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Modified Oligonucleotides: Strides towards Antisense Drugs#

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Abstract

Different types of modifications have been carried out on natural oligonucleotides. Some of the suitably modified oligonucleotides are currently approved drugs or under multiple clinical trials. After FDA approval of antisense drugs Vitravene and Kynamro, there is a rapid increase in the demand of modified oligonucleotides for antisense therapeutics. General chemical modifications on oligonucleotides include modifications of backbone, base and sugar functionalities. Many of such modified oligonucleotides exhibit improved therapeutic properties including high binding affinity to targeted RNA, efficiency of cellular uptake and improved metabolic stability. In this review article various modified oligonucleotides have been discussed, which are promising future candidates for antisense therapeutics.

Keywords: Antisense therapeutics, modified oligonucleotides, phosphate-backbone modification, phosphorothioate-linkage, sugar modified nucleosides, locked nucleic acid, peptide nucleic acid and phosphorodiamidate morpholino modification

Introduction

The era of antisense therapeutics was unleashed after Zamecnik and Stephenson reported that the replication of Rous sarcoma virus (RSV) can be inhibited by a synthetic 13mer oligodeoxynucleotide complementary to a specific mRNA of RSV.[1] They also suggested that such oligonucleotides could be stabilized by 3′- and 5′-terminal modifications. This novel approach to drug design was more alluring than the rational drug design in the sense that it can be used in sequence specific control of gene expression at the level of nucleic acid. Since then antisense strategy has been on a roller-coaster ride for the past 25 years with an aim to switch off the expression of a specific disease-associated protein. The first antisense oligonucleotide (AON) to enter the clinic was Vitravene[2] for the treatment of cytomegalovirus (CMV) infection followed by Kynamro[3] which has recently been approved by FDA for the treatment of a type of hypercholesterolemia (HoFH); both of these drugs involve modified phosphorothioate-backbone.

Use of nucleic acids composed of naturally occurring DNA or RNA nucleotides as therapeutics pose some limitations because of their poor binding affinity and low degree of nuclease resistance. To overcome these

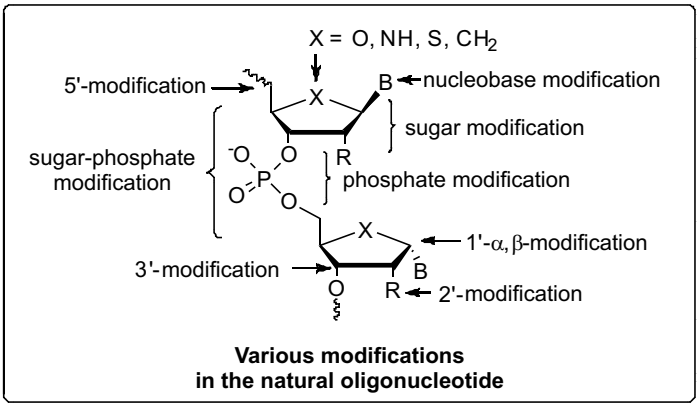

Figure 1. An overview of general modifications of antisense oligonucleotides (AONs)

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Dedicated to late Professor D. Loganathan, IIT Madras, Chennai, India

limitations, the ongoing synthetic studies have been focused on chemical modifications of the backbone,[4] base[5] and sugar[6] functionalities of the natural DNA/RNA and have resulted in significant progress towards establishing oligonucleotides as viable therapeutic agents (Figure 1). Consequently, the AONs are classified into three generations based on variation of these modifications.

First generation antisense oligonucleotides: The first generation of antisense agents contains backbone modifications such as replacement of oxygen atom of the phosphate linkage by sulphur (phosphorothioates)[7] or methyl group (methylphosphonates)[8] (Figure 2). Phosphorothioate oligonucleotides (PS-ONs) are the major representative of this generation and have been used most successfully for gene silencing. The introduction of Phosphorothioate (PS) linkage into ONs confers sufficient resistance to nuclease degradation, leading to higher bioavailability. In addition to nuclease resistance, PS-ONs form regular Watson-Crick base pairs, activate RNase H, carry negative charge for cell delivery and display attractive pharmacokinetic properties. However, their profiles of binding affinity to the target sequences, specificity and cellular uptake are less satisfactory.[9] Despite these disadvantages, FDA approved first antisense drug Vitravene, a first generation PS-modified AON for the treatment of AIDS-related cytomegalovirus (CMV) retinitis.[2]

Second generation antisense oligonucleotides: The problems associated with PS-ONs are poor binding affinity to the target RNA, lack of specificity and low cellular uptake. These problems were solved to some extent by the second generation oligonucleotides which mainly contains nucleotides with alkyl modifications at the 2' position of the ribose.[10] 2'-*O*-Methyl (2'-OMe) and 2'-*O*-methoxyethyl (2'-MOE) RNA are the most important members of this class (Figure 3).[11]

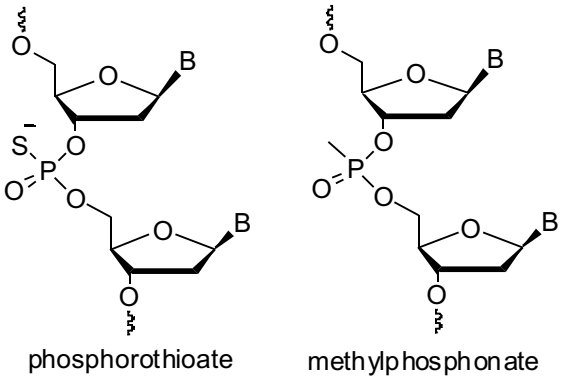


Figure 2. First generation antisense oligonucleotides (AONs).

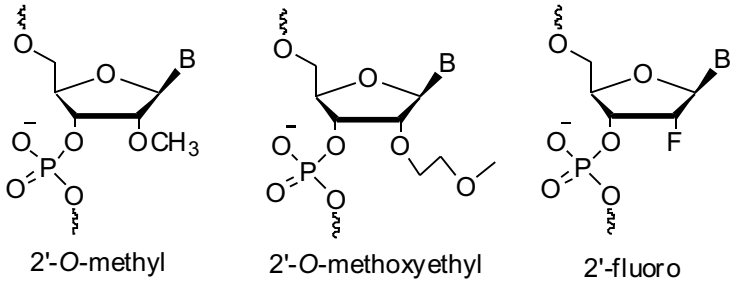
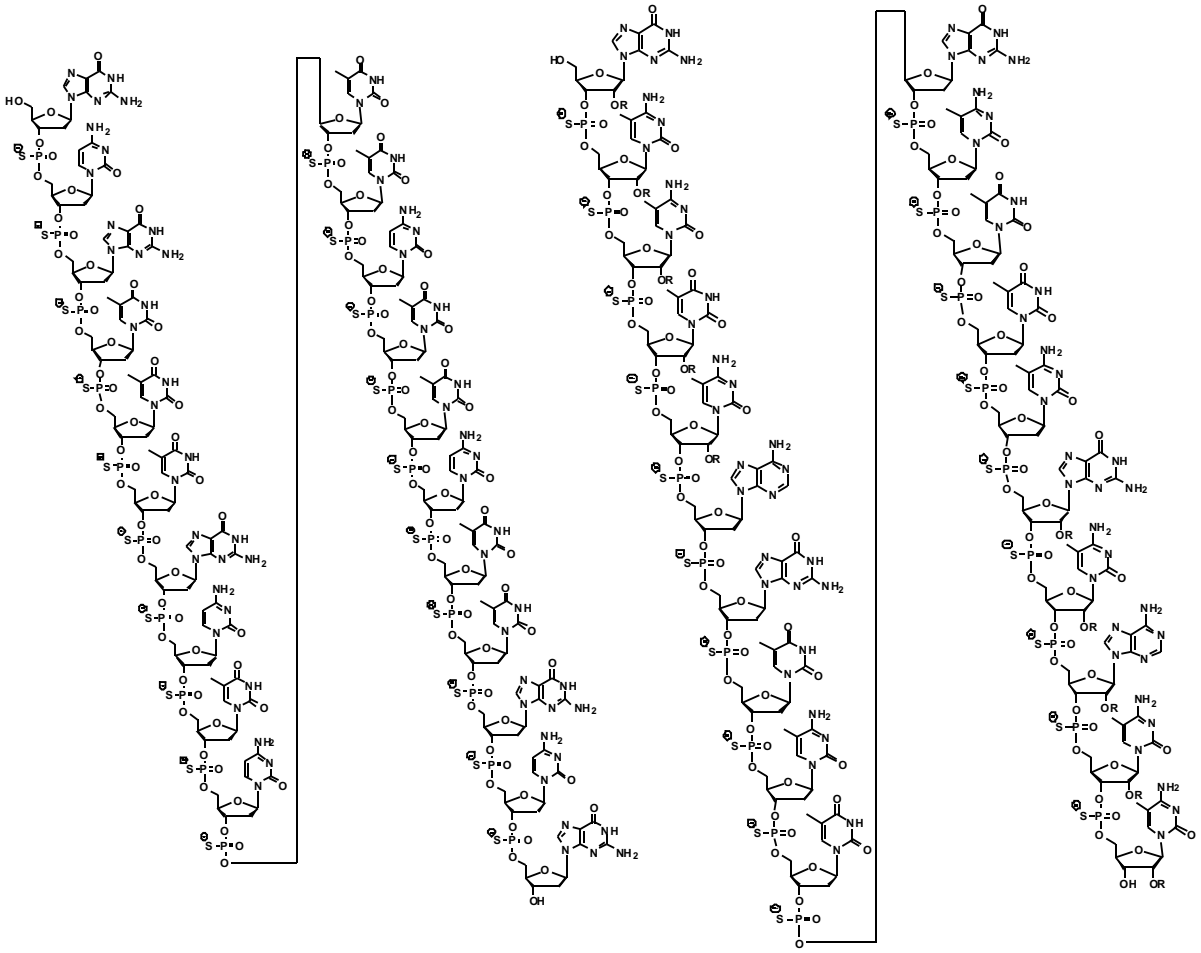


Figure 3. Second generation antisense oligonucleotides (AONs).

Although AONs made up of these building blocks are less toxic than PS-AONs and have slightly enhanced affinity towards their complementary RNAs, the efficiency to induce RNase H cleavage of the target RNA are the matter of concern regarding these second generation oligonucleotides.[12] Since RNase H cleavage is the most desirable mechanism for antisense effect and 2'-*O*-alkyl modifications are desirable for nuclease resistance and high binding affinity, a hybrid oligonucleotide construct incorporating both the characteristics has appeared in the form of the 'gapmer' antisense oligonucleotide.[13] A gapmer contains a central 'gap' of deoxynucleotides sufficient to induce RNase H cleavage, flanked by blocks of 2'-*O*-modified ribonucleotide 'wings' that protect the internal block from nuclease degradation. For example, 2'-*O*-methyl sugar modified nucleosides can be further combined with phosphorothioate-linkage (PS) as in Kynamro[3] (Figure 4), the second antisense drug approved by FDA to reduce low density lipoprotein-cholesterol (LDL-C), apolipoprotein-B, total cholesterol and non-high density lipoprotein-cholesterol in patients with homozygous familial hypercholesterolemia (HoFH). The 'gapmer' type chimera has been explored in numerous modified ONs undergoing multiple clinical trials to have improved therapeutic properties (Table 1).



5'-GCGTTTGCTCTTCTTCTTGCG-3'
Vitravene
First generation AON

5'-G^{Me}C^{Me}C^{Me}U^{Me}CAGT^{Me}CTG^{Me}CTT^{Me}CG^{Me}CA^{Me}C^{Me}C-3'
Kynamro
Second generation AON
(R = MOE; 2′-MOE chimera)

Figure 4. Structure of FDA approved antisense drugs Vitravene and Kynamro.

TABLE 1. Modified oligonucleotides as approved drugs/under phase III clinical trial for various diseases.

S.N.	Product	Chemistry	Disease	Target /*Mode of action*	Status (phase) / *Company*
1.	Fomivirsen[31] (Vitravene, ISIS-2922)	PS	CMV retinitis	Immediate-early 2 (IE2) gene / *Antisense*	Approved in 1998 /*ISIS Pharmaceuticals*
2.	Mipomersen[32] (Kynamro, ISIS-301012)	2'-MOE chimera*	Homozygous familial hypercholestero-lemia (HoFH)	Apolipoprotein B /*Antisense*	Approved in 2013 /*ISIS Pharmaceuticals*
3.	Pegaptanib[33] (Macugen)	2'-*O*-methylated purines and 2'-F-modified pyrimidines	Neovascular age-related macular degeneration (AMD)	Vascular endothelial growth factor (VEGF) /*RNA aptamer*	Approved in 2004 /*OSI Pharmaceuticals*
4.	Oblimersen[34] (Genasense, Augmerosen, G-3139)	PS	Chronic lymphocytic leukemia (CLL), malignant melanoma, multiple myeloma, non-small cell lung cancer (NSCLC), acute myeloid leukemia (AML)	B cell lymphoma (Bcl2) /*Antisense*	III /*Genta Incorporated & Aventis Pharma*
5.	Trabedersen[35] (AP-12009)	PS	Oncology-glioblastoma	Transforming growth factor beta 2 (TGF-β2) /*Antisense*	III /*Antisense Pharma*
6.	Aganirsen[36] (GS-101)	PS	Corneal neovascularization	Insulin receptor substrate-1 (IRS-1) / *Antisense*	III /*Gene Signal*
7.	Affinitak[37] (ISIS-3521, LY-900003, Aprinocarsen)	PS	NSCLC, breast cancer	Protein kinase C-α (PKC-α) / *Antisense*	III /*ISIS & Eli Lilly Pharmaceuticals*
8.	Custirsen[38] (OGX-011, ISIS-112989, TV-1011)	2'-MOE chimera	NSCLC, Prostate and breast cancer	Clusterin / *Antisense*	III /*OncoGenx*
9.	Drisapersen[39] (PRO-051, GSK-2402968)	2'-OMe chimera*	Duchenne muscular dystrophy	Dystrophin / *Antisense*	III /*Prosensa Therapeutics & GlaxoSmithKline*
10.	Bevasiranib[40] (Cand-5)	RNA	AMD	VEGF /*RNA interference (RNAi)*	III /*Opko Health (formerly Acuity)*
11.	Defibrotide[41]	Random mixture of single-stranded oligodeoxy-ribo-nucleotides derived from porcine mucosal DNA	Hepatic veno-occlusive disease (VOD)	Complications resulting after myeloablative chemotherapy / *Unknown*	III /*Gentium & Dana-Farber*
12.	ProMune[42] (CPG-7909, PF-3512676)	PS	NSCLC	Toll-like receptor 9 (TLR9) / *Immune-active*	III /*Pfizer*
13.	1018-ISS[43]	PS	Ragweed allergy, hepatitis B, non-hodgkin's lymphoma and colorectal neoplasms	TLR9 /*Immune-active*	III /*Dynavax Technologies*
14.	Alicaforsen[44] (ISIS-2302)	PS	Crohn's disease	Intercellular adhesion molecule-1 (ICAM-1) / *Antisense*	III /*ISIS Pharmaceuticals*
15.	AVI-4126[45] (Resten-NG/ Resten-MP)	PMO	Restenosis, Cancer and kidney diseases	C-myc mRNA / *Antisense*	II/III /*Sarepta Therapeutics*
16.	Edifoligide[46] (E2F Decoy)	PS decoy ODN	Atherosclerosis	Transcription factor (E2F) / *Decoy ON*	II/III /*Duke clinical research institute*

*2′-MOE chimera, 2′-methoxyethyl-DNA chimeric oligonucleotides with phosphorothioate-linkages; 2′-*O*-Me chimera, 2′-*O*-methyl-DNA chimeric oligonucleotide with phosphorothioate-linkages.

The major structural difference between DNA and RNA is the 2′-substitution on the furanose ring of RNA apart from the variation of the pyrimidine bases.[14] Hence, in order to improve the RNA binding behavior of antisense oligonucleotides, mimicking RNA structures by 2′-modified nucleosides had shown excellent results.[10] Substituents like fluorine and oxygen, which have high electronegativity, forces the furanose sugar to C3′-*endo* conformation.[15] This conformational rigidity may be due to preferred *gauche* orientation of the 2′-substituent and the ring oxygen (Figure 5). As a result RNA and 2′-modified nucleosides are found predominantly in the C3′-*endo* conformation that is exclusively present in A-type duplexes.[16]

Third generation antisense oligonucleotides: In order to further enhance target affinity, nuclease resistance, bio-stability and pharmacokinetics, a third generation of AONs was developed mainly by modifications of the furanose ring of the nucleotide. Peptide nucleic acid (PNA), locked nucleic acid (LNA) and phosphoro-diamidate morpholino oligomer (PMO) are the three most studied third-generation AONs (Figure 6).[17,18]

PNAs are dramatic alterations in which the phosphodiester backbone is replaced with a flexible pseudopeptide polymer *N*-(2-aminoethyl)glycine and nucleobases are attached to the backbone via methylenecarbonyl-linkage (Figure 6).[19,20] PNA is a non-charged nucleotide analogue that can hybridize

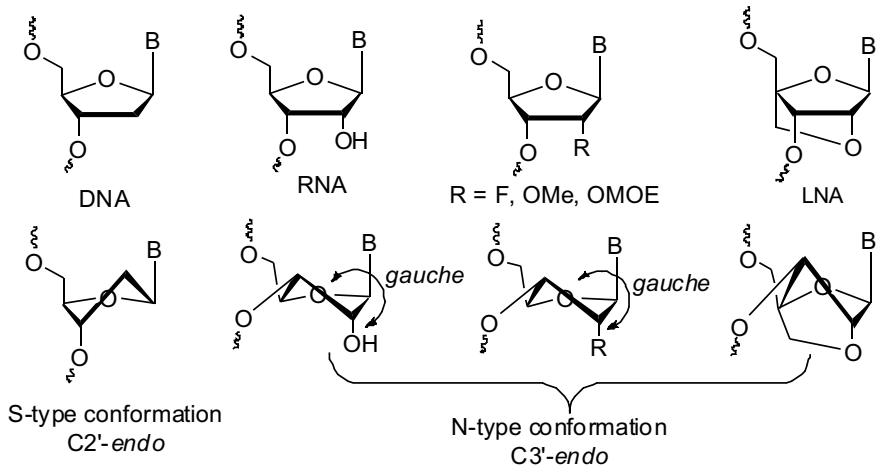


Figure 5. Structure of RNA-like 2′-substituted nucleosides; B = nucleobases

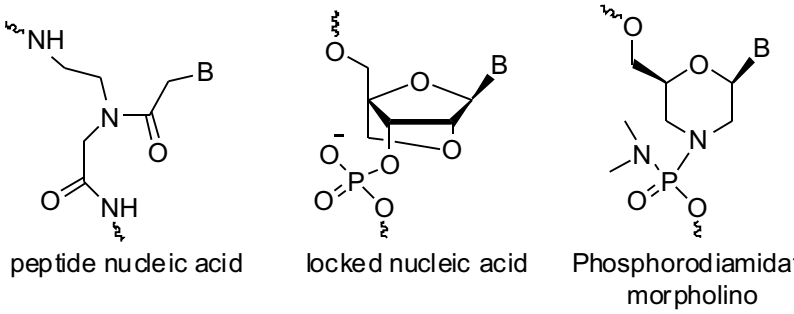


Figure 6. Third generation antisense oligonucleotides (AONs).

with complementary DNA or RNA with higher affinity and specificity than unmodified DNA-DNA and DNA-RNA duplexes. Further, PNA demonstrates high bio-stability in biological fluid because it is not degraded by nucleases or peptidases. Peptide nucleic acid exerts its antisense effect by forming a sequence-specific duplex with mRNA, which mainly causes steric hindrance of translational machinery leading to protein knockdown because it is not a substrate for RNase H .

Locked nucleic acid (LNA, Figure 6), contains a ribose ring which is locked in an N-type (C3′-*endo*) sugar puckering by the introduction of a 2′-*O*, 4′-*C* methylene-linkage (Figure 6).[6,21] Incorporation of one or more LNA monomer unit(s) into an ONs shows extraordinary thermal stability when hybridized with either DNA, RNA, or with LNA itself.[22,23] LNA offers key properties needed for successful therapeutic exploitation of oligonucleotides, including (1) unprecedented binding affinity towards RNA (and DNA), (2) excellent base pairing specificity, (3) high bio-stability (resistance towards nucleolytic degradation), (4) low toxicity (at least for many LNA oligonucleotides) in animals, and (5) convenient chemistry for manufacturing and modification. Like any other 2′-*O* ribose modification, LNA is not a substrate for RNase H. Notwithstanding, LNA monomers can be freely incorporated into RNA and DNA to form chimeric oligonucleotides resulting in restoration of RNase H-mediated cleavage of mRNA. It has been shown that the chimeric LNA/DNA/LNA gapmer with 7-10 PS-modified DNA central gaps flanked by three to four LNA oligomers on both ends provides highly efficient mRNA cleavage and nuclease resistance.[24]

Phosphorodiamidate morpholino oligomer (PMO) represents a non-charged AON agent in which the ribose sugar is replaced by a six-membered morpholino ring and the phosphodiester bond is replaced by a phosphorodiamidate-linkage (Figure 6).[25] Similar to the PNAs, these modifications do not activate RNase H and can be used only as steric blockers to inhibit gene expression in biological system. This chemical modification also confers excellent stability against nucleases and have similar target affinity to that of the isosequential unmodified AONs. Because backbone in PMOs is uncharged they are unlikely to have unwanted interactions with proteins but on the other hand it affects their cellular uptake.[26] PMO does not readily enter mammalian cells in culture, but a recent study using an arginine-rich peptide (ARP) conjugation to PMO markedly enhanced its cellular uptake and antisense potency by increasing the thermal stability of the ARP-PMO-mRNA heteroduplex.[27] Phosphoroamidate morpholino oligomer has demonstrated antisense efficacy in animal models *in vivo* and in human clinical trials.[28-30]

Modified oligonucleotides under clinical trial

In the past few years there has been an explosive growth in the number of modified oligonucleotides-related clinical trials. There are sixteen oligonucleotide drugs which are either approved or in the phase III clinical trials for various diseases. The majority of these drugs are second-generation PS chimeric that are designed to inhibit gene expression through antisense mechanism (Table 1). New opportunities such as the use of ribozymes and dsRNAs including siRNA and microRNA have emerged and are also the subjects of exciting progress. To the beginning of the antisense therapeutic era, two drugs have been approved by FDA and with continuous promising clinical trials new antisense drugs are expected to come in the near future.

Conclusion

Over the past two decades, the rapid advances in antisense technology have offered unlimited scope for the development of new and highly specific therapeutics. Suitably modified antisense oligonucleotides have various advantages over rational drugs for specific modulation of gene expression of selected targets, such as for cancer, leukemia, macular degradation, carcinoma, malignant melanoma, diabetes, duchenne muscular dystrophy, Crohn′s disease, multiple sclerosis, asthma, arthritis, etc. The results to date are very encouraging and platform of antisense-based therapeutics is therefore getting stronger.

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References:

1. Zamecnik, P. C.; Stephenson, M. L. *Proc. Natl. Acad. Sci., USA* 1978, *75*, 280.
2. Mulamba, G. B.; Hu, A.; Azad, R. F.; Anderson, K. P.; Coen, D. M. *Antimicrob. Agents Chemother.* 1998, *42*, 971.
3. McGowan, M. P.; Tardif, J.; Ceska, R.; Burgess, L. J.; Soran, H.; Gouni-Berthold, I.; Wagener, G.; Chasan-Taber, S. *PLoS One* 2012, *7*, e49006.
4. Corey, D. R. *J. Clin. Invest.* 2007, *117,* 3615.
5. Mickelfield, J. *Curr. Med. Chem*. 2001, *8*, 1157.
6. (a) Prakash, T. P.; Kawasaki, A. M.; Wancewicz, E. V.; Shen, L.; Monia, B. P.; Ross, B. S.; Bhat, B.; Manoharan, M. *J. Med. Chem*. 2008, *51*, 2766; (b) Wengel, J. *Acc. Chem. Res*. 1999, *32*, 301.
7. (a) Xie, X.; Liang, J.; Pu, T.; Xu, F.; Yao, F.; Yang, Y.; Zhao, Y. L.; You, D.; Zhou, X.; Deng, Z.; Wang, Z. *Nucleic Acids Res*. 2012, *40*, 9115. (b) Rahman, S. M.; Baba, T.; Kodama, T.; Islam, M. A.;

Obika, S. *Bioorg. Med. Chem.* 2012, *20*, 4098. (c) DeClerq, E.; Eckstein, F.; Merigan, T. C. *Science* 1969, *165,* 1137.
8. (a) Monn, S. T.; Schürch, S. *J. Am. Soc. Mass Spectrom.* 2007, *18*, 984. (b) Shoji, Y.; Akhtar, S.; Periasamy, A.; Herman, B.; Juliano, R. L. *Nucleic Acids Res*. 1991, *19*, 5543.
9. (a) Crooke, S. T. *Therapeutic applications of oligonucleotides, Austin, Ed.* Landes, R. G. 1995, 79. (b) Coughlin, J. E.; Pandey, R. K.; Padmanabhan, S.; O'Loughlin, K. G.; Marquis, J.; Green, C. E.; Mirsalis, J. C.; Iyer, R. P. *Drug Metab. Dispos*. 2012, *40*, 970.
10. (a) Yamada, T.; Okaniwa, N.; Saneyoshi, H.; Ohkubo, A.; Seio, K.; Nagata, T.; Aoki, Y.; Takeda, S.; Sekine, M. *J. Org. Chem.* 2011, *76*, 3042. (b) Cummins, L. L.; Owens, S. R.; Risen, L. M.; Lesnik, E. A.; Freier, S. M.; McGee, D.; Guinosso, C. J.; Cook, P. D. *Nucleic Acids Res*. 1995, *23*, 2019.
11. Prakash, T. P. *Chem. Biodivers*. 2011, *8*, 1616.
12. Sproat, B. S.; Lamond, A. I.; Beijer, B.; Neuner, P.; Ryder, U. *Nucleic Acids Res*. 1989, *17*, 3373.
13. (a) Seth, P. P.; Jazayeri, A.; Yu, J.; Allerson, C. R.; Bhat, B.; Swayze, E. E. *Mol. Ther. Nucleic Acids* 2012, *1*, e47. (b) Monia, B. P.; Lesnik, E. A.; Gonzalez, C.; Lima ,W. F.; McGee, D.; Guinosso, C. J.; Kawasaki, A. M.; Cook, P. D.; Freier, S. M. *J. Biol. Chem.* 1993, *268*, 14514.
14. Mesmaeker, A. D.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* 1995, *28,* 366.
15. Guschlbauer, W.; Jankowski, K. *Nucleic Acids Res*. 1980, *8*, 1421.
16. (a) Saenger, W. *Principles of nucleic acid structures, Springer-Verlag, New York* 1984. (b) Prakash, T. P.; Bhat, B. *Curr. Top. Med. Chem.* 2007, *7*, 641.
17. Kurreck, J. *Eur. J. Biochem*. 2003, *270*, 1628.
18. Gleave, M. E.; Monia, B. P. *Nat. Rev. Cancer* 2005, *5*, 468.
19. (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* 1991, *254*, 1497. (b) Banerjee, A.; Kumar, V. A. *Bioorg. Med. Chem*. 2013, *21*, 4092.
20. Nielsen, P. E. *Mol. Biotechnol*. 2004, *26*, 233.
21. Hildebrandt-Eriksen, E. S.; Aarup, V.; Persson, R.; Hansen, H. F.; Munk, M. E.; Orum, H. *Nucleic Acid Ther*. 2012, *22*, 152.
22. Veedu, R. N.; Wengel, J. *Chem. Biodivers*. 2010, *7*, 536.
23. Baraasch, D. A.; Corey, D. R. *Chem. Biol*. 2001, *8*, 1.
24. Kurreck, J.; Wyszko, E.; Gillen, C.; Erdmann, V. A. *Nucleic Acids Res*. 2002, *30*, 1911.
25. Schnell, F. J.; Crumley, S. L.; Mourich, D. V.; Iversen, P. L. *Biores. Open Access* 2013, *2*, 61.
26. Amantana, A.; Iversen, P. L. *Curr. Opin. Pharmacol*. 2005, *5*, 550.
27. Nelson, M. H.; Stein, D. A.; Kroeker, A. D.; Hatlevig, S. A.; Iversen, P. L.; Moulton, H. M. *Bioconjug. Chem.* 2005, *16*, 959.
28. McCaffrey, A. P.; Meuse, L.; Karimi, M.; Contag, C. H.; Kay, M. A. *Hepatology* 2003, *38*, 503.
29. Iversen, P. L.; Warren, T. K.; Wells, J. B.; Garza, N. L.; Mourich, D. V.; Welch, L. S.; Panchal, R. G.; Bavari, S. *Viruses* 2012, *4*, 2806.
30. Malerba, A.; Kang, J. K.; McClorey, M. G.; Saleh, A. F.; Popplewell, L.; Gait, M. J.; Wood, M. J.; Dickson, G. *Mol. Ther. Nucleic Acids* 2012, *1*, e62.
31. Perry, C. M.; Balfour, J. A. *Drugs* 1999, *57*, 375.
32. Hair, P.; Cameron, F.; McKeage, K. *Drugs* 2013, *73*, 487.
33. (a) Akar, E. E.; Oner, V.; Küçükerdönmez, C.; Aydın, A. Y. *Int. J. Ophthalmol.* 2013, *6*, 136. (b) Ng, E. W.; Shima, D. T.; Calias, P.; Cunningham, E. T. Jr.; Guyer, D. R.; Adamis, A. P. *Nat. Rev. Drug Discov.* 2006, *5*, 123. (c) Burnett, J. C.; Rossi, J. J. *Chem. Biol.* 2012, *19*, 60.
34. Borthakur, G.; O'Brien, S. *Blood and Lymphatic Cancer: Targets and Therapy* 2012, *2*, 137.
35. Jaschinski, F.; Rothhammer, T.; Jachimczak, P.; Seitz, C.; Schneider, A.; Schlingensiepen, K. H. *Curr. Pharm. Biotechnol.* 2011, *12*, 2203.
36. Cursiefen, C.; Bock, F.; Horn, F. K.; Kruse, F. E.; Seitz, B.; Borderie, V.; Früh, B.; Thiel, M. A.; Wilhelm, F.; Geudelin, B.; Descohand, I.; Steuhl, K. P.; Hahn, A.; Meller, D. *Ophthalmology* 2009, *116*, 1630.
37. Grossman, S. A.; Alavi, J. B.; Supko, J. G.; Carson, K. A.; Priet, R.; Dorr, F. A.; Grundy, J. S.; Holmlund, J. T. *Neuro-oncol.* 2005, *7*, 32.
38. Laskin, J. J.; Nicholas, G.; Lee, C.; Gitlitz, B.; Vincent, M.; Cormier, Y.; Stephenson, J.; Ung, Y.; Sanborn, R.; Pressnail, B.; Nugent, F.; Nemunaitis, J.; Gleave, M. E.; Murray, N.; Hao, D. *J. Thorac. Oncol*. 2012, *7*, 579.
39. *http://clinicaltrials.gov/ct2/results?term=GSK-2402968*
40. Singerman, L. *Retina* 2009, *29*, S49.
41. (a) *http://www.gentium.com/products/defibrotide.* b) Richardson, P. G.; Soiffer, R. J.; Antin, J. H.; Uno, H.; Jin, Z.; Kurtzberg, J.; Martin, P. L.; Steinbach, G.; Murray, K. F.; Vogelsang, G. B.; Chen, A. R.; Krishnan, A.; Kernan, N. A.; Avigan, D. E.; Spitzer, T. R.; Shulman, H. M.; Di Salvo, D. N.; Revta, C.; Warren, D.; Momtaz, P.; Bradwin, G.; Wei, L. J.; Iacobelli. M.; McDonald, G. B.; Guinan, E. C. *Biol. Blood Marrow Transplant* 2010, *16*, 1005.
42. (a) Kim, Y. H.; Girardi, M.; Duvic, M.; Kuzel, T.; Link, B. K.; Pinter-Brown, L.; Rook, A. H. *J. Am. Acad. Dermatol.* 2010, *63*, 975. (b) Murad, Y. M.; Clay, T. M.; Lyerly, H. K.; Morse, M. A. *Expert Opin. Biol. Ther.* 2007, *7*, 1257. (c) *http://www.im.org/AcademicAffairs/Aging/IGP/ExpandingResearchEfforts/Documents/1CPG%207909.pdf.*
43. Barry, M.; Cooper, C. *Expert Opin. Biol. Ther.* 2007, *7*, 1731.
44. Yacyshyn, B.; Chey, W. Y.; Wedel, M. K.; Yu, R. Z.; Paul, D.; Chuang, E. *Clin. Gastroenterol Hepatol*, 2007, *5*, 215.
45. (a) Sekhon, H. S.; London, C. A.; Sekhon, M.; Iversen, P. L.; Devi, G. R. *Lung Cancer* 2008, *60*, 347. (b) *http://investorrelations.avibio.com/phoenix.zhtml?c=64231&p=irol-newsArticle&ID=928275&highlight=*
46. (a) *http://www.drugfuture.com/chemdata/edifoligide-sodium.html.* (b) Hoel, A. W.; Conte, M. S. *Cardiovasc. Drug Rev*. 2007, *25*, 221.