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Novel lipoidal amine-based nanocarrier formulations for siRNA delivery

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Abstract

Background—Lack of safe and efficient delivery of siRNA remains the greatest hurdle for the therapeutic application of siRNA. This article reports synthesis and evaluation of novel lipoidal amine-based nanocarrier (LANC) formulations for siRNA delivery.

Method—Physicochemical properties were analyzed for LANC formulations. siRNA delivery efficiency of LANC–siRNA complexes was determined using a *luciferase* reporter gene assay. Cytotoxicity of the LANC–siRNA complexes was measured by the MTS assay. Finally, cellular uptake and cytoplasmic release of siRNA were analyzed using flow cytometry.

Conclusion—The LANC formulation facilitated siRNA uptake and release into the cytoplasm, mediating significant *luciferase* knockdown (70% inhibition).

RNAi has been shown to specifically and efficiently inhibit gene expression in mammalian cells [1]. To capitalize on the RNAi mechanism, siRNA molecules, alone or in combination with other small molecular drugs, have been explored as new therapeutic agents for the treatment of viral infections [2], cancer [3] and various other diseases [1]. However, inefficient delivery of siRNAs still limits their therapeutic application [4].

Cationic liposomes and other lipid nanoparticles are most commonly used as **nanocarriers (NCs) of siRNA** for *in vitro* and *in vivo* transfection [5]. Development of lipid-based

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Financial & competing interests disclosure

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formulations is ongoing. For example, novel lipid-like materials synthesized using combinatorial chemistry, called lipidoids, were shown to have remarkable siRNA delivery activity *in vitro* and *in vivo* [6,7]. The key structural elements for the lipidoids were the amino alcohols containing polar secondary/tertiary amino head groups and hydrophobic hydrocarbon tails [8,9]. Avridine, a lipoidal amine composed of two tertiary amino groups and two nonpolar hydrocarbon chains (Figure 1A), had been used in the preparation of liposomes to enhance the efficacy of immunizing agents [8]. Although Avridine® shares structural similarity with the lipidoids, it has yet to be incorporated in a cationic NC formulation for siRNA delivery.

In this study, novel lipoidal amine-based NC (LANC) formulations have been developed and evaluated as siRNA transfection vectors (Figure 1B). Avridine, as the cationic component, was formulated with 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) or sorbitan mono-oleate (Span 80) [9], plus D- α -tocopheryl polyethylene glycol-1000 succinate (TPGS). The physiochemical characteristics, *in vitro* transfection activity and cytoplasmic release of siRNA from the LANC–siRNA complexes were investigated.

Experimental

Materials

The antiluciferase siRNA (siLuc), negative control 1 siRNA (siNC), FAM-labeled negative control 1 siRNA (FAM-siRNA), RiboGreen RNA reagent, heat-inactivated fetal bovine serum, trypsin and antibiotics/antimycotics were obtained from Life Technologies Corporation (CA, USA). GAPDH mRNA targeting Cy3-MB [9,10] was purchased from MWG/Operon (AL, USA). MTS, cell lysis reagent and luciferase assay kit were purchased from Promega (WI, USA). Avridine was supplied by Tocris Bioscience (MO, USA). Span 80 and TPGS were purchased from Fisher Scientific Inc. (PA, USA). DOPE was obtained from Genzyme Pharmaceuticals (MA, USA). All other reagents were purchased from Sigma-Aldrich (MO, USA).

Preparation of LANC formulations

The LANC formulations, composed of Avridine/Span 80/TPGS or Avridine/DOPE/TPGS, were prepared by an ethanol injection method reported previously [9]. Lipids and surfactants were dissolved in ethanol at 20–50 mg/ml to prepare stock solutions of the lipid/surfactant. The stock solutions were combined to achieve the desired molar ratio of the compositions (Table 1). To prepare 1 ml LANC formulation, the lipid/surfactant mixture was diluted to 300 μ l using ethanol. Then, 300 μ l ethanol solution of lipids and surfactants mixture was rapidly injected to 700 μ l citric buffer (pH = 4.0), followed by a 30 s vortex at room temperature [11–13].

Preparation & characterization of LANC–siRNA complexes

To prepare LANC–siRNA complexes, LANCs solution was mixed with siRNA solution at a vector:siRNA weight ratio of 15:1 under vortexing. The LANC–siRNA complexes were incubated at room temperature for 15 min before $15 \times$ volume of HEPES buffer (20 mM HEPES; pH = 7.4) was added to neutralize the pH and dilute the ethanol concentration. The formed complexes were then used for physical characterization.

The particle size and ζ potential of the LANC–siRNA complexes were measured using a NICOMP Particle Sizer model 370 (Particle Sizing Systems, CA, USA) and a ZetaPALS (Brookhaven Instruments Corp., NY, USA), respectively. The volume-weighted mean diameter was reported. The siRNA complexation efficiency was determined by the Quant-iT™ RiboGreen® RNA Kit following the manufacturer's instructions [6,9].

Cell culture

SK Hep-1 cells with stable *luciferase* expression were grown in MEM with nonessential amino acids (5%), sodium pyruvate (5%), G418 (1.6%), antibiotics/antimycotics (5%) and FBS (10%). Cells were cultured at 37°C in a 5% CO₂ humidified air atmosphere.

In vitro siRNA transfection & cytotoxicity

For *in vitro* transfection, SK Hep-1 cells were seeded in 96-well plate at a density of 2×10^4 cells per well. After 24 h, cells were washed twice before transfection. LANCs and siLuc with a vector:siRNA weight ratio of 15:1 were complexed in serum-free medium. The complexes (final siRNA concentration 100 nM) were used to transfect the cells for 4 h at 37°C. After transfection, the medium was removed, and the cells were washed twice and replaced with fresh medium. 48 h after treatment, 20 µl MTS solution was added to each well and the plate was incubated for an additional 1 h. The absorption at 490 nm was measured with a Multiskan Ascent plate reader (MTX Lab Systems, Inc., VA, USA). The solution in the wells was then removed and replaced with 50 µl lysis buffer. The plate was placed on a shaker for 30 min for cell lysis at room temperature. Then, 50 µl luciferase assay kit solution was added to each well for measurement of the luciferase activity. The luminescence intensity was immediately read on a luminometer (TECAN, Switzerland). The luciferase activity of each well was first normalized by the cell number obtained from the MTS reading and then divided by the average of normalized luciferase activities of the untreated control to obtain the relative *luciferase* expression [14,15].

The cytotoxicity of LANC–siRNA complexes was evaluated by MTS assay. SK Hep-1 cells were transfected using the same protocol for *in vitro* transfection, while the siNC was used for cytotoxicity study. An MTS readout was obtained 48 h after treatment [14,15].

Flow cytometry analysis of the cellular uptake & cytoplasmic release of LANCs

SK Hep-1 cells with stable luciferase expression were seeded in a 24-well plate at a density of 8×10^4 cells/well. After 24 h, FAM-siRNA and Cy3-MB were mixed at 1:1 molar ratio and complexed with different LANC formulations. Then the complexes were used to transfect the cells for 1 h incubation. After transfection, cells were washed twice with PBS and treated with 0.25% trypsin/EDTA for 2 min at 37°C to be detached, and fixed by 4% formalin. The mean fluorescence intensity of FAM-siRNA and Cy3-MB in cells was analyzed by flow cytometry (FACS calibur, Becton Dickinson, NJ, USA) [15].

Statistical analysis

Student's t-test was used to detect statistically significant differences with Microsoft Excel 2003 software (Microsoft, WA). Results were considered significant for $p < 0.05$.

Results & discussion

Physical characteristics of LANC formulations

The LANC formulations containing different components were characterized by particle size, ζ potential and siRNA complexation efficiency. The LANC formulations had large particle size in the presence of 30% EtOH in citric buffer (Figure 2A). The particle size reduced dramatically after forming the LANC–siRNA complexes in low EtOH% and neutral pH buffer (Figure 2B). The size reduction was primarily due to lowering EtOH percentage, as 30% ethanol in the initial LANC formulations could destabilize the particles and increased their particle sizes [12]. In addition, Figure 2B indicated that increasing TPGS percentage in LANC–DOPE formulations reduced the particle size of the LANC–DOPE–siRNA complexes (~220 nm for LANC–DOPE and 5% TPGS). Furthermore, the standard

deviation of particle size distribution (error bars in Figure 2B) indicated that the distribution of the LANC–siRNA complexes was small. When Avridine was formulated with Span 80, which was recently reported as a helper component for siRNA vectors [9], LANC–Span 80 generally showed a much larger particle size than that of LANC–DOPE, and increasing TPGS percentage increased the particle size of the LANC–Span 80/siRNA complexes. The exact mechanism for the effect of TPGS on particle size was unclear. One possible explanation could be that the two types of formulations were different in stability of the lipid/surfactant bilayer and in siRNA complexation process in the presence of 30% ethanol. In addition, no clear correlation was observed between initial and final particle size of formulations, although the rank order of the formulations was maintained for both LANCs with Span 80 and LANCs with DOPE. Span 80 formulations had larger particle sizes than DOPE formulations, but the trend remained the same for both initial and final particle sizes.

All LANC formulations showed slightly positive ζ potential ($<+15$ mV) before and after complexing with siRNA (Figure 3A & B), which may be primarily due to the tertiary amine groups of Avridine [11]. The LANC formulations showed remarkably high siRNA complexation efficiency of approximately 75% (Figure 3B). No significant differences were observed among formulations with different helper components or percentages of TPGS. The high complexation efficiency of the LANCs in the absence of high positive charge on the surface was because the conditionally protonatable tertiary amine groups of Avridine became protonated at acidic pH (pH = 4.0), where siRNA complexation took place, while at physiological pH (pH = 7.4) Avridine was almost charge neutral [11,16]. The neutral surface charge was particularly important for longer systemic circulation time of the nanoparticles [11,17].

Cytotoxicity

Cell viability of LANC formulations was measured by the MTS assay. The data shown in Figure 4 reveal that LANC–siRNA complexes had no significant cytotoxicity at 100 nM siRNA concentration. When 1,2-dioleoyl-3-trimethylammonium-propane, a common cationic lipid, was formulated with DOPE and TPGS, significant cytotoxicity (~50% decrease in cell viability) was observed at 100 nM siRNA concentration [17]. The lack of cytotoxicity of LANC formulations could be due to their relatively low surface charge (<15 mV for LANCs vs 25–30 mV for 1,2-dioleoyl-3-trimethylammonium-propane formulations) [18].

Gene-silencing activity of LANC formulations

The luciferase silencing ability of LANC–siRNA complexes was evaluated by transfecting SK-Hep1 luc cells with LANC–siLuc complexes.

In the present study, we accessed RNAi-mediated *luciferase* gene silencing by measuring luciferase protein expression. To ensure the decrease of protein (luciferase) expression was due to RNAi, and not side effects of the transfection vector, such as cytotoxicity, transfection of siLuc and siNC were performed in parallel. LANC–siLuc complexes treatment decreased *luciferase* gene expression of the cells to 20–40% of untreated cells (Figure 5A). The LANC–Span 80 had a slightly higher gene-silencing activity than the LANC–DOPE; 80 and 70% gene-silencing effects were achieved with LANC–Span 80 and LANC–DOPE, respectively.

We also studied the transfection efficiency of LANC containing different percentages of TPGS. The results showed that LANCs with 5% TPGS were more efficient in knocking down the *luciferase* expression than the LANCs with 1% TPGS; although the differences were not statistically significant ($p > 0.05$). Previously, it has been shown that increased

polyethylene glycol (PEG)ylation density on the surface of NCs could reduce plasma protein binding, thus avoiding reticuloendothelial system uptake [19]. However, higher PEG content in the NCs may also compromise their transfection efficiency [9,20]. Herein, Figure 5A indicated that increasing the PEG content (TPGS) from 1 to 5% in the LANC formulations may slightly enhance the siRNA delivery activity (the differences were not statistically significant). This may be beneficial for *in vivo* applications due to the increased transfection efficiency and avoidance of extensive reticuloendothelial system uptake.

In addition, transfection of LANC–siNC complexes resulted in no *luciferase* downregulation (Figure 5B). Together with the cytotoxicity data, it is concluded that the gene-silencing effect of the LANC–siRNA complexes was clearly due to RNAi [9].

Cellular uptake & cytoplasmic release of LANC–siRNA complexes

The **cellular uptake** of siRNA mediated by different LANC formulations was investigated with flow cytometry measurements of MFI_{FAM-siRNA} (Figure 6). We also studied the cytoplasmic release of siRNA using the mimic of siRNA, the MB [10,14,21]. An MB is an oligo-deoxynucleotide with a hairpin structure and a fluorophore and a quencher on each end. When the MB binds to the target mRNA sequences in the cytoplasm, the quencher and the fluorophore are physically separated, and then the MB becomes fluorescent [9]. The MBs are analogues of siRNA molecules, due to their similar physiochemical properties such as the negative charge, molecular weight and molecular structure. The amount of MBs inside the cells has been shown to have a linear relationship with the MFI [9,14]. This enables the study of the cytoplasmic release of siRNA from LANC–siRNA complexes, because only those siRNAs released into the cytoplasm are biologically available for the RNAi pathway.

As shown in Figure 6A & C, LANC–siRNA complexes exhibited significant cellular uptake efficiency compared with untreated controls. The uptake of LANC–Span 80–siRNA complexes was more efficient than that of the LANC–DOPE–siRNA complexes (Figure 6A & C). More quantitatively, the MFI_{FAM-siRNA} obtained from flow cytometry analysis was also shown in Figure 6C. Figure 6C revealed that the uptake of the LANC–Span 80–siRNA complexes was approximately threefold higher than LANC–DOPE–siRNA complexes, although the gene-silencing activity for the LANC formulations were comparable (Figure 5). Previously, it has been shown that the siRNA taken up by cells is not completely released into the cytoplasm, thus, the overall cellular uptake of siRNA could be quite different from the amount of siRNA escaping from the endocytic vesicles and reaching the cytoplasm [9,11,22]. This explains why the overall cellular uptake of NCs does not correlate with gene-silencing activity.

Consequently, MFI_{MB}, a metric for the cytoplasmic delivery efficiency of the NCs, was used to quantify the amount of ‘bioavailable’ siRNAs. As shown in Figure 6B–D, small differences were observed among different LANC formulations in terms of MFI_{MB}, suggesting that all LANC formulations delivered comparable amounts of siRNAs into cytoplasm, despite the fact that LANC–Span 80–siRNA complexes were much more extensively taken up relative to LANC–DOPE–siRNA complexes. The MFI_{MB} data were consistent with the gene-silencing activity observed for the LANC formulations (Figure 5A). This indicated that siRNA release and subsequent silencing were correlated. As a result, the cytoplasmic delivery efficiency could predict the gene-silencing activity of siRNA.

Taken together, the results of cellular uptake, cytoplasmic release and gene silencing indicated that cellular uptake was not the rate-limiting step for gene-silencing activity of LANCs *in vitro*, as the level of cellular uptake showed little impact on the gene-silencing activity. LANC formulations with DOPE and Span 80, while taken up by cells at very

different extents, showed comparable gene-silencing activity. The data suggested that escape from the endocytic vesicles and intracellular release of siRNA are more critical for the gene-silencing activity than cellular uptake [11]. This also highlighted the importance of elucidating the mechanism behind cytoplasmic delivery of siRNA in formulation design.

Conclusion

In summary, the lipoidal amine, Avridine, was successfully formulated with the helper lipids DOPE and Span 80, as well as the PEG component, TPGS. The LANC–DOPE with 5% TPGS was selected as the optimal LANC formulation. It possessed favorable particle size, ζ potential and siRNA complexation efficiency. In addition, the LANC–DOPE with 5% TPGS mediated efficient delivery of siRNA into the cytoplasm, thus resulting in significant *Luciferase* gene knockdown (70% inhibition). Moreover, even when high PEG content was incorporated into the LANC–DOPE, which can potentially improve the *in vivo* pharmacokinetic and biodistribution profiles, the transfection efficiency of formulation remained high. In conclusion, novel LANC formulations were successfully synthesized and shown to efficiently mediate cytoplasmic delivery of siRNA and gene silencing. The present study provides a novel approach to co-formulating a pharmacological active agent and siRNAs, which offer a promising strategy for synergistic enhancement of therapeutic effect.

Future perspective

Although the novel LANC formulations have promising potential for *in vivo* delivery of siRNA, the optimal LANC formulation should be further investigated for transfection efficiency in the presence of serum in order to better predict the *in vivo* siRNA delivery capability of the LANCs [23]. This is because *in vivo* the NCs would be immediately covered by a rich protein corona upon injection into the bloodstream [24]. The protein coating of the complexes may have a profound impact on pharmacokinetics, biodistribution in target tissues or organs, and siRNA transfection efficiency of the LANCs [24]. The present study indicated that increase of PEG density on the LANCs does not affect the cytoplasmic delivery of siRNA and the genesilencing activity. Because PEG has been shown to improve the stability, pharmacokinetic and biodistribution profiles of gene-/oligo-delivery systems *in vivo* [25,26], the optimal LANC formulation is likely to have high siRNA transfection efficiency *in vivo*. Furthermore, Avridine, as a pharmacological active compound, has been reported to have immunoadjuvanticity [8,27,28] and interferon-stimulating activity [29]. Thus, coformulating Avridine and antiviral siRNA [2,30] in the LANCs will significantly alter the pharmacokinetic and biodistribution profiles of both molecules, which may be a potential strategy for combination therapy against viral infection. Unlike previous methods that are based on encapsulation of small-molecule drugs into NC formulations [31,32], the present study offers a new strategy that directly incorporates the small-molecule drug directly into the lipid bilayer. This approach takes advantage of the cationic charge of the small-molecule drug and avoids using the potentially cytotoxic cationic lipid [9], which could reduce the cytotoxicity of the NCs (Figure 4). However, due to the interferon-inducing properties and potential immunogenicity of siRNAs complexed to lipoidal amine [29,30], the dose and dosing regimen of LANC–siRNA complexes should be carefully selected for *in vivo* applications.

The present study is the first to investigate the distribution of effervescent inhalable NPs after pulmonary administration in a cancer-bearing mouse model by WBA and fluorescence microscopy. The autoradiograms showed that inhalable NPs carried in effervescent microparticles achieved deep lung deposition. Moreover, NPs disseminated to different parts of the lung and to some extrapulmonary tissues. The long retention time (24 h) of inhalable NPs in the lungs contributes to the efficacy and safety of inhalable NPs. CLSM images

indicated that DOX was released from the inhalable NPs after deep lung deposition and reached cell nuclei to exert anticancer activity. The ability of inhalable NPs to achieve deep lung deposition, to be actively released from microcarrier particles, to spread to different parts of the lung and to release DOX *in vivo*, all contribute to the efficacy of the delivery and localization of the effervescent inhalable NPs as a novel approach to cancer treatment. The current results, and previous studies of the safety and efficacy of inhalable effervescent NPs, support the use of this delivery system as a lung cancer treatment in humans. In the future, active targeting properties (antibodies or other targeting moieties) could be added to effervescent inhalable NPs to recognize cancerous cells in lung or extrapulmonary organs after pulmonary delivery. The effect of the effervescent reaction on the stability of the targeting moiety should be evaluated. The macrophage–NP relationship should be studied further, and effects of inhalable NPs on targeting or avoiding alveolar macrophages could be investigated.

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Key Term

Nanocarriers of siRNA	Colloidal particulate systems with 1–1000-nm particle size, which can carry siRNAs through the cellular membrane and release them into the cytoplasm
Cellular uptake	Vesicle-mediated process used by all cells to take up extracellular fluid, macromolecules and micro-/nano-particles

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Executive summary

- Novel lipoidal amine-based nanocarrier (LANC) formulations were developed to combine a pharmacological active agent, Avridine[®], and siRNAs within one nanocarrier delivery system.
- The optimal LANC formulation efficiently delivered siRNA into cytoplasm and mediated significant gene-silencing activity.
- The results of cellular uptake, cytoplasmic release and gene silencing suggested that escaping from the endocytic vesicles and intracellular release of siRNA were more critical for the gene-silencing activity of LANCs than cellular uptake.
- The LANC formulations offered a new methodology to directly incorporate the small-molecule drug into the lipid bilayer of siRNA nanocarriers.

Figure 1. Structures of the major components of lipoidal amine-based nanocarriers and the lipoidal amine-based nanocarrier-siRNA complexes

(A) Avridine, (B) DOPE, (C) Span 80 and (D) TPGS. (E) The lipoidal amine-based nanocarrier.

DOPE: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; PEG: Polyethylene glycol; Span 80: Sorbitan mono-oleate; TPGS: D- α -tocopheryl polyethylene glycol-1000 succinate.

Figure 2. Particle size of lipoidal amine-based nanocarrier formulations

Particle sizes of (A) lipoidal amine-based nanocarrier and (B) lipoidal amine-based nanocarrier-siRNA complexes were determined by dynamic light scatter. Error bars represent standard deviation of size distribution.

DOPE: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; LANC: Lipoidal amine-based nanocarrier; Span 80: Sorbitan mono-oleate; TP GS: D- α -tocopheryl polyethylene glycol-1000 succinate.

Figure 3. ζ potential and complexation efficiency of lipoidal amine-based nanocarriers
(A) ζ potential of lipoidal amine-based nanocarrier and **(B)** ζ potential of siRNA–lipoidal amine-based nanocarrier complexes and siRNA complexation efficiency of siRNA–lipoidal amine-based nanocarrier complexes. Error bars represent standard deviation of ζ potential. DOPE: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; LANC: Lipoidal amine-based nanocarrier; Span 80: Sorbitan mono-oleate; TPGS: D-α-tocopheryl polyethylene glycol-1000 succinate.

Figure 4. Cell viability of lipoidal amine-based nanocarrier formulations

The cells were treated with lipoidal amine-based nanocarrier formulations containing siRNA (vector:siRNA weight ratio of 15:1) and the cells' viability was measured by MTS assay. The concentration of siRNA in each well was 100 nM. Data are presented as the mean $\pm$ standard deviation (n = 3).

DOPE: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; LANC: Lipoidal amine-based nanocarrier; Span 80: Sorbitan mono-oleate; TPGS: D- α -tocopheryl polyethylene glycol-1000 succinate.

Figure 5. Luciferase gene silencing by lipoidal amine-based nanocarrier-siRNA complexes
 SK-Hep1 luc cells were transfected with lipoidal amine-based nanocarrier-siRNA complexes with (A) antiluciferase siRNA or (B) negative control 1 siRNA at 100 nM siRNA concentration. Data were represented as the mean $\pm$ standard deviation ($n = 3$, ** $p < 0.01$, lipoidal amine-based nanocarriers treated versus untreated control).
 DOPE: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; LANC: Lipoidal amine-based nanocarrier; Span 80: Sorbitan mono-oleate; TPGS: D- α -tocopheryl polyethylene glycol-1000 succinate.

Figure 6. Cellular uptake and cytoplasmic release of lipoidal amine-based nanocarrier-FAM-siRNA-Cy3-MB complexes

(A) Representative flow-cytometry histograms of cellular uptake and (B) cytoplasmic release of LANC-FAM-siRNA-Cy3-MB complexes. (C) MFI of FAM-siRNA and (D) Cy3-MB obtained from flow-cytometry measurement shown in (A) and (B). Data were represented as the mean $\pm$ standard deviation ($n = 3$).

DOPE: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; LANC: Lipoidal amine-based nanocarrier; MFI: Mean fluorescence intensity; Span 80: Sorbitan mono-oleate; TPGS: D- α -tocopheryl polyethylene glycol-1000 succinate.

Table 1

Compositions of lipoidal amine-based nanocarrier formulations.

Name	Lipid composition	Molar ratio
LANC-DOPE	Avridine/DOPE/TPGS	50/49/1
LANC-DOPE	Avridine/DOPE/TPGS	50/45/5
LANC-Span 80	Avridine/Span 80/TPGS	50/49/1
LANC-Span 80	Avridine/Span 80/TPGS	50/45/5

DOPE: 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; LANC: Lipoidal amine-based nanocarrier; Span 80: Sorbitan mono-oleate; TPGS: D- α -tocopheryl polyethylene glycol-1000 succinate.