T cell antigen engagement on healthy cells.<sup>148</sup> Potentially, these adverse effects could be avoided by applying technologies that employ a transient expression of CAR on T cells in the manufacturing process.<sup>107</sup>

Alternative production methods that allow such transient expression could be applied in the production of CAR-T cells. This would help mitigate the emergence of adverse effects, and significantly reduce the cost and manufacture time, thus broadening the accessibility of CAR-T cell therapy.<sup>7</sup> LNPs can be used as tools for RNA-based T cell engineering and, by screening novel ionizable lipids, their transfection can be enhanced as described by Billingsley et al.<sup>149,150</sup> Interestingly, by varying the molecular ratios of the components of LNPs, our group has shown improved transfection of T cells using pDNA. Another strategy, which in addition to varying lipids, is the incorporation of DNA-condensing agents, such as the P3000 reagent, during LNP synthesis has also been investigated as a strategy to enhance nucleic acid delivery for CAR-T therapy. This approach improves the efficiency of LNPs following multiple administrations in Jurkat cells, enabling sustained transient expression while preserving cell viability, even under repeated pDNA delivery at 48 h intervals.<sup>151</sup> This study provided valuable insights into a non-integrative method of cell engineering, while maintaining cell viability and improved CAR expression when compared to conventional methods such as electroporation and commercial transfection reagents.<sup>107</sup>

New possibilities of T cell engineering also arise from LNPs. Some platforms have been optimized for increased delivery *ex vivo*, while concomitantly being used to activate transformed cells. This may lead to significant reduction in the manufacture time, since cell expansion and activation are major bottlenecks of this process.<sup>150</sup> An innovative application of LNPs in CAR-T cell engineering is the *in vivo* delivery of mRNA, which overcomes the need of isolating and manipulating the cells *ex vivo*.<sup>70</sup> Recently, it was demonstrated that LNPs encapsulating CAR mRNA, targeted to the spleen and functionalized with anti-CD3 antibodies, enable the *in situ* engineering of T cells to express both CAR and IL-7.<sup>121</sup> This strategy generated CAR-T cells that effectively infiltrated and eradicated melanoma *in vivo* with high efficacy and safety, without inducing cytokine release syndrome.<sup>121</sup> This technology could improve CAR-T cell function by co-delivering mRNA for CAR expression and siRNA to downregulate exhaustion molecules such as PD-1.<sup>152</sup> Interestingly, recent advances on co-delivery have successfully generated CAR-T cells using a transposon-transposase system, which consolidates the applications of LNP platforms in ACT.<sup>116</sup>

*In vivo* co-delivery of minicircle DNA (mcDNA) encoding CAR together with transposase mRNA within functionalized nanoparticles bearing anti-CD3 and anti-CD7 demonstrated significant efficacy in leukemia models following a single administration.<sup>153</sup> These findings highlight the versatility of LNPs in enabling the efficient co-delivery of multiple genetic cargos while supporting transposase-mediated genomic integration.<sup>153</sup> Collectively, this approach offers a promising platform to democratize CAR-T therapies by reducing manufacturing costs, simplifying logistics, and expanding large-scale accessibility.

CAR-T cell therapies represent a highly personalized and potent strategy by reprogramming T cells to recognize and eliminate tumor cells, supported by significant advances in vector design, gene editing technologies, and manufacturing platforms that have expanded their clinical applicability and safety profile. Similarly, cancer vaccines aim to selectively activate these same T cells *in vivo*, leveraging tumor-associated antigens to elicit a robust and targeted antitumor immune response.

## Cancer Vaccines

Another way of leveraging the immune system to target tumor cells is to present peptides processed by APCs and activate antigen-specific cytotoxic T cells.<sup>10</sup> This form of immunotherapy, namely cancer vaccines, have been used in the form of cell-based platforms, such as Sipuleucel-T (a dendritic cell vaccine) with significant results against castration-resistant prostate cancer.<sup>154</sup> However, other cell-based candidates have shown limited efficacy in clinical trials.<sup>154</sup> As a vaccine platform, cancer vaccines could greatly benefit from novel delivery methods, such as LNPs, which emerge as alternatives to traditional delivery methods, and do not rely on cell or virus-based treatments.<sup>155,156</sup> With these platforms, nucleic acid delivery can be targeted towards APCs, triggering a more effective activation of antigen-specific cytotoxic cells.<sup>155–157</sup>

Furthermore, these LNPs can be chemically optimized or functionalized to improve their uptake by APCs. This has been investigated by BioNTech in a Phase I/II clinical trial.<sup>158</sup> In this approach, a lipoplex encapsulating an RNA for Claudin 6, used as tumor associated antigen, is given in combination with CAR-T cells targeting the same antigen.<sup>159</sup> Preclinical data suggested that this drives the expansion of CAR-T cells and opens an alternative to the treatment of

relapsed solid tumors.<sup>158</sup> The use of LNPs to enhance cancer treatment have been widely used as described in works using the HPV model of cancer, including the oropharyngeal form. In this way, it was described the success of delivering RNA and saRNA resulting in the decreasing of tumor volume.<sup>160,161</sup> Also was possible to observe the reduction in tumor volume of B16F10-OVA mice model when vaccinating the animals with OVA even in mRNA or with the protein associated to the LNP.<sup>60,162</sup> In summary, vaccines have been shown to be versatile tools when efficient antigen presentation is necessary. The delivery of nucleic acids (both DNA and RNA) by LNPs is also a proven tool for the fast development of vaccines, as evidenced by the strategies employed in the COVID-19 vaccines.<sup>13,17,163</sup> Therefore, LNP platforms may also be a practical way to tailor personalized cancer vaccines, which can be designed using sequencing data from the patient's own biopsies and trigger efficient T cell responses against antigens that would otherwise be difficult to target.

Furthermore, cytokines are described as key molecules that orchestrate the entire immune system and are intrinsically linked to the body's response to cancer. As such, they play a fundamental role in cancer treatment and can be used to enhance the immune response against cancer cells. In this context, the use of cytokines encapsulated in LNPs has emerged as a promising and widely explored strategy in cancer therapy, as we will discuss next.

## Cytokine-Based Immunotherapy

Cytokines have been fundamental in cancer immunotherapy due to their potent immunomodulatory effects. The first cytokine to be approved by the FDA for cancer treatment was IFN- $\alpha$  in 1986, for hairy cell leukemia.<sup>164</sup> This milestone was followed by the approval of IL-2 for metastatic renal cell carcinoma and advanced melanoma in the early 1990s.<sup>165</sup> Despite these achievements, the clinical application of cytokines has been hindered by their short half-life and systemic toxicity.<sup>166</sup> The necessity for high doses to achieve therapeutic concentrations within the TME has limited their efficacy and highlighted the need for innovative delivery systems that can localize cytokine activity to tumors while minimizing systemic exposure.<sup>167,168</sup>

The challenges associated with cytokine therapy stem from their mode of action and pharmacokinetics. Cytokines act in a paracrine or autocrine manner and have a relatively short half-life, necessitating large quantities to be systemically administered to achieve a sufficient concentration within the TME.<sup>169</sup> This often results in severe toxicities, limiting the dose that can be safely administered. For instance, IL-2 therapy is associated with capillary leak syndrome, a potentially life-threatening condition characterized by hypotension, edema, and organ dysfunction.<sup>170</sup> Similarly, IFN- $\alpha$  can cause flu-like symptoms, fatigue, and depression. The use of cytokines in cancer therapy has also been limited by their lack of specificity, leading to off-target effects.<sup>171</sup> Consequently, developing localized therapy that directly delivers immune-stimulatory cytokines to tumors is a promising strategy to overcome this dilemma.<sup>168</sup> Localized delivery can enhance the therapeutic index by maximizing the concentration of cytokines within the tumor while minimizing systemic exposure.<sup>172</sup> The administration of IL-21 and IL-7 mRNA in LNP was described as increasing the CD8<sup>+</sup> infiltrated in mouse model of colorectal cancer and breast cancer.<sup>74</sup> Using the same mouse model of colorectal cancer, when treating the animals with IL-2 mRNA in different LNPs, a reduction was observed in the size of tumor mass.<sup>173</sup> In addition, a recent study described the treatment of Balb/c animals injected with 4T1 cells and treated with IFN $\alpha$ 2 mRNA LNPs. They observed as a result, an increase of CXCL9 and CXCL10 chemokines, besides reduction on primary tumor.<sup>174</sup> Also, there is described an increase in CD8<sup>+</sup> T cells response after injection of IL-12 mRNA in animal model of melanoma.

Furthermore, the TME is a complex and immunosuppressive environment that can limit the efficacy of cytokine therapy.<sup>175</sup> Tumors often develop mechanisms to evade immune surveillance, including the production of immunosuppressive cytokines, such as transforming Growth Factor  $\beta$  (TGF- $\beta$ ), and the recruitment of immune suppressive cells, such as regulatory T cells.<sup>176</sup> Therefore, effective cytokine therapy must not only deliver immune-stimulatory cytokines to the tumor but also overcome the immunosuppressive TME.<sup>177</sup> Recent advances in our understanding of the TME have led to the development of novel strategies to modulate the immune response and enhance the efficacy of cytokine therapy.<sup>178</sup>

Genetic engineering and gene editing have enabled the development of novel cytokine-based therapies with improved specificity and reduced toxicity. For example, the use of CAR-T cells, which are engineered to also express specific cytokine receptors, have shown promise in enhancing the efficacy and specificity of cytokine therapy.<sup>179</sup> In addition, the

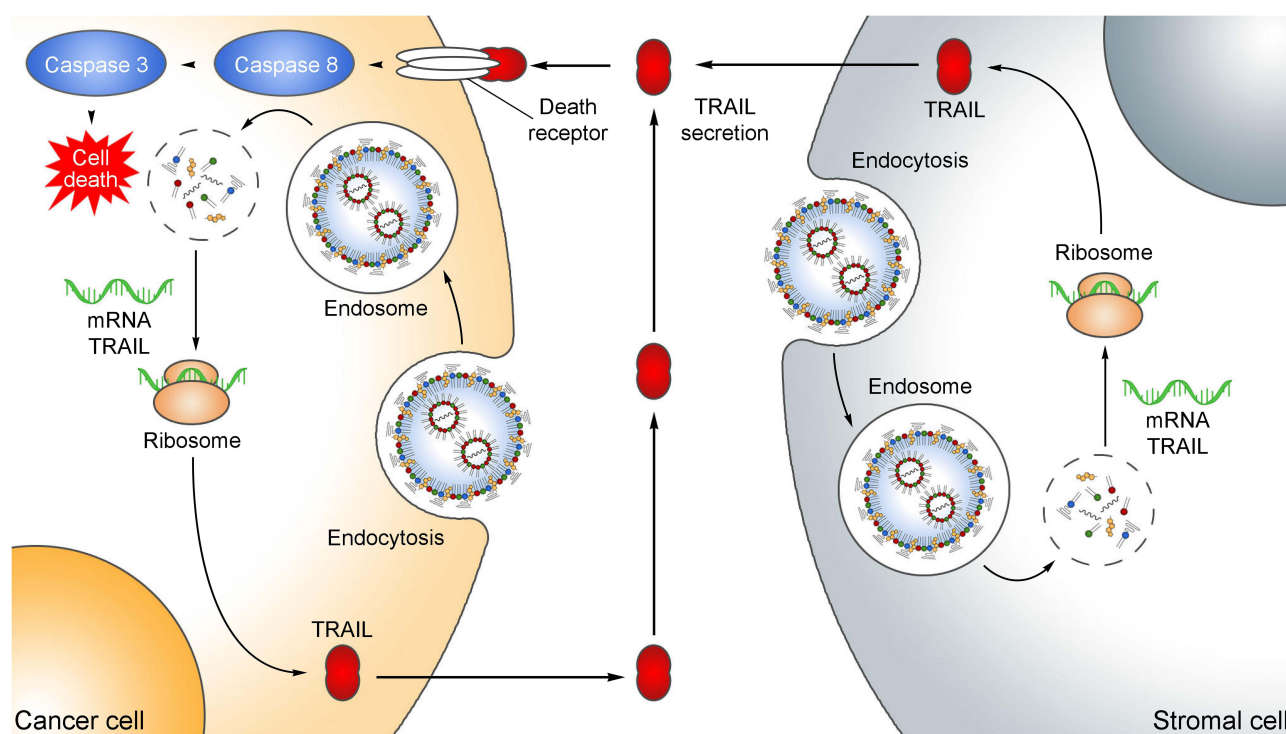
combination of cytokine therapy with other immunotherapies, such as checkpoint inhibitors, has shown potential in enhancing anti-tumor immune responses.<sup>168</sup> The use of cytokines to modulate the TME and enhance the efficacy of checkpoint inhibitors is an area of active research. Overall, the development of novel cytokine-based therapies with improved specificity and reduced toxicity is crucial for realizing the full potential of cytokine therapy in cancer treatment.<sup>180</sup>

Therefore, mRNA-based approaches enable the in-situ production of therapeutic proteins, including cytokines, directly within target tissues.<sup>181</sup> In line with this, LNPs have been shown to effectively deliver mRNA to a range of tissues, including the liver, spleen, and tumors.<sup>182</sup> The use of LNPs to deliver mRNA-encoded cytokines, such as IL-12, IL-15 and IL-10, has shown potential in enhancing anti-tumor immune responses and promoting tumor regression.<sup>73,183</sup>

Moreover, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a cytokine that has received significant interest in oncology due to its remarkable ability to selectively induce apoptosis in cancer cells while sparing healthy tissues.<sup>184</sup> This selective cytotoxicity is primarily mediated through TRAIL's binding to death receptors, particularly DR4 and DR5, which are overexpressed on the surface of many malignant cells.<sup>185</sup> Upon receptor engagement, TRAIL initiates a well-characterized intracellular signaling cascade that culminates in the activation of caspases and the execution of programmed cell death<sup>184,185</sup> (Figure 4).

Several investigations have demonstrated that mRNA-encoded TRAIL, when delivered using LNP platforms, retains its tumoricidal activity and promotes meaningful tumor regression.<sup>186–188</sup> Notably, a study employing LNP-mediated delivery of TRAIL mRNA in a murine model of colon cancer reported significant tumor reduction, confirming both the functionality of the expressed protein and the effectiveness of the delivery system.<sup>189</sup> These findings underscore the therapeutic promise of this approach and highlight its potential to revitalize interest in TRAIL-based therapies. Ongoing research aimed at optimizing LNP design, improving tumor-targeting efficiency, and enhancing the stability and safety of mRNA formulations will be crucial for the successful clinical translation of this promising therapeutic platform.

Although cytokines hold great promise as anticancer agents, their clinical application as recombinant proteins has been hampered by poor pharmacokinetics, including rapid clearance, short half-life, and limited stability, necessitating



**Figure 4** mRNA-TRAIL delivery mediated by LNPs. After endocytosis, the mRNA is released into the cytoplasm and translated into TRAIL protein, which is secreted and can act in both autocrine and paracrine manners by binding to death receptors on cancer cells, triggering apoptosis through caspase-8 and caspase-3 activation.

frequent high-dose administrations that increase the risk of systemic toxicity.<sup>182,190</sup> To overcome these limitations, mRNA–LNP platforms have emerged as a powerful alternative, enabling endogenous production of cytokines directly within target cells and ensuring sustained, localized expression in the TME.<sup>186</sup> This strategy bypasses the intrinsic instability of recombinant proteins while allowing more precise control of dosage and expression kinetics. Preclinical studies have demonstrated that LNP-mediated cytokine delivery can preserve biological activity and promote meaningful antitumor responses, underscoring the therapeutic potential of this approach.<sup>73,191</sup>

Clinical trials are exploring the use of mRNA-LNPs encoding cytokines for cancer treatment. For example, mRNA-2752, an LNP formulation encoding OX40L, IL-23, and IL-36 $\gamma$ , is being evaluated for intratumoral injection in patients with advanced malignancies (NCT03739931). Preliminary results indicate that such localized delivery can stimulate robust anti-tumor immune responses with manageable safety profiles.<sup>189</sup> These studies underscore the potential of mRNA-LNP platforms to deliver potent immunostimulatory agents directly to the TME, offering a promising approach for cancer therapy.<sup>189</sup>

The clinical development of mRNA-LNPs encoding cytokines is an active area of research, with multiple ongoing trials evaluating their safety and efficacy in various cancer types.<sup>192</sup> These trials are designed to assess the therapeutic potential of mRNA-LNPs in different cancer settings, including as monotherapy or in combination with other therapies, such as checkpoint inhibitors or chemotherapy.<sup>193</sup> The results of these trials will be critical in determining the future of mRNA-LNP therapeutics in cancer therapy. As our understanding of the immune system continues to evolve, it is likely that cytokine therapy will remain a vital component of cancer treatment, offering new hope for patients with advanced malignancies.<sup>194</sup>

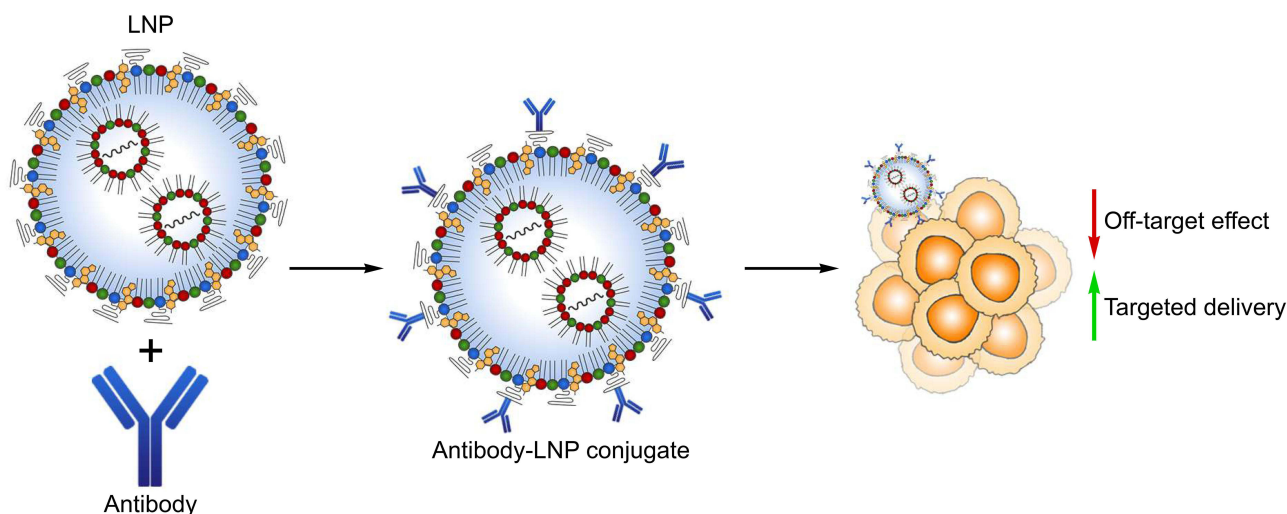
In summary, the importance of cytokines in orchestrating immune responses is well established, and this remains true in the context of cancer. The administration of these molecules via LNPs has shown promise in enhancing their immunomodulatory effects, contributing meaningfully to cancer therapy. Given the central role of the patient's immune system in therapeutic success, other approaches have also aimed to amplify this response, making treatment more multifactorial. Bispecific antibodies align with this strategy by promoting precise targeting of immune cells toward tumor cells. This technology is highly promising, and in the next section, we will explore how LNPs and bispecific antibodies can be combined to deliver targeted and innovative therapeutics.

## Bispecific Antibodies and LNP Functionalization

BsAbs represent a groundbreaking advancement in therapeutic antibody engineering, offering unique capabilities in targeting and treating a variety of diseases. BsAbs are a promising drug immunotherapy with significant potential to address a wide range of diseases, such as cancer and autoimmune disorders.<sup>195,196</sup> Unlike traditional monoclonal antibodies that bind to a single antigen, BsAbs are designed to simultaneously bind to two different antigens or epitopes. This dual binding capability enables them to bridge interactions between cells, providing enhanced therapeutic precision and efficacy.<sup>197</sup>

One of the primary applications of bispecific antibodies is in cancer treatment. BsAbs bind to tumor-associated antigens and CD3 subunits on T cells simultaneously. This binding recruits T cells to the tumor and T cells become activated and release cytolytic proteins that destroy the tumor cells.<sup>198,199</sup> BsAbs can enhance tumor specificity and immune system involvement, minimize collateral damage to healthy tissues and when combined with other therapies, such as chemotherapy, can promote anti-cancer activity.<sup>200,201</sup> BsAbs have been used to treat hematological malignancies and select solid cancers.<sup>197</sup> The clinical success of therapies like Blinatumomab, a BsAb approved for treating acute lymphoblastic leukemia, highlights the potential of this approach. Beyond oncology, bispecific antibodies are being explored for use in treating autoimmune diseases, infectious diseases, and even neurological disorders.<sup>202–204</sup> Their adaptability and modularity make them highly versatile tools in addressing complex disease mechanisms. Successfully developing BsAbs requires addressing the challenges of managing toxicities while understanding their binding kinetics, stability, and pharmacokinetics.<sup>197</sup>

Functionalization plays a crucial role in the design and optimization of BsAbs, enabling precise modulation of their biological activity and therapeutic performance by introducing chemical or molecular modifications. This can involve techniques like covalent or non-covalent attachment of dyes, nanoparticles, or other molecules to the antibody's



**Figure 5** Antibody-functionalized LNPs improve targeted delivery. Conjugation of antibodies to the LNP surface enhances delivery to specific cells (eg, tumor cells) while minimizing nonspecific uptake by non-target organs such as the liver, reducing off-target accumulation.

surface.<sup>205</sup> Advances in antibody engineering have made it possible to tailor BsAbs with improved stability, reduced immunogenicity, and enhanced pharmacokinetics. Functionalization techniques ensure that the dual-binding properties of these antibodies are precisely tuned for specific therapeutic goals.<sup>206</sup>

One approach in which BsAbs can improve LNP delivery is the functionalization of nanoparticles. This process aims to tailor LNPs for specific therapeutic applications, such as targeted drug delivery to tumors or enhancing mRNA delivery to the brain<sup>71,207</sup> (Figure 5). Molecules such as antibodies and peptides can be attached to the LNP surface in order to enhance targeted delivery to diseased cells, improve efficacy, reduce off-target effects, and increase payload delivery. To address issues with circulation time and nonspecific uptake, BsAbs have been used as bridges to target cell surface proteins like PD-L1, CD4, and GRP78. Embedding hemagglutinin antigen (HA) epitopes in LNPs enables BsAbs to enhance transfection efficiency, with notable *in vivo* and *ex vivo* improvements in cancer cells and human T cells.<sup>208–210</sup> This approach also demonstrates tumor-specific drug delivery with robust binding and therapeutic expression. BsAbs further facilitate targeted mRNA delivery to specific proteins like EGFR and PSMA, showcasing an adaptable, scalable strategy for advancing precision medicine and mRNA therapeutics.<sup>210</sup>

In summary, bispecific antibodies are a transformative class of immunotherapeutics, combining precision and versatility. When combined with innovative functionalization strategies using LNPs, their potential to reshape the treatment landscape across diverse diseases is vast, making them a centerpiece of modern biomedical research and clinical development.

## Outlook and Challenges

LNPs have established themselves as a highly promising platform for nucleic acid delivery, opening new horizons for vaccine development, cell therapies, and immunotherapy. Despite recent successes, as evidenced by mRNA-based COVID-19 vaccines, several challenges still limit their full potential. One major obstacle is the difficulty in targeting the LNPs to specific cell types and tissues, to reduce off-target effects and liver accumulation. In terms of manufacturing, although LNPs offer a more cost-effective alternative to viral vectors, issues related to reproducibility persist.

In immunotherapy, LNPs offer new strategies for *in vivo* engineering of immune cells, cancer vaccination, cytokine delivery, and the development of T cell engagers and bispecific antibodies. The future of LNP technology is moving toward the development of more biodegradable and less immunogenic lipids, the incorporation of targeting ligands for greater specificity, the integration of machine learning and artificial intelligence tools to optimize formulations. Advances

in these areas will be crucial to expanding the application of LNPs not only in cancer treatment but also in broader immunotherapeutic approaches, enabling more personalized, potent, and safer therapies.

Future research must focus on optimizing the design of these LNPs, refining their interaction with immune cells, and targeting the immunosuppressive TME to enable more effective therapies. By addressing these issues, the integration of LNP-based platforms into cancer immunotherapy holds the promise of creating more accessible, efficient, and personalized treatments for a wide range of cancers. Ultimately, continued innovations in both the design of delivery technologies and the fundamental understanding of immune system dynamics will pave the way for the next generation of cancer immunotherapies, offering new hope for patients worldwide.

## Conclusion

LNPs platforms have emerged as a transformative tool for enhancing the delivery of nucleic acids in cancer immunotherapy. Their ability to deliver mRNA and DNA safely and efficiently enables new possibilities for improving the precision, scalability, and accessibility of immunotherapeutic strategies. By supporting the development of engineered CAR-T cells, cancer vaccines, cytokine therapies, and bsAbs, LNPs offer a versatile and non-viral alternative capable of addressing key challenges such as systemic toxicity, poor biodistribution, and manufacturing complexity. Continued innovation in LNP design and functionalization will be critical to advancing both off-the-shelf and personalized immunotherapies, paving the way for more effective treatments across a wider range of cancers.

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## Disclosure

The authors declare no potential conflicts of interest in this work.

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