Special Report

Future Rare Diseases

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Research and development of oligonucleotide therapeutics in Japan for rare diseases

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Inherited gene mutations, insertions, deletions of single genes cause most of the rare diseases. Oligonucleotide therapeutics represent one of the most flexible platforms for developing drugs for rare diseases. Presently, 15 oligonucleotide therapeutics have been approved in the United States of America (USA) to treat various rare diseases and 4 oligonucleotide therapeutics (eteplirsen, golodirsen, viltolarsen and casimersen) are used to treat Duchenne muscular dystrophy. The progress of oligonucleotide therapeutics in Japan has emerged from several decades of basic research. In March 2020, viltolarsen, developed by Japanese companies, was approved as a treatment for Duchenne muscular dystrophy. This article discusses the research and development of oligonucleotide therapeutics for rare diseases from the viewpoint of the proprietary technologies in Japanese pharmaceutical and bio-venture companies.

Plain language summary: Recently, oligonucleotide therapeutics have received awareness following small molecule and antibody drugs. The research related to oligonucleotide therapeutics in Japan is based on the results of basic research accumulated over several decades. For example, viltolarsen (NS-065/NCNP-01) from Nippon Shinyaku was approved for Duchenne muscular dystrophy in Japan and USA in 2020. Here, we report the development of oligonucleotide therapeutics and the role of Japan.

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Keywords: antisense oligonucleotide • bio-venture • Duchenne muscular dystrophy • oligonucleotide therapeutics • rare diseases • small interfering RNA • viltolarsen

Overview of rare disease

There are approximately 350 million patients with rare diseases worldwide. While 80% of the cases are caused by inherited gene mutations, infectious and autoimmune diseases including those induced by environmental factors, cause the remaining 20% [1]. About 7000 inherited rare diseases have been identified; however, no therapeutic methods have been established for 95% of them. In addition, more than half of the patients are under 18 because of the juvenile-onset nature of the diseases [2]. The patients' quality of life is hindered by the severe persistent symptoms and fast progression of the diseases. For example, epidemiological statistics showed that about 30% of the patients with rare diseases die by the age of 5 because of the increased rate of disease progression and low rate of treatment successes [3].

The definition of a rare disease differs from country to country in the context of cultural backgrounds, resources and medical systems. Support from a government is necessary for developing therapeutic drugs for rare diseases, including oligonucleotides. Moreover, an in-depth scientific understanding of the onset mechanisms of a rare disease is essential for finding its cure.

Future Medicine

Inherited mutations, insertions and deletions of single genes cause most rare diseases. The responsible genes of many rare diseases have been discovered. However, it is difficult to develop therapeutics for rare diseases because the detailed mechanisms of many rare diseases remain unknown [4]. For example, the deletion of the region on chromosome 5 containing the *survival motor neuron 1* gene causes spinal muscular atrophy; however, there is no abnormal mRNA or protein in the patient [5].

Biological actions of oligonucleotide therapeutics

Oligonucleotide therapeutics [6] are classified as therapeutics that use antisense oligonucleotides [7] or small interfering RNAs (siRNAs) to target RNA or protein [8]. Oligonucleotide therapeutics inhibit the translation of certain proteins from mRNA, regulate the expression of microRNA (miRNA) that are complementary to certain mRNAs [8], and include decoys [9] that inhibit transcription activities by targeting transcription factors. They also function as aptamers [10] that specifically bind to various proteins to suppress intracellular signal transduction and as CpG oligos [11] that bind to Toll-like receptors to induce innate immunity [12]. Moreover, the research on enhancing protein translation with antisense long noncoding RNA by interacting with the translational machinery has begun recently [13].

On the other hand, splicing modulation is the mode of action of the antisense oligonucleotides used to treat Duchenne muscular dystrophy (DMD) patients. For example, Viltolarsen is the antisense oligonucleotide (ASO) that targets exon 53 of the *dystrophin* gene, which is responsible for DMD, to restore the amino acid reading frame by skipping exon 53 during the splicing process. Various intracellular actions of oligonucleotide therapeutics are illustrated in Figure 1A., and the modulation of RNA splicing by viltolarsen is described as an example in Figure 1B

Oligonucleotide therapeutics for rare diseases

Our understanding of how oligonucleotides modulate gene activities in a cell is traced back to the discovery of the double helical structure of DNA in the 1950s [14], followed by the adaptation of the central dogma, which describes how genomic DNA encodes proteins. Then, RNA interference (RNAi), a mechanism significantly different from those in the central dogma, was discovered in 1993. In RNAi, a double-stranded RNA is degraded by dicer, a nuclease, into small interfering RNA (siRNA) molecules of 21 to 23 nucleotides. siRNA and the RNA-induced silencing complex regulate the degradation of mRNA complementary to the siRNA using the nuclease activity of the complex. Therefore, the idea of ASO, siRNA, and decoy is based on the concepts of the central dogma and RNAi [15,16].

Rare diseases are often caused by mutations. For example, dyserythropoietic anemia and other impaired hematopoiesis are associated with a single intronic mutation in the *GATA-1* gene. The two separate symptoms of dyserythropoietic anemia and other impaired hematopoiesis are associated with a single intronic mutation in the *GATA-1* gene that results in a five amino acid insertion [17]. This intronic mutation, like single nucleotide polymorphism, shows us that there are still many unknown mechanisms of the rare disease onset. Because most oligonucleotide therapeutics are designed to target DNA or RNA sequences, more in-depth knowledge of the human genome is necessary.

Fifteen oligonucleotide therapeutics, including fomivirsen [10], pegaptanib [10], mipomersen [10], defibrotide [10], eteplirsen [10], nusinersen [18], inotersen [19], patisiran [20], volanesorsen [21], givosiran [22], golodirsen [23], viltolarsen [24], lumasiran [25], inclisiran [26] and casimersen [27], have been approved in various countries for a range of rare diseases (Table 1 & Figure 3). All these approved oligonucleotide therapeutics employ 20-mers or longer sequences with a molecular weight of approximately 6000 Dalton.

Chemistry & manufacturing of nucleic acid in Japan

The first nucleic acid applied to antisense nucleotide technology was the phosphorothioate backbone [28]. Improved stability and increased affinity have been achieved using nucleotides with a 2′-modified sugar [29], including 2′-fluoro (2′-F), 2′-*O*-methyl (2′-OMe), and 2′-*O*-methoxyethyl (2′-MOE) modifications [30], and a phosphorodiamidate morpholino oligomer (PMO) [31]. Such modifications are used in many approved drugs (Figure 2A) [32].

Japanese researchers have contributed substantially to nucleic acid chemistry. The bridged sugar in nucleic acids was developed primarily by Obika *et al.* [33]. In 2′-*O*, 4′-C-methylene-bridged or locked nucleic acids (2′,4′-BNA/LNA) [34], the furanose ring in the nucleic acid was fixed with an N-type conformation (Figure 2B). As a result, the conformation of the furanose ring is locked in the proper conformation, resulting in high RNA binding

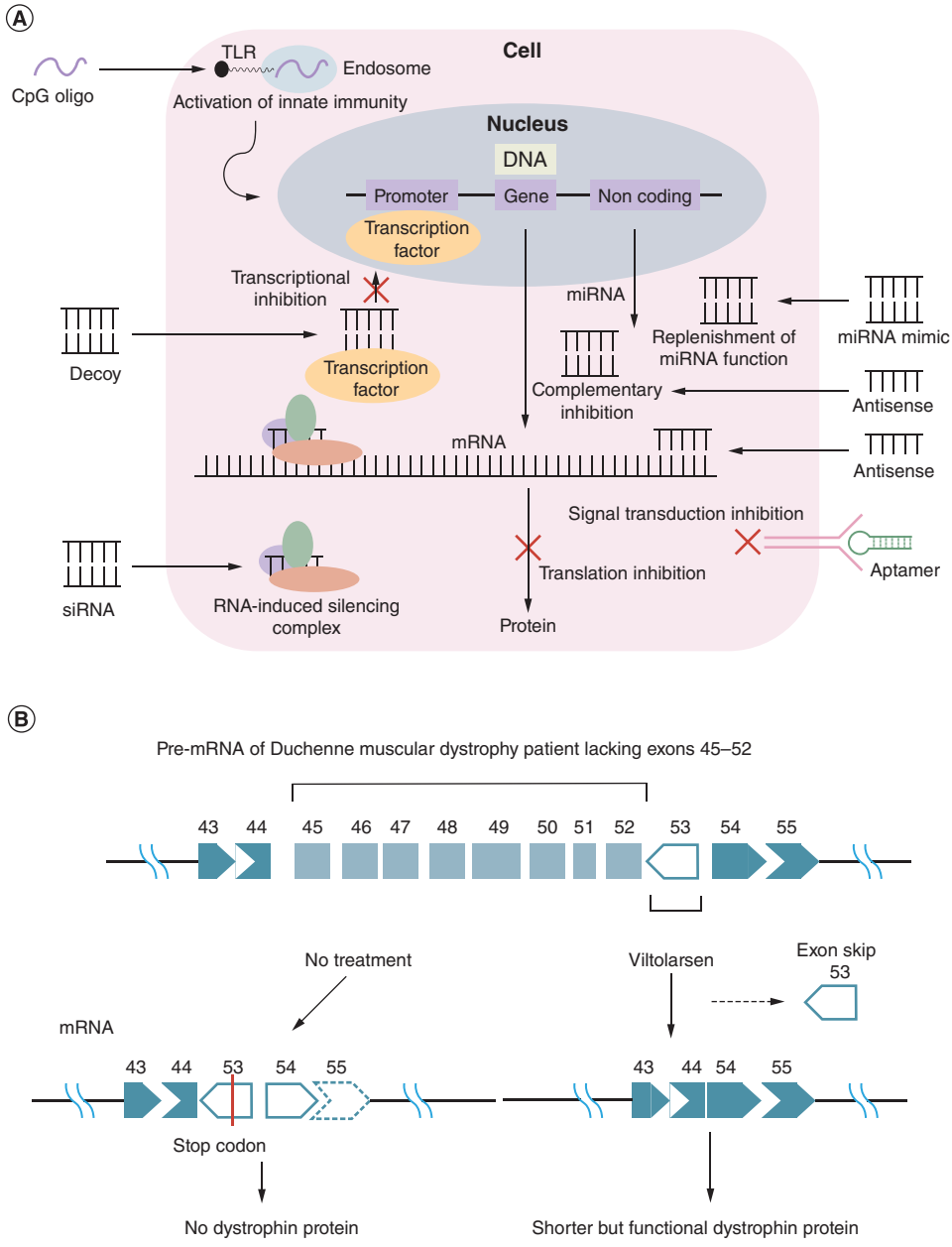


Figure 1. Basic mechanism of biological actions of oligonucleotide therapeutics. (A) Various intracellular biological actions of oligonucleotides. CpG, a cytosine triphosphate deoxynucleotide ('C'), a phosphodiester link between consecutive ('p'), and a guanine triphosphate deoxynucleotide ('G') nucleotide; miRNA: microRNA; siRNA: small interfering RNA. **(B)** The translation of *dystrophin* in patients with Duchenne muscular dystrophy lacking exon 45–52.

affinity. 2′-*O*, 4′-*C*-ethylene-bridged nucleic acids (ENA) [35] and a constrained ethyl cyclic nucleic acid (cEt) [36] are used as bridged sugars in these nucleic acids as a DMD therapeutic agent (DS-5141b) [37]. The bridged sugars in nucleic acids, amide-bridged nucleic acid (AmNA) [38], guanine-bridged nucleic acid (GuNA) [39] and 2′-*O*,4′-*C*-spirocyclopropylene (scpBNA) [40], maintain high-affinity binding to the target RNA and are more highly resistant to nucleolytic enzymes [41].

Meanwhile, the Bonac nucleic acid [42] and a DNA/RNA heteroduplex oligonucleotide (HDO) [43] were developed by Bonac and Rena Therapeutics, respectively. Bonac nucleic acid PnkRNA uses proline as a linker and is more chemically stable than nkRNA. In contrast, HDO is composed of an RNA strand complementary to a

Table 1. Approved oligonucleotide therapeutics at the end of December 2021.

Drug	Brand name	Year of approval	Indication/administration/target/type
Fomivirsen†	Vitravene	USA, 1998 EU, 1999	Cytomegalovirus retinitis/intravitreal *Cytomegalovirus IE2* (eye)/antisense oligonucleotide
Pegaptanib	Macugen	USA, 2004 EU, 2006 JP, 2008	Neovascular, age-related macular degeneration/intravitreal (eye)/aptamer
Mipomersen‡	Kynamro	USA,2013	Homozygous familial hypercholesterolemia/subcutaneous (liver)/antisense oligonucleotide
Defibrotide	Defitelio	USA,2016 JP, 2019	Hepatic veno-occlusive disease/intravenous Proteins, nonspecific§ (liver)
Eteplirsen	Exondys 51	USA, 2016	Duchenne muscular dystrophy/intravenous *Dystrophin* (exon 51) (muscle)/antisense oligonucleotide
Nusinersen	Spinraza	USA, 2016 EU, 2017 JP, 2017	Spinal muscular atrophy/intrathecal *SMN 2* (CNS)/antisense oligonucleotide
Inotersen	Tegsedi	EU, 2018 USA, 2018	Human hereditary transthyretin amyloidosis/subcutaneous (liver)/antisense oligonucleotide
Patisiran	Onpattro	USA, 2018 EU, 2018 JP, 2019	Human hereditary transthyretin amyloidosis/intravenous (liver)/siRNA
Volanesorsen	Waylivra	EU, 2019	Familial chylomicronemia syndrome/subcutaneous (liver)/antisense oligonucleotide
Givosiran	Givlaari	USA, 2019 EU, 2020	Acute hepatic porphyria/subcutaneous (liver)/siRNA
Golodirsen	Vyondys 53	USA, 2019	Duchenne muscular dystrophy/intravenous *Dystrophin* (Exon 53)/antisense oligonucleotide
Viltolarsen	Viltepso	JP, 2020 USA, 2020	Duchenne muscular dystrophy/intravenous *Dystrophin* (Exon 53)/antisense oligonucleotide
Lumasiran	Oxlumo	USA, 2020 EU, 2020	Primary hyperoxaluria type 1/subcutaneous (liver)/siRNA
Inclisiran	Leqvio	EU, 2020	Atherosclerotic cardiovascular disease and heterozygous familial hypercholesterolemia/subcutaneous *PC-9* (liver)/siRNA
Casimersen	Amondys 45	USA, 2021	Duchenne muscular dystrophy/intravenous *Dystrophin* (Exon 45)/antisense oligonucleotide

†The sale is currently discontinued.
‡It is still available on a very restricted basis due to side effects.
§The mechanism of action is complex and has not been completely elucidated.
JP: Japan.

DNA backbone with 12–20 bases and has a gapmer structure in which LNA is placed at both ends. As a result, HDO exhibits higher efficacy and lower toxicity in humans than the conventional oligonucleotide therapeutics.

On the other hand, the conventional solid-phase and liquid-phase syntheses of oligonucleotide therapeutics face problems in yield, purity and quality. Thus, Ajinomoto Healthy Supply has developed a proprietary liquid-phase manufacturing method, AJIPHASE [44], with an acceptable yield, high purity, lower cost and sufficient quality.

In this special report, we analyze the state of research and development of oligonucleotide therapeutics in Japan. In addition, we provide an update on the oligonucleotide therapeutics of interest to Japanese pharmaceutical and bio-venture companies with proprietary technologies.

Materials & methods

We extracted information relevant to Japanese bio-venture companies using the keywords 'oligonucleotide therapeutics' or 'oligonucleotide manufacture' in Internet search engines, such as Yahoo or Google. In addition, we used the websites of the Japan Medical Abstracts Society, the Japan Bioindustry Association and the Nucleic Acid Therapeutics Society of Japan. We also used a retrieval method that summarized the business contents based on oligonucleotide therapeutics including articles on oligonucleotide manufacturing related to these companies. Then, we confirmed the information from the home pages of the companies (Figure 3); the patent information (J-Plat-Pat) searched with the keywords 'oligonucleotide therapeutics,' 'oligonucleotide manufacture' and 'company name'; the scientific articles (National center for Biotechnology Information and Scifinder) searched using the same keywords

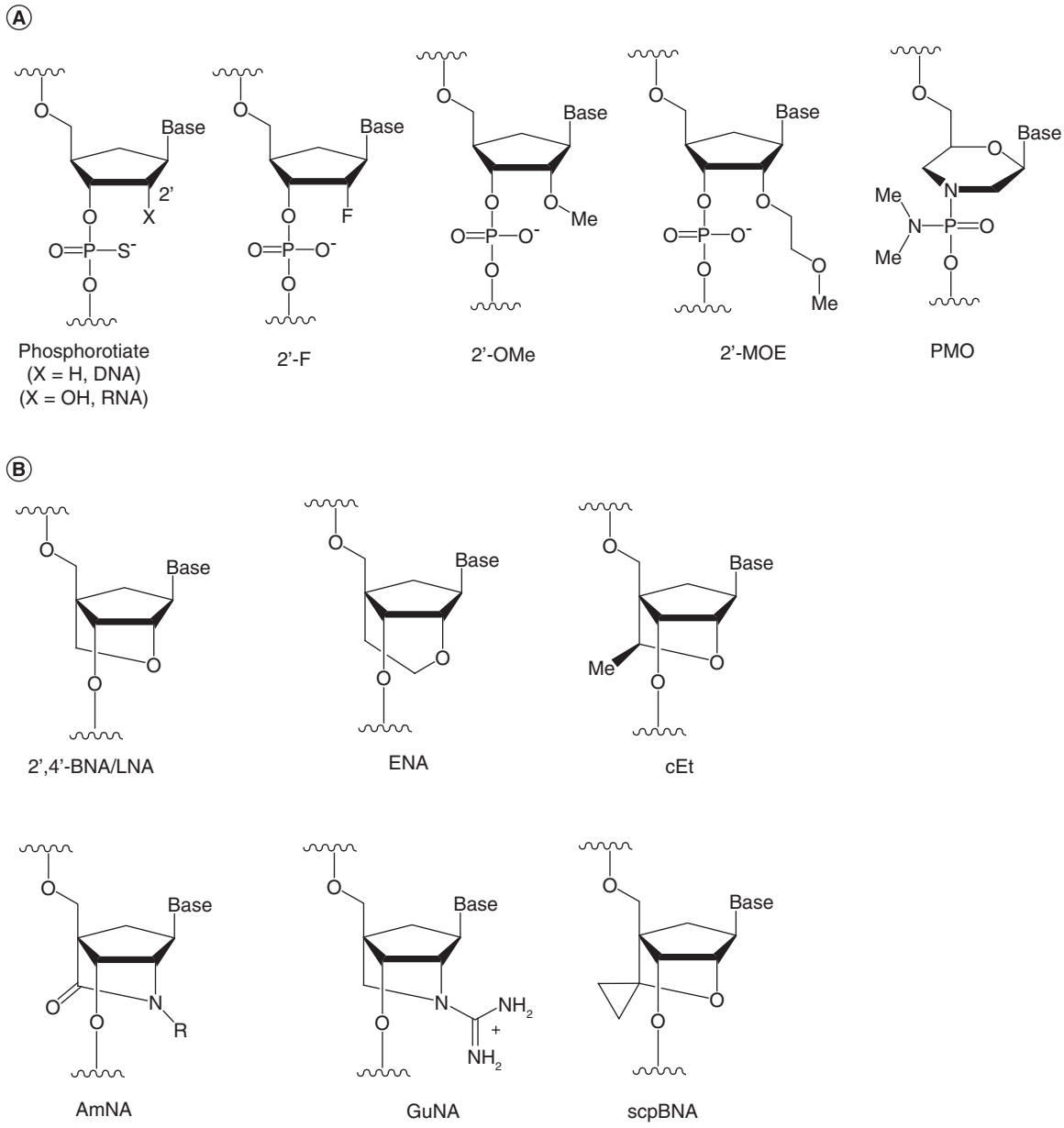


Figure 2. Structure modifications of the sugar for the stabilization of oligonucleotides. (A) The sugar modifications of oligonucleotides used in approved drugs. **(B)** The structures of cross-linked artificial nucleic acids. 2′-F: 2′-fluoro; 2′-OMe: 2′-O-methyl; 2′-MOE: 2′-O-methoxyethyl; 2′,4′-BNA/LNA: 2′-*O*, 4′-*C*-methylene-bridged nucleic acid/locked nucleic acid; AmNA: Amide-bridged nucleic acid; cET: Constrained ethyl cyclic nucleic acid; ENA: 2′-O, 4′-C-ethylene-bridged nucleic acid; GuNA: Guanine-bridged nucleic acid; PMO: Phosphorodiamidate morpholino oligomer; scpBNA: 2′-*O*,4′-*C*-spirocyclopropylene.

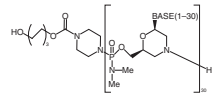
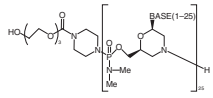
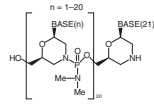
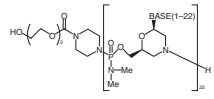
and clinical trials (ClinicalTrials.gov and JAPIC: Japan Clinical Trials Information) using the drug name, country (Japan, the USA and European Union (EU)), status (active, completed and terminated), study design (study type, actual enrollment, allocation, masking and criteria) and outcomes. The results are summarized in Table 2.

Japanese Pharmaceutical & Venture Companies of Oligonucleotide Therapeutics

Nippon Shinyaku

Nippon Shinyaku Co. Ltd. was established in 1911. It developed the oligonucleotide therapeutic NS-065/NCNP-01 (generic name: viltolarsen) in collaboration with the National Center of Neurology and Psychiatry in Japan. Subsequently, the Ministry of Health, Labor and Welfare (MHLW) approved viltolarsen in 2020, when oligonu-

Figure 3. Structure of approved oligonucleotide therapeutics at the end of December 2021.

Drug	Structure
Formivirsen	5′-GCGTTTGCTCTTCTTCTTGCG-3′- All phosphate bonds are phosphorothioate linkages.
Pegaptanib	R[1]-CGGAAUCAGUGAAUGCUUAUACAUCCGdT-3′
Mipomersen	5′-GCCUCAGTCTGCTTCGCACC-3′ All phosphate bonds are phosphorothioate linkages.
Defibrotide	Natural product*1 (MW: 13,000–20,000)
Eteplirsen	 5′-CTCCAACATCAAGGAAGATGGCATTTCTAG-3′
Nusinersen	5′-UCACUUUCAUAAUGCUGG-3′ All phosphate bonds are phosphorothioate linkages.
Inotersen	5′-UCUUGGTTACATGAAAUCCC-3′ All phosphate bonds are phosphorothioate linkages.
Patisiran	5′-dTdTCAUUGGUUCUCAUAAGGUA-3′ 3′-GUAACCAAGAGUAUUCCAUdTdT-5′
Volanesorsen	5′-TATTTdmCdGdAdmCdmCdTdGdTdTdmCTTmCGA-3′ All phosphate bonds are phosphorothioate linkages.
Givosiran	5′-CAGAAAGAGUGUCUCAUCUUA-triGalNAc-3′ 3′-U(s)G(s)GUCUUUCUCACAGAGUAGA(s)A(s)U-5′
Golodirsen	 5′-GTTGCCTCCGGTTCTGAAGGTGTTC-3′
Viltolarsen	 5′-CCTCCGGTTCTGAAGGTGTTC-3′
Lumasiran	5′-GACUUUCAUCCUGGAAAUAUA-triGalNAc-3′ 3′-A(s)C(s)CUGAAAGUAGGACCUUUAU(s)A(s)U-5′
Inclisiran	5′-A(s)C(s)A(s)AAAGCAAAACAGGUCUA(s)G(s)A(s)A-3′ 3′-triGalNAc-UGUUUUCGUUdTUGUCCAG(s)A(s)U(s)C-5′
Casimersen	 5′-CAATGCCATCCTGGAGTTCCTG-3′

*1: Polydeoxyribonucleotide sodium depolymerized from DNA isolated from porcine intestine mucosa. Abbreviations: A, adenine 2′-OMe ribonucleoside; A, adenine 2′-Fribonucleoside; A, adenine 2′-O-(2-methoxyethyl) ribonucleoside; A, adenine phosphorodiamidate morpholino nucleoside; C, cytosine 2′-OMe ribonucleoside; C, cytosine 2′-F ribonucleoside; C, cytosine 2′-O-(2-methoxyethyl) ribonucleoside; C, cytosine phosphorodiamidate morpholino nucleoside; d, 2′-deoxy; G, guanine 2′-OMeribonucleoside; G, guanine 2′-Fribonucleoside; G, guanine 2′-O-(2-methoxyethyl) ribonucleoside; G, guanine phosphorodiamidate morpholino nucleoside; m, 5-methyl; (s), phosphorothioate linkage; T, thymine 2′-O-(2-methoxyethyl) ribonucleoside; T, thyminephosphorodiamidate morpholino nucleoside; triGalNAc, triantennary N-acetylgalactosamine; U, uracil 2′-OMe ribonucleoside; U, uracil 2′-F ribonucleoside, U, uracil phosphorodiamidate morpholino nucleoside.

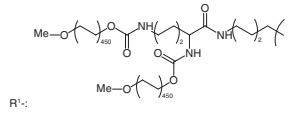


Figure 3. Structure of approved oligonucleotide therapeutics at the end of December 2021.
†: Polydeoxyribonucleotide sodium depolymerized from DNA isolated from porcine intestine mucosa.
A: adenine Adenine 2′-OMe ribonucleoside; A: adenine Adenine 2′-Fribonucleoside; A: adenine Adenine 2′-O-(2-methoxyethyl) ribonucleoside; A: adenine Adenine phosphorodiamidate morpholino nucleoside; C: cytosine Cytosine 2′-OMe ribonucleoside; C: cytosine Cytosine 2′-F ribonucleoside; C: cytosine Cytosine 2′-O-(2-methoxyethyl) ribonucleoside; C: cytosine Cytosine phosphorodiamidate morpholino nucleoside; d: 2′-deoxy; G: guanine Guanine 2′-OMeribonucleoside; G: guanine Guanine 2′-Fribonucleoside; G: guanine Guanine 2′-O-(2-methoxyethyl) ribonucleoside; G: guanine Guanine phosphorodiamidate morpholino nucleoside; m: 5-methyl; (s): phosphorothioate Phosphorothioate linkage; T: thymine Thymine 2′-O-(2-methoxyethyl) ribonucleoside; T: thyminephosphorodiamidate Thyminephosphorodiamidate morpholino nucleoside; triGalNAc: triantennary Triantennary N-acetylgalactosamine; U: uracil Uracil 2′-OMe ribonucleoside; U: uracil Uracil 2′-F ribonucleoside, U: uracil Uracil phosphorodiamidate morpholino nucleoside.

Table 2. Japanese pharmaceutical companies developing oligonucleotide therapeutics.

Pharmaceutical company	Code no.	Indication/administration target/type	Development phase	Patent no.
Orphan Disease Treatment Institute	DS-5141	Duchenne muscular dystrophy/subcutaneous *Dystrophin* (Exon 45)/antisense oligonucleotide	Phase I/II with no effect, study ongoing (Japan) NCT02667483	JP 2016-182122
TME Therapeutics	STNM01	Unresectable pancreatic cancer/injection *CHST15*/siRNA	Ongoing, Phase I study (Japan)	WO 2014/013535
		Esophageal stricture post endoscopic therapy/injection *CHST15*/siRNA	Non-clinical study	
AnGes	AMG0101	Atopic dermatitis/ointment *NF-κB*/decoy	Completed Phase III study (Japan) with no effect	JP 2011-50381
	AMG0101	Disc herniation/injection *NF-κB*/decoy	Recruiting subjects for Phase Ib study (US)	JP 2012-92104
Ribomic	RBM-007	Age-related macular degeneration/intravitreal FGF-2/aptamer	Recruiting subjects for Phase I/IIa→IIb study (US), Phase I study (Japan) NCT03633084	WO2008/059877
		Achondroplasia/subcutaneous FGF-2/aptamer	Ongoing, healthy adults for Phase I study (JapicCTI-205345)	
	RBM-003	Heart failure/under developing Chymase/aptamer	Non-clinical study	WO2010/035725
	RBM-004	Pain/under developing Nerve growth factor/aptamer	Non-clinical study	WO2010/143714
	RBM-006	Fibrosis/under developing Autotaxin/aptamer	Non-clinical study	WO2011/099576
	RBM-001	Undecided/under developing Midkine/aptamer	Non-clinical study	WO2015/163458
Nitto Nitto Biopharma	NBF-006	KRAS-mutated cancer/intravenous *GST-π*/siRNA	Recruiting, Phase I study (US) NCT03819387	JP 2016-127853
	ND-L02-s0201	Idiopathic pulmonary fibrosis/intravenous *HSP47*/siRNA	Recruiting, Phase II study (US) Ongoing, Phase II study (Japan) NCT03538301	JP 2018-65804 A
		Liver fibrosis/intravenous *HSP47*/siRNA	Completed Phase II study (US) NCT03538301	JP 2018-183163 A
SBI Biotech	GNKS356	Psoriasis, Rheumatism/under developing TLR7,8,9/DNA agonist	Licensing	JP 2019-500873
Quarkpharma	QPI-1002	Acute renal failure/intravenous *p53*/siRNA	Ongoing, Phase III study (US) NCT03510897	JP 2015-535174
3D Matrix	TDM-812	Drug-resistant breast cancer/injection *RPN2*/siRNA	Reporting, Phase I study (Japan)	JP 2016-101167
Bonac/Toray	BNC-1021/TRK-250	Idiopathic pulmonary fibrosis/inhalation *TGF-β1*/Bonac RNA	Recruiting, Phase I study (Japan and US), NCT03727802 Orphan drug approved from FDA	WO2016/098782
Bonac	BNC-1501	Cancer/under developing miR-34/Bonac RNA	Laboratory study	—
	BNC-1602	Fibrosis/under developing miR-29/Bonac RNA	Laboratory study	—
	BNC-1601	Uveitis, Diabetic retinopathy/instillation *PENR*/Bonac RNA	Non-clinical study	WO2017/073767
	BNC-1251	Cancer/under developing/ Bonac RNA	Laboratory study	WO2017/115872
TAK-Circulator	TAKC-02	Intractable asthma/under developing *Mex3B*/antisense oligonucleotide	Non-clinical study	JP 2018-11593
RenaTherapeutics	—	Under developing/under developing Under developing/hetero-double stranded DNA/RNA	Laboratory study	JP 2018-11593
TAGCyx	—	Under developing/under developing *INF-gamma* or *vWF*/Xenoligo aptamer	Non-clinical study	JP 2018-19697, 2017-547793, WO2017/073535, WO2017/073536

cleotide therapeutics created in Japan reached the approval stage for the first time. Viltolarsen was designed to skip the normal exon 53 of the *dystrophin* gene in patients with exon deletions, 43–52, 45–52, 47–52, 48–52, 49–52, 50–52 and 52, resulting in the synthesis of an in-frame *dystrophin* transcript and the production of a shorter but functional dystrophin protein (Figure 1B) [24]. This oligonucleotide therapeutic is used with a morpholino nucleic acid at the 5' end.

Clinical trials of viltolarsen for DMD with the exon 45–52 deletion have been conducted in Japan and USA. In Japan, Phase I clinical trials using an intravenous injection of two doses, 40 and 80 mg/kg, were also conducted in 16 male patients aged 5–12 for 24 weeks, and the potential adverse events were evaluated. The drug trials are ongoing, and only mild or medium-grade adverse effects have been observed. Additionally, the primary end point of efficacy, namely, the skipping of exon 53, was confirmed in all the patients and indicated by increased protein levels of intracellular dystrophin in 14 of the 16 patients tested [45].

In USA, NS Pharma Inc., a subsidiary of Nippon Shinyaku, and Dr. Paula Clemens at Pittsburgh University conducted a Phase II clinical trial. Sixteen boys aged 4–10 were divided equally into two dosage groups, 40 and 80 mg/kg, and received the drug intravenously once a week for 24 weeks. Increased levels of intracellular dystrophin were observed in all (16/16) patients. The increasing levels of dystrophin among the treated patients were on average 5.8% (1.1–14.4%) and such increase improves the DMD symptoms significantly. Furthermore, no serious drug-related adverse effects were observed, and all the adverse effects were mild or moderate in grade.

Orphan Disease Treatment Institute

Orphan Disease Treatment Institute, established in 2013 as a subsidiary of Daiichi Sankyo, has developed an antisense DNA oligonucleotide (DS-5141) as a DMD drug that is designed to skip normal exon 45 of the *dystrophin* gene to prevent the occurrence of a stop codon in exon 45 and restore the functional, short type of dystrophin [37]. The company is conducting a Phase II clinical study in Japan.

TME Therapeutics

TME Therapeutics Co. Ltd., established in 2018, has developed double-strand siRNA for some carbohydrate sulfotransferases (*CHST*) encoded by *CHST3*, *CHST11* and *CHST15* [46]. Among the developed therapeutics, STNM01 for *CHST15* was tested in a Phase I/IIa trial against unresectable pancreatic cancer and in non-clinical studies for esophageal stricture for post endoscopic therapies. Unfortunately, due to the results from the non-clinical studies, the development of STNM01 was discontinued due to low efficacy.

AnGes

This bio-venture company, established in 1999, is a pioneer in a type of oligonucleotide therapeutics known as decoys. AnGes designed a specific *Nuclear Factor-kappa B* (*NF-κB)* decoy to treat autoimmune or inflammatory diseases marked by *NF-κB* activation by inhibiting the binding of NF-κB to the binding sites on the promoter region of its downstream genes [9,47]. A Phase Ib clinical study with the same drug for intervertebral disk-related lumbago was conducted in USA to determine the optimal dosage.

Ribomic

Ribomic began an investigation of oligonucleotide therapeutics in 2003. Since then, it has developed its core technology, the Ribomic aptamer refined therapeutics (Ribo ART) system, which is deployed through Systematic Evolution of Ligands by Exponential Enrichment (SELEX), a self-developed platform to synthesize aptamers for target molecules. In addition, a Phase I clinical trial of the aptamer RBM-007 targeting FGF 2 (fibroblast growth factor 2) protein for aging-related macular degeneration was conducted in USA.

Nitto

Nitto began investigating oligonucleotide therapeutics in 2008. It conducts research, development, and clinical trials at a subsidiary company, Nitto Biopharma, in USA. Its oligonucleotide therapeutic, NBF-006, is composed of siRNA-targeting *glutathione S-transferase-Pi* (*GST-π*), which is highly expressed in carcinomas with *KRAS* mutations. A Phase I clinical study of NBF-006 for non-small-cell lung cancer is in progress in USA [48]. Another siRNA oligonucleotide, ND-L02-s0201, targets *heat shock protein 47* (*HSP47*) to treat idiopathic pulmonary fibrosis [49]. A Phase II clinical study of ND-L02-s0201 is ongoing in USA, and the reagent is licensed under

Bristol-Myers Squibb. *GST*-π siRNA and *HSP47* siRNA are packed in a vitamin A-conjugated Drug delivery system (DDS) and administered intravenously.

SBI Biotech

SBI, established in 2001, is a bio-venture company developing drugs that target cancers and autoimmune diseases. Its product, GNKS356, is a DNA antagonist of the Toll-like receptor 7/8/9 protein that suppresses the extracellular immunological signal pathway linked to persistent inflammation by inhibiting the nucleotide binding associated with psoriasis and rheumatic diseases. The SBI business model is to acquire revenue by licensing a development pipeline to a major pharmaceutical company. Therefore, Quark Pharmaceuticals was established as a subsidiary of SBI in USA to develop its oligonucleotide therapeutics and build manufacturing capacity [50].

3D Matrix

3D Matrix, Ltd., established in 2004, markets peptides developed by Dr Shuguang Zhang of the Massachusetts Institute of Technology. The original siRNA, targeting *RPN2* (*ribophorinII*) with the surfactant peptide A6K serving as a DDS, was developed to treat drug-resistant breast cancer. A Phase I study of the *RPN2* siRNA (TDM-812) used with A6K was conducted in Japan, and results are being analyzed [51,52].

Bonac

The Bonac Corporation was established in 2010. Its basic technology is oligonucleotide chemistry, including the research and development of the Bonac Nucleic Acids nkRNA and PnkRNA. Bonac RNA, targeting *TGF-β1* (*transforming growth factor-β1*), was developed with the Japanese chemical company Toray to treat idiopathic pulmonary fibrosis. A Phase I clinical study of this RNA as an orphan drug is ongoing in USA. Bonac is also developing new oligonucleotide therapeutics for COVID-19, with non-clinical and Phase I/II clinical studies conducted in collaboration with the Nagasaki University, Tokyo Medical University and the Fukuoka Prefecture in Japan.

TAK-Circulator

This bio-venture company, established in 2014, conducts genome and microbiome analysis using next-generation DNA sequencing and contract analysis for ovarian cancer diagnosis. In addition, the company has developed a *Mex3 RNA binding family member B* mRNA-degrading and gapmer-type antisense oligonucleotide (TAKC-02) to treat intractable asthma. In September 2019, they entered a partnership with NIPPON SHOKUBAI Co, Ltd. to develop TAKC-02.

Rena Therapeutics

Rena Therapeutics Co., Ltd. is a bio-venture company established in 2005 by the Tokyo Medical Dental University with the goal of creating oligonucleotide therapeutics through a platform-type business model. They developed a heteroduplex oligonucleotide (HDO) comprising active chain DNA, carrier chain RNA, and a ligand to transport the construct to target organs with high efficacy. This company is also developing the HDO technology to treat cancers, neurodegenerative diseases and genetic disorders.

TAGCyx

In 2007, Riken established TAGCyx Biotechnologies as a bio-venture company. TAGCyx has developed a DNA aptamer, known as Xenoligo [53,54], that was selected by the SELEX method. Xenoligo is intended for treating autoimmune diseases by targeting Interferon-gamma to inhibit cytokine production. It is also intended for blood clotting disorders by inhibiting the binding von-Willebrand Factor A1 domain to glycoprotein Ib. The aptamer is at the non-clinical development stage. The company's research collaboration with CAGE Bio Inc. (USA) for autoimmune skin diseases and GC Pharma (South Korea) for anti-coagulant and ischemic stroke began in 2020.

Conclusion

Oligonucleotide therapeutics have attracted attention as a new modality, following small-molecule and antibody drugs. Fifteen oligonucleotide therapeutics have been approved for various rare diseases, however, there is still a significant need to develop more drugs in the market. While Japan started developing oligonucleotide drugs later than EU and the USA, it has made significant progress. We provided an update on oligonucleotide therapeutics

of interest to Japanese pharmaceutical companies and bio-venture companies with proprietary technologies. We described viltolarsen, the therapeutics Duchenne muscular dystrophy that has been approved, and the drugs in clinical or non-clinical development.

Future perspective

The future direction of oligonucleotide therapeutics in Japan

There are many research and development programs for rare diseases in Japan. The MHLW has supported the research and development of pharmaceutical and medical products for orphan drugs since 1993. At the end of 2017, the MHLW revised the basic drug development policy to focus on oligonucleotide therapeutics and regenerative medicine as a means to guide oligonucleotide therapeutics development. At this time, Japanese pharmaceutical companies have begun to focus on oligonucleotide therapeutics and develop rapidly.

In addition, mRNA drug discovery in the field of oligonucleotide therapeutics is attracting attention. The idea of using mRNA as a drug has been around for a long time; however, it has been difficult to put it into practical use because of various problems, such as the extremely low stability of mRNA *in vivo*. However, due to the recent advances in RNA modification technology to improve RNA stability and DDS technologies, the practical application of mRNA drugs is progressing in the areas of rare diseases. mRNA drug therapy involves administering artificially produced mRNA to humans and producing treatment-related proteins from the mRNA in the human body to treat a disease. Since mRNA is administered, there is no need for viral vector-like gene therapies or concern that it will be incorporated into the patient's DNA.

The progress in domestically developed oligonucleotide therapeutics in Japan has been remarkable, although Japan started later than EU and the USA. Thus, the innovative oligonucleotide and DDS products in Japan will contribute substantially to the field of oligonucleotide therapeutics and cancer treatment. Although medical technologies have advanced, many intractable diseases remain unresolved by conventional medical treatment. Therefore, oligonucleotide therapeutics, an alternative type of drugs for rare diseases, will continue to be developed. In the future, oligonucleotide therapeutics may provide more advantages so that its application may supersede that of small molecule and antibody drugs.

Executive summary

The development of oligonucleotide therapeutics by Japanese venture companies

- Viltolarsen, approved for Duchenne muscular dystrophy (DMD) patients, is the only oligonucleotide therapeutics originating from Japan. Oligonucleotide therapeutics for 22 types of rare diseases are in development at the clinical and non-clinical stages from the viewpoint of proprietary technologies in Japanese pharmaceutical and venture companies. Therefore, more oligonucleotide therapeutics for various rare diseases can be approved in the future.
- Viltolarsen, comprising antisense oligonucleotides that target exon 53 of the *dystrophin* gene responsible for DMD, restores the amino acid reading frame.

The latest results on oligonucleotide therapeutics in Japanese venture companies

- Ribomic reported promising results on RBM-007, an oligonucleotide therapeutic drug for aging macular degeneration, in the interim report of its Phase II study in the United States of America.
- Rena therapeutics has succeeded in developing a blood-brain-barrier-crossing heteroduplex oligonucleotide capable of controlling gene expression in the brain.

The future direction of oligonucleotide therapeutics development in Japan

- The progress in oligonucleotide therapeutics development in Japan has been remarkable with the support of the government, Ministry of Health, Labor and Welfare.
- In the future, there will be a potential to provide more effective oligonucleotide therapeutics than small-molecule or antibody drugs.

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