Nucleic Acid Drugs Delivery Carriers

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Nucleic acid drugs are not readily permeable through cell membranes and often exhibit poor blood serum stability, rapid renal clearance and poor endosomal escape/cytoplasmic escape. Therefore, they are commonly used in combination with drug delivery system (DDS) carriers. The drug carrier plays an important role in the process of drug delivery.

nucleic acid drugs drug delivery system conjugate antibody

1. Introduction

For many years, the development of therapeutic drugs for cancer has been dominated by low molecular-weight chemical compounds. In this area cases exist in which drug discovery has been difficult, even when promising target molecules have been identified [1][2][3]. In recent years, however, nucleic acid drugs, such as antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs), have attracted attention as a new modality for cancer treatment [4][5][6]. These drugs can directly target genes and are potentially applicable to all types of diseases. Improvements in the technology used for artificial nucleic acid development have led to the successive approval of nucleic acid drugs for intractable and hereditary diseases; these drugs have been recognized worldwide for their therapeutic efficacy [4]. Nucleic acid drugs for cancer treatment are being actively developed, with many now at the clinical stage [7]. Thus, in the near future, it is expected that these drugs will contribute to the improvement of therapeutic outcomes as major cancer therapeutics.

Nucleic acid drugs are not readily permeable through cell membranes and often exhibit poor blood serum stability, rapid renal clearance and poor endosomal escape/cytoplasmic escape. Therefore, they are commonly used in combination with drug delivery system (DDS) carriers [8][9] (**Figure 1**). Initially, topically administered products for injection directly into the affected area were approved; however, subcutaneous and intravenous products are now being approved. ONPATTRO® (patisiran), a siRNA drug with a liposomal formulation, was approved in 2018, exactly 20 years after the discovery of RNA [10]. Because nucleic acid drugs can be chemically synthesized like small molecule drugs, ligand-conjugated oligonucleotides have also attracted attention in recent years. Given the success of ligand-conjugated nucleic acids, an *N*-acetylgalactosamine (GalNAc)-conjugated siRNA drug (GIVLAAR®, givosiran) has been developed by Alnylam (Cambridge, MA, USA), a leading company in the development of siRNA drugs [11]. This drug comprises tri-antennary GalNAc, a ligand of the asialoglycoprotein receptor that is highly expressed specifically in hepatic parenchymal cells, combined with siRNA; it can be transferred to hepatic parenchymal cells with high efficiency via subcutaneous administration and acts on a target gene [11][12][13]. Over the past years, development of siRNA drugs for cancer treatment have been conducted. To date, some of them, such as Atu027 [14][15][16] and siG12D-LODER [17][18][19], have passed or are currently in Phase II trials and they are expected to be eventually commercialized.

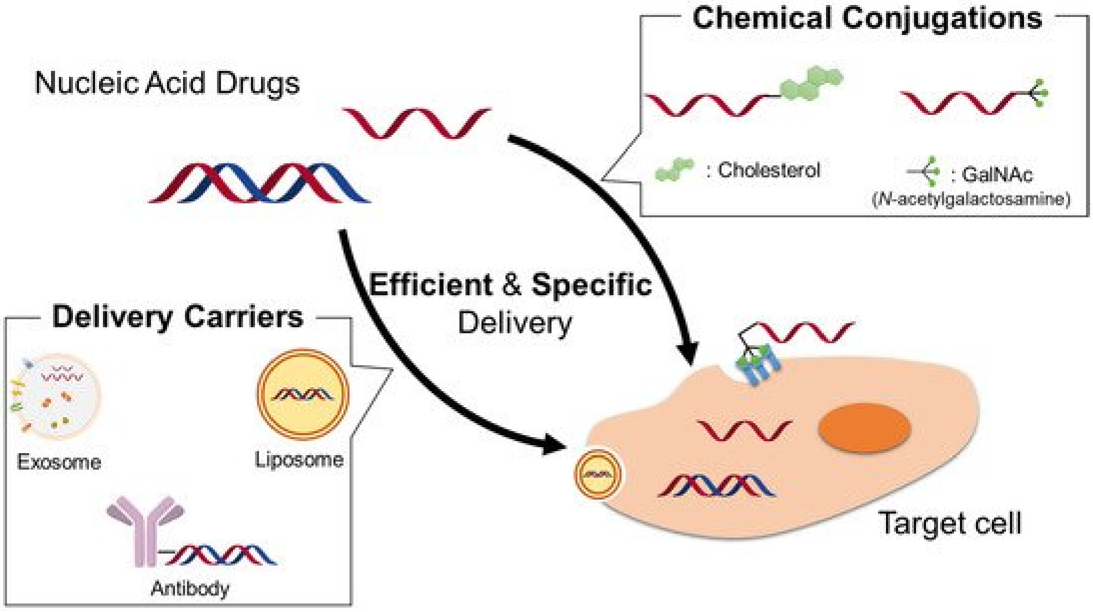

Figure 1. Schematic illustrations of delivery carriers and chemical conjugation strategies for nucleic acid drugs. Here shows typical delivery strategies: cholesterol conjugations [20][21][22], GalNAc conjugations [11][12][13][23][24][25][26], exosome [27][28][29][30][31][32][33][34][35][36], liposome [14][15][16][22][37][38][39][40][41][42], antibody [27][43][44][45][46][47][48].

2. Nonviral Drug Delivery Systems for Nucleic Acid Drugs

The delivery of nucleic acid drugs can be divided into two main strategies: viral and nonviral delivery. Viral vectors are exceptionally efficacious in delivering genetic material to cells because millions of years of evolution have shaped and optimized them for this purpose. Recently, owing to developments in vector design and safety, viral gene therapy strategies have progressed toward clinical use against many genetic disorders. However, depending on the type of vector, viruses will always retain some of their inherent weaknesses, which can include potential immunogenicity, tumorigenicity, limited cargo-carrying capacity, and complex production. Importantly, viral vectors are not universally applicable to all nucleic acid-based molecules; for example, they are not compatible with the delivery of short synthetic oligonucleotides.

It is difficult for nucleic acids and their analogs to permeate cell membranes due to their negative-charged nature. Therefore, various positively charged molecules have been used as intracellular delivery carriers of therapeutic nucleic acids. Protamines are arginine-rich polycationic nuclear proteins that replace histones late in the haploid phase of spermatogenesis; they allow for denser packaging of DNA in the spermatozoon than would be possible with histones. This property has enabled protamines to be used as carriers for therapeutic oligonucleotides. Junguhans et al. were the first to demonstrate the cellular uptake of phosphodiester anti-c-*myc* antisense oligonucleotides into human promonocytic leukemia cells using protamines and to report their antisense effects [49].

Antisense oligonucleotide/protamine complexes have also been used successfully to inhibit human immunodeficiency virus 1 (HIV-1) gene expression [50].

Lipid nanoparticle (LNP) systems are currently the leading nonviral delivery systems for realizing the clinical potential of genetic drugs. Cullis et al. were the first to demonstrate the utility of LNPs based on ethanol injection to encapsulate antisense oligonucleotides [37]. In recent years, the world's first siRNA drug and first nucleic acid drug with liposome implementation, namely patisiran, has been approved by the Food and Drug Administration (FDA) [10]. Patisiran consists of siRNA encapsulated in a LNP carrier (it was formerly known as a SNALP or "stable nucleic acid lipid particle") [38][39]. The accumulation of SNALP within tissues of clinical interest takes advantage of passive disease-site targeting.

3. Conjugation of Functional Molecules to Therapeutic Oligonucleotides

In recent decades, the derivatization of nucleic acid drugs has been studied extensively. The nucleic acid cargo can be covalently attached to functional carrier molecules or loaded into supramolecular delivery devices. Conjugations of uptake-enhancing or targeting ligands to oligonucleotides provide the advantage of generating a defined molecule that allows for traditional pharmaceutical quality assessment. Several molecules have been attached to therapeutic oligonucleotides to improve their delivery, biodistribution, and cellular uptake; some are detailed in this section.

GalNAc derivatives were first introduced to oligonucleotides by TsO's research group in 1995 [23]. They developed GalNAc neoglycopeptide (ah-GalNAc)-conjugated oligodeoxynucleoside methylphosphonate (ah-GalNAc-oligo-MP) and successfully showed that the uptake of ah-GalNAc-oligo-MP by human hepatocellular carcinoma cells (Hep G2) is cell-type specific and can be completely inhibited by the addition of a 100-fold excess of free (ah-GalNAc) 3 in the culture medium, indicating the cell uptake of ah-GalNAc-oligo-MP was ligand dependent.

This specific and enhanced cellular uptake of GalNAc-conjugated oligonucleotides was also confirmed in vivo by several research groups [11][24]. Prakash et al. reported that antisense oligonucleotides conjugated to tri-antennary GalNAc improve the potency of therapeutic oligonucleotides about 10-fold in mice [24]. Now, there are various kinds of chemical modifications of GalNAc-conjugated, and from these reports, it has been shown that the GalNAc introduced into oligonucleotides does not necessarily have a tri-antennary structure, and, surprisingly, even mono-anntenary GalNAc-conjugation was also found effective [25][26]. In the future, we expect to uncover more detailed mechanisms of action of these monomeric GalNAc-conjugated oligonucleotides.

Folic acid (vitamin B9) binds with high affinity to the folate receptor protein to trigger cellular uptake via an endosomal pathway. The presence of the folate receptor on many cancer types has prompted the use of folate in targeted therapy [51]. Indeed, it has been used on liposomes or polyplexes to effectively deliver oligonucleotides to cancer cells that have the folate receptor [52][53]. Dohmen et al. were the first to develop folate-conjugated oligonucleotides, however, tethering folate to siRNA results in specific uptake but not silencing of reporter genes

[54]. Folic acid– oligonucleotide conjugates are trapped in endosomes with insufficient endosomal escape to the cytosol for gene silencing. Later, Orellana's group succeeded in eliciting the gene inhibitory effects of folic acid-conjugated oligonucleotides by connecting folic acid and oligonucleotides with a cleavable linker [55].

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Cholesterol was tethered to siRNA in one of the first reports of endogenous gene silencing in vivo; this was conducted under physiological conditions with a normal pressure injection in mice [56]. Cholesterol can easily be attached to a controlled-pore glass support prior to oligonucleotide synthesis, and an aminocaproic acid pyrrolidine phosphate linker is often used between ligands and oligonucleotides. Results have shown that cholesterol–siRNA conjugates can reduce the mRNA of targeted apoB by around 50% while unconjugated siRNA has no effect; similar results have been reported for the lipid docosanyl and stearoyl ligands [57]. Cholesterol–siRNA conjugates can also be used for noncovalent association to polymers, as demonstrated by in vivo gene silencing in combination with a targeted engineered polymer [58].

In 2019, Alnylam Pharmaceuticals, the company that developed patisiran, succeeded in developing an siRNA drug called "GalNAc-conjugated siRNA (GIVLAAR®, namely gibosiran)" [11][12]. This technology utilizes the binding of GalNAc to asialoglycoprotein receptors (ASGPR) that appear on the cell surface of hepatic parenchymal cells. Givosiran can be administered systemically (subcutaneously) without a carrier, whereas patisiran, which is encased in LNPs, requires a time-consuming intravenous infusion, making givosiran more useful in clinical practice. In addition, from the perspective of manufacturing and quality control, such conjugates are considered to be more advantageous than the drugs of this class with delivery carriers which often have complex structures like LNPs. In 2020, another GalNAc-siRNA (OXLUMO®, namely lumasiran) has been also approved by FDA [13].

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Cell penetrating peptides (CPPs) can facilitate cellular uptake of their cargo, which is directly attached through covalent linkages or the formation of noncovalent complexes. When CPPs were first identified, they were derived from peptide sequences found in naturally occurring protein elements that exhibited inherent translocating properties. Some of these were important for subsequent CPP iterations including the transactivator of transcription from HIV [59], Penetratin-1 derived from the homeodomain of Antennapedia [60], transportan (a chimeric peptide derived from galanin and the wasp-venom peptide toxin mastoparan) [61], and cationic polyarginine and polylysine sequences such as Arg8 [62].

5. Conclusions

nucleic acid drugs, such as ASOs and siRNAs, which promote the “disappearance” or “loss of function” of the target protein and act by new mechanisms that utilize the inherent characteristics of oligonucleotides. Although there are no nucleic acid drugs approved for cancer treatment yet, recent results of several clinical trials suggest that anti-cancer nucleic acid drugs will probably be approved in the near future. Furthermore, it is expected that nucleic acid drugs will be developed and practically used in a coordinated manner according to the characteristics of cancer types.

References

1. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2018. CA Cancer J. Clin. 2018, 68, 7–30.
2. Lameire, N. Nephrotoxicity of recent anti-cancer agents. Clin. Kidney J. 2014, 7, 11–22.
3. Suter, T.M.; Ewer, M.S. Cancer drugs and the heart: Importance and management Eur. Heart J. 2013, 34, 1102–1111.
4. Levin, A.A. Treating disease at the RNA level with oligonucleotides. N. Engl. J. Med. 2019, 380, 57–70.
5. Wang, F.; Zuroske, T.; Watts, J.K. RNA therapeutics on the rise. Nat. Rev. Drug Discov. 2020, 19, 441–442.
6. Craig, K.; Abrams, M.; Amiji, M. Recent preclinical and clinical advances in oligonucleotide conjugates. Expert Opin. Drug Deliv. 2018, 15, 629–640.

7. Quemener, A.M.; Bachelot, L.; Forestier, A.; Donnou-Fournet, E.; Gilot, D.; Galibert, M.D. The powerful world of antisense oligonucleotides: From bench to bedside. Wiley Interdiscip. Rev. RNA 2020, 11, e1594.
8. Mukalel, A.J.; Riley, R.S.; Zhang, R.; Mitchell, M.J. Nanoparticles for nucleic acid delivery: Applications in cancer immunotherapy. Cancer Lett. 2019, 458, 102–112.
9. Fumoto, S.; Yamamoto, T.; Okami, K.; Maemura, Y.; Terada, C.; Yamayoshi, A.; Nishida, K. Understanding In Vivo Fate of Nucleic Acid and Gene Medicines for the Rational Design of Drugs. Pharmaceutics 2021, 13, 159.
10. Judge, A.D.; Robbins, M.; Tavakoli, I.; Levi, J.; Hu, L.; Fronda, A.; Ambegia, E.; McClintock, K.; MacLachlan, I. Confirming the RNAi-mediated mechanism of action of siRNA-based cancer therapeutics in mice. J. Clin. Investig. 2009, 119, 661–673.
11. Nair, J.K.; Willoughby, J.L.; Chan, A.; Charisse, K.; Alam, M.R.; Wang, Q.; Hoekstra, M.; Kandasamy, P.; Kel'in, A.V.; Milstein, S.; et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. J. Am. Chem. Soc. 2014, 136, 16958–16961.
12. Khvorova, A.; Watts, J.K. The chemical evolution of oligonucleotide therapies of clinical utility. Nat. Biotechnol. 2017, 35, 238–248.
13. Garrelfs, S.F.; Frishberg, Y.; Hulton, S.A.; Koren, M.J.; O'Riordan, W.D.; Cochat, P.; Deschênes, G.; Shasha-Lavsky, H.; Saland, J.M.; Van't Hoff, W.G.; et al. Lumasiran, an RNAi Therapeutic for Primary Hyperoxaluria Type 1. N. Engl. J. Med. 2021, 384, 1216–1226.
14. Santel, A.; Aleku, M.; Keil, O.; Endruschat, J.; Esche, V.; Durieux, B.; Löffler, K.; Fechtner, M.; Röhl, T.; Fisch, G.; et al. RNA interference in the mouse vascular endothelium by systemic administration of siRNA-lipoplexes for cancer therapy. Gene Ther. 2006, 13, 1360–1370.
15. Schultheis, B.; Strumberg, D.; Kuhlmann, J.; Wolf, M.; Link, K.; Seufferlein, T.; Kaufmann, J.; Feist, M.; Gebhardt, F.; Khan, M.; et al. Safety, Efficacy and Pharcacokinetics of Targeted Therapy with The Liposomal RNA Interference Therapeutic Atu027 Combined with Gemcitabine in Patients with Pancreatic Adenocarcinoma. A Randomized Phase Ib/IIa Study. Cancers 2020, 12, 3130.
16. Atu027 Plus Gemcitabine in Advanced or Metastatic Pancreatic Cancer (Atu027-I-02) (Atu027-I-02). ClinicalTrials.gov, Identifier: NCT01808638 ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01808638 (accessed on 7 July 2021).
17. Golan, T.; Khvalevsky, E.Z.; Hubert, A.; Gabai, R.M.; Hen, N.; Segal, A.; Domb, A.; Harari, G.; David, E.B.; Raskin, S.; et al. RNAi therapy targeting KRAS in combination with chemotherapy for locally advanced pancreatic cancer patients. Oncotarget 2015, 6, 24560–24570.
18. Shemi, A.; Khvalevsky, E.Z.; Gabai, R.M.; Gabai, R.M.; Hen, N.; Segal, A.; Domb, A.; Harari, G.; David, E.B.; Raskin, S.; et al. Multistep, effective drug distribution within solid tumors. Oncotarget

2015, 6, 39564–39577.

19. A Phase 2 Study of siG12D LODER in Combination with Chemotherapy in Patients with Locally Advanced Pancreatic Cancer (PROTACT). ClinicalTrials.gov, Identifier: NCT01676259 ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT01676259 (accessed on 7 July 2021).
20. Merkel, O.M.; Beyerle, A.; Librizzi, D.; Pfestroff, A.; Behr, T.M.; Sproat, B.; Barth, P.J.; Kissel, T. Nonviral siRNA delivery to the lung: Investigation of PEG-PEI polyplexes and their in vivo performance. Mol. Pharm. 2009, 6, 1246–1260.
21. Hussain, M.; Shchepinov, M.; Sohail, M.; Benter, I.F.; Hollins, A.J.; Southern, E.M.; Akhtar, S. A novel anionic dendrimer for improved cellular delivery of antisense oligonucleotides. J. Control. Release 2004, 99, 139–155.
22. Ko, Y.T.; Bhattacharya, R.; Bickel, U. Liposome encapsulated polyethylenimine/ODN polyplexes for brain targeting. J. Control. Release 2009, 133, 230–237.
23. Hangeland, J.J.; Levis, J.T.; Lee, Y.C.; Ts'O, P.O. Cell-type specific and ligand specific enhancement of cellular uptake of oligodeoxynucleoside methylphosphonates covalently linked with a neoglycopeptide, YEE(ah-GalNAc)3. Bioconjug. Chem. 1995, 6, 695–701.
24. Prakash, T.P.; Graham, M.J.; Yu, J.; Carty, R.; Low, A.; Chappell, A.; Schmidt, K.; Zhao, C.; Aghajan, M.; Murray, H.F.; et al. Targeted delivery of antisense oligonucleotides to hepatocytes using triantennary N-acetyl galactosamine improves potency 10-fold in mice. Nucleic Acids Res. 2014, 42, 8796–8807.
25. Rajeev, K.G.; Nair, J.K.; Jayaraman, M.; Charisse, K.; Taneja, N.; O'Shea, J.; Willoughby, J.L.; Yucius, K.; Nguyen, T.; Shulga-Morskaya, S.; et al. Hepatocyte-specific delivery of siRNAs conjugated to novel non-nucleosidic trivalent N-acetylgalactosamine elicits robust gene silencing in vivo. Chembiochem 2015, 16, 903–908.
26. Yamamoto, T.; Sawamura, M.; Wada, F.; Harada-Shiba, M.; Obika, S. Serial incorporation of a monovalent GalNAc phosphoramidite unit into hepatocyte-targeting antisense oligonucleotides. Bioorg. Med. Chem. 2016, 24, 26–32.
27. Yamayoshi, A.; Oyama, S.; Kishimoto, Y.; Konishi, R.; Yamamoto, T.; Kobori, A.; Harada, H.; Ashihara, E.; Sugiyama, H.; Murakami, A. Development of Antibody–Oligonucleotide Complexes for Targeting Exosomal MicroRNA. Pharmaceutics 2020, 12, 545.
28. Balwani, M.; Sardh, E.; Ventura, P.; Peiró, P.A.; Rees, D.C.; Stölzel, U.; Bissell, M.; Bonkovsky, H.L.; Windyga, J.; Anderson, K.E.; et al. Phase 3 Trial of RNAi Therapeutic Givosiran for Acute Intermittent Porphyria. N. Engl. J. Med. 2020, 382, 2289–2301.
29. Ray, K.K.; Wright, R.S.; Kallend, D.; Koenig, W.; Leiter, L.A.; Raal, F.J.; Bisch, J.A.; Richardson, T.; Jaros, M.; Wijngaard, P.L.J.; et al. Two Phase 3 Trials of Inclisiran in Patients with Elevated

LDL Cholesterol. N. Engl. J. Med. 2020, 382, 1507–1519.

30. Alvarez-Erviti, L.; Seow, Y.; Yin, H.; Betts, C.; Lakhal, S.; Wood, M.J.A. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat. Biotechnol. 2011, 29, 341–345.
31. Kooijmans, S.A.A.; Stremersch, S.; Braeckmans, K.; de Smedt, S.C.; Hendrix, A.; Wood, M.J.A.; Schiffelers, R.M.; Raemdonck, K.; Vader, P. Electroporation-induced siRNA precipitation obscures the efficiency of siRNA loading into extracellular vesicles. J. Control. Release 2013, 172, 229–238.
32. Didiot, M.C.; Hall, L.M.; Coles, A.H.; Haraszti, R.A.; Godinho, B.M.D.C.; Chase, K.; Sapp, E.; Ly, S.; Alterman, J.F.; Hassler, M.R. Exosome-mediated Delivery of Hydrophobically Modified siRNA for Huntingtin mRNA Silencing. Mol. Ther. 2016, 24, 1836–1847.
33. Zhao, L.; Gu, C.; Gan, Y.; Shao, L.; Chen, H.; Zhu, H. Exosome-mediated siRNA delivery to suppress postoperative breast cancer metastasis. J. Control. Release 2020, 318, 1–15.
34. Munagala, R.; Aqil, F.; Jeyabalan, J.; Kandimalla, R.; Wallena, M.; Tyagi, N.; Wilcher, S.; Yan, J.; Schultz, D.J.; Spencer, W.; et al. Exosome-mediated delivery of RNA and DNA for gene therapy. Cancer Lett. 2021, 505, 58–72.
35. Kamerkar, S.; LeBleu, V.S.; Sugimoto, H.; Yang, S.; Ruivo, C.F.; Melo, S.A.; Lee, J.J.; Kalluri, R. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. Nature 2017, 546, 498–503.
36. iExosomes in Treating Participants with Metastatic Pancreas Cancer With KrasG12D Mutation. ClinicalTrials.gov, Identifier: NCT03608631 ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT03608631 (accessed on 7 July 2021).
37. Semple, S.C.; Klimuk, S.K.; Harasym, T.O.; Dos Santos, N.; Ansell, S.M.; Wong, K.F.; Maurer, N.; Stark, H.; Cullis, P.R.; Hope, M.J.; et al. Efficient encapsulation of antisense oligonucleotides in lipid vesicles using ionizable aminolipids: Formation of novel small multilamellar vesicle structures. Biochim. Biophys. Acta 2001, 1510, 152–166.
38. Morrissey, D.V.; Lockridge, J.A.; Shaw, L.; Blanchard, K.; Jensen, K.; Breen, W.; Hartsough, K.; Machemer, L.; Radka, S.; Jadhav, V.; et al. Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. Nat. Biotechnol. 2005, 23, 1002–1007.
39. Judge, A.D.; Bola, G.; Lee, A.C.; MacLachlan, I. Design of noninflammatory synthetic siRNA mediating potent gene silencing in vivo. Mol. Ther. 2006, 13, 494–505.
40. A Study of NBF-006 in Non-Small Cell Lung, Pancreatic, or Colorectal Cancer. ClinicalTrials.gov, Identifier: NCT03819387 ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT03819387 (accessed on 7 July 2021).

41. Sahin, U.; Oehm, P.; Derhovanessian, E.; Jabulowsky, R.A.; Vormehr, M.; Gold, M.; Maurus, D.; Schwarck-Kokarakis, D.; Kuhn, A.N.; Omokoko, T.; et al. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. Nature 2020, 585, 107–112.
42. Trial With BNT111 and Cemiplimab in Combination or as Single Agents in Patients with Anti-PD1-refractory/Relapsed, Unresectable Stage III or IV Melanoma. ClinicalTrials.gov, Identifier: NCT04526899 ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT04526899 (accessed on 7 July 2021).
43. Song, E.; Zhu, P.; Lee, S.K.; Chowdhury, D.; Kussman, S.; Dykxhoorn, D.M.; Feng, Y.; Palliser, D.; Weiner, D.B.; Shankar, P.; et al. Antibody mediated in vivo delivery of small interfering RNAs via cell-surface receptors. Nat. Biotechnol. 2005, 23, 709–717.
44. Ma, Y.; Kowolik, C.M.; Swiderski, P.M.; Kortylewski, M.; Yu, H.; Horne, D.A.; Jove, R.; Caballero, O.L.; Simpson, A.J.G.; Lee, F.-T.; et al. Humanized Lewis-Y specific antibody based delivery of STAT3 siRNA. ACS Chem. Biol. 2011, 6, 962–970.
45. Xia, C.F.; Zhang, Y.; Zhang, Y.; Boado, R.J.; Pardridge, W.M. Intravenous siRNA of brain cancer with receptor targeting and avidin-biotin technology. Pharm. Res. 2007, 24, 2309–2316.
46. Arnold, A.E.; Malek-Adamian, E.; Le, P.U.; Meng, A.; Martínez-Montero, S.; Petrecca, K.; Damha, M.J.; Shoichet, M.S. Antibody-Antisense Oligonucleotide Conjugate Downregulates a Key Gene in Glioblastoma Stem Cells. Mol. Ther. Nucleic Acids 2018, 11, 518–527.
47. Sugo, T.; Terada, M.; Oikawa, T.; Miyata, K.; Nishimura, S.; Kenjo, E.; Ogasawara-Shimizu, M.; Makita, Y.; Imaichi, S.; Murata, S.; et al. Development of antibody-siRNA conjugate targeted to cardiac and skeletal muscles. J. Control. Release 2016, 237, 1–13.
48. Fishman, J.B.; Rubin, J.B.; Handrahan, J.V.; Connor, J.R.; Fine, R.E. Receptor-mediated transcytosis of transferrin across the blood-brain barrier. J. Neurosci. Res. 1987, 18, 299–304.
49. Junghans, M.; Kreuter, J.; Zimmer, A. Antisense delivery using protamine-oligonucleotide particles. Nucleic Acids Res. 2000, 28, E45.
50. Dinauer, N.; Lochmann, D.; Demirhan, I.; Bouazzaoui, A.; Zimmer, A.; Chandrac, A.; JörgKreuter; Briesena, H. Intracellular tracking of protamine/antisense oligonucleotide nanoparticles and their inhibitory effect on HIV-1 transactivation. J. Control. Release 2004, 96, 497–507.
51. Low, P.S.; Henne, W.A.; Doornereerd, D.D. Discovery and Development of Folic-Acid-Based Receptor Targeting for Imaging and Therapy of Cancer and Inflammatory Diseases. Acc. Chem. Res. 2008, 41, 120–129.
52. Zhou, W.; Yuan, X.; Wilson, A.; Yang, L.; Mokotoff, M.; Pitt, B.; Li, S. Efficient intracellular delivery of oligonucleotides formulated in folate receptor-targeted lipid vesicles. Bioconjug. Chem. 2002, 13, 1220–1225.

53. Wang, M.; Hu, H.; Sun, Y.; Qiua, L.; Zhang, J.; Guan, G.; Zhao, X.; Qiao, M.; Cheng, L.; Cheng, L.; et al. A pH-sensitive gene delivery system based on folic acid-PEG-chitosan—PAMAM-plasmid DNA complexes for cancer cell targeting. Biomaterials 2013, 34, 10120–10132.
54. Dohmen, C.; Fröhlich, T.; Lächelt, U.; Röhl, I.; Vornlocher, H.-P.; Hadwiger, P.; Wagner, E. Defined Folate-PEG-siRNA Conjugates for Receptor-specific Gene Silencing. Mol. Ther. Nucleic Acids 2012, 1, e7.
55. Orellana, O.; Tenneti, S.; Rangasamy, L.; Lyle, T.; Low, P.S.; Kasinski, A.L. FolamiRs: Ligand-targeted, vehicle-free delivery of microRNAs for the treatment of cancer. Sci. Transl. Med. 2017, 9, eaam9327.
56. Juliano, R.L.; Ming, X.; Nakagawa, O. Cellular uptake and intracellular trafficking of antisense and siRNA oligonucleotides. Bioconjug. Chem. 2012, 23, 147–157.
57. Wolfrum, C.; Shi, S.; Jayaprakash, K.N.; Jayaraman, M.; Wang, G.; Pandey, R.K.; Rajeev, K.G.; Nakayama, T.; Charrise, K.; Ndungo, E.M.; et al. Mechanisms and optimization of in vivo delivery of lipophilic siRNAs. Nat. Biotechnol. 2007, 25, 1149–1157.
58. Wong, S.C.; Klein, J.J.; Hamilton, H.L.; Chu, Q.; Frey, C.L.; Truvetskoy, V.S.; Hegge, J.; Wakefield, D.; Rozema, D.B.; Lewis, D.L. Co-injection of a targeted, reversibly masked endosomolytic polymer dramatically improves the efficacy of cholesterol-conjugated small interfering RNAs in vivo. Nucleic Acid Ther. 2012, 22, 380–390.
59. Frankel, A.D.; Pabo, C.O. Cellular uptake of the tat protein from human immunodeficiency virus. Cell 1988, 55, 1189–1193.
60. Derossi, D.; Joliot, A.H.; Chassaing, G.; Prochiantz, A. The third helix of the Antennapedia homeodomain translocates through biological membranes. J. Biol. Chem. 1994, 269, 10444–10450.
61. Pooga, M.; Hällbrink, M.; Zorko, M.; Langel, U. Cell penetration by transportan. FASEB J. 1998, 12, 67–77.
62. Futaki, S.; Suzuki, T.; Ohashi, W.; Yagami, T.; Tanaka, S.; Ueda, K.; Sugiura, Y. Arginine-rich peptides. An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. J. Biol. Chem. 2001, 276, 5836–5840.