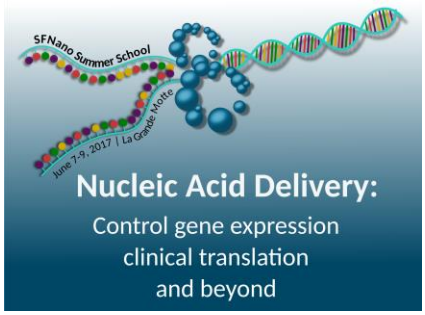

The SFNano (French Society for Nanomedicine) Summer School on "Nucleic acids-based strategies to control gene expression: principles, applications in biology, clinical translation and delivery issues" was held from June 7 to 9. Participants from 9 countries have had ample possibilities to learn about the latest developments in the booming field of nucleic acids therapeutics and to exchange experiences in the friendly environment of hotel Mercure in the La Grande-Motte beach resort.

The organization of the school has benefited from the involvement of SF Nano board members and from the generous financial support of AFM Téléthon, Institut de Recherches Servier, Cancéropôle Grand Sud-Ouest, InCellArt, Precision Nanosystems, Anton Paar, IPBS-CNRS Toulouse, Toulouse University and Montpellier University.

Introduction. Dr. S. Braun (Scientific Director, AFM Téléthon) reviewed the development of gene therapies in particular for Duchenne muscular dystrophy. As general for new therapies, initial successes were followed by a long period of skepticism. It is now over with many advanced clinical trials and the approval of nucleic acids-based drugs for the treatment of several human genetic and acquired diseases. More efficient and safer delivery vectors as well as the rapid emergence of genomic edition offer particularly interesting perspectives.

Genome edition using custom nucleases and in particular the easy-to-implement CRISPR-Cas9 nuclease provide exciting possibilities to introduce targeted genome modifications thus revolutionizing genetics. Underlying mechanisms, examples of applications and, importantly for non-specialists, present limitations of these strategies were pointed by Dr. C. Giovannangeli (Museum National d'Histoire Naturelle, Paris) in her review. They include

specificity, delivery issues and the poor efficiency of homologous recombination in adult tissues.

Messenger RNAs with potential to generate *in vivo* proteins (for vaccination in particular) has undergone recent and rapid developments as an alternative to pDNA transfection. The field was covered by Dr. P. Midoux (Centre de Biophysique Moléculaire, Orléans) and by Prof. K. Kariko (BioNTech RNA Pharmaceuticals, Mainz). P. Midoux focused on delivery and tissue targeting issues while K. Kariko reviewed mRNA modifications requested to reduce immunogenicity and to increase translatability. Both gave examples of the many potential clinical applications of a strategy that was barely envisaged as possible a few years ago.

Dr. S. Guo (Ionis Pharmaceuticals, Carlsbad) gave an overview of the various antisense strategies under development in her company since many years. Major advances have included the concept of gapmer antisense oligonucleotides (ONs) which combine several ON chemistries and more recently modifications allowing to target specific tissues. The most advanced are GalNac-modified ONs targeting the asialoglycoprotein receptors in hepatocytes with ongoing clinical trials. Other delivery issues are also being explored with for example aerosolized ONs for lung delivery.

Dr. T. Lehto (Karolinska Institute, Stockholm) reviewed the many applications of splice-switching oligonucleotides (SSO), a variant antisense strategy, from splicing defect correction to the production of novel protein isoforms. As pointed in several other lectures, their therapeutic potential has been demonstrated with the recently FDA approvals of SSOs for the treatment of Duchenne muscular dystrophy (developed by Sarepta) and Spinal muscular atrophy (developed by Ionis Pharmaceuticals with Biogen).

The emerging field of non-coding RNAs and their clinical applications was reviewed by Prof. M. Lindsay (University of Bath, UK). He gave an overview of the major classes of non coding RNAs whose existence was barely ignored

a decade ago and whose discovery was made possible with the emergence of extremely potent large-scale sequencing. Short ncRNAs (siRNAs and miRNAs) associate with mRNAs to promote translation inhibition and degradation, thus providing a new network of gene regulation. The roles of very recently discovered long ncRNAs are still being explored, but they mainly act as scaffolds for proteins binding and subsequent regulation of transcription as an example. Examples, taken from M. Lindsay's own research, of altered expression of these various ncRNA classes in inflammation were detailed.

Dr. Y. Sanghvi (Rasayan Inc, Encinitas, CA) reviewed the numerous improvements in ON solid phase synthesis (many of them due to its own contributions while working with Isis Pharm) which have allowed a dramatic decrease in synthetic ONs prices and their possible use in large scale clinical trials.

Major non-viral strategies to deliver therapeutic nucleic acids were introduced by Dr. M-P. Rols (IPBS-CNRS, Toulouse). She first presented the current clinical applications of electroporation in the field of cancer treatment and promising clinical trials in gene therapy. Electroporation is a physical method of molecules delivery in cells and tissues. Based on the use of electric field pulses, it induces the transient and localized permeabilization of cell membranes allowing the controlled and efficient transfer of biomolecules. The full knowledge of the mechanisms of nucleic acid delivery, addressed with different experimental models and microscopy tools, has allowed to define safe and efficient protocols.

Other physical methods that offer great potential for the controlled delivery of nucleic acids are ultrasound and microbubbles. Prof. C. Pichon (CBM-CNRS, Orléans) gave an overview of the fundamentals of sonoporation and addressed the different issues that have to be taken into account for successful delivery, in particular for the fine-tuning of microbubbles composition. She presented the underlying mechanisms and main data on nucleic acids delivery by sonoporation in different organs.

Dr. B. Pitard (Nantes University) described non-viral transfection agents for nucleic acids (developed in collaboration with the InCellArt Company in Nantes) and illustrated their potential with several examples. In the Nanospheres formulation, DNA is wrapped around the nanoparticles and enters cell by direct translocation across the plasma membrane. In the NanotaxiR formulation, nucleic acids (DNA, mRNA, siRNA) are combined with cationic lipids to form multilamellar particles. They enter cells by endocytosis but escape from endocytic vesicles by a lipid mixing phenomenon. Applications for the delivery of mRNA-based vaccines and for the delivery of erythropoietin mRNA have been described with very encouraging data in animal models.

Dr. N. Tagnaouti (Precision Nanosystem, Vancouver) described the NanoAssemblr™ platform developed by her company, a spin-out of British Columbia University, for the non-viral delivery of nucleic acids. This platform allows a fast and one-step formation of controlled and stable lipid nanoparticles using microfluidics cartridges. Several devices are commercially available covering researcher's needs from discovery (Spark®, Benchtop®) to clinic (with a GMP system). They are compatible with different kinds of nanoparticles. As an example, Zika mRNA LNPs have been evaluated in a phase I clinical trial.

Dr. J-L. Coll (Institute for Advanced Biosciences, Grenoble) reviewed difficulties encountered when nanoparticles used for the delivery of genes, ONs or siRNAs have to be re-directed to target tumors and not the original organs into which they normally accumulate. He also documented how non-invasive optical imaging in the near-infrared can help for such investigations.

Dr. M.J. Gait (Medical Research Council, Cambridge) comprehensively reviewed the use of cell penetrating peptides (CPP) for the delivery of ONs as well as their cellular trafficking. Charged ONs can be associated non covalently with such peptides leading to peptidic nanoparticles. However, most strategies have relied on the covalent CPP conjugation of uncharged ON analogues (PNAs or PMOs) with very encouraging data in animal models

of neuromuscular (DMD, SMA) diseases. Exciting developments in the Gait laboratory (in collaboration with the MDEX clinical consortium) have led to the design of new families of CPP-PMO conjugates called PPMOs with superior efficiency as compared to the Tat and Penetration CPPs. Current research focuses on the search for CPPs with a lower content in basic amino acids and with ability to pass the blood brain barrier (BBB).

Prof. M.J. Wood (Oxford University) first described their studies of PPMOs in animal models of DMD and SMA. Although more efficient than free PMOs, therapeutic indexes are still too low to allow clinical trials hence the ongoing search for less toxic CPP formulations. In a second part, he described the use of extracellular vesicles (exosomes) as an alternative strategy for the delivery of ONs across the BBB with an accent on the treatment of neurological diseases.

In overviews of ON delivery concepts and strategies lectured by Prof. R.L. Juliano (University of North Carolina, Chapel Hill) and Dr. J.A. Boutin (Institut de Recherches Servier, Suresnes). Dr. Juliano emphasized the many (endothelial, cellular and intracellular) biological barriers which have to be crossed by therapeutic nucleic acids to reach their intracellular targets. As rightly pointed, our understanding of ONs trafficking is still very poor. It is in particular admitted than most delivery strategies involve endocytosis leading to entrapment of ONs in endocytic vesicles. Dr.Juliano's presented original studies concerning the HTS screening of chemical libraries in search for low molecular weight drugs leading to an increased endosomal membrane permeability at non toxic doses.

Dr. Boutin listed problems to be faced as seen by a « big pharma » in the development of nucleic acids-based drugs which are much more complex chemical entities than most presently used low molecular weight drugs. He stressed the lack of tissue targeting as a major present limitation.