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Lipid-Based Nanoparticles for siRNA Delivery in Cancer Therapy: Paradigms and Challenges

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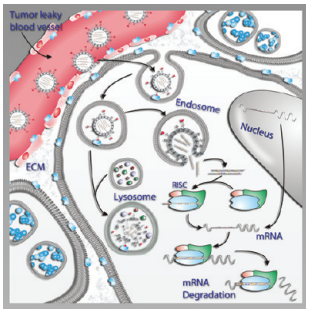
CONSPECTUS

RNA interference (RNAi) is a specific gene-silencing mechanism that can be mediated by the delivery of chemical synthesized small-interfering RNA (siRNA). RNAi might constitute a novel therapeutic approach for cancer treatment because researchers can easily design siRNA molecules to inhibit, specifically and potently, the expression of any protein involved in tumor initiation and progression.



Despite all the potential of siRNA as a novel class of drugs, the limited cellular uptake, low biological stability, and unfavorable pharmacokinetics of siRNAs have limited their application in the clinic. Indeed, blood nucleases easily degrade naked siRNAs, and the kidneys rapidly eliminate these molecules. Furthermore, at the level of target cells, the negative charge and hydrophilicity of siRNAs strongly impair their cellular internalization. Therefore, the translation of siRNA to the clinical setting is highly dependent on the development of an appropriate delivery system, able to ameliorate siRNA pharmacokinetic and biodistribution properties.

In this regard, major advances have been achieved with lipid-based nanocarriers sterically stabilized by poly(ethylene glycol) (PEG), such as the stabilized nucleic acid lipid particles (SNALP). However, PEG has not solved all the major problems associated with siRNA delivery. In this Account, the major problems associated with PEGylated lipid-based nanoparticles, and the different strategies to overcome them are discussed.

Although PEG has revolutionized the field of nanocarriers, cumulative experience has revealed that upon repeated administration, PEGylated liposomes lose their ability to circulate over long periods in the bloodstream, a phenomenon known as accelerated blood clearance. In addition, PEGylation impairs the internalization of the siRNA into the target cell and its subsequent escape from the endocytic pathway, which reduces biological activity. An interesting approach to overcome such limitations relies on the design of novel exchangeable PEG-derivatized lipids. After systemic administration, these lipids can be released from the nanoparticle surface. Moreover, the design and synthesis of novel cationic lipids that are more fusogenic and the use of internalizing targeting ligands have contributed to the emergence of novel lipid-based nanoparticles with remarkable transfection efficiency.

1. The Challenge: Systemic Delivery of siRNA

The discovery of RNA interference (RNAi) as a potent sequence-specific post-transcriptional gene silencing mechanism has opened an avenue of opportunities to study gene function and potential novel forms of therapeutic intervention in several genetic diseases.

In mammalian cells, RNAi can be mediated intracellularly by small-interfering RNAs (siRNAs), 21−23 nucleotide long double-stranded RNAs, which inhibit the expression of a target gene through specific cleavage of perfectly complementary mRNA.[1] This siRNA-based technology has an enormous potential to become a novel therapeutic strategy, because it can modulate the expression of any protein, even undruggable target proteins (e.g., transcription factors), with higher specificity than traditional drugs. Moreover, novel siRNA sequences can be rapidly and rationally designed when the mRNA sequence is known, being the manufacturing process rapid and scalable.[2] Oncology is

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one of the medical areas that can benefit most from this new therapeutic strategy, because it can modulate the expression of any gene involved in tumor initiation and growth and metastasis formation.

However, the clinical application of siRNAs has been impaired by their limited cellular uptake, low biological stability, and unfavorable pharmacokinetics. Indeed, naked siRNAs are easily degraded by blood nucleases and rapidly eliminated by the kidneys.[3] Furthermore, at the level of the target cells, the negative charge and hydrophilic nature of siRNAs strongly impair their cellular internalization.[3] Therefore, their translation to the clinical setting is largely dependent on the development of an appropriate delivery system, able to ameliorate siRNA pharmacokinetic and biodistribution properties.

Ideally, a siRNA nanocarrier should provide protection from blood nucleases and extended blood circulation, which ultimately would increase the possibility of accumulation in the primary tumor or metastasis. Moreover, at the tumor level, the leaky and discontinuous tumor endothelium together with the poor lymphatic drainage facilitates the accumulation of 100−200 nm nanocarriers, a phenomenon known as "enhanced permeability and retention" (EPR) effect.[4] The delivery system containing the siRNA must then be internalized by the target cells (cancer or supporting stromal cells), and upon receptor-mediated endocytosis, it must be able to escape from the endosomal compartment into the cell cytoplasm, where the RNAi machinery and the corresponding mRNA are located, while avoiding degradation by lysosomal enzymes (Figure 1).[3] Furthermore, delivery systems for the systemic administration of siRNA ought to be well tolerated upon administration. They should be safe and nonimmunogenic, enabling multiadministration treatment modalities required for improved clinical outcomes.[2] From a development point of view, production of large batches with reproducible specifications is also desirable.

Many different types of nanomaterials have been developed to provide solutions to successful tumor targeting, and lipid-based nanoparticles are by far the most widely used and studied nanocarriers for cancer therapy.[3] Among lipid-based nanoparticles, cationic liposomes have been the most extensively used system for the delivery of nucleic acids, including siRNA.[5] Complexation of cationic liposomes with the negatively charged siRNA generates lipoplexes with high ability to mediate siRNA transfection.[5] Albeit lipoplexes represent a valuable tool for *in vitro* purposes and some success has been achieved *in vivo*, for example, in bladder cancer following intravesical administration,[6] they are not suited for the systemic delivery of siRNAs. In fact, upon intravenous administration, positively charged nanoparticles interact with the negatively charged serum proteins forming aggregates that accumulate mainly in the lungs, liver, and spleen.[5] Moreover, lipoplexes often induce pulmonary toxicity associated with complement activation and inflammation.[5] Therefore, alternative formulations have been developed with adequate properties for the systemic administration of siRNAs.

In this respect, significant advances have been achieved with the development of different types of poly(ethylene glycol) (PEG)-grafted nanocarriers.[3] The hydrophilic nature of PEG provides an aqueous shield around the nanoparticle surface, thus decreasing the extent of opsonization and the subsequent recognition by the macrophages of the mononuclear phagocytic system, which ultimately leads to an increase of the nanoparticle blood residence time.[3] Consequently, PEG has been extensively used by the pharmaceutical industry to improve the pharmacokinetics properties of different therapeutic agents and drug nanocarriers.

2. The "PEG Dilemma"

Although the development of PEG-derivatized lipids has brought significant advances to the design of nanoparticles for intravenous administration, the experience accumulated over the last years has revealed some important limitations, especially in what concerns nucleic acid-containing nanocarriers.[7]

2.1. Immunogenicity and Accelerated Blood Clearance of PEGylated Liposomes. In a clinical setting, multiple administrations of the therapeutic agent are usually required for improved therapeutic outcome. Initially, it was believed that nanoparticles sterically stabilized by PEG were not immunogenic. However, several reports have demonstrated that upon subsequent administrations of PEGylated liposomes, an immune response could be elicited, leading to a rapid blood clearance or undesirable side effects, which further compromised the nanoparticle clinical utility. Dams et al.[8] were the first to report that systemic administration of "empty" PEGylated liposomes significantly altered the pharmacokinetic profile of subsequently injected liposomes, given up to 4 weeks after the first injection. It was further demonstrated that the observed accelerated blood clearance was due to the production of anti-PEG IgM followed by activation of the complement system and enhanced liposomal uptake by Kupffer cells.[9]

2.2. Adjuvant Effect of Nucleic Acids on PEG Immunogenicity. The nature of the drug encapsulated in the PEGylated liposomes has shown to have a strong influence on the

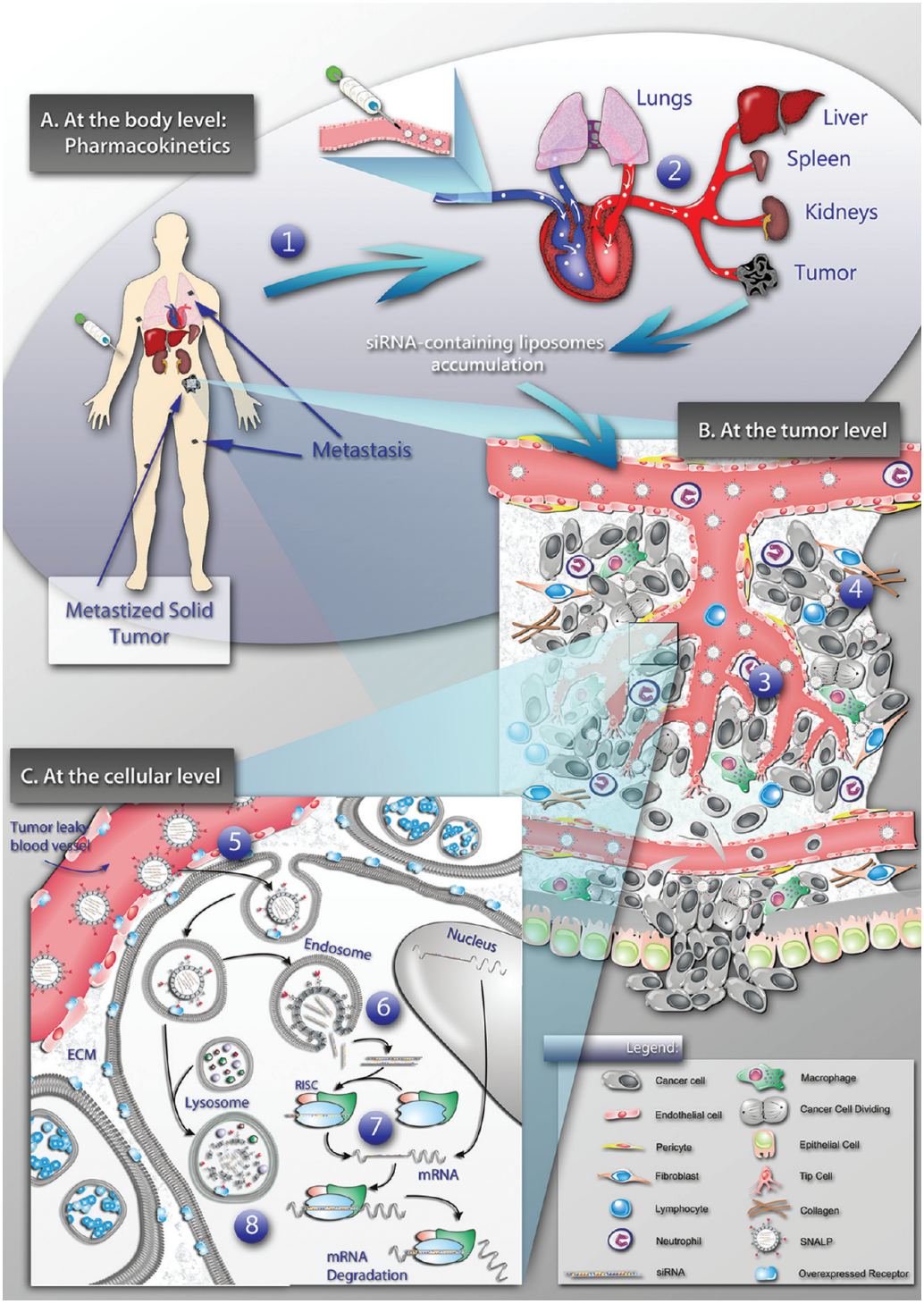

FIGURE 1. Schematic representation of barriers faced by tumor-targeted siRNA-containing nanoparticles, following intravenous administration. (1) The clinical use of siRNA-based therapies is largely dependent on the development of nanocarriers with adequate features for systemic administration. (2) They should avoid the uptake by the mononuclear phagocytic system to achieve long circulation times in the blood, (3) aiming at increasing the extent of extravasation through endothelial fenestrations of angiogenic tumor blood vessels. (4) At the tumor level, nanoparticles must diffuse throughout the extracellular matrix, spreading all over the tumor mass, including tumor cells located in hypoxia regions. (5) Upon tumor accumulation, an existing targeting moiety at the nanoparticle surface should be recognized by receptors overexpressed on the surface of cancer or other stromal cells, leading to an active internalization of the siRNA-containing nanoparticles. (6) Intracellularly, the siRNA has to escape from the endocytic pathway into the cell cytoplasm and (7) be incorporated into the RNAi machinery while (8) avoiding lysosomal destruction.

rate of blood clearance. With a small drug-like doxorubicin, the accelerated blood clearance has not been elicited in a multiadministration schedule, owing to its antiproliferative effect on splenic B cells, which reduced the extent of production of anti-PEG IgM.[10] This is in contrast to payloads like nucleic acids.

Judge et al.[11] demonstrated that multiadministration of stabilized plasmid lipid particles (SPLP) with an extended blood circulation time resulted in a significant loss of transgene expression at the tumor level and increased expression in the liver. As in the reports previously referenced, the decrease in the extent of tumor targeting was elicited by a strong antibody response (IgM and IgG) against PEG, following the first administration.[11]

The PEG-derivatized lipids used in the mentioned work had the ability to diffuse out of the lipid bilayer, at a rate determined by the length of the lipid anchor. The shorter the length of the acyl chain of the lipid anchor, the faster PEG dissociated from the lipid bilayer, resulting in particles with higher transfection efficiency than those containing PEG−lipid conjugates with longer lipid anchors. The use of exchangeable lipids will be further discussed in section 2.4.

In fact, in the work of Judge et al.[11] the replacement of the PEG-derivatized lipid with an 18C alkyl chain by the 14C counterpart led to 10-fold reduction in the production of anti-PEG IgM, which was explained by its higher diffusion rate. Despite the 2-fold lower transgene expression provided by the latter, it enabled equivalent levels of tumor transgene expression after repeated administrations, thus indicating that the accelerated blood clearance phenomenon was in fact prevented.[11] Similar results were reported with stabilized antisense lipid particles encapsulating antisense oligonucleotides (SALP) or ribozymes.[12]

Tagami et al.[13] showed that systemic administration of siRNA-associated PEG-coated lipoplexes, regardless of the presence of immune stimulatory motifs, caused anti-PEG IgM production, which further mediated accelerated blood clearance of a second dose injected 5 days later. This effect was also observed with PEG-coated pDNA-associated lipoplexes administered systemically in a multiadministration schedule and was enhanced by the presence of the immunostimulatory pDNA CpG motifs, which induced proliferation of splenic cells including B cells.[14]

Interestingly, when the siRNA was encapsulated into the core of PEGylated liposomes, rather than complexed, the anti-PEG IgM levels were dramatically reduced.[15] This observation points out an important benefit of siRNA encapsulation when multiple systemic administrations of siRNA are required. However, this effect was solely verified with nonimmunostimulatory siRNA.[15]

Overall, these observations demonstrated the importance of using nonimmunostimulatory nucleic acids to prevent the production of anti-PEG antibodies and the consequent accelerated blood clearance, which ultimately might compromise the clinical utility of siRNA-containing lipid-based nanosystems upon repeated administrations.

2.3. The Effect of PEGylation on Cellular Internalization and Endosomal Escape. The prolonged blood circulation time is a critical factor for any nanotechnology-based strategy aiming at targeting disease sites beyond the liver, like solid tumors. In this respect, the use of a component-like PEG is unavoidable. However, the final therapeutic outcome is a sum of different events that, in the case of gene silencing strategies, is also dependent on the access of the nucleic acid to the cytoplasm of the target cell, in its intact form. At the cell level, PEG significantly hinders the cellular uptake of the liposomes and the endosomal escape of the nucleic acid, because it limits the contact between membranes. This effect was observed with nucleic acids (either plasmids or as ODN) when delivered by nanoparticles of different nature (either lipoplexes[16] or polyethylenimine- or cyclodextrin-based nanoparticles[17]). Overall, the design of any nanocarrier aimed at the successful systemic delivery of siRNAs to distant organs or entities such as solid tumors should rely on a balance between the transfection efficiency and favorable pharmacokinetics.

2.4. Overcoming the Adverse Effects of PEG. PEGylated liposomes for siRNA delivery should be designed in a way that circumvents the PEG-associated limitations, which strongly influence two major factors of the overall pharmacodynamics of the delivered nucleic acid: the blood circulation half-life and the intracellular bioavailability.

An interesting approach to overcome these problems consists in the use of exchangeable PEG-derivatized lipids, as already exemplified in section 2.2. This strategy relies on the use of different lengths of the acyl chain of the lipid anchor, typically ceramides (PEG−Cer)[18] or diacylglycerols (PEG−S−DAGs).[19] As the length of the acyl chain increases, the residence time of the PEG-derivatized lipid on the nanoparticle surface also increases, which is of high relevance, for instance, when the target tissue is a distant extra-hepatic tumor. This was clearly evidenced with SPLP prepared with $CerC_{20}$−PEG_{2000} (exchange rate $t_{1/2} \geq 13$ days)[20] which, upon replacement by $CerC_8$−PEG_{2000} (exchange rate $t_{1/2} \leq 1.2$ min), resulted in efficient tumor gene expression *in vitro* and *in vivo*, after intraperitoneal administration.[21]

Nevertheless, the use of the fast exchangeable $CerC_8$–PEG_{2000} is not adequate for a systemic administration because the particles rapidly expose their positive charges, thus becoming very unstable.

Fast and simple synthesis is the main advantage associated with the use of PEG–S–DAGs, relative to the PEG–ceramide counterparts. SPLP incorporating PEG–S–DMG (14C), PEG–S–DPG (16C), or PEG–S–DSG (18C) presented an exchange rate $t_{1/2}$ in the bloodstream of 1, 6, or 15 h, respectively. Indeed, upon intravenous administration, SPLP incorporating PEG–S–DMG (14 C) predominantly accumulated in the organs of the mononuclear phagocytic system, while the extent of tumor accumulation (in a subcutaneous model) was very low. This pattern of distribution was reverted as the acyl chain length increased to 16C (PEG–S–DPG) or 18C (PEG–S–DSG), enabling longer blood circulation times and subsequent tumor accumulation. This was further correlated with increased efficiency of gene expression at the tumor level, despite the fact that, *in vitro*, the results were the opposite trend. Therefore, when designing a lipid-based nanoparticle for the delivery of siRNA, the choice of the PEG-derivatized lipid is a balance among prolonged blood residence time, lack of immunogenicity, and transfection efficiency. It was demonstrated that both PEG–Cer and PEG–S–DAGs were fairly unstable upon storage of the liposomes in their suspension form, which strongly compromises the commercial viability of such lipid-based nanoparticles. An extent of degradation greater than 60% of PEG–S–DMG (14C) was observed upon storage for 2 months at 40 °C (accelerated stability study). Novel lipids without the hydrolyzable carboxylic ester bonds (where the succinate linker was replaced by a carbamate linker) were recently synthesized, namely, PEG–C–DSA (18C) and its analogue PEG–C–DMA (14C), which were stable in the lipid bilayer at 40 °C for more than 6 weeks.[22]

At the intracellular level, one of the most common strategies to enhance the nucleic acid bioavailability is the incorporation of fusogenic lipids such as 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). Intercalation of amphiphilic molecules containing a protonatable acidic group (negatively charged at physiological pH) among phosphoethanolamine (PE) molecules allows the formation of bilayer structures, leading to liposome formation at physiological pH and temperature. This latter approach constitutes the basis for the biophysical mechanisms underlying the pH-sensitivity exhibited by PE-containing liposomes. While stable liposomes are formed at physiological pH, upon progressive acidification of the endocytic pathway, the carboxylic groups of the amphiphiles are protonated, reducing their stabilizing effect as the PE molecules revert into their inverted hexagonal phase.[23,24] This process promotes the liposomal destabilization, which further facilitates the access of the siRNA to its intracellular site of action, the cell cytoplasm.

Recently, novel cationic lipids with increased fusogenic properties have been designed. From the ionizable cationic lipid 1,2-dioleyloxy-*N*,*N*-dimethyl-3-aminopropane (DODMA), which has one double bond per alkyl chain, Heyes et al.[25] synthesized a series of cationic lipids with different degrees of saturation: zero (1,2-distearyloxy-*N*,*N*-dimethyl-3-aminopropane (DSDMA)), two (1,2-dilinoleyloxy-*N*,*N*-dimethyl-3-aminopropane (DLinDMA)), or three (1,2-dilinolenyloxy-*N*,*N*-dimethyl-3-aminopropane (DLenDMA)) double bonds per alkyl chain. Upon incorporation onto stabilized nucleic acid lipid particle (SNALP) formulations containing anti-luciferase siRNA, the authors demonstrated that the lamellar ($L\alpha$) to reversed hexagonal (H_{II}) phase transition temperature is, to some extent, inversely proportional to the number of double bonds.[26] As the number of double bonds increased from 0 to 2, a significant improvement of the transfection efficiency was observed. Nevertheless, the incorporation of DLenDMA did not bring any additional benefit. Recently, the linker and headgroup of DLinDMA were optimized upon introduction of a ketal ring linker and methylene group into the headgroup, giving rise to DLin-KC2-DMA, which provided a 10-fold increase in the gene silencing activity at the liver level.[26]

Another interesting approach to overcome PEG limitations relies on the development of new cleavable PEG-derivatized nanoparticles taking advantage of pH-,[27] redox potential-,[28,29] or enzyme-responsive[30] chemistry. PEG cleavage from the nanoparticle surface improves the transfection ability of the former toward cancer cells.

The aforementioned examples illustrate how important a deep understanding of the lipid structure–activity relationship is for the rational development of lipid-based nanoplatforms for the systemic administration of siRNAs. Indeed, a balance between prolonged blood circulation times and high transfection efficiency is mandatory to achieve significant therapeutic results.

3. "Stabilized Nucleic Acid Lipid Particles" (SNALPs)

Numerous formulations of PEG sterically stabilized liposomes have been described for the systemic delivery of nucleic acids for gene silencing, one of the most noteworthy

advances being achieved with the development of "stabilized nucleic acid lipid particles" (SNALPs) by Tekmira Pharmaceuticals Corporation. Typically, SNALPs have a mean size of 100 nm and are formed with an ionizable cationic lipid such as DLinDMA or more recently DLin-KC2-DMA, cholesterol, a lipid with a high transition temperature such as 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and a PEG-derivatized lipid like the previously mentioned diacylglycerols (DAGs). Indeed, it was the rational combination of a fusogenic and ionizable cationic lipid with lipids that stabilize the lipid bilayer (DSPC and cholesterol) and a PEG-derivatized lipid that enabled the development of a transfection-competent nanoparticle with prolonged blood circulation.

Morrissey et al.[31] were the first to confirm the suitability of SNALPs for the systemic delivery of siRNA. In a mouse model of hepatitis B virus (HBV) infection, intravenous injection of SNALPs containing an anti-*HBV* siRNA (3 mg/kg) during 3 consecutive days led to a prominent inhibition of virus replication that lasted for up to 7 days after dosing. Zimmerman et al.[32] successfully demonstrated in cynomolgus monkeys that such strategy enabled a rapid onset and a long lasting effect. Forty-eight hours following a single intravenous administration of 2.5 mg/kg of anti-apolipoprotein B (*ApoB*) siRNA, encapsulated in SNALPs, reduced *ApoB* mRNA in the liver by 80−90%, which was associated with 65% cholesterol reduction. Remarkably, *ApoB* silencing was persistent for at least the 11 days of the study.

In the work of Geisbert et al.,[33] complete postexposure protection against a lethal Zaire Ebola virus (ZEBOV) challenge was also attained with repeated administrations of SNALPs encapsulating a pool of four siRNAs against the RNA polymerase L gene of ZEBOV, both in guinea pigs (1 mg/kg) and in rhesus macaque (2 mg/kg).

The versatility of SNALPs as a delivery platform for siRNA has been extended to the down-regulation of polo-like kinase 1 gene (*PLK1*), which is involved in the deregulated proliferation of cancer cells of tumors of diverse histological origin. Intravenous administration of 2 mg/kg of anti-*PLK1* siRNA encapsulated in SNALPs twice a week for 3 weeks in mice bearing established Hep3B orthotopic liver tumors caused significant suppression of tumor growth but not complete eradication. It is important to emphasize that this treatment schedule was devoid of immune responses, and it was experimentally demonstrated for the first time that the therapeutic outcome was the result of RNAi-specific mRNA cleavage in cancer cells.[34]

The success generated in each of the previously mentioned reports has boosted the clinical evaluation of each of the referred liposomal (SNALP) siRNAs for the treatment of liver cancer, hypercholesterolemia, and Ebola virus infection. These clinical trials were identified as NCT01262235, NCT00927459, and NCT0151881, respectively, in the ClinicalTrials.gov database.

A careful look at the majority of the successful results associated with the systemic delivery of siRNA, either with SNALPs or any other siRNA delivery platform, reveals that they are almost exclusively related to liver-associated diseases. This is not a mere coincidence but rather an exploitation of the favored liver accumulation, which is in part explained by its good perfusion and fenestrated endothelium, as well as by its role in the mononuclear phagocytic system, being the primary site of accumulation of foreign bodies, such as delivery platforms. Therefore, the distinctive liver physiology creates an attractive opportunity for the development of siRNA delivery systems targeting liver-associated diseases, while it poses a major challenge to targeting disease sites beyond the liver.

Recently, Akinc et al.[35] demonstrated that, once inside the vascular compartment, SNALPs are opsonized by apolipoprotein E (ApoE), which acts as an endogenous targeting ligand. Upon recognition by low-density lipoprotein receptors, ApoE mediates an active receptor-mediated endocytosis by the target cells, the hepatocytes, while escaping from the uptake by the Kupffer cells.

Therefore, the endogenously formed ApoE-targeted SNALPs, together with the specific SNALP lipid composition, high extent of accumulation at the liver level, and potency of the delivered siRNA, are behind the success of this strategy against liver-associated diseases.

4. Ligand-Mediated Targeting

Effective delivery of siRNA toward extrahepatic targets like solid tumors still represents an enormous challenge that has not been successfully addressed. In this context, one of the most promising strategies involves the covalent attachment of an internalizing targeting ligand at the extremity of PEG chains grafted onto a delivery system, which will specifically interact with antigens or receptors overexpressed on the surface of the target cancer cells.[36]

However, an improved cellular internalization is not necessarily synonymous of an efficient gene silencing. The work of Santos et al.[37] is one of such example. Antagonist G-targeted SNALPs (130 nm), formed with 10 mol % $CerC_{16}$−PEG_{2000} and containing anti-*BCL2* siRNA, presented an uptake into small cell lung cancer cells that was 20-fold higher than that of the nontargeted counterpart but failed to

down-regulate the target protein. As discussed previously, this lack of biological activity was likely due to the presence of high amounts of PEG.

Alternatively, it is interesting to notice that the obstacles imposed by the presence of PEG, can be overcome through the selection of targeting ligands with fusogenic properties, like transferrin.[38] In fact, Mendonça et al.[39] have developed transferrin-targeted SNALPs (180 nm), incorporating 8 mol % PEG, which *in vitro* resulted in enhanced cellular internalization and further improvement of *BCR-ABL* silencing at the mRNA and protein levels in two leukemia cell lines.

In the work of Di Paolo et al.,[40] coated cationic liposomes (CCL), a PEGylated type of liposomes, were targeted to GD2-overexpressing neuroblastoma cells with a GD2-targeted monoclonal antibody or its Fab′ fragment, resulting in liposomes with a mean size of 159 and 143 nm, respectively. *In vitro* studies demonstrated improved cellular internalization and further silencing of the anaplastic lymphoma kinase (ALK) gene. This effect was further correlated with *in vivo* tumor growth inhibition and *ALK* down-regulation at the tumor level.

Recently, the first in-human phase I clinical trial using targeted nanoparticles for the systemic delivery of siRNA was conducted in three patients with metastatic melanoma. In tumor sections from patients' biopsies, a transferrin-targeted cyclodextrin-based nanoparticle containing a siRNA against *RRM2* with a mean size of 70 nm (CALAA-01, from Calando Pharmaceuticals) presented a dose-dependent accumulation and was heterogeneously distributed in the tumor tissue but not in the adjacent epidermis.[41] *RRM2* silencing, both at mRNA and at protein levels, was detected in two of the three patients. Moreover, detection of the mRNA cleavage products by RACE-PCR demonstrated for the first time that RNAi could be achieved in solid tumors in patients, following systemic delivery of siRNA-containing targeted nanoparticle.[41]

5. Concluding Remarks

The perspective of tackling the molecular basis, at the mRNA level, of a disease associated with protein overexpression like cancer, opens a wide range of therapeutic options that have not been available with existing low molecular weight drugs or monoclonal antibodies. Nevertheless, the confirmation of the promises of gene silencing-based medicines, siRNAs being a prominent representative, is still strongly dependent on the capacity to manipulate the spatial and temporal delivery of these charged and unstable 21−23 nucleotides long double-stranded RNA molecules. In this regard, nanotechnology-based approaches have paved the way for a successful clinical implementation.

When one designs a nanocarrier for the systemic delivery of siRNA, multiple parameters have to be carefully considered aiming at attaining the desirable prolonged blood circulation and the ability to overcome, at the tumor level, every extra- and intracellular barrier that separates the siRNA from its molecular target, the mRNA. The increased understanding of the mechanistic basis of how the body interacts with the developed delivery platforms containing siRNA has enabled the rationale design of lipid-based nanoplatforms. The deep knowledge about the structure−activity relationship of the lipidic components and improvements performed on their chemistry, complemented with the use of internalizing targeting ligands, have enabled us to overcome issues related to immunogenicity and to improve the intracellular bioavailability of the delivered siRNA. Overall, solid steps are being taken toward an siRNA delivery system that is safe, efficient, and increasingly specific toward the tumor. Once this delivery platform is accomplished, the encapsulation of siRNAs targeting the molecular specificities of the cancer to be treated, will give rise to a medical care with major benefits for cancer patients.

L. C. Gomes da Silva is a student of the international Ph.D. program on Biomedicine and Experimental Biology from the Center for Neuroscience and Cell Biology, University of Coimbra, and recipient of a fellowship from the Portuguese Foundation for Science and Technology (FCT) (ref no. SFRH/BD/33184/2007). Nuno Fonseca is recipient of a fellowship from FCT (ref no. SFRH/BD/64243/2009). The work performed by the authors has the support of the Portugal-Spain capacitation program in Nanoscience and Nanotechnology (ref no. NANO/NMed-AT/0042/2007).

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Vera Moura has a degree in Pharmaceutical Sciences and a Ph.D. in Pharmaceutical Technology by the Faculty of Pharmacy of

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Maria C. Pedroso de Lima received her Ph.D. degree in Chemistry from Imperial College of Science and Technology at the University of London in 1977. She is currently a Full Professor at the Department of Life Sciences and the Head of the Group of Vectors and Gene Therapy at CNC. Her current research activity is focused on the design of lipid-based systems for nucleic acid delivery to target tissues or cells and to specific molecular targets in gene therapy approaches towards cancer and neurodegenerative diseases. Dr. Pedroso de Lima is the author of over 100 publications in peer-reviewed journals and holds 1 patent on nonviral vectors for gene delivery, approved in the USA. She has edited 1 book published by Springer-Verlag and has been responsible for collaborative research projects funded by the NATO Scientific Affairs Division, EU, and FCT.

Sérgio Simões is currently a Professor of Pharmaceutical Biotechnology at the University of Coimbra and a contributing lecturer for the MIT-Portugal Program. His research activities include the participation in several national and international projects funded by the FCT, EU, ADI, SIME, PRIME, QREN, and private companies. He is the author/coauthor of 7 book chapters, 90 scientific articles having been the co-inventor of 7 international patents. Moreover, his activities span to the entrepreneurial sector where he is currently the Vice-President of Bluepharma and the shareholder of other pharmaceutical technology-based companies operating in the field of research, development, and production of medicines, such as Pharmagen, Luzitin, and Treat U.

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FOOTNOTES

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The authors declare no competing financial interest.

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