RNAi-Based Therapy: Prospect as Cancer Treatment

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Abstract

Cancer is one of the deadliest form of diseases in humans, with the annual deaths ranging in the millions. Conventional treatments including chemotherapy, radiotherapy, or surgery has their limitations, including common off-target and non-specific effects. Ribonucleic acid interference (RNAi) offers a new strategy for treating cancer by silencing specific genes to prevent gene expression. This review highlights the application of RNAi-based approach in targeting cancer, discusses its potential advantages and limitations, summarizes the existing clinical trials and provides a greater understanding of RNAi-based therapy in cancer.

Introduction

Cancer is one of the leading causes of mortality worldwide, accounting for nearly 10 million deaths in 2020 (nearly one in six deaths), with the most common cancers being breast, lung, colon, rectum, and prostate cancers.[1] Globally, the incidences of cancer have been constantly rising and are expected to reach almost 22 million per year by 2030.[2] Conventional cancer treatment usually involves surgery, chemotherapy, radiotherapy or a combination of those three modalities. However, their limitations hinder those conventional treatments, including the non-specific and off-target effects. The challenge of managing difficulties in treating numerous types of cancer necessitates the need for developing new strategies to improve treatment outcomes.

Cancer treatment through ribonucleic acid interference (RNAi) is an emerging field, which has shown promising evidence. Its therapeutic use is mediated through non-coding RNAs (ncRNAs), such as small interfering RNAs (siRNAs) and microRNAs (miRNAs), that act as gene-specific silencer. Those ncRNAs will bind and activate the RNA-induced silencing complex (RISC), a multi-protein complex. Subsequently they will bind to target messenger RNAs (mRNAs) and will degrade and cleave target mRNAs before their translation becoming proteins. This mechanism will interfere gene expression, resulting in post-transcriptional gene silencing.[3]

One advantage of RNAi-based treatment is its precision and accuracy, hence able to target the expression of specific target genes.[4] This means that it could potentially circumvent the hurdles commonly experienced among patients receiving chemotherapies, such as the rapid development of drug resistance and the risk of systemic toxicity due to the lack of specificity.[5] However, despite its promising applications, an issue that needs to be addressed is the issue regarding the ease of degradability of such RNA molecules.[6] Thus, the current focus of RNAi treatment is to look for the proper delivery methods, which ensures the appropriate concentration of the RNA reaching the target sites, where it can exert its therapeutic effect.

In this review, the application of RNAi-based approach in targeting cancer will be discussed in detail. In particular, this review will discuss its potential advantages and limitations, summarize the existing number of clinical trials and overall provide a greater understanding of RNAi-based therapy in cancer.

Principles of RNAi

As shown in **Figure 1**, the ncRNA molecules (e.g., miRNA or siRNA), which are double-stranded RNAs, are cleaved into shorter fragments by an enzyme called Dicer in the cytoplasm. Once this occurs, the miRNAs or siRNAs are loaded into the RISC, which acts as the key intermediate in the whole process. Each of miRNA or siRNA is unwound and split into two single-stranded RNAs, named the passenger and the guide strands, respectively. While the passenger strand is subsequently cleaved by the Ago 2 protein and degraded, the guide strand is incorporated into the RISC. The RISC then binds to target mRNA (through the assistance of the guide strand), before it cleaves and degrades target mRNA, resulting in inhibition of the gene expression.[7]

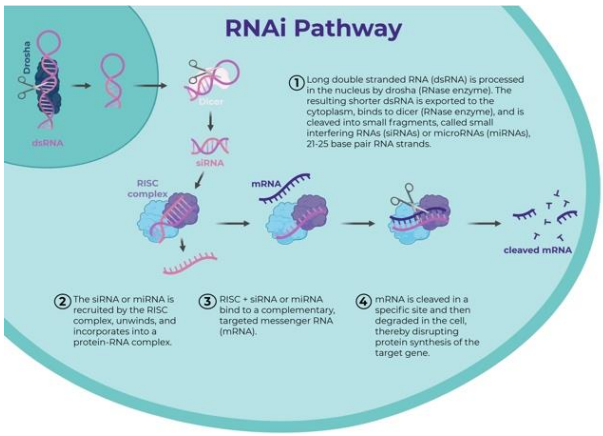

Figure 1. The action mechanism of RNAi. The picture was obtained from https://www.umassmed.edu/rti/biology/rna/how-rnai-works/

The use of RNAi as therapeutic agent

Due to its ability to target and silence specific genes, RNAi provides a novel opportunity to treat various diseases by targeting the expression of certain genes that are involved in the pathological processes.[8] Recent therapeutic approaches that targeting proteins, such as small-molecule inhibitors and immunotherapy, have been proven to be successful in several cases. However, even for protein-based drugs that are highly specific in nature (e.g., monoclonal antibodies), they are limited to targeting either freely circulating proteins or cell-surface receptors. In contrast, RNAi can overcome this limitation as RNAi can interfere with the expression of specific genes by blocking translation of their mRNA transcripts.[4]

RNAi-based therapy has been used in neurodegenerative diseases, cardiovascular diseases, infectious diseases and particularly cancer.[9] With the recent advancements in high-throughput sequencing technologies, the profiling of gene expression in cancer cells has facilitated the discovery of various dysregulated genes found in cancer cells. For this reason, RNAi therapy has been considered as an ideal strategy in silencing the expression of oncogenes.[8]

Strengths and challenges of RNAi

The application of RNAi-based therapy in the treatment of cancers offers several advantages. First, it can specifically target any gene within the cancerous cells.[10] This is crucial especially in the field of oncology, where many important therapeutic targets are currently undruggable using conventional drugs and immunotherapy.[11] Second, it can be easily synthesized and modified.[12] Third, it has good safety and efficacy profiles with low immunogenicity and it is highly specific with minimum off-target effects.[12] Indeed, RNAi-based therapy has shown promising *in vitro* tumor growth suppression and antiproliferative effect via the stat6 pathway and PLK1[13], angiogenesis suppression through inhibition of receptors such as VEGFs and VEGFR-1[14], as well as inhibition of tumor invasion and metastasis through chemokines CXCL8 and CXCL11.[15]

RNAi-based therapy has shown a great potential in terms of its therapeutic applications in several diseases including cancer.[9] However, there are issues that hinder RNAi-based therapy from being fully accepted into the clinical practice, such as its low stability and its delivery into cells.[16] For example, unprotected and unmodified RNA molecules are very easily degraded by endonucleases and can also trigger an immune response within the body.[16] Furthermore, since RNA-based therapy

relies on the cytoplasm entrance to exert its therapeutic effect, there are various challenges encountered during its delivery.[17]

Delivery methods of RNAi

As mentioned, one main limitation of RNAi-based therapy is that RNAs are extremely vulnerable to be degraded by ribonuclease presents in the body. Following their administration, RNA molecules are quickly degraded by the abundant sera ribonucleases, contributing to its short half-life *in vivo*. Even though double-stranded RNAs possess greater resistance towards ribonuclease degradation than single-stranded RNAs, the difference is insignificant.[18] Therefore, new methods are required to improve the resistance of ncRNA. This could be done by chemically modifying the RNA molecules to improve their stability. These chemical modifications can also help to solve other issues, such as minimizing immunogenicity and reducing off-target effects.[19] Currently, the common types of chemical modification which are studied in designing siRNA and miRNA are (i) locked and unlocked nucleic acids[20]; (ii) ribose 2′-OH group modification; and (iii) phosphorothioate modification.[19] Thus, depending on the desired function, different RNA modifications may be utilized.

Targeted delivery is another major obstacle in ensuring successfulness of RNAi-based therapy. Due to the inherently unstable nature of RNA, appropriate delivery vehicles are required that could protect RNA from degradation by ribonuclease. In addition, miRNA and siRNA have an intracellular site of action and thus require the entry of the RNA into the cytoplasm. However, due to their high molecular weight (~14–15 kDa) and polar nature, the overall properties of RNAi makes them poorly permeable across cellular membranes.[21] Therefore, novel delivery vehicles are required to deliver the ncRNA to the target site, protect ncRNA molecules from premature degradation by ribonuclease as well as to aid the cellular uptake of ncRNA into the cytoplasm, where it will exert its therapeutic effect.[22]

There have been numerous efforts put into designing and developing various delivery vehicles. Briefly, the viral vector and non-viral vector will be discussed as the RNA-delivery vehicles.

1. Viral Vectors

The usage of viral vectors, which encode either siRNAs or miRNAs, have been shown to trigger gene-silencing effects in several studies.[23] The main advantage of using viral vectors is their efficient ability to introduce the RNA-encoding genes into the cellular nucleus and ensure high expression of ncRNA. This facilitates the expression of multiple copies of ncRNA molecule from a single transcript, resulting in sustained gene silencing.[23] Several viruses have been utilized for this purpose, including lentiviruses[24], adenoviruses[25] as well as adeno-associated viruses (AAVs).[26]

However, there are several concerns in regards to the use of viral vectors, such as its high immunogenicity and the risk of viral genome insertion into the host's genome (i.e., insertional mutagenesis). These issues, along with the low packaging capacity and high production cost have limited the clinical applications of such viral vectors.[23] As a result, the use of non-viral vectors in delivering synthetic siRNAs and miRNAs is currently considered to be a better alternative due to their better safety profile and lower production cost.[27]

2. Non-viral vectors

Two main types of non-viral delivery systems are lipid-based and polymer-based systems

A. Lipid-based vector

A common lipid-based vector is liposomes, which are small spherical vesicle and are composed of a lipid membrane with a hydrophilic core. They protect the contents from degradation and have beneficial properties due to their low toxicity and immunogenicity.[28]

Furthermore, due to their hydrophilic core and hydrophobic lipid membrane, liposomes are able to fuse with the cellular membrane, which has a similar

characteristic. Following its fusion, the contents within the liposomes are released into the cytoplasm.[29] Liposomes can form a lipid complex with nucleic acid through electrostatic interaction, which aids in improving the overall stability and transfection efficiency. In this case, liposomes are given a positive charge to bind to the negatively charged nucleic acid content.[30] Cationic liposomes generally possess a greater encapsulation's efficiency when compared to their negatively charged counterparts. In addition, liposomes prolong the half-life of RNA in the blood by reducing its degradation.[30]

One modification to cationic liposomes is the addition of polyethylene glycol (PEG) to their surface to reduce the overall positive charge. This reduces the cytotoxicity and immunogenicity of liposomes as well as helps in prolonging the half-life of RNA in blood's circulation.[31] In the case of cancer, this modification has been shown to induce the uptake of lipid complexes into tumor cells as well.[9]

Besides liposomes, recent research has been focusing on the use of solid lipid nanoparticles (LNPs) as transporters. Solid LNPs address the issues found in liposomes, such as the stability and easier mass production due to the homogeneity of the particles.[32] Solid LNPs are composed of cationic lipids, cholesterol and polyethylene glycol (PEG), which still able to carry RNA and to protect it from degradation.[31] Solid LNPs generally have more complex internal structures as well as greater physical stability when compared to the less complex liposome.[32] An example of the usage is Patisiran, which is an siRNA-based therapy used to treat hereditary transthyretin-mediated amyloidosis.[33]

B. Polymer-based vector

This refers to the wide range of compounds, which are composed of monomers that interact with each other to form complex structures.[34] These materials commonly include synthetic polymers, such as poly(lactic-co-glycolic acid) (PLGA), polylactic acid (PLA), polyethylenimine (PEI), but can be composed of natural polymers, such as chitosan.[35] Similar to the lipid-based vector, these polymers are cationic structures, which can form complexes with negatively charged RNA through electrostatic interactions. Several advantages of such delivery systems are that in addition to the longer half-life, these polymers can be refined for delivering specific compounds through chemical modifications, hence allowing for a better control over the content's release.[25]

Chitosan is an example of a widely used and naturally derived vector. It is a polysaccharide composed of β-(1–4)-linked d-glucosamine and N-acetyl-d-glucosamine monomers. Due to its positive charge, chitosan and several of its derivatives have been extensively studied regarding its application as a vector for the delivery of both RNA and DNA.[36] Chitosan/siRNA nanoplex formulations have been studied for the delivery of PDGF-D and PDGFR-β–specific siRNA, efficiently reducing proliferation and invasion of breast cancer cells.[37] Oligochitosan nanoparticles have also been utilized in delivering siRNA against myeloid leukemia 1 (MCL1).[38]

PEI is one of the most used synthetic cationic polymers for delivering siRNA and miRNA, with both straight and branched forms in various molecular weights. PEI has high cationic charge density, which is an advantageous property as it can form small and compact complexes with nucleic acids.[39] It has also shown promising evidence of delivering siRNA to silence target gene expression.[40] Complexes composed of PEI and HER-2 receptor-specific siRNA have been shown to produce gene-silencing and anti-tumor effects in mice.[41] However, the cytotoxic effect of PEI in its unmodified state limits its clinical application. Thus, PEI should be combined with other polymers, such as hyaluronic acid, chitosan and PEG, in order to reduce its cytotoxicity and improve its siRNA delivery capabilities.[42]

RNAi-based therapy in cancer

Application of RNAi in cancer is mainly seen through the inhibition of tumor anti-apoptosis genes, inhibition of angiogenesis-related factors, inhibition of

oncogenes, as well as reduction of tumor drug resistance and other hallmark traits of cancer.

1. RNAi in Breast Cancer

Breast cancer is one of the most common forms of cancer, with 1 in 7 women being diagnosed in their lifetime.[53] Breast cancer can be divided into three subtypes based on the expression of hormone receptors: (i) hormone receptor positive (estrogen receptor positive and/or progesterone receptor positive), (ii) human epidermal growth factor receptor 2/HER2-enriched (with estrogen receptor negative and progesterone receptor negative), and (iii) triple negative (estrogen receptor negative, progesterone receptor negative and HER2 negative).[43] Accounting for 15-25% of invasive breast cancer, the HER2-enriched subtype has the worst prognosis among all subtypes. Although Trastuzumab, a HER2-target treatment based on monoclonal antibody, has subsequently improved patients' prognosis, drug resistance cases are still present and common among patients. The utilization of siRNA is postulated to be superior to antibodies or small molecule inhibitors because siRNA can intervene in an earlier stage of protein production, compared to antibodies or small molecule inhibitors, which bind to the protein to prevent its function.[44] Ngamcherdtrakul *et al*., 2015 showed that knockdown of HER2 using siRNA(siHER2) was able to increase numbers of apoptosis in HER2-enriched cancer cells *in vitro,* compared to HER2-targeted antibody (Trastuzumab) and a small molecule inhibitor (lapatinib).[45] Therefore, this method can potentially become an alternative treatment for HER2-enriched breast cancer.

2. RNAi in Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDAC) is amongst the deadliest types of cancers and is projected to become the second leading cause of cancer-related death.[46] Unlike other forms of solid cancer, effective PDAC treatment is unavailable thus far. The standard care of PDAC using chemotherapy treatments (FOLFIRINOX and gemcitabine/nab-paclitaxel) only yields a median overall survival of less than a year. A siRNA-based drug (Atu027) is under development to silence the expression of PKN3, a downstream effector of PI3 kinase signaling, in the vascular endothelium.[47,48] If it works, this treatment can potentially inhibit the tumor's metastatic spread.

3. RNAi in Lung Cancer

Lung cancer remains the deadliest form of the disease, affecting both men and women. In 2018, approximately 30,023 new cases and 26,095 deaths due to lung cancer were reported in Indonesia.[49] An involved gene in lung cancer is cyclooxygenase-2 (COX-2) which functions to regulate tumor progression, metastasis, and anti-tumor immunity. However, existent COX-2 inhibitors are ineffective to treat lung cancer. An alternate approach was designed to knockdown the delta-5-desaturase (D5D), which will limit the formation of arachidonic acid, a substrate for COX-2.[50] Preclinical studies have explored the possibility of using RNAi-based therapy for this purpose. Combining epithelial cell adhesion molecule (EpCAM) aptamers with D5D-specific siRNA and nanoparticles that binds D5D-specific siRNA showed a target-specific accumulation by suppressing the YAP1/TAZ axis.[51] Inhibited proliferation and induced apoptosis of lung cancer cells were indeed observed in lung cancer cell lines and murine models.[51] Thus, the utilization of nanoparticle-mediated RNAi-based therapy has the potential to overcome the constraints of conventional chemotherapy by selectively silencing the oncogenes and multi-drug resistant genes as well as minimizing the adverse risk towards healthy cells.[52]

Table 1. Clinical trials of RNAi-based therapy for cancers.

Drug Name	Clinical Phase	Condition	Targets	NCT ID	References
Rintatolimod	Approved in 2017	Chronic fatigue syndrome (CFS)	TLR3	NCT00215813	[54]
Lefitolimod (MGN1703)	Phase III	Metastatic Colorectal Cancer	TLR9	NCT02077868	[55]
STP705	Phase II	Basal cell carcinoma; Cutaneous squamous cell carcinoma; Hepatocellular carcinoma	TGF-β1 & COX-2	NCT04669808 & NCT04293679	[56]
Atu027	Phase II	Solid carcinoma; Pancreatic ductal carcinoma	PKN3	NCT01808638	[57]
siG12D LODER	Phase I	Pancreatic cancer; Pancreatic ductal adenocarcinoma	KRAS G12D	NCT01676259	[58]
APN401	Phase I	Pancreatic cancer; Colorectal cancer; Metastatic melanoma	Cbl-b	NCT03087591	[59]
TargomiRs	Phase I	Malignant pleural mesothelioma; Non-small cell lung cancer	miR-16	NCT02369198	[60]
DCR-BCAT	Pre-clinical	Hepatocellular cancer; Colorectal cancer	CTNNB1	N/A	[61]

Clinical trials of RNAi-based therapy for cancer

RNAi-based therapy has made remarkable progress in recent years, with more and more RNAi-based-drugs entering the market with each passing year. There are three FDA approved siRNA-based-drugs for non-cancer diseases thus far. Onpattro® (Patisiran) was approved in 2018 for treatment of polyneuropathy. Givlaari® (Givosiran) targeting mRNA of ALAS1 was approved in 2019 for treating acute hepatic porphyria (AHP). Oxlumo® (Lumasiran) targeting the HAO1mRNA as the treatment of primary hyperoxaluria type 1 was approved in 2020.[53]

Currently there is only one RNAi-based therapy in the market that has a potential to treat tumors. First launched in 2017 in Argentina, Rintatolimod, with a tradename of Ampligen® in the United States, is a mismatched double-stranded RNA molecule with immunomodulatory properties that acts as a Toll-Like Receptor 3 agonist.[62] Rintatolimod was able to reactivate the local immune response whilst stimulated the production of interferons and tumor necrosis factors.[63] Although this drug is licensed as a treatment for severe myalgic encephalomyelitis/chronic fatigue syndrome, it is currently tested in ongoing phase II/III clinical trials to evaluate its potential to treat stage II-IV HER2-enriched breast cancer, pancreatic cancer, renal cell carcinoma and other solid form tumors. Concurrently, *in vitro* studies suggested that the activation of TLR3 was able to induce apoptosis in lung cancer cell lines.[62]

Other RNAi-based therapy that has been determined to be safe in humans and are currently tested in advanced cancers include Atu027 for solid carcinoma and pancreatic ductal carcinoma; Lefitolimod for metastatic colorectal cancer; and TargomiRs for mesothelioma. The summary of currently tested RNAi-based therapy is shown in **Table 1**.

Conclusions

Cancer remains to be one of the deadliest and leading cause of human mortality. The malignant cell's ability to adapt and evade drug-induced cell death and immune responses render many medical treatments void. The search for a more precise and efficacious treatment is at the utmost importance. The silencing properties of RNAi offers potential in combating cancerous cells. As of now, many pharmaceutical companies are investigating the prospect of using RNAi-based therapy as cancer treatment.

References

1. World Health Organization (WHO). Fact Sheets: Cancer [Internet]. World Health Organization. World Health Organization. 2022. https://www.who.int/news-room/fact-sheets/detail/cancer

2. Bray F, Jemal A, Torre LA, Forman D, Vineis P. Long-term realism and cost-effectiveness: primary prevention in combating cancer and associated inequalities worldwide. J Natl Cancer Inst. 2015;107(12). https://doi.org/10.1093/jnci/djv273

3. Agrawal N, Dasaradhi PV, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK. RNA interference: Biology, mechanism, and applications. Microbiol Mol Biol Rev. 2003; 67(4):657–85. https://doi.org/10.1128/mmbr.67.4.657-685.2003

4. Kim Y-K. RNA therapy: rich history, various applications and unlimited future prospects. Exp Mol Med. 2022;54(4):455–65. https://doi.org/10.1038%2Fs12276-022-00757-5

5. Svoboda P. Key mechanistic principles and considerations concerning RNA interference. Front Plant Sci. 2020;11.

6. Wang Y. Delivery systems for RNA interference therapy: Current technologies and limitations. Curr Gene Ther. 2020; 20(5):356–72. https://doi.org/10.2174/1566523220666201005110726

7. Neumeier J, Meister G. siRNA specificity: RNAi mechanisms and strategies to reduce off-target effects. Front Plant Sci. 2021;11. https://doi.org/10.3389/fpls.2020.526455

8. Chen X, Mangala LS, Rodriguez-Aguayo C, Kong X, Lopez-Berestein G, Sood AK. RNA interference-based therapy and its delivery systems. Cancer and Metastasis Rev. 2017;37(1):107–24. https://doi.org/10.1007/s10555-017-9717-6

9. Tian Z, Liang G, Cui K, Liang Y, Wang Q, Lv S, et al. Insight into the prospects for RNAi therapy of cancer. Front Pharmacol. 2021;12. https://doi.org/10.3389/fphar.2021.644718

10. Cao S, Lin C, Liang S, Tan CH, Er Saw P, Xu X. Enhancing chemotherapy by RNA interference. BIO Integration. 2020;1(2):64–81. http://dx.doi.org/10.15212/bioi-2020-0003

11. Roscigno G, Scognamiglio I, Ingenito F, Chianese RV, Palma F, Chan A, et al. Modulating the crosstalk between the tumor and the microenvironment using siRNA: A flexible strategy for breast cancer treatment. Cancers. 2020;12(12):3744. https://doi.org/10.3390%2Fcancers12123744

12. Jiang Y, Huo S, Hardie J, Liang X-J, Rotello VM. Progress and perspective of inorganic nanoparticle-based siRNA delivery systems. Expert Opin Drug Deliv. 2016;13(4):547–59. https://doi.org/10.1517/17425247.2016.1134486

13. Binnemars-Postma K, Bansal R, Storm G, Prakash J. Targeting the STAT6 pathway in tumor-associated macrophages reduces tumor growth and metastatic niche formation in breast cancer. FASEB J. 2018;32(2):969–78. https://doi.org/10.1096/fj.201700629r

14. Song Y, Tang C, Yin C. Combination antitumor immunotherapy with VEGF and PIGF siRNA via systemic delivery of multi-functionalized nanoparticles to tumor-associated macrophages and breast cancer cells. Biomaterials. 2018;185:117–32. https://doi.org/10.1016/j.biomaterials.2018.09.017

15. Hwang HJ, Lee Y-R, Kang D, Lee HC, Seo HR, Ryu J-K, et al. Endothelial cells under therapy-induced senescence secrete CXCL11, which increases aggressiveness of breast cancer cells. Cancer Lett. 2020; 490:100–10. https://doi.org/10.1016/j.canlet.2020.06.019

16. Vicentini FT, Borgheti-Cardoso LN, Depieri LV, Abelha TF, Petrilli R, Bentley MV. Delivery Systems and local administration routes for therapeutic siRNA. Pharm Res. 2013;30(4):915–31. https://doi.org/10.1007%2Fs11095-013-0971-1

17. Hattab D, Gazzali AM, Bakhtiar A. Clinical advances of siRNA-based nanotherapeutics for cancer treatment. Pharmaceutics. 2021;13(7):1009. https://doi.org/10.3390%2Fpharmaceutics13071009

18. Watts J, Deleavey G, Damha M. Chemically modified siRNA: Tools and applications. Drug Discov Today. 2008;13(19–20):842–55. https://doi.org/10.1016/j.drudis.2008.05.007

19. Layzer JM, McCafrey AP, Tanner AK, Huang Z, Kay MA, Sullenger BA. In vivo activity of nuclease-resistant siRNAs. RNA. 2004;10(5):766–71. https://doi.org/10.1261/rna.5239604

20. Damase TR, Sukhovershin R, Boada C, Taraballi F, Pettigrew RI, Cooke JP. The limitless future of RNA therapeutics. Front Bioeng Biotechnol. 2021;9. https://doi.org/10.3389/fbioe.2021.628137

21. Malburet C, Leclercq L, Cotte J-F, Thiebaud J, Bazin E, Garinot M, et al. Size and charge characterization of lipid nanoparticles for mRNA vaccines. Anal Chem. 2022;94(11):4677–85. https://doi.org/10.1021/acs.analchem.1c04778

22. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG. Non-viral vectors for gene-based therapy. Nat Rev Genet. 2014;15(8):541–55. https://doi.org/10.1038/nrg3763

23. Couto LB, High KA. Viral vector-mediated RNA interference. Curr Opin Pharmacol. 2010;10(5):534–42. https://doi.org/10.1016/j.coph.2010.06.007

24. Kasar S, Salerno E, Yuan Y, Underbayev C, Vollenweider D, Laurindo MF, et al. Systemic in vivo lentiviral delivery of mir-15a/16 reduces malignancy in the NZB de novo mouse model of chronic lymphocytic leukemia. Genes & Immun. 2011;13(2):109–19. https://doi.org/10.1038/gene.2011.58

25. Lou W, Chen Q, Ma L, Liu J, Yang Z, Shen J, et al. Oncolytic adenovirus co-expressing miRNA-34a and IL-24 induces superior antitumor activity in experimental tumor model. J Mol Med. 2013;91(6):715–25. https://doi.org/10.1007/s00109-012-0985-x

26. Borel F, Kay MA, Mueller C. Recombinant AAV as a platform for translating the therapeutic potential of RNA interference. Mol Ther. 2014;22(4):692–701. https://doi.org/10.1038/mt.2013.285

27. Lam JK, Chow MY, Zhang Y, Leung SW. siRNA versus miRNA as therapeutics for gene silencing. Mol Ther Nucleic Acids. 2015;4(9). https://doi.org/10.1038%2Fmtna.2015.23

28. Chen Y, Zhu X, Zhang X, Liu B, Huang L. Nanoparticles modified with tumor-targeting scFv deliver siRNA and miRNA for cancer therapy. Mol Ther. 2010;18(9):1650–6. https://doi.org/10.1038/mt.2010.136

29. Pattni BS, Chupin VV, Torchilin VP. New developments in liposomal drug delivery. Chem Rev. 2015;115(19):10938–66. https://doi.org/10.1021/acs.chemrev.5b00046

30. Young SW, Stenzel M, Jia-Lin Y. Nanoparticle-siRNA: A potential cancer therapy? Crit Rev Oncol Hemato. 2016;98:159–69. https://doi.org/10.1016/j.critrevonc.2015.10.015

31. Tokatlian T, Segura T. siRNA applications in nanomedicine. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2010;2(3):305–15. https://doi.org/10.1002/wnan.81

32. Naseri N, Valizadeh H, Zakeri-Milani P. Solid lipid nanoparticles and nanostructured lipid carriers: Structure, preparation and application. Adv Pharm Bull. 2015;5(3):305–13. https://doi.org/10.15171%2Fapb.2015.043

33. Hoy SM. Patisiran: First global approval. Drugs. 2018;78(15):1625–31. https://doi.org/10.1007/s40265-018-0983-6

34. Ilarduya CT, Düzgüneş N, Sun Y. Gene delivery by lipoplexes and polyplexes. Eur J Pharm Sci. 2010;40(3):159-70. https://doi.org/10.1016/j.ejps.2010.03.019

35. Xin Y, Huang M, Guo WW, Huang Q, Zhang L Zhen, Jiang G. Nano-based delivery of RNAi in cancer therapy. Mol Cancer. 2017;16(1). https://doi.org/10.1186%2Fs12943-017-0683-y

36. Singh A, Trivedi P, Jain NK. Advances in siRNA delivery in cancer therapy. Artif Cells Nanomed Biotechnol. 2017;46(2):274–83. https://doi.org/10.1080/21691401.2017.1307210

37. Mao S, Sun W, Kissel T. Chitosan-based formulations for delivery of DNA and siRNA. Adv Drug Deliv Rev. 2010;62(1):12–27. https://doi.org/10.1016/j.addr.2009.08.004

38. Noh SM, Han SE, Shim G, Lee KE, Kim C-W, Han SS, et al. Tocopheryl oligochitosan-based self assembling oligomersomes for siRNA delivery. Biomaterials. 2011;32(3):849–57. https://doi.org/10.1016/j.biomaterials.2010.09.027

39. Şalva E, Özbaş S, Alan S, Özkan N, Ekentok-Atıcı C, Kabasakal L, et al. Combination therapy with Chitosan/siRNA nanoplexes targeting PDGF-D and pdgfr-β reveals anticancer effect in breast cancer. J Gene Med. 2022;25(2). https://doi.org/10.1002/jgm.3465

40. Zhang S, Zhao B, Jiang H, Wang B, Ma B. Cationic lipids and polymers mediated vectors for delivery of siRNA. J Control Release. 2007;123(1):1–10. https://doi.org/10.1016/j.jconrel.2007.07.016

41. Urban-Klein B, Werth S, Abuharbeid S, Czubayko F, Aigner A. RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA in vivo. Gene Ther. 2004;12(5):461–6. https://doi.org/10.1038/sj.gt.3302425

42. Kim Y-K, Minai-Tehrani A, Lee, Cho C-S, Cho M-H, Jiang H-L. Therapeutic efficiency of folated poly(ethylene glycol)-chitosan-graft-polyethylenimine-PDCD4 complexes in H-RAS12V mice with liver cancer. Int J Nanomedicine. 2013;8: 1489-98. https://doi.org/10.2147/ijn.s42949

43. Johnson KS, Conant EF, Soo MS. Molecular subtypes of breast cancer: A review for Breast Radiologists. J Breast Imaging. 2020;3(1):12–24. http://dx.doi.org/10.1093/jbi/wbaa110

44. Ngamcherdtrakul W, Yantasee W. siRNA Therapeutics for breast cancer: Recent efforts in targeting metastasis, drug resistance, and immune evasion. Transl Res. 2019;214:105–20. https://doi.org/10.1016/j.trsl.2019.08.005

45. Ngamcherdtrakul W, Morry J, Gu S, Castro DJ, Goodyear SM, Sangvanich T, et al. Cationic polymer modified mesoporous silica nanoparticles for targeted siRNA delivery to $HER2^{+}$ breast cancer. Adv Funct Mater. 2015;25(18):2646–59. https://doi.org/10.1002/adfm.201404629

46. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: Globocan sources and methods. Int J Cancer. 2018;144(8):1941–53. https://doi.org/10.1002/ijc.31937

47. Schultheis B, Strumberg D, Kuhlmann J, Wolf M, Link K, Seufferlein T, 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(11):3130. https://doi.org/10.3390%2Fcancers12113130

48. Schultheis B, Strumberg D, Santel A, Vank C, Gebhardt F, Keil O, et al. First-in-human phase I study of the liposomal RNA interference therapeutic ATU027 in patients with advanced solid tumors. J Clin Oncol. 2014;32(36):4141–8. https://doi.org/10.1200/jco.2013.55.0376

49. Suraya A, Nowak D, Sulistomo AW, Icksan AG, Berger U, Syahruddin E, et al. Excess risk of lung cancer among agriculture and construction workers in Indonesia. Ann Glob Health. 2021;87(1):8. https://doi.org/10.5334%2Faogh.3155

50. Khan P, Siddiqui JA, Lakshmanan I, Ganti AK, Salgia R, Jain M, et al. RNA-based therapies: A cog in the wheel of lung cancer defense. Mol Cancer. 2021;20(1):54. https://doi.org/10.1186/s12943-021-01338-2

51. Pang L, Shah H, Wang H, Shu D, Qian SY, Sathish V. EPCAM-targeted 3WJ RNA nanoparticle harboring delta-5-desaturase siRNA inhibited lung tumor formation via DGLA peroxidation. Mol Ther Nucleic Acids. 2020;22:222–35. https://doi.org/10.1016/j.omtn.2020.08.024

52. Magalhães M, Alvarez-Lorenzo C, Concheiro A, Figueiras A, Santos AC, Veiga F. RNAi-based therapeutics for lung cancer: Biomarkers, micrornas, and nanocarriers. Expert Opin Drug Deliv. 2018;15(10):965–82. https://doi.org/10.1080/17425247.2018.1517744

53. Kara G, Calin GA, Ozpolat B. RNAi-based Therapeutics and tumor targeted delivery in cancer. Adv Drug Deliv Rev. 2022;182:114113. https://doi.org/10.1016/j.addr.2022.114113

54. Jiang Q, Wei H, Tian Z. Poly I:C enhances cycloheximide-induced apoptosis of tumor cells through TLR3 pathway. BMC cancer. 2008. 8:12. https://doi.org/10.1186/1471-2407-8-12

55. Cunningham D, Salazar R, Sobrero A, Ducreux MP, Van Cutsem E, Scheithauer W, et al. Lefitolimod vs Standard of Care (SOC) for patients with metastatic colorectal cancer (mcrc) responding to first-line standard treatment: Results from the Randomized Phase III IMPALA trial. Ann Onc. 2019;30:v868–9. https://doi.org/10.1093/annonc%2Fmdz394.022

56. Kuźbicki Ł, Brożyna AA. Expression of cyclooxygenase-2 in human epithelial skin lesions. Appl Immunohistochem Mol Morphol. 2021. 29(3):163-174. https://doi.org/10.1097/pai.0000000000000871

57. Schultheis B, Strumberg D, Kuhlmann J, Wolf M, Link K, Seufferlein T, et al. Safety, efficacy and pharmacokinetics 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(11):3130. https://doi.org/10.3390/cancers12113130

58. Golan T, Khvalevsky EZ, Hubert A, Gabai RM, Hen N, Segal A, et al. RNAi therapy targeting KRAS in combination with chemotherapy for locally advanced pancreatic cancer patients. Oncotarget. 2015;6(27):24560–70. https://doi.org/10.18632/oncotarget.4183

59. Wolf D, Baier G. IFNγ helps CBLB-deficient CD8+ T cells to put up resistance to Tregs. Cancer Immunol Res. 2022;10(4):370–370. https://doi.org/10.1158/2326-6066.cir-22-0080

60. Reid G, Pel ME, Kirschner MB, Cheng YY, Mugridge N, Weiss J, et al. Restoring expression of miR-16: A novel approach to therapy for malignant pleural mesothelioma. Ann Oncol. 2013;24(12):3128–35. https://doi.org/10.1093/annonc/mdt412

61. Ganesh S, Cyr W, Koser M, Chopda G, Chipumuro E, Siddiquee Z, et al. Abstract 3827: Preclinical characterization of DCR-BCAT as a component of combination therapy. Cancer Res. 2016;76(14_Supplement):3827–3827. http://dx.doi.org/10.1158/1538-7445.AM2016-3827

62. Bianchi F, Alexiadis S, Camisaschi C, Truini M, Centonze G, Milione M, et al. TLR3 expression induces apoptosis in human non-small-cell lung cancer. Int J Mol Sci. 2020;21(4):1440. https://doi.org/10.3390%2Fijms21041440

63. Mitchell WM. Efficacy of rintatolimod in the treatment of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). Expert Rev Clin Pharmacol. 2016;9(6):755–70. https://doi.org/10.1586/17512433.2016.1172960