

# *Preface*

Oligonucleotides are a class of biomacromolecule with great potential for research and therapeutic applications. By definition, oligonucleotides consist of approximately ten to 100 DNA or RNA monomers or chemically modified analogs thereof. Although being rather homogeneous in composition, their mechanisms of action are extremely diverse. It is the aim of this volume to illuminate these different aspects of oligonucleotides. The story of their use is a rollercoaster of enthusiasm and depression. Recent progress made in clinical testing, as well as the hope that the most recently discovered RNA interference technology will help to overcome efficacy problems of the previous approaches, are the basis for this collection of contributions from leading experts in the different fields of application for oligonucleotides.

Three decades ago, in 1978, Paul Zamecnik and Mary Stephenson initiated the era of antisense research when they reported on the inhibition of virus replication in cell culture by a DNA oligonucleotide complementary to the target RNA. This finding launched the expectation that oligonucleotides might be employed to treat any disease that results from deleterious gene expression. We now know that this view was too optimistic, since only one antisense drug, Vitravene (to treat cytomegalovirus-induced retinitis) has been approved for the market to date. In Chapter 2 of this volume, Cy Stein and colleagues give a critical review about another advanced candidate, Genasense, which is an antisense oligonucleotide targeted against Bcl-2 and which has been tested in Phase III clinical trials to treat patients with cancer.

Most of the first-generation antisense oligonucleotides consist of phosphorothioate linkages. It soon became clear, however, that modified nucleotides with improved properties are desirable for many types of application of oligonucleotides, as outlined in Chapter 1. Several examples of antisense oligonucleotides consisting of modified building blocks are discussed in the first part of the book. Hong and Jon Moulton describe in Chapter 3 the use of phosphorodiamidate morpholino oligomers (PMOs) that have already been tested in clinical trials for different indications. Michael Gait and colleagues introduce

peptide nucleic acids (PNAs) in Chapter 4. Both PMOs and PNAs act as steric blocks of RNA cellular processes and, in each case, they can be coupled to peptides to improve their cellular uptake. In recent years, locked nucleic acids (LNAs) have been considered as very promising analogs. Troels Koch *et al.* describe in Chapter 5 their properties as well as the first results from clinical applications. This chapter also addresses another point with major relevance. For clinical applications, large amounts of oligonucleotides must be produced under current good manufacturing practices (cGMP) conditions at reasonable costs. Another recently developed application of LNAs that is dealt with by Koch and colleagues is the inhibition of endogenously expressed microRNAs.

Single-stranded oligodeoxynucleotides, however, do not only act as inhibitors of gene expression. CpG sequence motifs activate the innate immune response in mammals. This unwanted side effect of antisense oligonucleotides, which had been apparent for many years, is now exploited to deliberately stimulate the human immune system against viral infections and cancer, as outlined by Eugen Uhlmann in Chapter 6.

Another breakthrough in the field of oligonucleotide research was the finding that certain RNA molecules possess the ability to catalyse hydrolysis of phosphodiester bonds. For this unexpected discovery of *ribonucleotides* with *enzymatic* activity, now known as ribozymes, Thomas Cech and Sydney Altman were awarded the Nobel Prize in Chemistry for 1989. In recent years, a number of new types of ribozymes were found in nature or identified by *in vitro* selection approaches. Several ribozymes have been tested in clinical trials to treat viral infections and cancer. The outcomes of these approaches, however, did not meet expectations. Thus, ribozymes can still be considered to be a fascinating area of research, but their relevance for therapeutic applications has decreased somewhat. One current approach is to combine a ribozyme with other types of RNA-based drugs to treat patients infected with human immunodeficiency virus-1 (HIV-1) and is described by John Rossi in Chapter 14 (see also below).

While antisense oligonucleotides and ribozymes usually act as single-stranded molecules, decoy oligonucleotides represent a class of double-stranded DNA molecules that can be employed to modulate gene expression. As described by Marcus Hecker and colleagues in Chapter 7, decoy oligonucleotides mimic promoter-binding regions to bind specifically and inactivate transcription factors. Several decoy oligonucleotides have been tested in clinical trials primarily for the treatment of inflammatory diseases.

In 1990, the groups of Larry Gold and Jack Szostak simultaneously came up with the concept of *in vitro* selection of oligonucleotides with a high affinity to a target molecule. This class of oligonucleotides, referred to as aptamers, can be obtained by an iterative process of selection and amplification called SELEX (systematic evolution of ligands by exponential enrichment). It was a great breakthrough for the field, when the aptamer Macugen was approved for the treatment of age-related macular degeneration in 2004. Nigel Courtenay-Luck and Donald Miller describe the clinical development of an anticancer aptamer targeting nucleolin (Chapter 8). To obtain highly stable molecules, a method was developed that results in aptamers in the unnatural L-form of nucleotides.

This sophisticated technology is described by Sven Klussmann and colleagues in Chapter 9.

In the late 1990s double-stranded RNA (dsRNA) molecules were found to efficiently inhibit expression of homologue genes in *Caenorhabditis elegans*, a phenomenon nowadays known as RNA interference (RNAi). For this discovery, Craig Mello and Andrew Fire were awarded the Nobel Prize in Physiology or Medicine for 2006. Interest in oligonucleotide-based approaches dramatically increased when Thomas Tuschl's group was able to specifically silence genes in mammalian cells with short dsRNA molecules, dubbed small or short interfering RNAs (siRNAs). This new technology not only revolutionized life-science research, but also opened the road for new therapeutic strategies.

Since siRNAs (as well as antisense oligonucleotides) act inside cells, a major hurdle for their successful application remains delivery of the highly charged molecules into cells. Two chapters therefore address this problem. Christian Reinsch *et al.* give a general introduction into strategies for systemic delivery of antisense oligonucleotides and siRNAs in Chapter 10. In the following chapter, Ian MacLachlan focuses on one of the most advanced systems for the efficient delivery of siRNAs, stable nucleic acid lipid particles (SNALPs). An alternative to the use of chemically pre-synthesized siRNA is the endogenous expression of short hairpin RNAs (shRNAs). This methodology, which is described in detail in Chapter 12, allows long-term inhibition of target gene expression, regulation of the silencing, and delivery by viral vectors.

In 2004, the first clinical trials based on RNAi were initiated. Two companies are developing siRNAs targeting the vascular endothelial growth factor (VEGF) and its receptor, respectively, to treat age-related macular degeneration, a major ocular disease. In another trial, the safety of siRNAs against respiratory syncytial virus is being evaluated. While these approaches involve chemically synthesized siRNAs that are delivered locally, the last two chapters of this volume deal with vector-based approaches to deliver shRNAs against HIV-1. In Chapter 13, Olivier ter Brake and Ben Berkhout describe their strategy to use vectors, which express multiple shRNAs against different sites in the HIV genome simultaneously, to prevent the development of escape mutants. John Rossi and colleagues (Chapter 14) follow a slightly different strategy. They combine three different oligonucleotide-based principles to cope with the problem of viral escape. In their approach, a lentiviral vector is employed to express simultaneously an shRNA targeting the viral *rev* and *tat* mRNAs, a nucleolar-localizing transactivation response element (TAR) RNA decoy and a ribozyme to inhibit the expression of the CCR5 receptor in the host cell. As outlined in this chapter, a clinical trial to use the triple vector for *ex vivo* delivery to haematopoietic progenitor cells of HIV-1-infected patients has just commenced.

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