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Advances in Lipid-Based Codelivery Systems for Cancer and Inflammatory Diseases

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Combination therapy targeting multiple therapeutic targets is a favorable strategy to achieve better therapeutic outcomes in cancer and inflammatory diseases. Codelivery is a subfield of drug delivery that aims to achieve combined delivery of diverse therapeutic cargoes within the same delivery system, thereby ensuring delivery to the same site and providing an opportunity to tailor the release kinetics as desired. Among the wide range of materials being investigated in the design of codelivery systems, lipids have stood out on account of their low toxicity, biocompatibility, and ease of formulation scale-up. This review highlights the advances of the last decade in lipid-based codelivery systems focusing on the codelivery of drug–drug, drug–nucleic acid, nucleic acid–nucleic acid, and protein therapeutic-based combinations for targeted therapy in cancer and inflammatory diseases.

1. Introduction

Increasing resistance to monotherapy has become a serious challenge globally due to multiple issues, such as the emergence of drug resistance, an increase in the development and frequency of target site mutations, or the ability of the cells to compensate for the target through multiple available redundancy mechanisms.[1] The involvement of interconnected signaling pathways, complex molecular networks, and the crosstalk between them mitigates the efficacy of drugs acting on a single molecular target.[2] In complex diseases such as cancer, targeted cytotoxic monotherapy inadvertently leads to intense evolutionary selection pressure on the surviving tumor cells, thereby promoting development of drug-resistant populations.[3] Combination therapy has shown promise to tackle these problems.[4] Combination therapy refers to the strategy of taking advantage of the potential synergistic action of two or more therapeutic agents or approaches with the hope that these may succeed where monotherapies fail.[5] In general, combination therapy involves using multiple therapeutics acting on different pathways or multiple therapeutics targeting different components of the same pathway.[6] Using more than one drug or therapeutic modality in combination offers several advantages, including delaying the emergence of resistance, reducing treatment duration, improving therapeutic outcomes, and allowing the use of a lower concentration of drugs, thus reducing the toxic effects.[7] The combination of two drugs can usually achieve favorable outcomes through additive, synergistic or potentiation effects. The combination is said to be additive when the therapeutic effect is equal to the sum of the individual therapeutics in the combination and synergistic, when the combined effect exceeds the sum of the individual therapeutics. Potentiation refers to the improvement of therapeutic efficacy of one drug by another through regulation of its pharmacokinetic profile.[8] Additionally, in certain cases, combining antagonistic drugs can also be fruitful.[9] A detailed discussion on the different mechanisms of combination therapy can be found in an excellent review by Hu et al.[8]

Widespread efforts are being undertaken recommending combination therapy as a standard first-line treatment for multiple cancers and inflammatory diseases.[10–13] The plethora of cancer research has unequivocally established the benefits of combination therapy and the importance of targeted delivery systems for the said combination therapy. In contrast, delivery systems for combination therapies to treat inflammatory diseases are relatively unexplored and warrant further attention. Inflammatory diseases can be systemic (e.g., systemic lupus nephritis) or tissue-specific [e.g., inflammatory bowel disease (IBD), psoriasis, and rheumatoid arthritis (RA)], and the treatment usually involves immunosuppressants, anti-inflammatory drugs, and corticosteroids either alone or in combination.[14] Combination therapy with a tumor necrosis factor-α (TNF-α) inhibitor (infliximab) and an immunosuppressor (azathioprine) led to superior therapeutic outcomes in treatment naïve patients, as seen in the UC SUCCESS trial for ulcerative colitis and SONIC trial for crohn's disease.[15,16] Similarly, multiple clinical trials have demonstrated the superiority of combination therapy compared to monotherapy for various other inflammatory diseases.[15,17]

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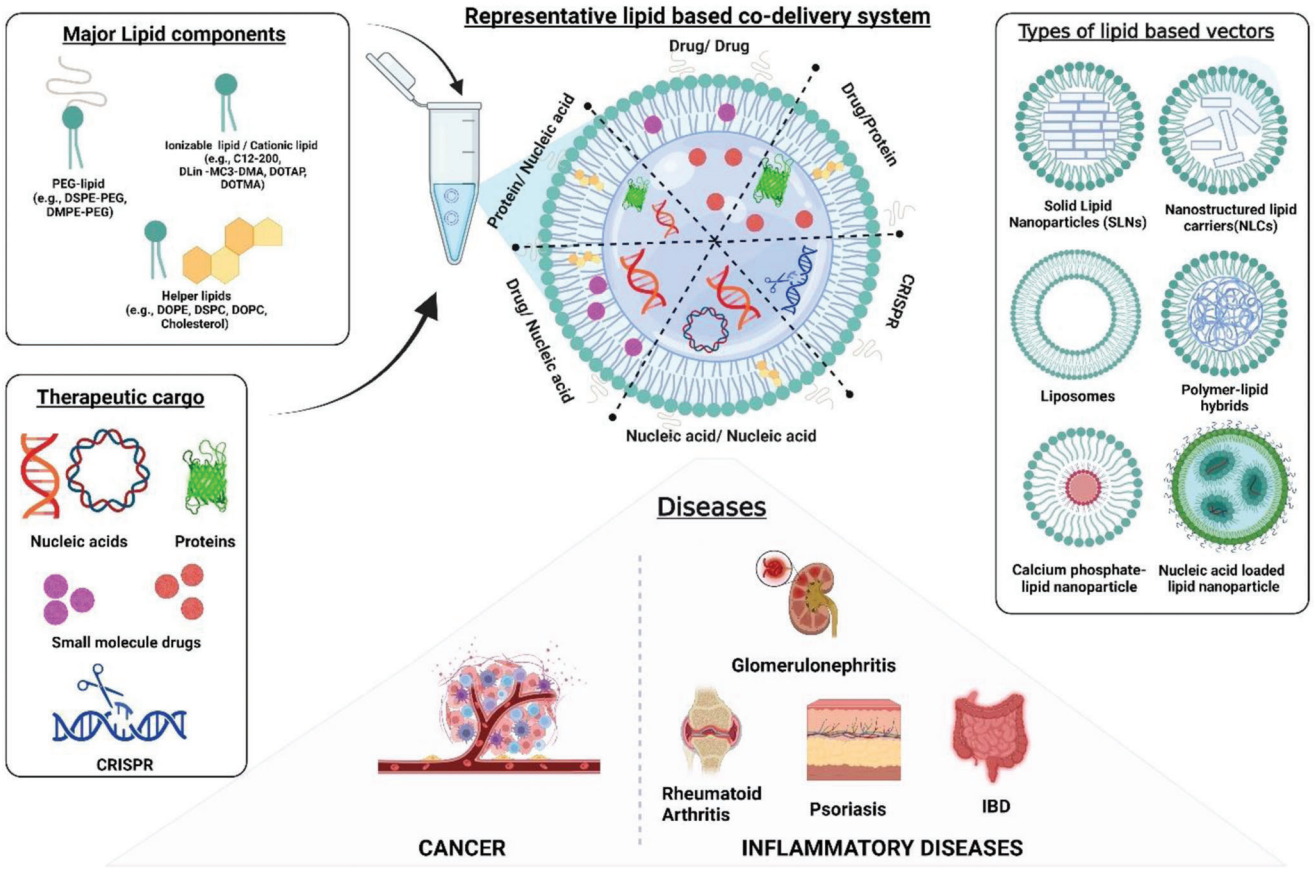

Figure 1. Schematic overview of the review. The central lipid nanoparticle represents the different types of lipid-based carriers as well as the different combinations of therapeutics discussed in this review. Major types of lipid-based vectors are shown on the right. Boxes on the left show the major lipid components involved in formulation and the various classes of therapeutics that can be codelivered for combination therapy. Finally, the section at the bottom represents the two major classes of diseases that have been discussed in this review. (DSPE-PEG: 1, 2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-Poly(ethylene glycol), DMPE-PEG: 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphoethanolamine-Poly(ethylene glycol), DOTAP: 1,2-Dioleoyl-3-trimethylammonium propane, DOTMA: 1,2-*di*-*O*-octadecenyl-3-trimethylammonium propane, DOPE: dioleoyl phosphatidylethanolamine, DSPC: 1,2-Distearoyl-*sn*-glycero-3-phosphocholine, DOPC: 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine). Created with BioRender.com.

However, with all its advantages, conventional combination therapy is limited by several issues, such as obtaining an optimum therapeutic ratio at the target site, drug stability, differences in pharmacokinetics, drug interactions, and the possible cumulative toxicity of the combination. It has also been reported that drugs in a combination oftentimes distribute unequally to all affected regions of the body. This can greatly diminish the chances of treatment success by creating regions with therapeutically relevant concentrations of only one drug from the combination. Such conditions provoke cells to develop resistance to individual drugs in the combination, eventually leading to multidrug resistance.[18] Advances in research and the emergence of RNA interference (RNAi) have updated the therapeutic cargo in combination therapy to include nucleic acids (NAs), peptides, antibodies, and proteins besides traditional small molecule drugs. Proteins and NAs are unstable and need to be protected from degradation until they reach the target site. Hence, there is a tremendous need for advancing traditional formulations to safely deliver such therapeutic agents to their desired site of action within the body.

Codelivery is a subfield of drug delivery that aims to achieve combined delivery of diverse therapeutic cargoes within the same delivery system, thereby ensuring delivery to the same site and providing an opportunity to tailor the release kinetics as desired.[19] Chemically diverse cargo such as NAs and small-molecule drugs can be delivered in the same carrier to address issues associated with conventional combination therapy. Codelivery systems have been designed using several formulations that have been traditionally used for the delivery of single drugs. However, systems designed for the delivery of a single therapeutic cargo have some limitations in codelivery, and much of the effort today is focused on developing novel delivery systems capable of codelivering cargos having varied properties, improving coloading efficiencies, and controlling release rates.[19] Among the plethora of materials used to deliver drugs and biologicals simultaneously, lipids have stood out due to their excellent safety profile, high biocompatibility, and ease of scale-up.[20] This review attempts to highlight the developments in lipid-based codelivery systems for targeted therapy in cancer and inflammatory diseases over the past decade (**Figure 1**).

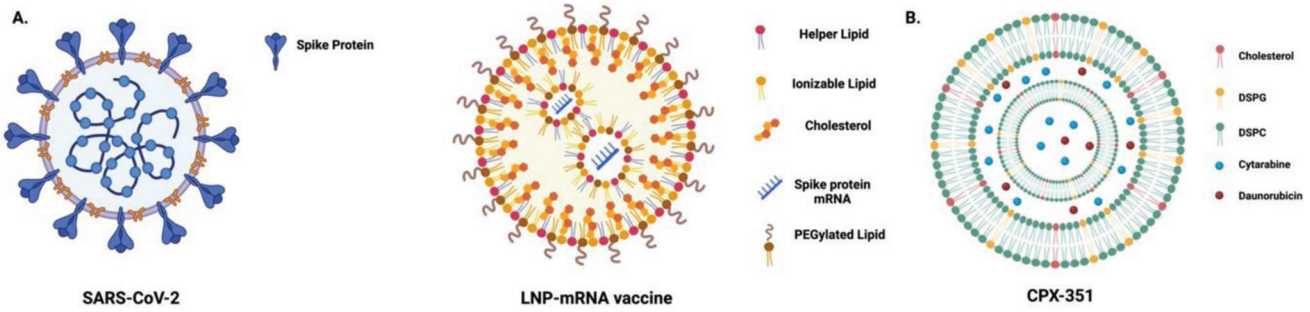

Figure 2. A) Lipid nanoparticle (LNP)-based mRNA vaccine formulation against SARS-CoV-2. B) Diagrammatic illustration of CPX-351 liposome. The liposomes are 100 nm in diameter and are made up of 2 concentric phospholipid bilayers. 1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC), distearylphosphatidylglycerol (DSPG), and cholesterol in a molar proportion of 7:2:1 make up the lipid membrane. Cytarabine and daunorubicin are enclosed in the aqueous spaces at a 5:1 molar ratio. Created with BioRender.com.

Lipids are hydrophobic or amphipathic substances usually soluble in organic solvents.[21] Traditionally lipids have been extensively used to improve the oral bioavailability of drugs with low solubilities.[22,23] Ease of processing and favorable physicochemical properties have permitted the use of lipids in the design of new delivery systems, and today, lipids are among the most widely explored materials for the development of codelivery platforms.[24] Lipid-based delivery systems stand out in the crowd due to multiple advantages such as the potential to deliver both hydrophilic and hydrophobic drugs, capacity to codeliver vastly dissimilar cargo like drugs and NAs, ease of surface modification, low toxicity, ability to be administered through multiple delivery routes, and allowing control of release kinetics.[25] Lipid-based systems have the best chances for clinical translation, with liposomes representing the maximum number of clinically approved nanoscale formulations, including Doxil—the first FDA approved liposome.[26] In addition to small molecule drugs, Patisiran/Onpattro is a formulation developed by Alnylam Pharmaceuticals, consisting of siRNA encapsulated in a lipid nanoparticle.[27] Patisiran received FDA approval in 2018 and is the first FDA-approved nanoparticle for the delivery of RNAi therapeutics. Apart from these well-known examples, a number of other lipid-based systems are presently under different stages of clinical trials.[28] Moreover, the recently approved two COVID-19 vaccines, mRNA-1273 and BNT-162b2 have been formulated using lipid nanoparticles (LNP) containing ionizable lipids to deliver mRNA capable of encoding the spike protein of SARS-CoV-2. Formulation of the LNP-based vaccine is described in **Figure 2**A.[29]

With phenomenal success already seen in the delivery of monotherapeutics, lipid-based delivery systems were naturally the preferred choice for the design of codelivery systems. Recently, a bilamellar liposomal formulation (size 100 nm) comprising daunorubicin and cytarabine in a 1:5 synergistic ratio was developed and marketed as VYXEOS/CPX-351 by Jazz Pharmaceuticals. VYXEOS received FDA approval for the treatment of acute myeloid leukemia and is the first FDA approved codelivery system for the simultaneous delivery of two free drugs (Figure 2B).[30,31] In a pivotal efficacy study, VYXEOS demonstrated better efficacy at lower collective daunorubicin and cytarabine doses, further cementing the benefits of codelivery systems.[32] Additionally, a Phase I clinical trial of two mRNAs encoding fragments of an anti-chikungunya antibody is currently underway.[33]

Apart from clinically established liposome-based systems and lipid nanoparticles, nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), and polymer-lipid hybrids represent additional classes of lipid-based systems being widely investigated in several preclinical studies for the codelivery of therapeutics.[34–36] Additionally, combining the lipid prodrug technique with nanoparticles has emerged as an effective way to improve solubility and stability of poorly soluble drugs while enabling codelivery of additional therapeutic cargo. Lipids such as fatty acids, triglycerides, and phospholipid compounds have been widely used for conjugation to small-molecule drugs.[37,38] LNPs incorporating a small-molecule prodrug have also been used for codelivery of biologics, such as nucleic acids.[39,40] With increasing understanding about the molecular basis of diseases and the increasing emphasis on individualized therapeutics and precision-based approaches, the therapeutic landscape has evolved over the past few decades. Small molecule drugs are no longer the primary class of therapeutics, and new generations of therapeutics have evolved to include NAs, proteins, peptides, and monoclonal antibodies. These new therapeutic classes have brought about additional challenges in the formulation of delivery systems and have allowed a multitude of permutations and combinations in the therapeutic cargo involved in combination therapeutics. Advances in biomaterial sciences have allowed the codelivery of these combinations, especially in various cancer types and inflammatory diseases. Lipid-based codelivery systems for the delivery of these therapeutic combinations are discussed below.

2. Lipid-Based Codelivery Systems for Drug–Drug Combination Therapy

The strategy of using multiple drugs simultaneously for the treatment of diseases is not new; it has been known for a long time that combination therapy with two or more drugs having complementary effects leads to improved therapeutic outcomes compared to monotherapy.[4,41] Combination therapy to treat cancer was first postulated in 1965 when Frei and co-workers explored the possibility of combining methotrexate (MTX), 6-mercaptopurine, vincristine (VCR), and prednisone to treat children with acute lymphocytic leukemia.[42] The regimen was successful in decreasing tumor burden and extending remission. Since then, combination therapy has come a long way and is now

Table 1. Lipid-based drug–drug codelivery systems.

Nanocarrier	Therapeutic agents	Disease	Refs.
Liposomes	Quercetin and VCR	Breast cancer	[46]
	DOX and DHA	Colon cancer	[49]
	DOX and CQ	Breast cancer	[50, 51]
	LY294002 and 5-FU	Esophageal squamous cell carcinoma	[52]
	Resveratrol and PTX	Breast cancer	[53]
	Simvastatin and PTX	Lung cancer	[55]
	Epirubicin and ascorbic acid	Breast cancer	[57]
	Dexamethasone and diclofenac	Osteoarthritis	[77]
Solid lipid nanoparticles	DOX and α-tocopherol	Breast and ovarian cancer	[58]
	Aspirin, curcumin, and sulforaphane	Pancreatic cancer	[60]
	Dexamethasone and butyrate	IBD	[71]
Nanostructured lipid carriers	DOX and docosahexaenoic acid	Breast cancer	[62]
	DOX and VCR	Lymph cancer	[63]
	Calcipotriol and MTX	Psoriasis	[73]
Lipospheres	Tacrolimus and curcumin	Psoriasis	[74]
Liposomal gel	Trans retinoic acid and betamethasone	Psoriasis	[75]
Cerosomes	MTX and nicotinamide	Psoriasis	[76]

the primary treatment for several cancer types. Taking inspiration from its success in cancer, a number of inflammatory diseases are now regularly treated using combination therapy.[41,43] Drugs used in combination should have a positive outcome. To analyze the interaction between two potential drugs to be coadministered, isobolographic and combination index analyses are used.[44] Although, in theory, a combination of two or more drugs has advantages over monotherapy, utilizing the full potential of combination therapy is still hindered by dissimilar pharmacokinetics, biodistribution, and physicochemical characteristics of individual drugs in the combination. Lipid-based delivery systems promise to solve these problems (**Table 1**).

2.1. Drug–Drug Combinations to Treat Cancer

Development of anticancer small molecules has flourished in the recent years. The majority of these attempts have focused on delivering anticancer therapeutics as combinations to explore a multitarget treatment approach to treat cancer. Conventionally, combination therapy to treat cancer involves the use of multiple chemotherapeutics acting via different mechanisms to achieve synergistic effects. Traditional drug combinations include MTX, anthracyline- and paclitaxel (PTX)-based combinations.[45] Using novel drug delivery systems has benefitted traditional combinations by overcoming the differences in their physicochemical properties. Combinations of hydrophobic and hydrophilic drugs can now be codelivered in a single nanocarrier. Similarly, achieving ratiometric drug loading led to a significant impact on their efficacy. Wong and co-workers explored the liposomal formulations of quercetin and VCR for the treatment of estrogen-negative breast cancer.[46] The formulation was intended to overcome the low water solubility of quercetin. Additionally, they carried out work to achieve a synergistic molar ratio (1:2) of quercetin and VCR, which were coencapsulated in egg sphingomyelin-based liposomes. The formulation exhibited ≈78% encapsulation efficiencies for both drugs with a combination index of 0.113 and a dose-reduction index of 115 (at ED_{50} for VCR), respectively. Furthermore, an ≈9 times enhancement in quercetin solubility (in comparison with pristine quercetin) was achieved by adopting the liposomal formulation. Similarly, newer combinations such as FOLFIRINOX are also benefitted by advances in lipid-based drug delivery systems.[47] A schematic presentation of FOLFIRINOX encapsulated multiple layer-by-layer lipid-based nanoparticles has been presented in **Figure 3**.

However, the evolution of multi drug resistance (MDR) has stymied the positive effects seen with these traditional combinations. To overcome this, combinations with chemosensitizers and chemotherapeutic drugs have been explored. Chemosensitizers act in a potentiation effect and enhance tumor cell sensitivity toward chemotherapeutic drugs.[48] The development of resistance to chemotherapeutics is mainly regulated by the transmembrane efflux pumps, which are overexpressed in most MDR cancers, and pump out chemotherapeutics from the cell, thereby reducing their efficacy. A number of efflux pump inhibitors and MDR protein down regulators such as curcumin, verapamil, and cyclosporine are now being coadministered in combination with chemotherapeutic drugs to improve their efficacy. Kang et al. developed a mannosylated liposomal formulation to deliver doxorubicin (DOX) and dihydroartemisinin (DHA) for the treatment of drug-resistant colon cancer.[49] The presence of DHA resensitized colon cancer cells to DOX and inhibited Bcl-xl, a vital pathway in drug resistance leading to an antitumor effect. Enhanced expression of autophagic responses in the tumor cells is thought to be involved in MDR development in cancer cells. Hence, combination therapy with autophagy inhibitors and cytotoxic chemotherapeutic drugs has been proven to be effective in overcoming MDR. Recently, chloroquine (CQ) has been repurposed as an autophagy inhibitor. Gao et al. formulated a liposome codelivering DOX and CQ, and analyzed the mechanism by which it reversed drug

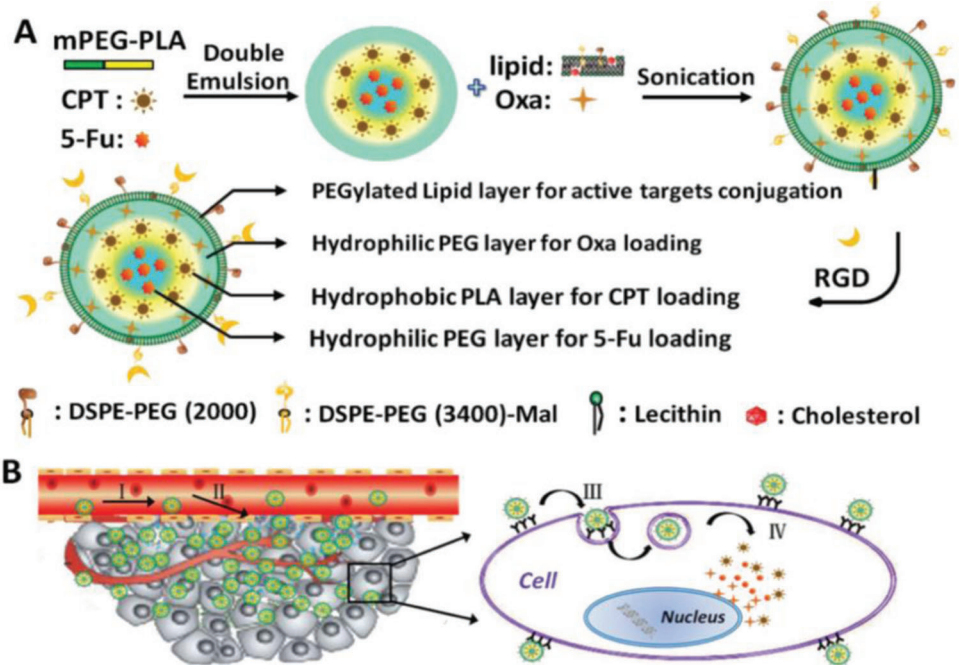

Figure 3. A) Schematic representation of FOLFIRINOX encapsulated lipid-based multiple layer-by-layer nanoparticles. FOLFIRINOX is a combination of folinic acid, oxaliplatin (Oxa), 5-fl uorouracil (5-Fu), and irinotecan (Camptosar, CPT). The mPEG-PLA helps in encapsulation of 5-Fu. 1, 2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-Poly(ethylene glycol) (DSPE-PEG) on the corona of the nanoparticle leads to the prolonged circulation in vivo, and the surface conjugated RGD polypeptide leads to the higher tumor accumulation. B) Diagrammatic representation of the transport of nanoparticles and their accumulation at the tumor site followed by the release of FOLFIRINOX. Reproduced with permission.[47] Copyright 2014, Wiley-VCH GmbH.

resistance in DOX-resistant MCF-7/ADR cells and HL60/ADR cells which overexpress P-gp and MRP1, respectively.[50,51] The enhanced antitumor effect was validated in vitro in tumor spheroids and in vivo in tumor-bearing zebrafish. A cell viability assay revealed that the IC_{50} of the liposome codelivering DOX and CQ in MCF7/ADR cells was ≈6-fold less compared to free DOX, whereas it was 19.5-fold less in HL60/ADR cells. In the presence of CQ, the levels of autophagy-associated protein LC3-II were elevated, indicating that inhibition of autophagy might be involved in the mechanism of MDR reversal. Working on similar principles, Feng et al. developed a PEGylated liposome coencapsulating an autophagy inhibitor LY294002 (LY) and 5-fluorouracil (5-FU) and tested its efficiency against esophageal squamous cell carcinoma.[52] The liposome demonstrated a programmable release, with LY being released much faster than 5-FU. The initial release of LY led to autophagy inhibition and an improved response of tumor cells to 5-FU, leading to enhanced cell death. Resveratrol, a well-known phytoestrogen, has been suggested to reverse MDR in breast cancer. Meng et al. developed PEGylated liposomes coencapsulating resveratrol and PTX to overcome the challenge of MDR in breast cancer.[53] The liposomes were assessed in mouse models of both drug-sensitive and drug-resistant breast cancer. In both cases, the coloaded liposome formulation showed about 80% of inhibition of tumor growth. In contrast, resveratrol only or PTX only liposomes barely showed any inhibition effect. This proved that codelivery of drugs could possibly overcome drug resistance in vivo. Cell penetrating peptides decorating the surface of nanocarriers is an important strategy to improve tumor penetration.[54] Jin et al. reported development of a liposome decorated with a cell penetrating peptide for combined delivery of simvastatin and PTX. Simvastatin functioned to reverse the epithelial-mesenchymal transition associated resistance in lung cancer. The system showed enhanced efficacy against drug-resistant nonsmall cell lung cancer.[55] Ascorbic acid in addition to being cytotoxic to cancer cells, potentiates the antineoplastic activity of a number of chemotherapeutic agents, especially anthracyclines in breast carcinoma cells.[56] Lipka et al. developed a novel formulation based on vitamin C driven loading of epirubicin in liposomes. Enhanced antineoplastic effect was seen attributable to the combination of epirubicin and ascorbic acid in the 4T1 murine mammary cancer orthotopic model.[57]

Oliveria et al. developed SLNs for the combined delivery of DOX and α-tocopherol succinate and evaluated their potential to subdue drug resistance and improve antitumor efficacy in cancer cell monolayer as well as in spheroids. The SLNs were coloaded with an encapsulation efficiency of >95%. The SLNs led to a notable decrease in spheroid volume when compared to free DOX.[58] Grandhi et al. formulated orally administered SLNs for combination delivery of aspirin, curcumin, and sulforaphane. The effective inhibitory doses were reduced 10-fold in a Syrian golden hamster pancreatic carcinogenesis model when the drugs were coadministered in SLNs.[59] Thakkar et al. further evaluated the efficacy of this system in a transgenic mouse model of pancreatic cancer and provided an in vivo proof of concept.[60] NLCs show a higher loading capacity for drugs mainly due to a less organized matrix courtesy of blending a liquid lipid with a solid lipid.[61] Mussi et al. formulated NLCs for the codelivery of DOX and docosahexaenoic acid and evaluated its ability to combat drug resistance and improve antitumor efficacy in a drug-resistant breast cancer cell line. Formulated NLCs had a size

of ≈80 nm and demonstrated a DOX encapsulation efficiency of nearly 100%. Furthermore, in comparison with free DOX, coloaded NLCs showed enhanced cytotoxicity on MCF-7/ADR monolayers and spheroids.[62] Similarly, Dong and coworkers reported NLCs coloaded with DOX and VCR to combat drug resistance and prevent lymph cancer relapse. The coloaded NLCs demonstrated a sustained release of DOX and VCR up to 16 and 48 h, respectively, leading to an enhanced synergistic antitumor effect.[63] NLCs have further been developed to impart targeting properties as well. Using an appropriate linker, targeting moieties can be attached to the NLC surface.[35] Few examples of such applications include cisplatin and PTX coloaded NLCs targeting folate receptor for head and neck cancer, temozolomide, and VCR containing NLCs targeting lactoferrin receptors in glioblastoma, and gemcitabine and PTX coloaded NLCs targeting glucose receptors in nonsmall cell lung cancer.[64–67]

2.2. Drug–Drug Combinations to Treat Inflammatory Diseases

The combined use of multiple anti-inflammatory agents with different mechanisms of action has made it possible to simultaneously target various pathways that drive the pathogenesis of inflammatory diseases. Drug combinations in RA and psoriasis include combinations of immunosuppressants, nonsteroidal anti-inflammatory drugs, corticosteroids, and disease modifying antirheumatic drugs. There have been limited reports for delivery of small molecule combinations using lipid nanocarriers for treating IBD. This is mainly because the focus has been on strategies that increase the colonic specificity of the delivery systems. Majority of the applications reported in literature have harnessed pH-responsive polymers to ensure efficient delivery of therapeutics.[68] Exploratory delivery systems have either been individual small molecule drugs loaded into nanoparticles coated with pH-responsive or mucoadhesive polymers or associated with polymers to form degradable or nondegradable polymer-drug conjugates.[69,70] Dianzani et al. developed SLNs coloaded with dexamethasone (DEX) and butyrate and assessed their efficacy in a mouse model of dextran sulfate sodium salt (DSS)-induced colitis. They reported that coloaded SLNs at concentrations of 2.5 nmol L^{-1} and 0.1 µmol L^{-1} for DEX and butyrate, respectively, exerted a synergistic effect. A significant reduction in inflammation-inducing cytokines and histopathological indicators of inflammation was seen in vivo.[71] Serpe et al. evaluated the same drug combination coloaded in SLNs on peripheral blood mononuclear cells obtained from human IBD patients. The coloaded SLNs showed a significant reduction in IL-1β and TNF-α with a contrasting increase in IL-10 as compared to free drugs at highest tested concentration, indicating a positive anti-inflammatory effect.[72]

Psoriasis represents a challenging application for combination therapy due to the inconvenience of administration. This is because antipsoriatic drugs usually have extremely different polarities making it difficult to incorporate them within a single vehicle. Lin and co-workers explored codelivery of calcipotriol and MTX using Precirol and squalene based NLCs for topical delivery to treat psoriasis. Calcipotriol and MTX have a log P of 4.6 and 2.2, respectively, making their polarities significantly different. As a result, higher incorporation of calcipotriol was observed into the Precirol of the NLCs. Since MTX is hydrophilic, it was observed that its release was not dependent on the composition of NLCs. However, the ability of both the drugs to permeate the stratum corneum (SC) was enhanced when coloaded into NLCs.[73] This was one of the works that showed promise of NLCs in treating psoriasis via topical administration. With a similar approach, Jain and co-workers investigated codelivery of tacrolimus and curcumin using lipospheres, which are solid lipid particles up to 50 nm in size.[74] The rationale behind co-encapsulation of drugs was to overcome issues with solubility, skin penetration, and absorption. Lipospheres contained a 1:1 molar ratio of the drugs with a total loading content of 7.5%. Lipospheres were further suspended in hydroxyl propyl methyl cellulose-based gel and demonstrated deeper skin penetration and sustained drug release compared to free drugs in a similar gel. Enhanced antipsoriatic activity of the gel with coloaded particles was seen in an imiquimod (IMQ) induced psoriasis mouse model. The animals treated with the particles also showed lower levels of proinflammatory cytokines. Wang et al. developed a liposomal gel coloaded with trans-retinoic acid and betamethasone for psoriasis treatment. They reported a particle size of >70 nm with almost 99% encapsulation efficiency for both drugs. To further increase the applicability of the liposomes, they were incorporated into a carbomer gel for topical application. Cellular studies showed time-dependent uptake of the drugs. They observed that the liposomes in the gel showed enhanced skin permeation as compared to free drugs in the gel or coloaded liposomes. Enlargement of the spleen, which is a characteristic of immunological diseases, was observed in mice affected by psoriasis. In contrast, the group that received the coloaded liposomal gel showed a reduction in spleen weight and size, indicating a positive impact. Histopathological evaluation showed a reduction in psoriatic characteristics in mice treated with liposomal gels in comparison to control animals. This was evidenced by a reduction in epidermal thickness, keratosis, and decreased immune cell infiltration in the epidermis. The animals treated with the liposomal gels also showed a reduction in inflammation-inducing cytokines, TNF-α, and IL-6.[75] Ceramides are sphingolipids and are prominent in the stratum corneum, and ceramide-based topical delivery systems have been widely explored. Yang et al. explored the therapeutic effects of ceramide niosomes for the codelivery of MTX and nicotinamide in an IMQ induced psoriasis-like mouse model. Nicotinamide has anti-inflammatory properties and has been widely used as an adjuvant in topical formulations. Nicotinamide also works as a hydrotrope to improve the solubility of lipophilic drugs. Nicotinamide effectively improved MTX solubility which subsequently led to improved skin permeability and better therapeutic outcomes in the treatment of psoriasis. In addition, the blank cerosomes exhibited moderate anti-inflammatory activity indicating their contribution to the synergistic effect seen with the system.[76]

Chang et al. investigated the efficacy of hyaluronan loaded DEX and diclofenac liposomes for treating osteoarthritis.[77] They hypothesized that this formulation would exhibit sustained release and enhanced anti-inflammatory activity. The prepared liposomes had a diameter of ≈100 nm. The DEX and diclofenac content were 22.5% and 2.5%, respectively. Data indicated that the drugs took 4 h to reach effective concentration, and a prolonged drug release could be seen at least up to 168 h. The liposomes

Table 2. Lipid-based drug-gene codelivery systems.

Nanocarrier	Drug	Gene	Disease	Refs.
Liposome	Sorefenib	VEGF siRNA	Hepatocellular carcinoma	[91]
	PTX	Survivin siRNA	Lung cancer	[93]
	Docetaxel	BCL-2 siRNA	Lung cancer	[94]
	DOX	SATB1 shRNA	Gastric cancer	[97]
	PTX	Antagomir 10b	Breast cancer	[101]
	DOX	miR-375	Hepatocellular carcinoma	[102]
	Curcumin	STAT3 siRNA	Skin cancer	[105]
	Methotrexate	NF-kB siRNA	Rheumatoid arthritis	[123]
	Dexamethasone	NF-κB decoy ODNs	Rheumatoid arthritis	[124]
	DHA	HMGB1 siRNA	Lupus nephritis	[126]
Nanostructured lipid carrier	DOX/PTX	BCL-2/MRP-1 siRNA	Lung cancer	[95]
	Tacrolimus	TNF-α siRNA	Psoriasis	[121]
Lipid-polymer hybrid	DTX	miR-34a	Breast cancer	[103]
	Capsaicin	TNF-α siRNA	Psoriasis	[118]
	Dexamethasone	MCL-1 siRNA	Rheumatoid arthritis	[122]
Lipoplex	Docetaxel	SIRT1 shRNA	Breast cancer	[98]
Solid lipid nanoparticle	PTX	miR-34a	Skin cancer	[104]
Lipoid nanoparticle	PTX	VEGF siRNA	Lung cancer	[92]
Lipid nanocarriers	Erlotinib	IL36α siRNA	Psoriasis	[119]

showed a higher proliferation of articular chondrocyte cells without eliciting cytotoxicity. In vivo imaging studies showed a significant reduction in knee joint inflammation in osteoarthritis-affected mice over 4 weeks. The liposomal formulation exhibited ≈78% reduction in inflammation volume over the span of those 4 weeks after a single injection.

3. Lipid-Based Codelivery Systems for Drug-Nucleic Acid Combination Therapy

Combination therapy has seen great success in treatment of complex diseases due to its major advantage of improved efficacy. Although a synergistic effect could be achieved with the appropriate combination of therapeutic drugs, the emergence of MDR has blunted the efficacy of traditional small molecule drug combinations. MDR in cancers is usually due to the presence of efflux pumps on tumor cells and enhanced resistance to apoptosis. RNAi is a promising strategy to tackle these problems.[78] Advances in many high throughput "omic" techniques have led to the identification of several genes/proteins involved with inflammation and the development of cancer. NAs can act in a supportive role when coadministered with drugs and downregulate the proteins involved in developing chemoresistance and subsequently enhance the accumulation and thus the efficacy of anticancer drugs.[79] Similarly, in inflammation, siRNA can target the proinflammatory cytokines in combination with traditional anti-inflammatory and immune-modulatory drugs to achieve a synergistic effect. Primary strategies currently involved in the codelivery of NAs and chemotherapeutics involve knocking down proteins associated with drug efflux, blocking cell survival pathways and inhibiting antiapoptotic factors. Also, knockdown of genes involved in angiogenesis has shown great promise in codelivery of chemotherapeutic agents with NAs.[80] Utilizing RNAi in the treatment of inflammatory diseases is a relatively less explored area and most of the research till date has been focused on targeting genes in the inflammatory cascade.[81–83]

The vast structural differences in small molecule drugs and NAs, lead to vastly different pharmacokinetics and other complexities which limits their use. This complexity of variation has been overcome by codelivering the drugs and NAs in a single nanocarrier either by coencapsulation or by synthesizing bioactive cationic polymers which can then complex the NAs. This ensures the colocalization of the cargos and overcomes the pharmacokinetic issues, maximizing the chances of achieving the highest synergistic efficacy.[84–87] Also, in cases where NAs are used to inhibit the expression of genes involved in the development of drug resistance; silencing of genes temporarily sensitizes the cells to the anticancer drug. This creates a short timeframe in which the drug is effective. Therefore, vital to the success of this codelivery strategy will be the design of a carrier system that allows tuning of cargo release to enable the RNAi to take effect before the drug is released.[88,89] Since small molecule drugs and NAs have vastly dissimilar biological and physicochemical properties, developing a successful formulation codelivering drugs and NAs is difficult. While a few lipid-based RNAi formulations have already become clinical realities, these carriers have been formulated for delivery of NAs as the only therapeutic cargo and are not suitable for codelivery of drugs and NAs. Inclusion of an additional cargo in the form of a drug requires a complete overhaul of the nanoparticle design.[90] Selective successful examples of drug-NA codelivery systems have been summarized in **Table 2**.

3.1. Drug-Nucleic Acid Combinations to Treat Cancer

Efforts to improve the therapeutic outcome of chemotherapy by inhibiting or bypassing the various chemoresistance

mechanisms via the combined delivery of NAs and anticancer drugs have shown promising results over the past couple of years. A sufficient supply of blood to the tumor vessels is essential for the growth and progression of tumors, and hence, suppressing angiogenesis is considered to be a promising strategy to tackle tumor progression. Vascular endothelial growth factor (VEGF) is regarded as one of the major players in the regulation of angiogenesis, and its inhibition effectively suppresses tumor angiogenesis and growth. Yao et al. formulated a pH-responsive liposome to suppress the VEGF signaling pathway through combined delivery of sorafenib, a multikinase inhibitor blocking VEGF receptor and a siRNA targeting VEGF. The liposomes could disassemble in the slightly acidic environment of the tumors and could synergistically downregulate the VEGF expression significantly compared to single loaded liposomes.[91] A similar synergistic effect was demonstrated by Zhang et al., who developed novel tripeptide lipoid nanoparticles for the codelivery of PTX and anti-VEGF siRNA.[91,92]

Overexpression of antiapoptotic genes like survivin and BCL-2 is involved in the generation of resistance to chemotherapeutic agents. Silencing these antiapoptotic genes is expected to reduce tumor growth, chemosensitize resistant tumors, and improve treatment outcomes. Zhang et al. formulated a cationic liposome for the codelivery of PTX and survivin siRNA to decrease the threshold of apoptosis in lung cancer cells and improve PTX efficacy at lower doses. The IC_{50} of the formulation was indeed found to be 3.5 times lower against NCI-H460 cells than that of PTX, suggesting a positive synergistic outcome.[93] Qu et al. used PEGylated liposomes to codeliver docetaxel and siRNA against BCL-2. The targeted delivery of the liposomes achieved a synergistic effect in an A549 tumor xenograft model.[94] Moving away from the commonly used liposomes, Taratula et al. developed LHRH (luteinizing hormone-releasing hormone) decorated NLCs for the codelivery of siRNAs against MRP-1/BCL-2 and DOX/PTX for lung cancers in vivo. MRP-1 and BCL-2 were chosen to suppress both, efflux pump mediated and nonpump mediated resistance in lung cancer cells.[95]

While siRNAs represent the simplest approach to RNAi, their irregular transfection efficiencies have limited their wide-spread use. Similarly, siRNA concentration gets gradually diluted with each cell division cycle, therefore siRNA induced gene silencing is short lived and prolonged knockdown of the target gene is unfeasible. DNA vector mediated synthesis of short hairpin RNAs (shRNAs) within the cell attempts to overcome these drawbacks of siRNAs.[96]

Peng et al. formulated novel thermosensitive magnetic liposomes to codeliver DOX and a shRNA encoding plasmid targeted to the special AT rich binding protein (SATB1) and assessed its synergistic efficacy against gastric cancer. The liposomal system could be directed to the tumors by a magnetic field followed by hyperthermia responsive DOX release at the tumor site. The combination therapy enhanced apoptosis and inhibited tumor growth in a murine MKN-28 induced orthotopic tumor model compared to all other groups tested.[97] Swami et al. developed a pH-sensitive lipoplex system to codeliver shRNA against SIRT1 and docetaxel. The combination therapy improved survival and reduced tumor growth in rats with 7, 12 – dimethylbenz[*a*]anthracene (DMBA) induced breast cancer.[98]

MicroRNAs (miRNAs) are a subtype of noncoding RNAs that are upregulated in several cancers and are involved in the progression of metastasis. Antagomirs are synthetic oligonucleotides used for silencing miRNAs.[99,100] Zhang et al. developed liposomes decorated with a pH-dependent antimicrobial peptide for the codelivery of antagomir-10b (metastasis inhibitor) and PTX for treating metastatic breast cancer. The combination therapy slowed down 4T1 primary tumor growth, while simultaneously reducing pulmonary metastases in vivo.[101]

Tumor-suppressive miRNAs are another therapeutic approach for gene alteration in cancer cells. Combinations of miRNAs and small-molecule drugs take advantage of the miRNA targeting several oncogenic pathways combined with the cytotoxic small molecule. The miR-375 is known to be downregulated in hepatocellular carcinoma (HCC) and enabling its expression could suppress the progression of the cancer by targeting major oncogenes such as AEG-1, YAP1, and ATG7. Fan et al. formulated a liposome for the codelivery of miR-375 and DOX and evaluated its synergistic effects in treating HCC. The combination therapy demonstrated improved efficacy in HepG2 and SMMC-7721 cell lines in vitro and in a murine xenograft HCC model in vivo. Furthermore, a dramatically decreased resistance to DOX was observed due to the reduced expression of MDR1 by the action of miR-375 on AEG1, further reinforcing the potential of drug-miRNA combinations in treating cancer.[102] miR-34a is known to augment the therapeutic efficacy of a number of chemotherapeutic drugs, including docetaxel. Sharma et al. designed hybrid lipopolymeric nanoplexes to codeliver miR-34a and DTX to achieve better therapeutic outcomes against mammary tumors. The system exhibited a high DTX encapsulation of 94.8% and demonstrated enhanced antitumor efficacy in MDA-MB-231 and 4T1 cells with favorable pharmacokinetics in vivo. The hybrid lipopolymeric nanoplexes are a viable delivery strategy for the codelivery of hydrophilic NAs and hydrophobic drugs, and further research is required to realize the translational potential of the delivery system.[103] Shi et al. developed SLNs to codeliver miR-34a and PTX and assessed their synergistic efficacy in B16F10-CD44+ cells in vitro and in vivo. The SLNs exhibited enhanced anticancer efficacy by regulating the CD44 expression combined with the cytotoxicity of PTX. Also, a decreased PTX dose could be used with the combination therapy, thus reducing the propensity of toxic side effects.[104] Topical administration of chemotherapeutics overcome unwanted toxic effects commonly seen with systemic delivery and help localize drug deposition. However, the skin forms a formidable barrier due to the stratum corneum. Several physical techniques have been used to enhance the penetration of nanocarriers. Jose et al. used iontophoresis to achieve better dermal penetration of deformable liposomes codelivering siRNA and curcumin against STAT3 to treat melanoma. The liposomes demonstrated enhanced suppression of A431 tumor cell growth in comparison to individual treatments.[105]

3.2. Drug-Nucleic Acid Combinations to Treat Inflammatory Diseases

The prolonged use of anti-inflammatory, immunosuppressive, and steroidal drugs can cause a number of unwanted side effects, including generalized immunosuppression. Since these agents

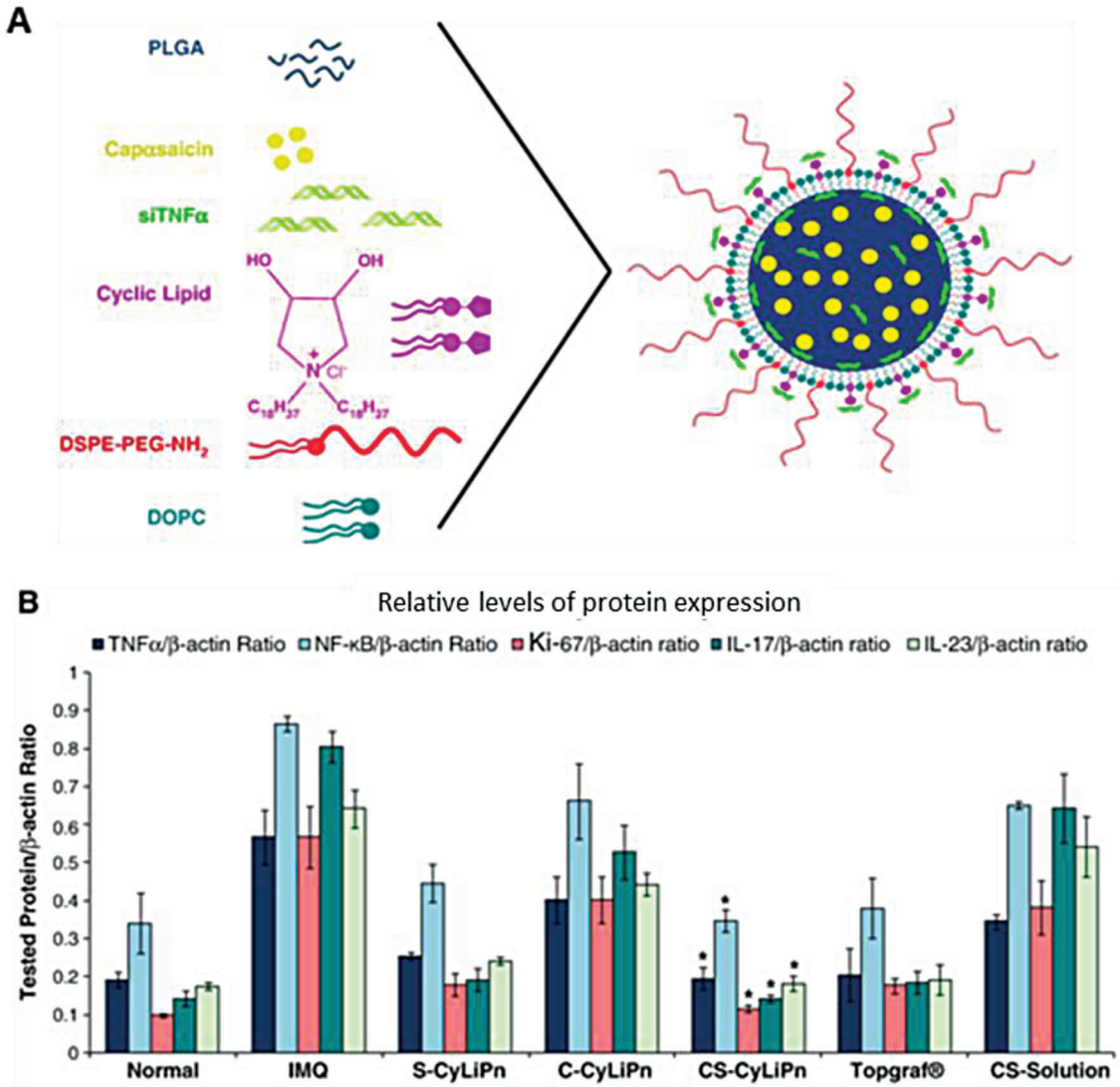

Figure 4. A) Diagrammatic representation of the lipid-polymer hybrid nanoparticles codelivering siTNF-α and Capsaicin. The nanoparticles consist of the hydrophobic poly(lactic-*co*-glycolic acid (PLGA) core for encapsulating the poorly water-soluble drug. The corona of the nanoparticles was constructed with 2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol)] (DSPE-PEG-NH_2), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), and the cyclic cationic lipid which helps in condensing siRNA. The novel cyclic pyrrolidinium head helps in skin penetration. B) Synergistic anti-inflammatory effects of the siRNA and capsaicin as seen by the relative levels of major anti-inflammatory proteins. Reproduced with permission.[118] Copyright 2013, Elsevier.

are the preferred therapeutic interventions for a majority of inflammatory diseases, reduction in their dose, treatment period, or bolstering their effect with additional therapeutic modalities is the need of the hour. RNAi seems to be the obvious choice since inflammation is mediated by a number of overexpressed genes regulating various inflammatory pathways. The success of NA therapy in reducing inflammation by targeting major proinflammatory cytokines, such as TNF-α, majority of the interleukins (ILs), or signaling pathways such as NF-kB, Notch1 in RA and psoriasis has already been proven.[106–112] Specifically, IL-based siRNA delivery systems have been found to be effective to treat psoriasis like skin diseases.[111] The astounding success achieved by combinatorial therapy in combating cancer led to an upsurge in studies trying drug-NA combinations in several inflammatory diseases.[113–116] An improvement in the pharmacokinetic profile of anti-TNF-α agents was seen with the coadministration of immunosuppressive agents such as MTX and thiopurines. This led to better therapeutic outcomes in the treatment of IBD.[15] Also, topical delivery of NAs has been receiving attention to treat diseases including psoriasis.[117] Lipid-based formulations, especially liposomes, have shown advantages over other nanoformulations to topically deliver small molecule drugs in combination with biologics either passively or coupled with penetration enhancing techniques, such as iontophoresis.[105] However, the stratum corneum is the primary obstacle in the transdermal penetration of NAs and proteins. To overcome this limitation, lipid-polymer hybrid nanoparticles were developed by Desai et al. to deliver capsaicin and TNF-α siRNA to treat psoriasis (**Figure 4**).

The lipids had a cyclic pyrrolidinium head group which resembled a well-established skin permeating agent-azone that helped overcome the skin barrier. The lipid-polymer hybrid nanostructures consisted of a poly (lactic-*co*-glycolic acid) (PLGA) core to entrap the hydrophobic capsaicin, followed by a cationic lipid layer to complex siRNA and a hydrophilic DSPE-PEG_{2000} polymer shell for improving siRNA penetration into the skin. The formulated nanoparticles displayed a moderate synergistic effect when tested in an IMQ-induced psoriasis mouse model. The synergistic effect of the nanoparticles could be attributed to the enhanced skin permeation of the therapeutic agents combined with an inhibition of the two distinct therapeutic targets of capsaicin and siTNF-α.[118] The developed lipid-polymer hybrid was further employed for codelivery of erlotinib and IL36-α siRNA in the treatment of psoriasis. The combined delivery effectively reduced the psoriatic plaques in C57BL/6 mice and suppressed the intrusion of dermal cytokines which play a major role in inducing chronic inflammation in psoriasis.[119] The antipsoriatic effects of tacrolimus in combination with TNF-α siRNA codelivered via NLCs were studied by Viegas et al. Psoriasis is skin disease associated with the immune system. Tacrolimus is a macrolide immunosuppressive drug that inhibits calcineurin and TNF-α is an overexpressed proinflammatory cytokine in psoriasis.[120] The NLC was formulated using the hot homogenization method. Polyethyleneimine (PEI), a widely used cationic transfection agent electrostatically complexed the siRNA on the surface, while tacrolimus was encapsulated in the lipid core. The in vivo therapeutic effect of the NLC was evaluated in an IMQ-induced psoriasis model. A significant suppression of TNF-α expression followed by pronounced anti-inflammatory response was seen which could be due to the synergistic combination of tacrolimus and TNF-α siRNA.[121]

Activated macrophages secrete inflammation inducing cytokines and are involved in the pathogenesis of RA. The expression of the CD44 receptor is significantly increased in activated macrophages. Hyaluronic acid (HA), a natural polysaccharide binds specifically to CD44. Li et al. developed HA-coated, pH-responsive lipid-polymer hybrid nanoparticles (HNPs/MD) encapsulating DEX and siRNA against MCL-1 for RA treatment. Here, DOTAP- a cationic surfactant was used to complex the siRNA, whereas DEX was encapsulated by an emulsion-solvent evaporation method. Diminished erythema, swelling of the paw, and a reduction in the cytokine levels were seen in animals treated with HNPs/MD as compared to nanoparticles loaded with either of the therapeutic agents. This indicates that the codelivery of DEX and MCL-1 siRNA leads to better therapeutic outcomes in RA than existing monotherapies by inducing apoptosis of activated macrophages in several different pathways, at the same time.[122] Blocking macrophage polarization to the M1 phenotype by silencing the NH-kB pathway is a promising strategy to raise the levels of anti-inflammatory M2 macrophages. To overcome the toxicity issues associated with most cationic materials used to complex siRNA, Duan et al. developed calcium phosphate nanoparticles (CaP) to deliver siRNA against NF-kB. To improve stability, the siRNA/CaP complex was entrapped liposomal core and MTX was enclosed in the lipid bilayer. The PEGylated liposome was further attached to folic acid to target the folate receptor β in activated macrophages, overexpressed in RA. The liposomes demonstrated excellent uptake in vitro under inflammatory conditions induced by lipopolysaccharide (LPS) in RAW 264.7 macrophage cells. The codelivery with folate conjugated liposomes could significantly suppress inflammation in a collagen induced arthritis mouse model. Combination therapy led to decreased levels of proinflammatory cytokines compared to treatment with either siRNA or drug alone.[123] Going a step further, Xue et al. developed liposomes with three anti-inflammatory agents for combined therapy of RA. The therapeutic cargo was made up of DEX, NF-kB decoy oligodeoxynucleotides (ODNs), and gold nanorods (GNRs), which were loaded into liposomes modified with folate (FA-lip (DEX + GNRs/ODNs)). The liposomal coformulation in combination with laser treatment, decreased the clinical scores for arthritis, cytokine levels in the serum, and prevented cartilage damage in adjuvant induced arthritis mice.[124]

Systemic lupus erythematosus can lead to lupus nephritis (LN) which is characterized by massive inflammation in the kidneys. Combination therapy is an efficacious approach to treat LN.[125] Hence, Diao et al. developed PEGylated cationic liposomes modified with the TAT-peptide to codeliver siRNA against anti-high-mobility group box 1 (HMGB1) and dihydroartemisinin (DHA) to improve therapeutic outcomes in LN. HMGB1 acts as an inflammatory trigger and is an important part of the TLR4 signaling axis. DHA is known to inhibit the TLR4 pathway and thus reduce inflammation. The anti-inflammatory efficacy of the liposomes was tested in vitro in RAW 264.7 cells stimulated with LPS. The liposome-based codelivery system demonstrated a synergistic effect which resulted in a major decrease in multiple proinflammatory cytokines compared to the individual treatment groups.[126]

4. Lipid-Based Codelivery Systems for Nucleic Acid–Nucleic Acid Combination Therapy

NA therapy leads to alterations in the gene expression profiles of target cells, addressing the root cause of diseases with a genetic alteration. Cancer is fundamentally caused by genetic dysregulation, and inflammation is associated with the activation of a highly organized gene expression system, making them the prime targets for NA therapy.[127] Research on NA therapy has seen a tremendous boost in the recent years, with multiple NA-based therapies already in the clinic. However, their use as a stand-alone therapy is limited because of multiple gene mutations during the progression of disease along with other complexities. Limitations of single gene-targeted therapy can be overcome by using a combination of NAs targeting two or more of the multiple genes involved in various distinct pathological pathways, and simultaneous gene silencing has shown promising results.[128–130] Combinational strategy has greatly increased the number of possible NA combinations that could be used target heterogenous tumors or a group of cytokines in the inflammatory cascade.[131] Combinations that have been explored so far include siRNA-pDNA, multiple siRNAs, miRNA-siRNA, siRNA-mRNA, and shRNA-siRNA.[132–142] A summary of several potential NA-NA co-delivery systems can be seen in **Table 3**. Codelivery of distinct NAs acting via different mechanisms has its own advantages. For example, siRNA and shRNA down-regulate gene expression by different pathways. The delivery of shRNA via shRNA incorporating plasmid offers a delayed RNAi effect

Table 3. Lipid-based gene-gene codelivery systems.

Nanocarrier	Therapeutic agents	Therapeutic targets	Disease	Refs.
Lipidoid nanoparticle	siRNA and mRNA	siRNA against factor VII, firefly luciferase, and mCHERRY mRNA	Cancer	[143]
Liposomes	siRNA and siRNA	BCL-2 and PKC-ι	Breast cancer	[144]
Liposomes	siRNA and miRNA	c-Myc, MDM2, VEGF siRNAs, and mi-34a	Skin cancer	[145]
Liposome	siRNA and siRNA	p38α MAPK and p65 siRNA	Glomerulonephritis	[146]
Lipid nanoparticle	siRNA and siRNA	TNF-alpha and STAT3	Psoriasis	[147]
Lipoplex	siRNA and siRNA	IL-1, IL-6, and IL-18	Rheumatoid arthritis	[148]

and long-term silencing of genes. In contrast, siRNA directly interacts with mRNA and promptly initiates RNAi, however, its prolonged efficacy is impaired by enzymatic degradation and by its dilution effect during cell proliferation. Hence, administered together, siRNA can trigger early gene silencing followed by shRNA-induced gene silencing enabling prolonged gene knockdown to enhance the antitumor efficacy.[141]

4.1. Nucleic Acid–Nucleic Acid Combinations to Treat Cancer

Decreased expression of tumor suppressor genes and the simultaneous overexpression of oncogenes is a characteristic feature of many cancer types. Combined delivery of NAs could benefit such cancers by enabling the synchronous silencing of oncogenes and the expression of desired genes. Combining delivery of siRNA and mRNA in a formulation increases chances of successful transfection of both NAs, maximizing the chances of achieving the desired therapeutic effect. In addition, a single delivery vehicle decreases the cost of production and is more patient compliant compared to two distinct formulations. Given the considerable difference in molecular characteristics between two different types of NAs, a successful codelivery system requires a nanoparticle design that can potentially accommodate both the therapeutic agents. The 100-fold difference in the molecular weights of siRNA and mRNA (10^4 vs 10^6 g mol^{-1} and differences in stability and molecular conformation need to be factored in the design of the delivery system. To achieve codelivery, Ball et al. developed a lipidoid nanoparticle to deliver siRNA against factor VII along with firefly luciferase or mCHERRY mRNA. The lipidoid nanoparticle was constructed by combining an ionizable and biodegradable lipidoid 3060_{i10}, a helper lipid, cholesterol, and PEG2000. Lipidoid nanoparticle-based codelivery demonstrated an improvement in inhibition of the factor VII gene by 44–87% (in vivo) in comparison with siRNA only formulation. Moreover, coformulation induced the luciferase protein expression three times higher than the mRNA only formulation, indicating effective improvement of mRNA delivery.[143] BCL-2 is a prominent tumor suppressor gene and is involved in tumorigenesis by blocking programmed cell death, and PKC-ι contributes to cancer development and metastasis. Kim et al. developed cationic liposomes decorated with an anti-EGFR aptamer to codeliver quantum dots and siRNAs against BCL-2 and PKC-ι to suppress the growth and metastasis of triple-negative breast cancer with enhanced EGFR expression. The liposome was constructed by using a cationic lipid-O, O′-dimyristyl-*N*-lysyl glutamate (DMKE), cholesterol, and DSPE-mPEG2000. siRNAs were encapsulated in the inner hydrophilic layer and hydrophobic quantum dots were enclosed in the lipid bilayer of the liposomes. Codelivery of anticancer siRNAs (against PKC-ι and BCL-2) synergistically suppressed cancer progression and metastasis, and the quantum dots allowed the tracking of liposomes inside the body. The established nanoformulation offered a novel theranostic delivery system capable of therapeutic effect along with tumor imaging ability (**Figure 5**).[144] Several miRNAs are also known to be upregulated in cancers, and combinations of siRNAs and miRNAs could have possible synergistic effects. Chen et al. designed a liposome-polycation-hyaluronic acid (LPH) nanoparticle decorated with a single-chain antibody fragment (scFV) targeting the tumor cells for codelivery of siRNA and miRNA to treat pulmonary metastases in a B16F10 melanoma model in mice. The LPH nanoparticles were prepared by electrostatic interactions. An anionic mixture of siRNA, miRNA, and HA was complexed with protamine followed by encapsulation into the cationic liposome and conjugation with scFV through a disulfide linker. The siRNA efficiently downregulated the target genes, while apoptosis was induced by miR-34a, in addition to blocking survivin expression and suppressing the MAPK pathway. Overall, the combined delivery led to an enhanced anticancer effect when compared to treatment with siRNA or miRNA alone.[145]

4.2. Nucleic Acid–Nucleic Acid Combinations to Treat Inflammatory Diseases

In the context of inflammatory diseases, a few pathways have been found to be a common factor in almost all inflammatory pathologies and using combinatorial RNAi to silence multiple genes in these pathways has been explored extensively. Wang et al. targeted two key inflammation associated proteins p38 MAPK and p65 that are implicated in the development of glomerulonephritis. p65 is involved in initiating inflammation and may also be a part of p38 MAPK pathway. Hence, the hypothesis was that the simultaneously suppression of p38 MAPK and NF-κB pathways might induce a synergistic effect and lead to better inhibition of inflammation. To achieve this, a glomerulus targeted liposomal formulation was developed with PEI to complex dual siRNAs against p65 and p38 MAPK. The delivery system could markedly downregulate both the proteins and reduced inflammation, extracellular matrix deposition, and proteinuria when compared to monotherapy by either of the siRNAs in a mouse model of IgA nephropathy (**Figure 6**).[146] Psoriasis is another disease that has benefitted from combinatorial nucleic acid therapy. Based on their previous work,

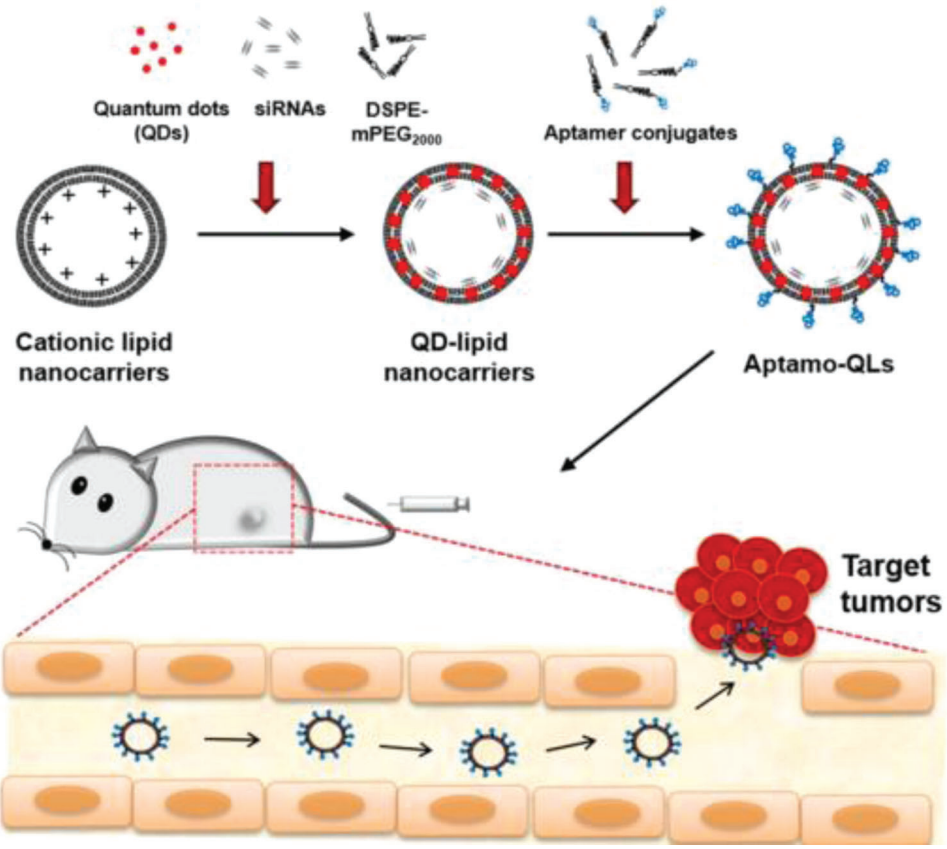

Figure 5. Schematic presentation of quantum dots (QDs) and anticancer BCL-2 and PKC-ι siRNAs encapsulated lipid nanocarrier conjugated with antiepidermal growth factor (EGF)-receptor targeting aptamer. The nanocarrier with quantum dots enables tumor imaging and siRNAs inhibit tumor growth. Reproduced with permission.[144] Copyright 2019, Ivyspring.

Marepally et al. designed a new fusogenic cationic amphiphile with a heterocyclic pyrrolidinium head group and oleoyl hydrophobic domain. The developed fusogenic NA lipid particle (F-NALP) was used to codeliver siRNAs against TNF-α and STAT3 to improve topical delivery to treat psoriasis-like plaques. The formulation consisted of the novel amphiphile—DOPyCl, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine,1,2-di-(9Z-octadecenoyl)-*sn*-glycero-3-phosphocholine, and 2-distearoyl-*sn*-glycero-3-phosphoethanolamine-PEG 2000. The formulation demonstrated a synergistic effect in an IMQ-induced psoriasis model in C57BL/6 mice. Codelivery with F-NALPs reduced severity index scores and decreased the levels of inflammatory proteins in comparison to either of the siRNAs alone and a commercial tacrolimus formulation-Topgraf. The synergistic anti-inflammatory effect can be attributed to better penetration of F-NALPs and the mechanistic cross-signaling between TNF-α and STAT3 where TNF-α modulated the synthesis of STAT3 by inducing activation of JAK1 and Tyk2.[147] Since, about 40% of patients with RA are unresponsive to anti-TNF biotherapies, Khoury et al. came up with a replacement therapeutic strategy for this subset of patients. They targeted inflammation-inducing cytokines IL-1, IL-6, and IL-18 and administered siRNAs formulated as lipoplexes weekly in a collagen-induced arthritis mouse model. The most significant results were seen with a combination of the 3 siRNAs delivered together. The siRNA blend led to an improvement in all pathological features of RA, including inflammation, joint damage, and the Th1 response. Although not developed as a codelivery system, this study provides further proof of the advantage of using combination NA therapy to treat inflammatory diseases.[148]

5. Lipid-Based Delivery Systems for Codelivery of Protein Therapeutics

Protein therapeutics have emerged as important strategies to combat cancer and inflammatory diseases, and their use has dramatically increased since the first protein therapeutic—human insulin, was approved more than 40 years ago. Treatment strategies for inflammatory diseases include the use of biologics, such as antileukotrienes and proinflammatory cytokine inhibitors like anti-TNF-α antibodies that inhibit recruitment of inflammatory cells.[149] Proteins with direct anticancer effects via activation of apoptotic pathways (e.g., tumor necrosis factor related apoptosis inducing ligand (TRAIL)) or with indirect effects such as the regulation of immune responses (e.g., anti PD-1/PD-L1 therapeutics) have been widely used in the treatment of cancer.[150,151] Combining protein therapies with chemotherapeutic drugs has had a notable synergistic benefit in the treatment of cancer. For instance, combining standard chemotherapeutics like PTX, DOX, and cisplatin with monoclonal antibodies

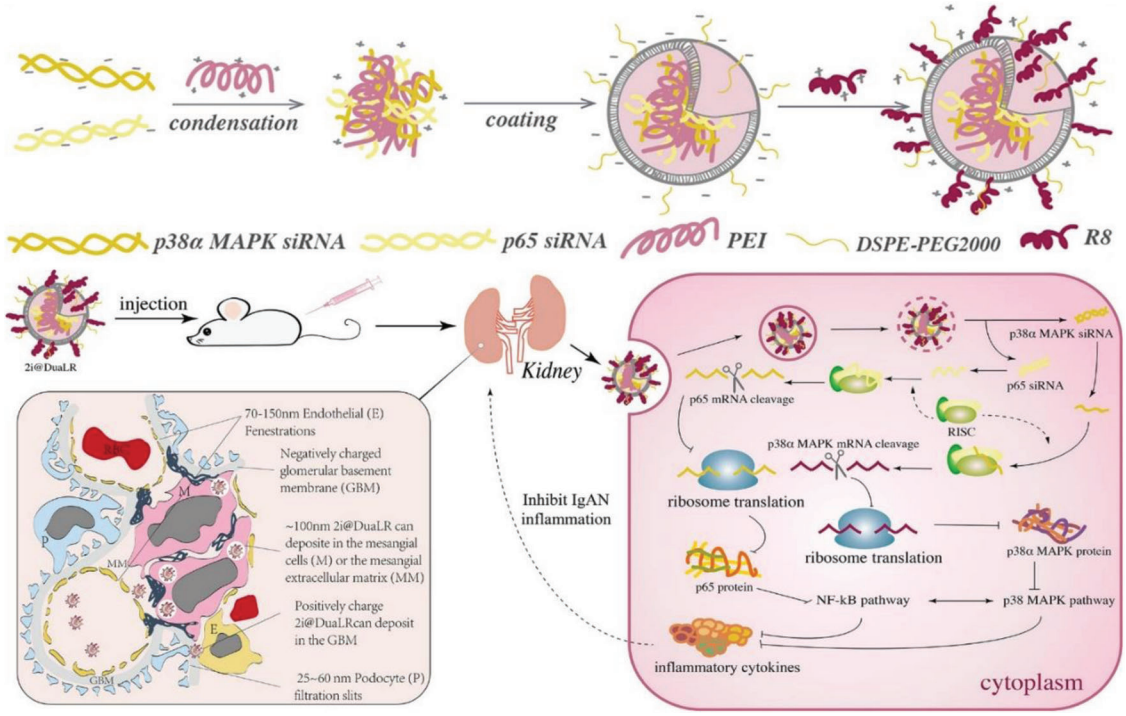

Figure 6. Schematic presentation of p38α MAPK siRNA and p65 siRNA encapsulated liposomes. The liposome surface was modified with neutrally charged 1, 2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-Poly(ethylene glycol) (DSPE-PEG) and positively charged peptide, octa-arginine (R8). The liposomes demonstrated efficient silencing of both p38α MAPK and p65 expression which resulted in a substantial reduction of inflammation in an immunoglobulin A nephropathy mouse model. Reproduced with permission.[146] Copyright 2020, Elsevier.

Table 4. Lipid-based delivery systems for codelivery of protein-based therapeutics.

Nanoparticle	Therapeutic agents	Disease	Refs.
Liposome	Protein (TRAIL) and small molecule drug (PTX)	Melanoma	[154]
Liposome	Protein (TRAIL) and small molecule drug (PTX)	Melanoma	[155]
Liposome	Protein (cytochrome C) and pDNA (p53)	Breast cancer	[156]
Liposome	Peptide (RA-V) and small-molecule drug (DOX)	Cervical cancer	[158]
Liposome	Peptide (RA-V) and antisense oligonucleotide HIF-1α inhibitor	Colon cancer	[159]
Solid lipid nanoparticle	Antibody (Etanercept) and small-molecule drug (MTX)	Rheumatoidarthritis	[161]

resulted in increased anticancer efficacy in numerous studies.[152] Additionally, significant improvement in was seen in patients with nonsquamous nonsmall-cell lung cancer with a combination of an antibody against PD-L1, atezolizumab, and platinum based chemotherapeutics in a recently concluded phase 3 clinical trial.[153] However, clinical outcomes from combinations of free drugs and proteins still suffer from drawbacks, mainly due to protease mediated degradation of proteins, short half-lives, mismatched pharmacokinetics, and insufficient distribution to the target site. To overcome these limitations, efforts have been directed to design codelivery systems incorporating protein therapeutics with other classes of therapeutic agents, such as nucleic acids and small molecule drugs. The following section elaborates on several protein-based codelivery systems focusing on the treatment of cancer and inflammatory disease (**Table 4**).

TRAIL is an antitumor cytokine that specifically kills cancer cells, while sparing healthy cells by interacting with death receptors DR4 and DR5 which are upregulated on the surface of most tumor cells. Preclinical studies have established the beneficial effects of TRAIL in multiple cancer types, however, progress in the development of TRAIL-based therapeutics has been slow due to the lack of appropriate delivery systems and low death-receptor expression levels on certain cancer types.[150] Combining TRAIL with other chemotherapeutics and ensuring targeted delivery using a delivery vector has shown promise in improving treatment efficacy. Huang et al. developed tumor microenvironment responsive liposomes (TRAIL-[Lip-PTX]$^{C18\text{-}TR}$) to codeliver PTX and TRAIL in melanoma. PTX was encapsulated inside the liposome with the cationic TRAIL being attached to the anionic surface of the liposome by electrostatic attraction. Finally, a pH sensitive cell penetrating peptide that selectively attaches to the integrin receptor $\alpha v\beta 3$ for melanoma targeting was attached to the liposomal surface. TRAIL-[Lip-PTX]$^{C18\text{-}TR}$ led to 93.8% tumor inhibition in vivo due to the combined effect of TRAIL induced death

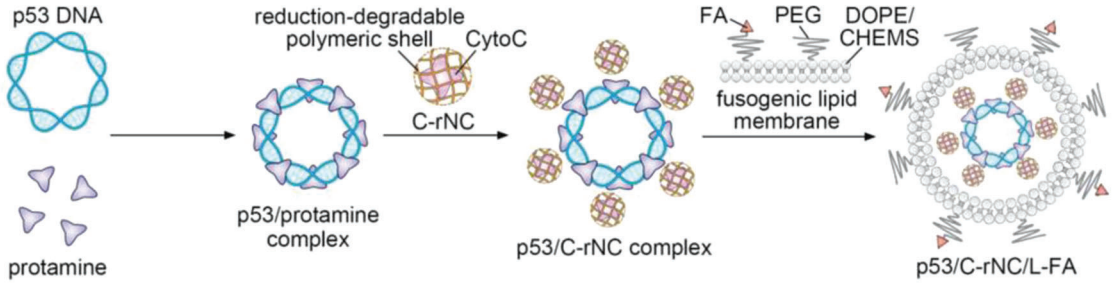

Figure 7. Diagrammatic representation p53/C-rNC/L-FA made up of a DNA/protein complex core and a liposomal shell containing dioleoylphosphatidylethanolamine (DOPE), cholesteryl hemisuccinate (CHEMS), DSPE-PEG, and folic acid-modified PEG-conjugated distearoylphosphoethanolamine (DSPE-PEG-FA). Reproduced with permission.[156] Copyright 2019, Wiley-VCH GmbH.

receptor mediated exogenous apoptosis and the PTX induced endogenous apoptosis and necrosis.[154] In addition, a similar results were seen in a study by Huang et al. who fabricated fibronectin targeting liposomal nanodisks for codelivery of TRAIL and PTX to tackle lung melanoma metastasis.[155] Chen et al. developed a nanoassembly comprising a core made up of a DNA/protein complex and a liposomal shell for targeted codelivery of an apoptotic protein cytochrome C (CytoC) and a tumor-suppressor gene (p53). A cationic p53/protamine complex was formed by electrostatic complexation. CytoC was loaded in an anionic nanocapsule (C-rNC) which degrades in a reducing environment. CytoC loaded nanocapsule was attached to the p53/protamine complex electrostatically forming p53/C-rNC complex. The complex was then enclosed into a folic acid-decorated PEGylated liposome (p53/C-rNC/L-FA) (**Figure 7**). The polymeric coating of C-rNC breaks down in the reducing environment of the cytoplasm, releasing CytoC which further moves away from the p53 complex due to electrostatic repulsion and brings about caspase-induced apoptosis. The protamine complex enables intranuclear p53 DNA delivery leading to tumor-suppressing activity, which combined with CytoC induced apoptosis leads to improved anticancer activity. A significant reduction in tumor growth was seen with p53/C-rNC/L-FA in an orthotopic MCF-7 tumor mouse model.[156]

Cytotoxic cyclic peptides, typically isolated from plants have emerged as novel chemotherapeutics with excellent selectivity and off-target toxicity.[157] RA-V is a cytotoxic cyclic peptide isolated from *Rubia cordifolia* L. known to have anticancer efficacy. Chen et al. developed pH/CytoC dual-responsive nanoparticles with a dendrimer-based poly(L-lysine) core and a shell made up of a liposome for organelle selective sequential release of a cytotoxic peptide RA-V and DOX. The system could also monitor real-time CytoC release in the apoptosis process. The nanoparticles designated as DGLipo NPs, have DOX incorporated into the DNA duplex having a CytoC aptamer, which was then condensed by dendritic poly(L-lysine) to form self-assembling nanostructures (Dox/DGL). A mitochondria-penetrating peptide (MPP) was attached to Dox/DGL (DOX/MPP-DGL). DOX/MPP-DGL was enclosed in the core and RA-V was loaded into the pH-sensitive liposomal shells. Finally, the delivery system was modified with a peptide c(RGDfK) to further improve cancer selectivity. DGLipo NPs could enter $\alpha_v\beta_3$ rich cells through endocytosis wherein the acidic microenvironment of the lysosomes could break down the liposomes to release RA-V and DOX/MPP-DGL. DGL can act as a proton sponge leading to lysosomal escape of DOX/MPP-DGL which then translocates to the mitochondria by virtue of MPP wherein DOX is released. DGLipo NPs could almost completely inhibit tumor growth in HeLa tumor bearing mice by virtue of the combined cytotoxicity of RA-V peptide and DOX in comparison with monotherapy.[158] Yao et al. developed a pH-sensitive liposome to codeliver the chemotherapeutic RA-V peptide and an antisense oligonucleotide HIF-1α inhibitor (RX-0047) to attenuate tumor hypoxia and enhance RA-V induced apoptosis. Anti-DR5 antibodies were attached to the surface to enable selective targeting to the colorectal tumor cells. Additionally, a caspase-8 activation probe was encapsulated in the liposome to allow real-time monitoring of caspase-8 activation and hence apoptosis. The liposome could selectively target the tumor in HCT-116 tumor bearing mice. Successful downregulation of HIF-1α led to inhibition of hypoxia and improved therapeutic efficacy of the RA-V peptide leading to a significant decrease in the tumor volume.[159]

Etanercept is a TNF inhibitor approved by the FDA that is widely used for psoriasis treatment. It binds to TNF-α thereby lowering its free concentration and hence prevents activation of the inflammatory system. Combining etanercept with other therapeutics such as MTX has improved therapeutic outcomes in psoriasis.[160] Ferreira et al. developed cetyl palmitate SLNs coloaded with etanercept and MTX which were further loaded in a carbopol hydrogel for topical administration. A sustained release of MTX was seen from the formulation with improved skin penetration.[161]

5.1. Lipid-Based Delivery Systems for Codelivery of Genome Editing Agents

A rapidly growing subfield in the sector of gene expression manipulation is genome editing, which attempts to overcome the off-target effects and unpredictable knockdown efficiencies of RNAi.[162] Genome editing platforms currently being explored include the CRISPR-Cas9 systems, transcription activator-like effector nucleases (TALENs), and zinc-finger nucleases (ZFNs). All these strategies generate breaks in both strands of the DNA, and the subsequent repair leads to modifications of the original sequence. Both TALENs and ZFNs generate sequence recognition specificity via protein-DNA interactions and targeting new sequences requires extensive protein engineering. Here we focus on the CRISPR-Cas9 system which in contrast, uses a sgRNA

Table 5. Lipid-based delivery systems for codelivery of genome editing agents.

Nanoparticle	Therapeutic agents	Therapeutic target	Disease	Refs.
Liposome templated hydrogel	Cas9 protein and sgRNA	PLK1	Brain cancer	[166]
Lipid-polymer hybrid	Cas9 plasmid and sgRNA	VEGFA	Osteosarcoma	[167]
Lipid core–shell nanocarrier	Cas9 protein and sgRNA plasmid	PLK1	Melanoma	[170]
Lipid nanoparticle	Cas9 mRNA and sgRNA	PLK1	Multiple cancers	[169]

to achieve DNA sequence specificity and a nuclease, commonly Cas9, to cleave the genome. Unfavorable side effects associated with the traditionally used viral vectors led to the development of nonviral vectors, especially lipids to codeliver the Cas9 protein and the sgRNA or mRNA/DNA encoding for either one or both of the components. All components of the CRISPR system are biomacromolecules and must overcome several barriers to be delivered to the target cell. Similar to RNAi, plasmid DNA, and mRNA need to be delivered to different locations within the cell to function. The DNA needs to enter the nucleus, while the mRNA acts in the cytoplasm, further complicating the requirements for codelivery. Also, the large size and the varied charges of the Cas9 protein, plasmid DNA, and mRNA pose formidable challenges for their effective encapsulation. Unlike most proteins, the commonly used Cas9 protein has a positive charge and hence the commonly used cationic lipids and polymers that electrostatically complex the anionic NAs cannot be used to complex the Cas9 protein.[163] There have been studies reporting that mRNA encoding Cas9 should preferably be delivered prior to the sgRNA to overcome differences in expression kinetics of the two components and achieve optimum efficiency. However, the requirement of both NAs to reach the same cell is most effectively achieved by their codelivery in a single nanoparticle and the preference for sequential administration was overcome by the authors by administering a higher ratio of Cas9 mRNA to sgRNA in the same nanoparticle.[164,165]

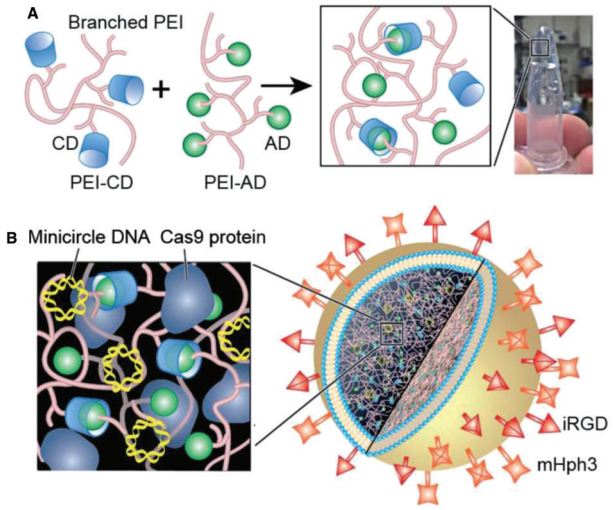

Figure 8. Formulation of Liposome-Templated Hydrogel Nanoparticles (LHNPs). A) Formation of PEI hydrogel via cyclodextrin (CD)-grafted PEI and adamantine (AD)-grafted PEI induced interactions. B) Design of LHNPs. The cationic lipid, 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP) liposomes form the shell while the PEI hydrogel forms the core of the NPs. Reproduced with permission.[166] Copyright 2017, Wiley-VCH GmbH.

Lipids represent one of the most successful materials employed to deliver CRISPR-Cas9 systems (**Table 5**). Chen et al. designed liposome-templated hydrogel nanoparticles (LHNPs) to achieve genome editing in cancer.[166] Cas9 delivered as a protein leads to optimum efficiency and prevents off target effects and hence, the LHNPs were used to codeliver Cas9 protein and sgRNA against polo-like kinase 1 (PLK1), a regulator of cell division showing enhanced expression in cancer cells. Polyethyleneimine (PEI) hydrogel encapsulating the Cas9 protein formed the core of the nanoparticles. The shell was made up of positively charged 1,2-dioleoyl-3-trimethylammonium-propane chloride salt (DOTAP) lipids for the complexation of the genetic material. The core PEI hydrogel was formed by noncovalent crosslinking between cyclodextrin (CD)-grafted PEI and adamantine (AD)-grafted PEI. The resulting hydrogel improved the encapsulation of the Cas9 protein, thereby overcoming the low encapsulation disadvantage of the DOTAP liposome. Furthermore, the hydrogel formed by CD-AD host–guest interaction crosslinks without the use of initiators or ultraviolet radiation, which are conditions usually necessary for the formation of hydrogels but are harmful for the proteins. Surface conjugation of the liposomes with the cell-penetrating peptide mHph3 significantly increased the delivery efficiency compared to commercially available lipofectamine. The formulation was effective in inhibiting cell growth in two human brain cancer stem cell lines (GS5 and U87), reduced the tumor volume, and increased the median survival time in mice with intracranial U87 tumors. Moreover, surface decoration with Lexiscan, a small molecule that can temporarily improve blood–brain barrier permeability, led to an increase in the intracranial tumor accumulation (**Figure 8**). Utilizing the advantages of both, lipids and polymers, Liang et al. developed PEG-PEI-Cholesterol (PPC)—a lipid conjugated polymer made up of covalently bound polyethyleneimine, methoxypolyethyleneglycol, and cholesterol which demonstrated excellent safety in a Phase II clinical trial. The lipopolymer was used to encapsulate gRNA against VEGA and Cas9 plasmid. An aptamer LC09, which targets osteosarcoma cells was attached to it to achieve tumor selective delivery. This led to successful modification of VEGFA thereby inhibiting malignancy in a murine orthotopic osteosarcoma model developed by the intraosseous inoculation of K7M2 cells.[167] Gold nanoclusters (GNs) are a rapidly rising form of inorganic nanomaterials due to their large surface area, stability, and excellent biocompatibility. Wang et al. developed a lipid core–shell nanocarrier combined with gold nanoclusters (LGCP) for codelivering Cas9 protein and Plk1 gene targeted sgRNA plasmid to tumor cells (**Figure 9**A). A TAT pep-

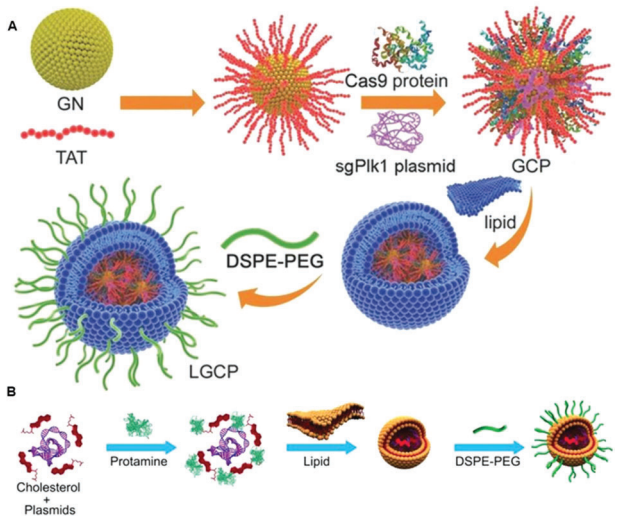

Figure 9. A) Schematic presentation of the formulation of lipid core–shell nanocarrier combined with gold nanoclusters (LGCP) nanoparticle, constructed with polyethylene glycol-lipid/gold nanocluster/Cas9 protein/sgPlk1 for cancer therapy. Reproduced with permission under the terms of the CC-BY license.[168] Copyright 2017, the Authors. Published by Wiley-VCH GmbH. B) Schematic presentation of the formulation of Cas9-sgRNA plasmid using chondroitin sulfate, protamine, 2-dioleoyl-3-trimethylammoniumpropane (DOTAP), dioleoylphosphatidylethanolamine (DOPE), and DSPE-PEG. Reproduced with permission.[170] Copyright 2017, Springer Nature.

tide was attached to the GNs to target the nucleus. The cationic TAT-GNs electrostatically complexed the anionic proteins (Cas9) and NA (sgRNA plasmid), the ternary complex was then enclosed in a lipid shell. The nanoparticles led to about 25% Plk1 genomic modification in A375 cells, which was significantly more than transfection with traditionally used plasmids. Plk1 genome-editing led to significant suppression of tumor development in a subcutaneous A375 cells induced melanoma model.[168] Rosenblum et al. developed a novel lipid nanoparticle to achieve codelivery of sgRNA and Cas9 encoding mRNA. To overcome limitations associated with traditional LNPs, a series of novel ionizable amino lipids was developed. 5-methoxyuridine modified mRNA and nontraditional sgRNAs (IDT sgRNA XT) were employed to further improve stability of the RNA and minimize activation of the immune system. The LNPs achieved excellent gene editing in multiple cancer types in vitro and in vivo.[169] Apart from delivery of sgRNA and Cas9 as two separate components, a single plasmid encoding for both has also been delivered using lipid-based core–shell delivery systems (Figure 9B).[170] Lipid-based delivery systems thus create new possibilities for the use of genome editing as a promising therapeutic strategy and bring us one step close to achieve clinical translation of the CRISPR-Cas9 technology.

6. Conclusion

Combination therapies are now an indispensable part of treatment strategies for many complex human diseases, particularly those resulting from alterations or dysregulation in genes or signaling pathways. Drug combination therapy has been explored ever since the pivotal study describing the synergistic effect of drugs was published by Loewe in 1928. However, most of the combinations being used in clinic today were discovered in an empirical manner or were a serendipitous result of other research. With growing knowledge about genomic sequencing, systems biology, disease modeling, and material science, a fresh outlook toward choosing combinational therapeutics and their delivery strategies is needed. Also, the molecular complexity of the diseases and the patient-to-patient variance in the aberrant genomic landscape means that a combination that works in a small subset of patients will probably not work in rest of the population. Individualized combinations customized to patients' unique genetic profile are needed to sufficiently address the vast complexity and incongruity of diseases. Systematic high throughput methods, disease modelling, and computational approaches using artificial intelligence can help optimize combination therapies. Improving preclinical studies with proper evaluation of combination index, reproducibility of data and using techniques such as predictive modelling can help select the most promising candidates which are least likely to fail in larger clinical studies. Similarly, although advances in multi-functional, sophisticated delivery platforms capable of delivering multiple therapeutic agents have shown great promise, we are still far from the goal of developing personalized nanotherapeutics which can be tailored to an individual's unique pathophysiology to achieve maximum therapeutic efficacy with minimal chances of failure. To achieve this, the limitations of using traditional animal models have to be recognized and newer methods better mimicking human biology need to be developed. Similarly, although lipid based nanocarriers have a proven track record of safety, no system is perfect and lipid-based carriers have certain drawbacks. Cationic lipids are associated with a concentration dependent cytotoxicity and certain lipids have been found to be inherently immunogenic. Predicting toxicity based on lipid structures is complex and careful in vivo screening remains crucial.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

cancer, codelivery, combination therapy, inflammatory bowel disease, lipid nanoparticles, psoriasis

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