



HAL
open science

Antisense strategies: delivery of oligonucleoides by nonviral nanoparticles

Claude Malvy, Christine Vauthier

► To cite this version:

Claude Malvy, Christine Vauthier. Antisense strategies: delivery of oligonucleoides by nonviral nanoparticles. Joshua D Ramsey; M Laird Forrest. Nanoparticles for Biotherapeutic Delivery, 1, Future Science Ltd, pp.6-21, 2015, 978-1-910420-40-9. 10.4155/fseb2013.14.142 . hal-03196151

HAL Id: hal-03196151

<https://hal.science/hal-03196151>

Submitted on 12 Apr 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ANTISENSE STRATEGIES: DELIVERY OF OLIGONUCLEOTIDES BY NON-VIRAL NANOPARTICLES.

Claude Malvy¹, Christine Vauthier^{2,3}

1 CNRS UMR 8203, Institut G. Roussy, rue Camille Desmoulins, 94800, Villejuif Cedex, France

2 Univ Paris-Sud, Faculté de Pharmacie, 5, rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France

3 CNRS UMR 8612, Institut Galien Paris-Sud, 5, rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France

Published in: Nanoparticles for Biotherapeutic Delivery (Volume 1). Joshua D Ramsey & M Laird Forrest, Editors. Future Science Book Series., ISBN (online): 978-1-910420-40-9. 2015; Chapter 1, Pages 6-21. DOI: 10.4155/fseb2013.14.142

Corresponding author

Claude Malvy

CNRS UMR 8203, Institut G. Roussy, rue Camille Desmoulins, 94800, Villejuif Cedex, France

E-mail : claude.malvy@igr.fr

Abstract

Gene silencing approaches have demonstrated high promise for the development of new treatments of diseases caused by the abnormal overexpression of a known gene. However, the *in vivo* delivery of the gene silencing mediating agents (antisense oligonucleotides and siRNA) is a bottleneck for the success of these innovative treatments. Solutions for their delivery are emerging from the nanomedicines thanks to the extremely active research aiming to find suitable delivery methods. This paper summarizes the main characteristics in term of their composition and structure of the most advanced carriers designed for siRNA and antisense oligonucleotide delivery. It also summarizes the results of the activity produced *in vivo* by several of these systems. The conclusion highlights open questions and bottlenecks that remain on the way to clinical development at a large scale.

Biography of the authors

Claude Malvy

Claude Paul Malvy, aged 66, is Professor of Biochemistry at the University Paris-Sud. He is in charge of a research group working on artificial regulation of gene expression by oligonucleotides in a CNRS laboratory located inside the Gustave Roussy Institute which is a prestigious european center devoted to treatment and research on cancer.

In the last 15 years he has focused his research on the inhibition by nucleic acids of junction oncogenes, created by chromosomal translocation, taking Ewing sarcoma as a model of junction oncogene cancer. The targeting of the junction at the RNA level is indeed very specific of the tumor cells and this research is now extended to many cancers. He is author and co-author of more than 100 publications in international scientific journals. He is a member of Nucleic Acids Therapeutic's board. Claude P Malvy is the head of the Institute for Higher Biomedical Studies located in Paris-area (IFSBM, <http://www.igr.fr/ifsbm>). The aim of this Institute is to give a formation in biology and medicine to high standard PhD students with an intial degree in hard sciences (physics, mathematics, informatics, chemistry). As a professor he is mainly teaching the basis of cellular biology to IFSBM students.



Christine Vauthier.

Christine VAUTHIER, PhD, is Director of Research at the CNRS (Centre National de la Recherche Scientifique). She received her Ph. D. in polymer chemistry in 1986 from the University Louis Pasteur at Strasbourg, France. She joined the University of Paris South, Faculty of Pharmacy in 1987 as a research assistant where she started working on nanomedicines. During her carrier, she was a visiting scientist at the Center for Chemical Controlled Delivery, University of Utah, USA and invited to spend some months at the Federal University of Pernambuco, Recife, Brazil. Currently, her research is focused on the design of biodegradable polymer nanoparticles for mucosal and intravenous administration of drugs including antisense oligonucleotides and the siRNA for developing new anticancer therapies.



Introduction

Antisense oligonucleotides (AS-ODN) and small interfering RNA (siRNA) are very promising molecules for the development of specific treatment against cancer [1-4]. However, their systemic in vivo delivery is challenging. The active molecules need to be protected against degradation by nucleases while they also need to be helped to go across the many biological barriers found on their way between the site of administration and the cell cytoplasm where the target molecules are located [2]. Chemical modifications of AS-ODN and siRNA are known to improve their stability in vivo but this is often at the expense of a loss of their activity [2,5]. Chemical modifications on their own are not sufficient to let the molecule to go across all barriers and to penetrate into cells. Alternative methods consist in associating the natural molecules which corresponds to the phosphodiester oligonucleotide with a carrier that will serve as transporter and transfection agent. Nucleic acids as anticancer drugs offer many possibilities. According to the properties of nucleic acids most of their applications are either using the coding properties of nucleic acids or the Watson Crick hybridization properties of nucleic acids. Coding properties fall in the Gene Therapy category and hybridization properties in the oligonucleotide (siRNA, AS-ODN) category. In cancer therapy the gene delivery in cancer cells inside a tumor requires a very high transfection efficiency which can be obtained only by using modified viruses to deliver genes (foreign genes have to be expressed in all cells of the tumor). Oligonucleotide with much shorter sizes than genes can be delivered at thousands copies in these same cells by using chemical vectors with lower transfection efficiency but which do not present the risks endogenous to modified viruses.

The number of proposed systems is impressive in relation to the youth of this area of research [6-12, 52]. In general, they achieved the first challenge which is to protect the nucleic acid fragments against the degradation by nucleases and other provided data are a rather rough description of their physicochemical characteristics. Only few systems were evaluated ahead to demonstrate their potential to deliver siRNA or AS-ODN producing an anticancer effect in vivo using appropriate animal models. The occurrence of a reduction of tumor growth was related to the expected antisense activity of the antisense agent associated with the carrier. The results were extremely promising motivating the rapid development of clinical tests in human with the few formulations that have passed all the preclinical evaluations stages [12-14]. It remains a very hot topic that is developing very fast through the identification of new potential targets and improving the delivery methods [15]. In this review, the first part we will

give a brief description of the different systems that are now in clinics in human or close to clinical testing. The second part described the biological activity of only few systems that are not yet in clinics but showed very promising effect on cancers caused by junction oncogenes.

Delivery systems for siRNA and AS-ODN by the systemic route that have reached advanced stages of development.

Only few synthetic delivery systems have reached an advanced stage of development. The table 1 and 2 give a description of their composition and their structure is described in the figure 1. As indicated in Table 1 and 2, several were already included in clinical trials in human for the delivery of siRNA and AS-ODN by the systemic route. It is noteworthy that the different proposed systems displayed a large variety of composition and structures that matched different groups of nanotechnologies developed as nanomedicine. Those made of polymers including nanospheres and nanocapsules were designed by polymerization of alkylcyanoacrylates used as monomers [16-18] and by methods based on the precipitation of an hydrophobic polymer in very well defined conditions including emulsification-followed by solvent evaporation [19,20]. It is noteworthy that many systems form thanks to the self assembly of their basic components. This can lead to the formation of particles having well defined structures and even, in some cases, internal crystal-like structures (Figure 1).

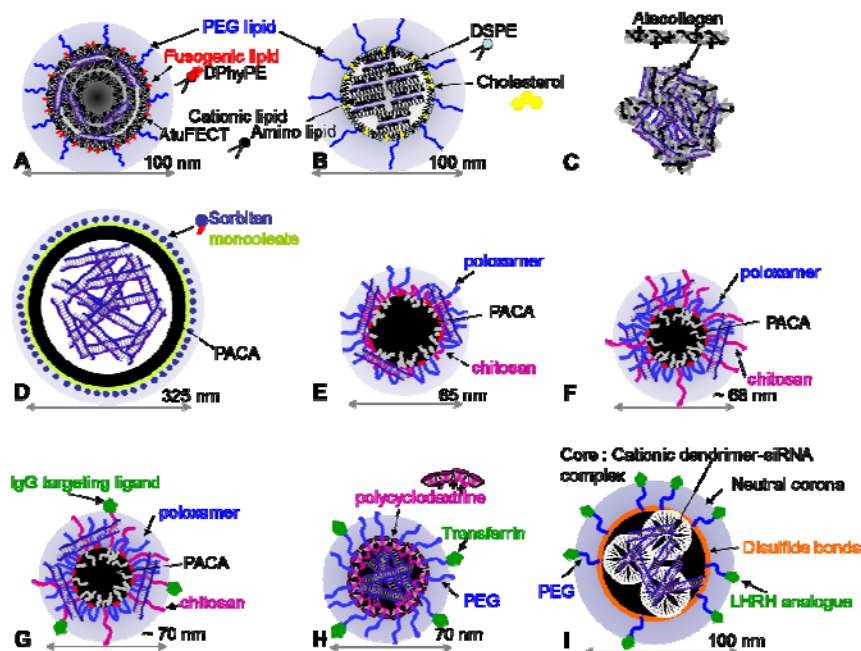


Figure 1: Schemes of the structures of several systems for the delivery of siRNA as they are proposed in the literature. A: AtuPLEX [30], B: Stable Nucleic Acid Lipid Nanoparticles (LNP) [43], C: Atecollagen-siRNA complexes [40], D: Poly(alkylcyanoacrylate) (PACA) nanocapsules [17,48], E: PACA nanospheres type I [16,51], F: PACA nanospheres type II [18,29,51], G: PACA nanospheres type II targeted [29], H: Nanospheres with cyclodextrines [25], I: Nanospheres with dendrimers [45]. DPhyPE: 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine

Table 1: More advanced Lipid based carriers developed for in vivo delivery of siRNA and ODN

Type of carrier/Name of technology/ Company	Composition	Figure	Nucleic acid	Disease (Status of development)	References
Cationic lipoplexe/ AtuPLEX lipoplex/ Silence Therapeutics	Atu027 Atufect01: multivalent cationic lipid DPhyPE: fusogenic helper lipid, MPEG 2000-DSPE Molar ratio: 50/49/1	A	23mer siRNA against PKN3 Delivery to endothelial cells	Solid Cancer Clinical trials	[30]
Neutral liposomes/ LNP liposomes/ Alnylam Pharma Tekmica Pharma	Amino lipid chosen among more than 50 molecules, DSPC, cholesterol PEG-lipid Molar ratio: 50/10/38.5/1.5	B	Already several targets located in the liver - siRNA targeting hepatic transthyretin (TTR) (ALN-TTR01) - siRNA targeting VEGF (vascular endothelial growth factor) and KSP (kinesin spindle protein) (ALN-VSP02) - siRNA targeting the polo-like kinase 1, (TKM-PLK1) - siRNA targeting apolipoprotein B, (TKM-ApoB) - siRNA targeting PCSK9 (ANL-PCS02) - Viral RNA, Ebola infections (TKM-EBOLA) - siRNA targeting the hepatitis B virus HBV	Transthyretin Amyloide (clinical trial) Liver cancer (clinical trial) Solid cancer and lymphoma (clinical trial) Hypercholesterolemia (clinical trials) Viral infections (Clinical trial)	[26,34]
Lipidoids	- 98N ₁₂ -5(1): five-tailed isomer of triethylenetetramine– laurylaminopropionate with a free internal amine - Cholesterol - mPEG ₂₀₀₀ -C14 Glyceride. Molar ratio: 42:48:10	NP**	Cationic lipid nanoparticle siRNA against PD-L1 gene, double stranded RNA (dsRNA) activating expression gene P21.	Antiviral immunity in kupffer cells Prostate cancer	[35-37]
Squalenoylated particles	Nanoaggregate formed from the self-assembly of squalenoylated siRNA (prodrug)	NP**	siRNA against a junction oncogene Ret/PTC 1	Radio induced thyroid cancer	[38,39]

*DPhyPE: 1,2-diphytanoyl-sn- glycerol-3-phosphoethanolamine

** NP : Not published yet

Table 2: More advanced polymer-based carriers developed for in vivo delivery of siRNA and ODN

Type of carrier/Name of technology	Composition	Ref. to Figure 1	Nucleic acid	Disease (Status of development)	References
Atelocollagen-siRNA complex:	Complexes of atelocollagen (product obtained from the peptidolysis of collagen) with siRNA	C	21-23 mer siRNA, siRNA directed against the alpha p110 subunit of human phosphoinositide 3-hydroxykinase;	metastatic tumors in bone tissue	[40]
Miniature biodegradable matrix Silenseed Ltd	Complex between siRNA and LODER polymer, nature not disclosed	NP*	SiRNA against K-RAS G12D,	Pancreatic cancer, Intratumoral administration	[41]
polyplexe	Poly-electrolyte complex between chitosan or modified chitosan and siRNA	NP*	Very active research on various targets.	Various diseases	[10,21-23]
Nanocapsules PACA	Polymer vesicles Poly(alkylcyanoacrylate) envelope entrapping AS-ODN	D	AS-ODN against the junction oncogene EWS-Fli 1.	Ewing sarcoma (pediatric bone cancer) (Research)	[17]
Nanospheres	Chitosan decorated nanoparticle made of poly(D,L-lactide-co-glycolide) (PLGA). Chitosan was adsorbed on the PLGA core in the course of formation by nanoprecipitation.	NP*	decoy-ODN targeted nuclear factor kappa B (NF- κ B) (proinflammatory cytokine)	Ulcerative colitis (inflammatory disease of the bowel) Oral administration	[20]
Nanospheres	Core-corona nanoparticles made of Poly(alkylcyanoacrylate)-chitosan copolymers	E,F	siRNA against the junction oncogene Ret/PTC AS-ODN or siRNA against EWS-Fli1	Radio-induced thyroid cancer (Research) Ewing sarcoma (pediatric bone cancer) (Research)	[16,18,29]
Targeted nanospheres	Core corona nanoparticle decorated with antibodies	G	siRNA against the junction oncogene EWS-Fli 1.	Ewing sarcoma (pediatric bone cancer) (Research)	[29]
Targeted nanospheres / Calando Pharma	Self-assembly of 4 components siRNA, polycyclodextrine-including positively charged linkaged between cyclodextrins, PEG-adamantane, Transferrin-PEG-adamantane	H	siRNA reducing expression of RRM2 (previously denoted siR2B15) (CALAA-01)	Myeloid Leukemia clinical trial registration number NCT 00689065	[25, 42]

Table 2: More advanced polymer-based carriers developed for in vivo delivery of siRNA and ODN (Continued)

Type of carrier/Name of technology	Composition	Ref. to Figure 1	Nucleic acid	Pathologies	References
Targeted nanospheres	Complex of cationic dendrimers (poly(propyleneimine) (PPI) or Polyamidoamine (QPAMAM)) with siRNA caged by surface crosslinking with disulfide bonds. Surface is neutral due to addition of PEG. A synthetic analogue of Luteinizing hormone releasing hormone (LHRH) confers specificity to cancer cells.	I	siRNA against BCL2 mRNA	Cancer (Research)	[44,45]

* NP : not published yet

The association of the nucleic acid is achieved by promoting ionic interactions between their negative charged given by the phosphate group and positively charged components of the carrier [10,21-23] . The whole structure is also assembled by strong hydrophobic interactions between components which include fatty acid chains of lipids [13], the squalene motive of the squalenoylated siRNA [24] and the use of cyclodextrines [25].

To be active in vivo, nanomedicines designed to deliver gene silencing effectors need to remain stable in biological fluids during transportation from the site of administration down to the target site while it should dissociate to release the nucleic acid once it has reached the interior of the cell. The stability of systems needs to be tuned very precisely to achieve the right balance between these contradictory properties. For instance, over 50 different amino lipids were tested in the formulation of the nucleic acid lipid nanoparticles (LNP) which are developed as a delivery platform for siRNA to find the optimal compromise [26]. This system is now under evaluation in clinics for various siRNA targets to treat diseases in the liver [27]. In the case of the ATUPlex, the second advanced delivery platform which is also based on a lipid self assembly SiRNA are inserted into bilayers of cationic lipids. The whole complex which occurs as a very small particle is stabilized by chains of poly(ethylene glycol) (PEG). Although used to insure the colloidal stability of the nanoparticles, PEG was used to decorate the surface of many types of carriers to confer them stealth properties. It is a method for camouflaging nanomedicine surfaces to hamper recognition of by the immune system of the host organism. In this way, the delivery system circulates in the blood compartment and reaches tumors thanks to the enhanced permeation and retention effect or it can be used to target vascular endothelial cells. Recently it was shown that PEG is not the only hydrophilic polymer that can be used to camouflage the surface of nanomedicines. Polysaccharides can be used too to design stealth nanoparticles provided that the chains adopt a hair configuration on the surface of the nanomedicine and that the grafting density is high enough [18,28,29].

To improve delivery into cells, several systems were including a ligand to promote transfection by fusion with the cell membrane. For instance, the fusion peptide GALA was inserted in the structure of the squalenoylated delivery platform using a cholesterol moiety grafted on this peptide [24]. In the AtuPLEX, the fusogenic molecule is the 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPhyPE) [30]. Another type of decoration of the particle surface includes the insertion of a targeting ligand. It was transferrin in the case of the cyclodextrin-based system [25] or a monoclonal antibody associated on the surface of poly(alkylcyanoacrylate) nanoparticles [29]. The platforms were designed in such a way that the targeting moiety can be interchangeable. In the cyclodextrin based system, cyclodextrins are exhibited on the surface of the carrier and serve as anchor for the targeting ligand onto

which an adamantane residue is grafted (Figure 2A). Attachment of the targeting ligand on the poly(alkylcyanoacrylate) nanoparticles was achieved using a biotin-avidin-biotin sandwich system [31]. The platform consists in biotinylated nanoparticles and targeting ligands are biotinylated as well. The assembly between these two partners is achieved by simple incubation with avidin (Figure 2B). The number of targeting antibody molecules associating with a single nanoparticle was low, 3 to 5 units, but this low number was sufficient to increase the delivery of the cargo to target sites *in vivo* after intravenous injection and to improve treatment efficacy [32]. The low number of targeting ligand required is an advantage regarding the modification of surface properties of the carrier introduced by the addition of the targeting ligands. Indeed, it remained low enough to preserve the stealthiness of the nanoparticles as acknowledged by the low capacity to activate the complement system of both the targeted and the parent nanoparticles and by the increased delivery of the cargo to tumor with the targeted nanoparticles. Surface properties of carriers are determinant for the *in vivo* fate of the nanoparticles.

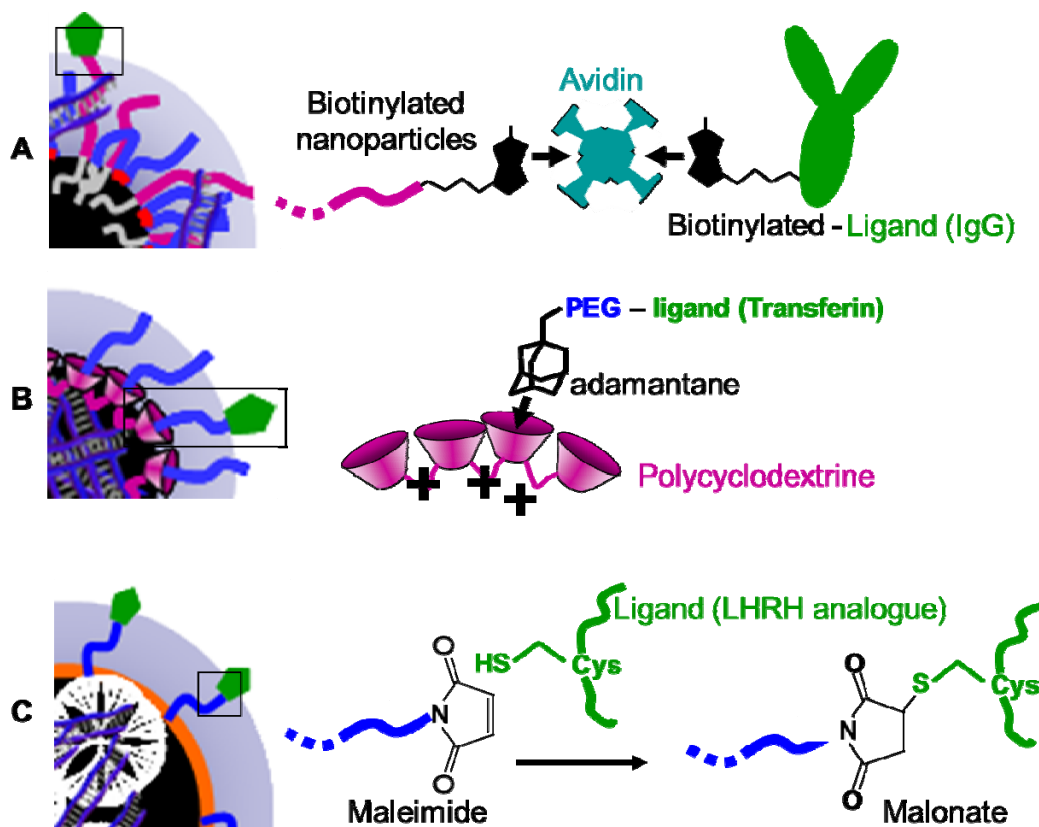


Figure 2: Mode of association of a targeting ligand to delivery systems for siRNA. A: Biotin-avidin-biotin sandwich system [29,31], B: adamantane-cyclodextrin association [25], C: chemical coupling [45]. Targeting ligands are easily interchangeable on the different delivery platforms.

Although surface properties must be designed precisely to achieve the correct biodistribution of the cargo, results from an extensive study of the influence of surface properties on the cytotoxicity and genotoxicity of carriers have pointed out that they can compromise the safety of the carrier [33]. A clear correlation was highlighted between the surface charge of the particles and their cytotoxicity. The same observation was reported regarding the evaluation of the genotoxicity of the particles through the formation of micronuclei in cells in contact with non cytotoxic concentration of the particles. It was suggested that newly designed carriers should display neutral or negatively charged surface to reduce such a risk of genotoxicity.

Examples of biological activity

All the nanoparticles-siRNA or antisense oligonucleotide listed in the table have been used successfully in biological systems either in cell culture or in vivo or both. Taking into account the diversity of their chemical nature it is very difficult to describe a unified common mechanism of action in vivo. However, in the cancer field one common point could be the Enhancement of Permeability Retention effect (EPR effect) which facilitates the uptake of nanoparticles by tumors taking into account the fenestrated nature of the tumor vascular endothelium. As examples we describe here some of the biological results obtained with squalenes, poly(alkylcyanoacrylate) nanoparticles and lipoplexes

A siRNA targeting the fusion oncogene RET/PTC1, which is the most prevalent type of gene rearrangement found in papillary thyroid carcinoma, at the level of the mRNA junction of RET and PTC1 was coupled to squalene. It formed 200 nm nanoparticles as already shown [38]. Ali *et al.* [24] found that these nanoparticles carrying the siRNA inhibit in vivo with specificity RET/PTC1 at the level of mRNA and protein as well as tumor growth. The in vivo model used was a mouse xenograft of human BHP 10-3 SC mice cells expressing the RET/PTC1 oncogene. The total dose of siRNA delivered intravenously was 0.5 mg/Kg. At the same dose the same siRNA without squalene has no effect on tumor growth and expression of RET/PTC1.

Nanospheres of PIBCA coated with chitosan and carrying a siRNA targeting RET/PTC1 were used by De Martimprey to treat mice having received a xenograft of tumorigenic murine cells stably expressing the human RET/PTC1. Either by intratumor [46] or by intravenous delivery [18] inhibition of RET/PTC1 expression and of tumor growth was obtained at a low dose of siRNA. Similar nanospheres have been used to carry either antisense oligonucleotide [16] or a siRNA [29] anti EWS/Fli-1 in preclinical models of Ewing sarcoma. With antisense oligonucleotides tumor (NIH3T3 cells expressing EWS/Fli-1) inhibition takes place after

intratumor injection (5 mg/Kg total oligonucleotide dose) [16] or after intravenous injection (30 mg/Kg total oligonucleotide dose) [47]. With siRNA a 40 % inhibition of EWS/Fli1 in the tumor (A673 human Ewing cells) is obtained with the nanospheres but is increased to near 80 % when the nanospheres are coupled to an antibody against CD99, a biomarker of Ewing cells (100 µg total dose siRNA by IV injection). Interestingly, results from the group of Malvy [47] show that, in addition to delivery, nanoparticles can sometimes erase unspecific polyanionic effects of nucleic acids.

PIBCA nanocapsules were used with antisense oligonucleotides targeting EWS/Fli-1 in cell culture (NIH3T3 cells expressing EWS/Fli-1). Nanocapsules greatly increased the cellular uptake of oligonucleotides allowing the inhibition of EWS/Fli-1 and of the cell proliferation [48]. The same nanocapsules were used to deliver siRNA targeting EWS/Fli-1 directly in the xenografted tumor [17]. At 1 mg/Kg total siRNA dose inhibition of EWS/Fli-1 expression as well as inhibition of tumor growth were observed.

Aleku et al. have used a siRNA targeting the protein kinase N3 in endothelial cells. This siRNA is associated to a lipoplex and the corresponding siRNA nanoparticle is called Atu027. Antimetastatic activity in different types of cancers in preclinical models has been shown by Aleku *et al.* [49] and Santel *et al.* [50].

Conclusion

Gene silencing approaches have now demonstrated their promise to develop efficient treatments of diseases caused by the abnormal over expression of a known gene. In vivo delivery of gene silencing mediating agents that include antisense oligonucleotides and siRNA remains the bottleneck for the success of treatments, but solutions are emerging thanks to the extremely active research aiming to find suitable delivery methods. As pointed out in this paper, several types of carriers have been identified and their clinical development is already on the way. Although tremendous progresses were made in a very short time, several challenges remain as we are still in the infant age developing therapeutic strategies based on gene silencing approaches. For instance, mechanisms by which the gene silencing activity is achieved in vivo need to be elucidated. It is also not understood so far why in vitro effect did not always correlate well with the observed in vivo effect and can even be contradictory in the sense that activity was only observed in vivo. We also need to improve our understanding of the biodistribution of nanocarriers that are designed to deliver antisense oligonucleotides and siRNA and their interactions with the immune system of the host. Another bottleneck can be the finding of relevant gene silencing mediators improving their specificity on the expression of the target gene and avoiding off target effects. In this regard, antisense oligonucleotides

and siRNA targeting junction oncogenes that are unique in the diseased cells and not present in the healthy cells are probably advantageously targeted for the development of therapeutic approached based on this method. Today, many hopes are based on research conducted in this area. For sure nanomedicines will contribute further to the development of these compounds and their success proposing new treatments to patients.

References

- 1 Burnett JC, Rossi JJ. RNA-based therapeutics: current progress and future prospects. *Chem. Biol.* 19(1), 60-71 (2012).
- 2 Gavrillov K, Saltzman W.M. Therapeutic siRNA: principles, challenges, and strategies. *Yale J. Biol. Med.* 85(2), 187-200 (2012).
- 3 Miele E, Spinelli GP, Miele E *et al.* Nanoparticle-based delivery of small interfering RNA: challenges for cancer therapy. *Int J Nanomedicine.* 7, 3637-3657 (2012).
- 4 Sen M, Grandis JR. Nucleic acid-based approaches to STAT inhibition. *JAKSTAT.* 1(4), 285-291 (2012).
- 5 Watts JK, Deleavey GF, Damha MJ. Chemically modified siRNA: tools and applications. *Drug Discov. Today.* 13(19-20):842-855 (2008).
- 6 Toub N, Malvy C, Fattal E *et al.* Innovative nanotechnologies for the delivery of oligonucleotides and siRNA. *Biomed. Pharmacother.* 60(9), 607-620 (2006).
- 7 Morille M, Passirani C, Vonarbourg A *et al.* Progress in developing cationic vectors for non-viral systemic gene therapy against cancer. *Biomaterials.* 29(24-25), 3477-3496 (2008).
- 8 Wang QZ, Lv YH, Diao Y *et al.* The design of vectors for RNAi delivery system. *Curr. Pharm. Des.* 14(13),1327-1340 (2008).
- 9 De Martimprey H, Vauthier C, Malvy C *et al.* Polymer nanocarriers for the delivery of small fragments of nucleic acids: oligonucleotides and siRNA. *Eur. J. Pharm. Biopharm.* 71(3), 490-504 (2009).
- 10 Howard KA. Delivery of RNA interference therapeutics using polycation-based nanoparticles. *Adv. Drug Deliv. Rev.* 61, 710-720 (2009).
- 11 Rudzinski WE, Aminabhavi TM. Chitosan as a carrier for targeted delivery of small interfering RNA. *Int. J. Pharm.* 399, 1-11 (2010).
- 12 Zhou J, Shum KT, Burnett JC *et al.* Nanoparticle-Based Delivery of RNAi Therapeutics: Progress and Challenges. *Pharmaceuticals (Basel).* 6(1), 85-107 (2013).
- 13 Bruno K. Using drug-exipient interactions for siRNA delivery. *Adv. Drug Deliv. Rev.* 63, 1210-1226 (2011).
- 14 Lee JM, Yoon TJ, Cho YS. Recent developments in nanoparticle-based siRNA delivery for cancer therapy. *Biomed. Res. Int.* 2013, 782041 (2013).
- 15 Maier MA, Jayaraman M, Matsuda S *et al.* Biodegradable lipids enabling rapidly eliminated lipid nanoparticles for systemic delivery of RNAi therapeutics. *Mol. Ther.* 21(8),1570-1578 (2013).

- 16 Maksimenko A, Villemeur M, Elhames H *et al.* In vivo potentialities of EWS-FLI-1 targeted antisense oligonucleotides-nanospheres complexes. *Ann. N.Y. Acad. Sci.* 1058, 52-61 (2005).
- 17 Toub N, Bertrand JR, Tamaddon A *et al.* Efficacy of siRNA-nanocapsules targeted against the EWS-FLI-1 oncogene in Ewing Sarcoma. *Pharm. Res.* 23, 892-900 (2006).
- 18 De Martimprey H, Bertrand JR, Malvy C *et al.* New core-shell nanoparticles for the intravenous delivery of siRNA to experimental thyroid papillary carcinoma. *Pharm. Res.* 27(3), 498-509 (2010).
- 19 Nafee N, Taetz S, Schneider M *et al.* Chitosan-coated PLGA nanoparticles for DNA/RNA delivery: effect of the formulation parameters on complexation and transfection of antisense oligonucleotides. *Nanomedicine* 3:173–183 (2007).
- 20 Tahara K, Samura S, Tsuji K *et al.* Oral nuclear factor-kB decoy oligonucleotides delivery system with chitosan modified poly(D,L-lactide-co-glycolide) nanospheres for inflammatory bowel disease. *Biomaterials* 32, 870-878 (2011).
- 21 Garcia-Fuentes M, Alonso M.J.. Chitosan-based drug nanocarriers: where do we stand? *J. Control. Release.* 161(2), 496-504 (2012).
- 22 Al-Qadi S, Grenha A, Remunan-Lopez C. Chitosan and its derivatives as nanocarriers for siRNA delivery. *J. Drug Del. Sci. Technol.* 22(1), 29-42 (2012).
- 23 Vauthier C, Zandanel C, Ramon AL. Chitosan-based nanoparticles for in vivo delivery of interfering agents including siRNA. *Curr. Opin. Coll. Interface Sci.* 18(15), 406-418 (2013).
- 24 Ali HM, Maksimenko A, Urbinati G *et al.* Effects of silencing RET/PTC1 oncogene in papillary thyroid carcinoma by siRNA-squalene nanoparticles with and without fusogenic companion GALA-Cholesterol. *Thyroid*. Published on line Jul 25, 2013.
- 25 Davis ME, Zuckerman JE, Choi CHJ *et al.* Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature.* 464, 1067-1070 (2010).
- 26 Jayaraman M, Ansell SM, Mui BL *et al.* Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew. Chem. Int. Ed. Engl.* 51(34), 8529-8533 (2012).
- 27 Haussecker D. The Business of RNAi Therapeutics in 2012. *Mol. Ther. Nucleic Acids.* 2, e8 (2012).
- 28 Alhareth K, Vauthier C, Bourasset F *et al.* Pharmacokinetics and tissue biodistribution in rats of doxorubicin loaded Poly(isobutylcyanoacrylate) nanoparticles prepared by redox radical emulsion polymerization. *Eur. J. Pharm. Biopharm.* 81(2), 453-457 (2012).
- 29 Ramon AL, Bertrand JR, de Martimprey H *et al.* siRNA associated with immunonanoparticles directed against cd99 antigen improves gene expression inhibition in vivo in Ewing's sarcoma. *J. Mol. Recognit.* 26(7), 318-329 (2013).
- 30 Strumberg D, Schultheis B, Traugott U *et al.* Phase I clinical development of Atu027, a siRNA formulation targeting PKN3 in patients with advanced solid tumors. *Int. J. Clin. Pharmacol. Ther.* 50(1), 76-78 (2012).
- 31 Segura-Sánchez F, Montembault V, Fontaine L *et al.* Synthesis and characterization of functionalized poly(γ -benzyl-L-glutamate) derivatives and corresponding nanoparticles preparation and characterization. *Int. J. Pharm.* 387(1-2), 244-252 (2010).

- 32 Ramon AL. New targeted approaches for the treatment of Ewing's sarcoma family of tumors, PhD Thesis University of Paris Sud - Paris XI (06/06/2012), Available on <http://tel.archives-ouvertes.fr/tel-00840226>
- 33 Shah V, Taratula O, Garbuzenko OB *et al.* Genotoxicity of different nanocarriers: possible modifications for the delivery of nucleic acids. *Curr. Drug Discov. Technol.* 10(1),8-15 (2013).
- 34 Morrissey DV, Lockridge JA, Shaw L *et al.* Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. *Nat. Biotechnol.* 23(8),1002-1007 (2005).
- 35 Dolina JS, Sung SS, Novobrantseva TI *et al.* Lipidoid Nanoparticles Containing PD-L1 siRNA Delivered In Vivo Enter Kupffer Cells and Enhance NK and CD8(+) T Cell-mediated Hepatic Antiviral Immunity. *Mol. Ther. Nucleic Acids.* 19(2):e72 (2013).
- 36 Akinc A, Goldberg M, Qin J *et al.* Development of lipidoid-siRNA formulations for systemic delivery to the liver. *Mol. Ther.* 17(5), 872-879 (2009).
- 37 Place RF, Wang J, Noonan EJ *et al.* Formulation of Small Activating RNA Into Lipidoid Nanoparticles Inhibits Xenograft Prostate Tumor Growth by Inducing p21 Expression. *Mol. Ther. Nuc. Acids* 1, e15 (2012).
- 38 Raouane M, Desmaele D, Gilbert-Sirieix M *et al.* Synthesis, characterization, and in vivo delivery of siRNA-squalene nanoparticles targeting fusion oncogene in papillary thyroid carcinoma. *J. Med. Chem.* 54, 4067-4076 (2011).
- 39 Massade L. Characterization and vectorization of siRNA targeting RET/PTC1 in human papillary thyroid carcinoma cells. *Biopol. Cell.* 27(5), 390-393 (2011).
- 40 Takeshita F, Minakuchi Y, Nagahara S *et al.* Efficient delivery of small interfering RNA to bone-metastatic tumors by using atelocollagen in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 102(34), 12177-12182 (2005).
- 41 Silenseed web site, <http://www.silenseed.com/article/42>, Consulted 27/11/2013
- 42 Zuckerman JE, Choi CH, Han H *et al.* Polycation-siRNA nanoparticles can disassemble at the kidney glomerular basement membrane. *Proc Natl Acad Sci U S A* 109(8),3137-3142 (2012)
- 43 RNAtherapeutic web site, <http://rnaitherapeutics.blogspot.com.br/2012/12/snlp-structure-reconsidered.html>, consulted 1 December 2013
- 44 Patil ML, Zhang M, Taratula O *et al.* Internally cationic polyamidoamine PAMAM-OH dendrimers for siRNA delivery: effect of the degree of quaternization and cancer targeting. *Biomacromolecules.* 10(2),258-266 (2009).
- 45 Taratula O, Garbuzenko OB, Kirkpatrick P *et al.* Surface-engineered targeted PPI dendrimer for efficient intracellular and intratumoral siRNA delivery. *J. Control. Release.* 140(3), 284-293 (2009).
- 46 De Martimprey H, Bertrand JR, Fusco A *et al.* siRNA nanoformulation against the ret/PTC1 junction oncogene is efficient in an in vivo model of papillary thyroid carcinoma. *Nuc. Acids Res.* 36(1), e2 (2008).
- 47 Elhames H, Bertrand JR, Maccario J *et al.* Antitumor vectorized oligonucleotides in a model of Ewing Sarcoma : Unexpected role of nanoparticles *Oligonucleotides* 19(3), 255-264 (2009).
- 48 Toub N, Bertrand JR, Malvy C *et al.* Antisense oligonucleotides nanocapsules efficiently inhibit EWS-Fli-1 expression in a Ewing's sarcoma model. *Oligonucleotides* 16, 158-168 (2006).

- 49 Aleku M, Schulz P, Keil O *et al.* Atu027, a liposomal small interfering RNA formulation targeting protein kinase N3, inhibits cancer progression, *Cancer Res.* 68(23), 9788–9798 (2008).
- 50 Santel A, Aleku M, Roder N *et al.* Atu027 prevents pulmonary metastasis in experimental and spontaneous mouse metastasis models. *Clin. Cancer Res.* 16(22), 5469–5480 (2010).
- 51 Zandanel C, Vauthier C. Poly(isobutylcyanoacrylate) Nanoparticles Decorated with Chitosan: Effect of Conformation of Chitosan Chains at the Surface on Complement Activation Properties. *J. Coll. Sci. Biotechnol.* 1(1), 68-81 (2012).
- 52 Shu D, Shu Y, Haque F, et al. Thermodynamically stable RNA three-way junction for constructing multifunctional nanoparticles for delivery of therapeutics. *Nature Nanotech.* 6, 658-667 (2011)

Key terms

Antisense strategy: Classical drugs are protein inhibitors interacting directly with the active site of a protein. Antisense strategies aim at inhibiting with specificity the synthesis of a given protein. It can be triple helices at the level of DNA or double helices at the level of mRNA. Usually, these double helices lead to the mRNA cleavage and degradation through either RNaseH or RISC complex

Oligonucleotides: Short fragments of nucleic acids occurring as oligodesoxynucleotides and ribonucleic acids. These fragments can interfere with the expression of a specific gene thanks the complementarity of their sequence with the target gene or the target mRNA. In the cells, they are part of physiologic mechanisms occurring for the regulation of gene expression.

Antisense Oligonucleotide (AS-ODN): Short fragment of a single chain of oligodesoxynucleotide having a sequence complementary to a target gene or a target mRNA. They can be used to block the expression of the gene in the nucleus by formation of a triple helix on the complementary sequence of DNA found on the corresponding chromosome. They can compromise the production of a protein by blocking the translation of the mRNA forming a complex with the complementary sequence found on the target mRNA. In this case, the translation of the mRNA into the protein is compromised either because the mRNA is then degraded by the RNaseH or because the AS-ODN-mRNA complex formed hindered the association or the progression of the ribosome which is required for the synthesis of the protein.

Small interfering RNA (siRNA): Short fragment of double strands ribonucleic acid. One of the strand, the antisense strand, has a complementary sequence to that of the target mRNA. This strand associates with a group of proteins to form the RISC complex while the second strand, the sense strand, is degraded. Once formed, the RISC complex displays an RNase activity against the mRNA complementary to the antisense strand of the siRNA. This leads to the degradation of the mRNA targeted by the siRNA hence to the inhibition of the production of the corresponding protein.

Non-viral nanoparticles: In contrast with drug carriers designed from viruses, non-viral nanoparticles are drug carriers obtained by designing artificial nanoparticles from a drug and eventually other materials that include in general either lipids or polymers.

Chapter summary

- Gene silencing approaches have demonstrated high promise for the development of new treatments of diseases caused by the abnormal expression of a known gene.
- Non-viral nanoparticles including lipid and polymer-based nanoparticles were identified to make possible in vivo delivery of biologically active gene silencing agents.
- Clinical developments have started with several systems considering development of treatments against various cancers, viral infections and hypercholesterolemia.
- The development of therapeutic strategies based on gene silencing approaches is still in its infant age and many challenges remain