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Advances in Nucleic Acid Therapeutics

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Foreword

The initial foundation for using either DNA or RNA (oligonucleotides) as therapeutic drugs was formulated by Zamecnik and Stephenson in a classic paper published several years ago (P. C. Zamecnik and M. L. Stephenson, *Proc. Natl. Acad. Sci. U. S. A.*, 1978, 75, 280). Many of us considered that this concept would prove to be a new, refreshing approach for providing revolutionary drugs useful in the treatment of nondruggable diseases. Over time these expectations have been realized as several oligonucleotide drugs (Macugen, Fomivirsen, Mipomersen, Eteplirsen, Nusinersen, Inotersen, and Patisiran) are currently available for treating a diverse group of diseases. There are also a large number of additional oligonucleotides in various stages of drug development.

The path towards identifying drugs in the nucleic acids therapeutic arena has involved many unexpected revelations. Initially the focus was strictly on using antisense oligonucleotides. Over time, new research has opened possibilities for oligonucleotide drugs in such diverse fields as interfering RNA, microRNA, noncoding RNA, splicing modulation of RNA transcripts, targeting toxic repeats in RNA and DNA, investigating RNA and DNA aptamers and ribozymes for treating various disease states, and formulating synthetic agonists of Toll-like receptors. In this book the editors, through various chapters, provide a broad and complete perspective on the history of these fields. As each chapter unfolds, the reader discovers the logic behind why various DNA and RNA analogues were developed, how they were applied in clinical studies, and the limitations and advantages of these analogues. Also clearly presented are details on the unexpected side effects with some of these being very serious, such as the Toll receptor problem, the retention and targeting of oligonucleotides in tissues, and the variation of clinical studies with animal models.
Drug development in the nucleic acids field continues at an increasing rate. Moreover so do the challenges that are important for successfully identifying an oligonucleotide therapeutic drug. This book provides an excellent road map for navigating all that has come before and also outlines for the reader a multitude of possible directions on how to proceed with further research. The editors have assembled an excellent set of authors who are experts in the science as presented in various chapters. This would be expected as both Michael Gait and Sudhir Agrawal are among the most highly respected and experienced research scientists in this field. Thus it is not at all surprising that the science is both current and focused on important concepts. I can enthusiastically recommend this book as both a reference and also as a guide for further research in the nucleic acids therapeutics arena.

Marv Caruthers
University of Colorado
Preface

Nucleic acids therapeutics are now recognized as the third major drug discovery platform, in addition to small molecules and proteins. In the past three decades, tremendous progress has been made towards the realization of the potential of nucleic acids therapeutics for the treatment of a broad range of diseases. In addition, multiple mechanisms of actions have been elucidated. Recently, several nucleic acid drugs have been approved for clinical use.

Chemical modifications of the three components of nucleic acids – heterocyclic bases, five-membered sugars, and internucleotide linkages – as well as the nucleotide sequences themselves are the key drivers for the creation of nucleic acid drugs. Rational combinations of these have provided drug-like properties. Further advances in the chemistry of nucleic acids and additional insights into their mechanisms of action have expanded their applications to include antisense targeting of mRNA, microRNA, non-coding RNA and splicing modulation, ribozymes, RNA interference (RNAi) and short interfering RNA (siRNA), gene editing, aptamers, and the modulation of immune responses. During the past ten years, since the excellent publication of Jens Kurreck’s book (Therapeutic Oligonucleotides), progress in this field has been so rapid and broad that we felt it was appropriate now to document the key developments in the field in the form of a new book.

In Chapter 1 we provide a brief history of the development of nucleotide analogues, early experience in the use of modified antisense oligonucleotides (ONs) from preclinical studies to human trials, as well as the importance of nucleotide sequence and its implications in interaction with innate immune receptors. The next three chapters provide updates on applications of antisense technology. In Chapter 2, David Corey and Zhongtian Liu discuss various mechanisms of action of antisense ONs. In Chapter 3, Eric Swayze and Punit Seth describe the medicinal chemistry of RNase H-activating antisense
ONs and in Chapter 4, Richard Geary, Brenda Baker and Brett Monia provide an update on and experience of the application of antisense ONs in clinical development.

During the development of antisense technology, it was realized that subcutaneous delivery of antisense ONs led to activation of host immune responses. Initially, this was thought to be a side effect but soon the discovery of the family of Toll-like receptors (TLRs) led to an understanding of immune activation triggered by receptor-mediated engagement. Tremendous progress has been made in translating these observations into a novel therapeutic platform. In Chapter 13, Shin-Ichiroh Saitoh and Kensuke Miyake provide a detailed background on immune receptors that are known to recognize nucleic acid sequence, patterns, and motifs. In Chapters 5 and 14, one of us (S.A.) and Ekambar Kandimalla have discussed the chemistry of novel nucleic acid compounds and how they modulate receptor-mediated immune responses, along with their therapeutic applications, including clinical proof of concept trials.

A more recent therapeutic application of antisense involves splicing modulation to affect the translation of the targeted pre-mRNA. In Chapter 6, Elena Daoutsali and Annemieke Aartsma-Rus provide an up to date survey on this subject through a variety of examples. Similarly, applications of antisense have been expanded to targeting toxic RNA repeats (Chapter 7 by Derick Wansink and colleagues), microRNA (Chapter 8 by Anna Malinowksi and Jonathan Hall) and long non-coding RNA (Chapter 9 by Claes Wahlestedt and colleagues).

In parallel, significant advances have also been made in RNAi technology for therapeutics. In Chapter 10, Anastasia Khvorova and colleagues discuss in detail the challenges of delivery of RNAi-based therapeutics and how these obstacles have been addressed. In Chapter 11, Muthiah Manoharan and Kalanthottathil Rajeev describe the clinical development of siRNA candidates targeted to liver. In Chapter 12 Jiehua Zhou and John Rossi describe the application of RNAi for treatment of HIV infection. Therapeutic application of ribozyme technology had shown early promise, but has now been found to have significant limitations. In Chapter 18, Darko Balke and Sabine Müller describe novel ribozyme constructs in the search for potential therapeutic applications.

In the past few years, we have also seen explosive growth in the development of gene editing using nucleic acids towards therapeutics. In Chapter 17, Carine Giovannangeli and colleagues provide details on this subject. Synthetic nucleic acids have been studied as aptamers to target proteins and other cellular targets and their clinical evaluation is reviewed in Chapter 15 by Paloma Giangrande and colleagues and in Chapter 16 by Gerald Zon. Through understanding the various mechanisms of actions of nucleic acids, extensive experience has been gained on their safety and pharmacokinetics, both in preclinical and in clinical use. In Chapter 20, Cathaline den Besten and Patrik Andersson discuss this topic in detail and provide their analysis.
To maintain successes in the field, significant advances have also been made in manufacturing and quality control, discussed in Chapter 19 by Yogesh Sanghvi.

We are immensely grateful to all these co-authors for their outstanding contributions that provide a detailed story of their respective subjects along with a current bibliography. We are also grateful to the editorial team at the Royal Society of Chemistry (Katie Morrey and Drew Gwilliams, Rowan Frame and Robin Driscoll) for their timely publishing and encouragement and members of the Royal Society of Chemistry staff for their assistance, as well as the many members of the Oligonucleotide Therapeutic Society, the premier professional society in the field, who have contributed to this book, which we hope will become a manual for the state of the art.

In this relatively young field of nucleic acid therapeutics, the use of their sequences to target drugs very precisely in cells and \textit{in vivo} and the development of nucleic acids chemistry have been paramount and resulted in a substantial broadening of their applications. Rapidly developed and newly approved drugs are now available for the treatment of some rare diseases and other more prevalent diseases are surely following. Despite some setbacks, the list of RNA targets and approved drugs is expanding quickly. We are excited at the future prospects for this field.

Sudhir Agrawal and Michael J. Gait
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CHAPTER 1

History and Development of Nucleotide Analogues in Nucleic Acids Drugs

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1.1 Introduction

Forty years ago, Zamecnik and Stephenson proposed the therapeutic use of antisense oligonucleotides on the basis of their finding that Rous sarcoma virus (RSV) replication could be inhibited by a synthetic oligonucleotide complimentary to the RSV genome.\textsuperscript{1} This concept opened up a new approach to drug discovery, namely an oligonucleotide binding sequence-specifically \textit{via} Watson–Crick base-pairing to a complementary target RNA.

Since then, continuous progress has been made towards realizing the potential of this novel scientific approach and this has led recently to the approval of five antisense drugs. While the underlying concept of antisense is very simple, a rigorous understanding of the chemistry of nucleic acids had to be developed for its use in humans. In this chapter we discuss the history of this chemistry of oligonucleotides in antisense and the lessons
learned from preclinical studies and clinical trials that have guided the development in conferring drug-like properties.

In parallel to the development of antisense (see Chapters 2–4), the application of synthetic oligonucleotides as therapeutic agents has evolved into broad applications involving multiple modalities. These applications include ribozymes (see Chapter 18), small interfering RNA (siRNA, see Chapters 10, 11 and 12), microRNA (see Chapter 8), aptamers (see Chapters 15 and 16), non-coding RNA (see Chapter 9), splicing modulation (Chapter 6), targeting toxic repeats (Chapter 7), gene editing (Chapter 17), and immune modulations (see Chapters 5, 13 and 14).

The common feature of these applications is that drug candidates are composed of natural nucleosides or nucleoside analogues linked via phosphodiester or modified linkages.

1.2 The Antisense Concept

In 1976, RSV was the only purified virus for which a sufficient quantity was available for potential sequencing. Maxam and Gilbert sequenced this RNA virus and noted that both ends of the linear viral genome bore the same primary sequence and were in the same polarity. It occurred to Zamecnik that the new piece of DNA synthesized by reverse transcription at the 5′-end of this retrovirus might circularize and hybridize with the 3′-end. Thus he considered the possibility of inhibiting viral replication by adding a piece of synthetic DNA to the replication system to block the circularization step by hybridizing specifically with the 3′-end of the viral RNA in a competitive way.

This experiment led to startling observations, including the inhibition of new virus particles and the prevention of transformation of chick fibroblasts into sarcoma cells. In a cell-free system, translation of the Rous sarcoma viral message was also dramatically impaired. These observations were the first to show proof of the antisense concept.1,2

Not much further progress was made in the field up to 1985, primarily for three reasons. First, there was still widespread disbelief that oligonucleotides could enter eukaryotic cells. Second, there was very little DNA (or RNA) genomic sequence available for targeting by antisense, and third, efficient automated methodologies to synthesize oligonucleotides in sufficient quantities were only just beginning to become established.

1.3 Developments in Oligonucleotide Synthesis

Although the principle of solid-phase oligonucleotide synthesis was first introduced by Letsinger and Mahadevan in 1965,3 development of more efficient methods of oligodeoxynucleotide (ODN) synthesis on solid support took place from 1975 in the Gait laboratory by the phosphodiester chemistry4 and from 1979 by the phosphotriester method in the Itakura laboratory5 and the Gait laboratory.6,7 These methods were superseded by