Chapter

RNA Therapeutics for Cancers

Michiko Kurikawa, Marimu Sakumoto and Akihide Yoshimi

Abstract

RNA therapeutics represent a promising class of drugs and some of the successful therapeutics have been recently transformed into clinics for several disorders. A growing body of evidence has underlined the involvement of aberrant expression of cancer-associate genes or RNA splicing in the pathogenesis of a variety of cancers. In addition, there have been >200 clinical trials of oligonucleotide therapeutics targeting a variety of molecules in cancers. Although there are no approved RNA therapeutics against cancers so far, some promising outcomes have been obtained in phase 1/2 clinical trials. We will review the recent advances in the study of cancer pathogenesis associated with RNA therapeutics and the development of RNA therapeutics for cancers.

Keywords: nucleic acid therapeutics, antisense oligonucleotide, cancer, aptamer, clinical trial

1. Introduction

Nucleic acid plays a central role in biology and it is an attractive tool for therapeutic applications due to multiple reasons. One of the major obstacles is the low *in vivo* stability of nucleic acid therapeutics due to nuclease sensitivity. Numerous synthetic oligonucleotides have been developed to overcome this obstacle using chemical modifications, phosphate backbone, and many other technologies. Some of these technologies have been shown to potently protect the oligonucleotides from degradation and enable efficient cellular uptake, which could be translated into the clinic. In fact, some RNA therapeutics have shown dramatic effects on neurodegenerative disorders such as spinal muscular atrophy and amyotrophic lateral sclerosis. Although there has been no approved RNA therapeutics in oncology so far, researchers have obtained a number of promising results from preclinical and clinical studies. In this chapter, we will concisely summarize the general characteristics of RNA therapeutics and review recent advances in the development of RNA therapeutics in the oncology field.

2. RNA therapeutics

RNA therapeutics represents a therapy with the use of RNA-based molecules to modulate molecular and biological processes to cure a specific disease or improve symptoms. There are multiple classes of RNA therapeutics and each of them has its own strengths which would be difficult to achieve by using other drug modalities.

2.1 Classification of RNA therapeutics

Oligonucleotide therapeutics that have been investigated in clinical trials include antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), microRNAs (miRNAs) and aptamers (**Figure 1**).

2.1.1 Antisense oligonucleotide (ASO)

ASOs are small (~18–30 nucleotides), synthetic, single-stranded nucleic acid polymers that are complementary to the specific RNA through Watson-Crick base-pairing [1]. They are highly sensitive to degradation by nucleases in their naked form. In addition, their phosphodiester backbone makes it difficult to go through the plasma membrane. To resolve these issues, numerous efforts have been made to improve these situations by chemically modifying ASOs. As a result, there are currently three generations of modified ASOs. Chemical modifications and pharmacological profiles were reviewed in detail elsewhere such as in [2]. The main mechanisms of approved ASOs are classified into the following two categories [3]:

i. ASOs in the first category induce the cleavage of a target mRNA by binding to the target sequence. When this category of ASOs binds to the target mRNA, RNase H endonuclease recognizes the RNA-DNA heteroduplex, degrades the mRNA and downregulates gene expression.

ii. ASOs in the second category regulate splicing of pre-mRNAs generally by blocking the binding of splicing factors to cis-element such as splice sites,

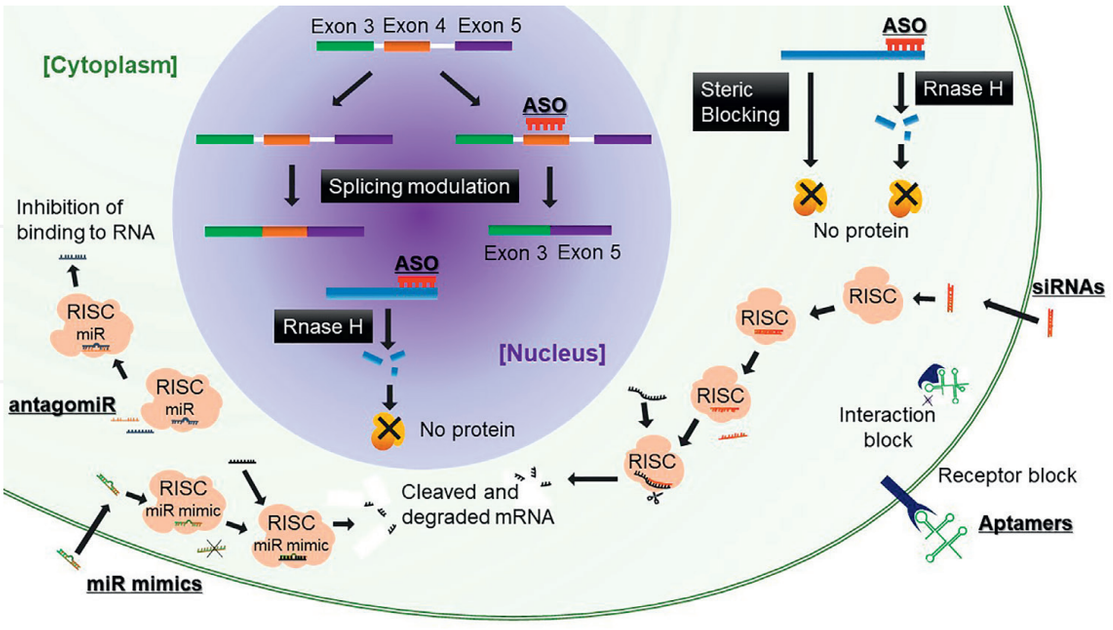

Figure 1.
A variety of RNA therapeutics and their mechanisms. Endocytosis is the main pathway for oligonucleotides to enter cells. Antisense oligonucleotides (ASOs) block the translation of target messenger RNA (mRNA) in RNase H-dependent and -independent manners. ASOs are also able to modulate RNA splicing. Mature mRNA is targeted by small interfering RNAs (siRNAs). The roles of microRNAs (miRNAs) are mainly classified into two types: miRNA mimetics that restore the levels of miRNAs and antagomiRs that suppress expression levels of target miRNA. Aptamers functions to block receptors, protein-protein interactions, etc. like antibodies, but they are smaller in size and easier to pass through the cell membrane compared to antibodies.

exonic splicing enhancer (ESE) and intronic splicing silencer (ISS). This category of ASOs is the most widely used strategy.

2.1.2 Small interfering RNA (siRNA)

siRNAs are non-coding RNAs that degrade the mRNA of the targeted gene. Exogenous double-stranded precursor siRNAs are taken up into the cell and processed by Dicer into 20–25 bp long, which are passed to Argonaut (Ago) protein and the sense strand is released [4]. The remaining antisense strand and Ago then form an RNA-induced silencing complex (RISC). Finally, the RISC seeks out and binds to the target mRNA and degrades it [5].

2.1.3 microRNA (miRNA)

In addition to siRNA, miRNA is another RNA therapy based on RNA interference. miRNA is a small non-coding RNA that degrades mRNA in the same way as siRNA. However, its mechanism is slightly different from that of siRNA. Transcripts expressed from miRNA genes are single-stranded RNAs, taking a hairpin structure. In the nucleus, miRNA transcripts undergo primary processing by Dorsha [6], which has an RNase III domain, and after that, Exportin5 transports them to the cytoplasm. In the cytoplasm, miRNAs receive secondary processing by Dicer and are cleaved as double-stranded miRNAs [7]. As with siRNAs, the single-stranded miRNAs then bind to Ago protein and form RISC. In addition, GW182 protein is required for target RNA degradation [8]. Via GW182, some RNA degrading enzymes assemble on the RNA and RISC destabilizes RNA instability.

2.1.4 Aptamers

The other category is RNA therapy targeting proteins. Aptamers are short single-stranded nucleic acids that bind to proteins. Its properties are achieved by its tertiary structures. Aptamer can have a wide range of functions including agonists [9, 10], antagonists [11, 12], bispecific aptamers [13, 14] and carriers for other drugs [15, 16]. Although its function is similar to antibodies, RNA aptamers are smaller in size and easier to pass through the cell membrane.

2.2 Advantages of RNA therapeutics

RNA therapy has several valuable strengths, which make the development of RNA technologies a worthwhile investment. These advantages could be summarized below:

2.2.1 Targeting the untargetable, treating the untreatable

One of the greatest advantages of RNA therapeutics is nicely condensed in the phrase above. RNA drugs can target "undruggable" molecules that are difficult or impossible to target with small molecule-based drugs or other modalities. Only about one-third of proteins can be targeted by common drugs such as small molecules and antibodies [17]. In addition, many proteins share similar structures, which makes it difficult to target specific proteins. On the other hand, as RNA drugs can indirectly act on proteins before the translation, they function independently of protein structure. Furthermore, small molecules and monoclonal antibodies exert their effects

by binding to the active site pocket of receptors or enzymes. For this reason, it is impossible for conventional drugs to target non-coding RNAs that are not translated. RNA drugs can target non-coding RNAs and are expected to greatly expand the range of therapeutic targets in the future [3]. We will review some examples of previously "undruggable" targets for which clinical trials are currently ongoing.

2.2.2 Quick production

As we all enjoyed the significant benefits from mRNA vaccines for coronavirus disease-2019 (COVID-19) in recent years, the next important advantage of RNA therapeutics is that RNA drugs can be designed and synthesized rapidly for clinical tests. Given that the development of small molecule or antibody-based drugs takes several years, this characteristic of RNA therapy is the biggest reason that we were able to control the COVID-19 pandemic by significantly reducing the rate of infection and the severity of the disease. By simply changing the sequence of RNA drugs according to the target genes/diseases, researchers can quickly create a novel RNA therapeutic for further testing within a short period of time. This leads to another advantage below.

2.2.3 Patient-customized therapy

Pharmaceutical companies generally hold the back investment for rare diseases as the market is small and the cost-benefit ratio is normally not attractive. However, RNA therapy might be a game changer in this scenario. A landmark trial of patient-customized ASO therapy for neuronal ceroid lipofuscinosis 7 (CLN7), a fatal neurodegenerative disorder (a form of Batten's disease) was reported in 2019 [18]. In this case, a mutation located in intron 6 of MFSD8 creates a novel acceptor, leading to a cryptic exon with a premature stop codon. The authors developed a tailored ASO to rescue the mis-splicing event and delivered it to the patient within 1 year after first contact with the patient. This led to a reduction in seizures without any serious adverse events. The fact that rare diseases affect approximately 30 million persons in the United States alone [19] highlights the importance of such rapid development of patient-customized treatments.

3. Current advances in the development of RNA therapy for cancers

Targeted therapies have greatly improved cancer management by specifically targeting the genetic alterations and consequent molecular disturbances that play an essential role in cancer initiation and maintenance. One of the major therapeutic successes would be inhibitors that specifically target constitutively active tyrosine kinases, such as imatinib and its second- and third-generation inhibitors specifically targeting BCR-ABL against Philadelphia chromosome-positive chronic myeloid leukemia (CML) [20] and acute lymphoblastic leukemia (ALL) [21]. Before the development of imatinib, treatment with interferon alfa plus cytarabine was standard care for patients with CML. In the landmark clinical trial of imatinib, newly diagnosed chronic-phase CML patients were treated with either imatinib or interferon alfa plus cytarabine. After a median follow-up of 19 months, the major cytogenetic response was 87.1% in the imatinib group versus 34.7% in the combination therapy group ($P < 0.001$) [20]. Based on the clearly superior therapeutic outcome, imatinib became the first-line therapy in newly diagnosed chronic-phase CML. Other successful targeted therapies include vemurafenib for the constitutively active form of the

BRAF kinase ($BRAF^{V600E}$) in BRAF-V600E mutated metastatic melanoma [22] and the blocking antibodies such as anti-EGFR antibody for metastatic colon cancer [23] and anti-HER2 antibody for breast cancer with HER-2 amplification) [24].

On the other hand, targeted therapies remained to be developed for many other cancer-associated genes, especially for other 'undruggable' targets such as RAS and MYC. Although there has been no approved RNA therapy for cancers so far, extensive efforts have been focused on targeting such 'undruggable' targets by using a variety of RNA therapeutics, which will be introduced in this section.

3.1 ASO therapy

Targeted therapies through ASO have been most actively studied among RNA therapeutic and approximately half of ongoing clinical trials on RNA therapeutics are classified as this modality. Recently developed ASO therapies against cancers are summarized in **Table 1**.

NCT Number	Phase	RNA therapy	Target	Start date	Status
NCT05267899	Phase 1	Cancers	AKT1	2022/8/1	Not yet recruiting
NCT02144051	Phase 1	Prostate cancer	AR	2014/5/1	Completed
NCT03300505	Phase 1/2	Prostate cancer	AR	2019/5/31	Suspended
NCT04072458	Phase 1	Lymphoid malignancies	BCL2	2020/11/5	Recruiting
NCT04504669	Phase 1	Cancers	FOXP3	2020/8/18	Recruiting
NCT02781883	Phase 2	AML	GRB2	2016/5/1	Recruiting
NCT02923986	Phase 1/2	Ph-ALL	GRB2	2017/9/1	Withdrawn
NCT04196257	Phase 1	Cancers	GRB2	2022/7/1	Not yet recruiting
NCT01780545	Phase 2	Bladder cancer	HSP27	2013/4/1	Completed
NCT02423590	Phase 2	Squamous cell lung cancers	HSP27	2014/6/1	Unknown status
NCT04485949	Phase 2	Glioblastoma	IFG-1R	2022/12/1	Not yet recruiting
NCT03101839	Phase 1	Cancers	KRAS	2017/5/15	Completed
NCT01563302	Phase 1/2	Cancers	STAT3	2012/2/27	Completed
NCT01839604	Phase 1	Hepatocellular carcinoma	STAT3	2013/5/1	Completed
NCT02417753	Phase 2	Cancers	STAT3	2015/4/3	Terminated
NCT02549651	Phase 1	DLBCL	STAT3	2016/7/13	Completed
NCT04862767	Phase 1	Cancers	TGF-β2	2021/3/9	Recruiting
NCT02243124	Phase 1	MDS	TP53	2014/9/1	Terminated

Abbreviations: AR, androgen receptor; AML, acute myeloid leukemia; Ph-ALL, Philadelphia-chromosome positive acute lymphoblastic leukemia; DLBCL, diffuse large B-cell lymphoma; MDS, myelodysplastic syndromes. [*] *The table does not include all the recent clinical trials on ASO therapies in oncology. This equally applies to* **Tables 2**–**4**.

Table 1.
Recent ASO therapy in clinical trials. [*]

Some of the landmark trials in this field were performed or are currently performed as follows:

3.1.1 ASO therapy against MYB

Historically, the first clinical trial of ASO in oncology was a phase II study back in 1993, which evaluated G4460, an ASO targeting MYB in CML (NCT00002592). *MYB* is a proto-oncogene that encode a transcription factor. As evidenced by the discovery of translocations and duplications of *MYB* in a subset of T-cell acute lymphoblastic leukemia (T-ALL) [25, 26], MYB activation was shown to contribute to the leukemogenesis via differentiation block [25]. In addition, early studies using an antisense oligodeoxynucleotide and dominant-negative form of MYB have demonstrated that MYB activation is important for the proliferative capacity of myeloid malignancies such as AML and CML. Another study indicated that an oligomer complementary to the sequence of *MYB*-encoded mRNA resulted in significant growth inhibition in several leukemic cell lines [27, 28]. Based on these observations, G4460 was designed to bind the MYB mRNA and trigger RNase H-dependent degradation [29]. In a pilot study, $CD34^+$ marrow autografts were purged with G4460 in allograft-ineligible CML patients. Although the clinical efficacy of G4460 could not be assessed in this pilot study, MYB mRNA levels were significantly reduced in approximately 50% of patients, suggesting the feasibility of transplanting G4460-treated autografts [29]. As described above, the standard treatment strategy for CML has been dramatically changed since imatinib and other tyrosine kinase inhibitors were developed. Nonetheless, MYB is an attractive target, considering that overexpression of MYB is associated with cellular proliferation and differentiation in multiple cancers including several types of leukemias and breast cancers [30].

3.1.2 ASO therapy targeting BCL2

BCL2 family of proteins have long been identified for their roles in apoptosis. BCL2 was initially discovered in the context of B-cell lymphoma in the 1980s, followed by the identification of a variety of homologous proteins [31–33]. The role of the BCL2 family is typically understood as the anti-apoptotic and pro-apoptotic members. By regulating outer mitochondrial membrane (OMM) integrity and function, BCL2 facilitates oncogenesis through cell death resistance [34]. In cancer, increased expression of BCL2 protein is frequently found [35] and is commonly associated with reduced susceptibility to chemotherapy and increased radioresistance [36]. These observations provided a rationale to target BCL2 in a variety of cancers.

Genasense (oblimersen, G3139) would be a representative ASO targeting BCL2, which targets codon 1–6 of BCL2 mRNA and triggers RNase H-dependent degradation [37]. More than 40 clinical trials have been performed on this ASO in a variety of types of cancers and Genasense obtained orphan drug designation for CLL in 2001. However, overall and progression-free survival was not affected and the primary endpoint was not reached by the treatment of Genasense in the following eight phase III studies. For example, combined fludarabine + cyclophosphamide + Genasense therapy resulted in a better response (complete + partial response) rate over fludarabine + cyclophosphamide therapy in CLL, which fulfilled only the second endpoint of the NCT00024440 trial [38]. Following these unsatisfactory results, Genasense was not approved and the production of Genasense was ceased.

Several other ASOs such as SPC2996 and PNT2258 have been developed to target BCL2. SPC2996 is a gapmer that targets the first six codons of the BCL2 mRNA. Although the phase 1/2 trial for evaluating SPC2996 was performed in CLL, approximately 40% of patients experienced painful inflammatory reactions [39]. PNT2258 is a liposome-encapsulated ASO that targets the BCL2 promoter to suppress its transcription. Although the safety of PNT2258 was confirmed in the phase 1 study, the following phase 2 trial targeting patients with diffuse large B-cell lymphoma (DLBCL) resulted in an unsatisfactory outcome with a very low response rate of 8.1%.

Following these failures of ASOs targeting BCL2, the development of ASOs against BCL2 slowed down. In 2016, the selective BCL2 inhibitor ABT-199 (venetoclax), a BH3 mimetic was approved as the first small molecule drug targeting a protein-protein interaction for chronic lymphocytic leukemia (CLL) [40]. Venetoclax has been also approved for the treatment of AML in combination with other chemotherapeutic agents such as DNA demethylating agents and low-dose cytarabine [41].

3.1.3 ASO therapy targeting IFG-1R

Results from a unique clinical study were reported in 2021 [42]. In this phase IB clinical trial, the safety and efficacy of IMV-001, an antisense oligodeoxynucleotide against IGF type 1 receptor (IGF-1R) mRNA were evaluated in adults with newly diagnosed glioblastoma. Glioblastoma is one of the most aggressive forms of brain cancer which represents approximately 15% of all brain tumors [43]. Despite intensive treatment, glioblastoma almost always recurs, leading to a dismal prognosis with a median survival of 10–13 months [44]. On the other hand, IFG-1R is highly expressed in a variety of malignancies, which regulates transformation and anti-apoptotic effects and are essential for the survival and progression of malignant cells [45–48]. However, previous efforts to target IGF-1R alone were not successful [48]. Interestingly, IMV-001 had an off-target effect to activate Toll-like receptor 9 (TLR9) in antigen-presenting cells [49, 50], which stimulates the immune response. Therefore, the research group from Thomas Jefferson University designed a phase IA trial of IGV-001 to use an autologous cell combination product therapy [51]. More specifically, 12 patients underwent MRI-based image-guided tumor resection (which resulted in partial resections in all the cases). After diagnostic confirmation, an abdominal acceptor site between the rectus sheath and rectus abdominis muscle was created. On the other hand, the resected tumor cells were treated with IMV-001 *ex vivo* and encapsulated in several chambers. Immediately after irradiation to the tumor cells, chambers were implanted in the acceptor site and removed after 24 h (**Figure 2**).

While 3 of 12 patients were re-treated after the approval from FDA was obtained, 8 patients received no other treatment except surgical resection and/or best support care (and the other one exceptional case received temozolomide). As a result, there were no unexpected treatment-related complications except deep vein thrombosis, which was successfully managed by enoxaparin prophylaxis. Post-treatment observation identified two and four patients with complete and partial responses, respectively, which were atypical for the nature of aggressive glioblastoma. Among the patients with these responses with disease recurrence, three patients had unusual regression spontaneously or after surgical resection. Interestingly, perivascular lymphocytic infiltration was observed in some patients who did not have such infiltration at diagnosis, strongly suggesting a contribution of the immune response. Based on these results, IGV-001 was granted Orphan Drug designation by FDA in 2017. A total 33 newly diagnosed patients with glioblastoma were enrolled in the subsequent Phase

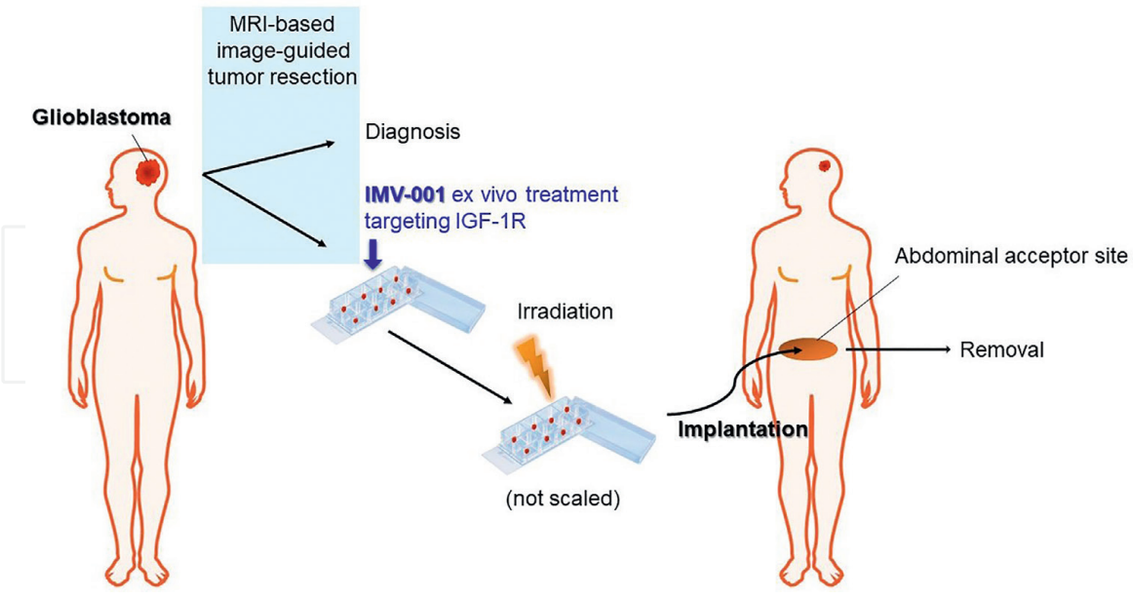

Figure 2.
Study design for the IGV-001 treatment. After MRI-based image-guided tumor resection and diagnostic confirmation, an abdominal acceptor site between the rectus sheath and rectus abdominis muscle was created. The resected tumor cells were treated with IMV-001 ex vivo and encapsulated in several chambers. Immediately after irradiation to the tumor cells, chambers were implanted in the acceptor site and removed.

IB study (ClinicalTrials.gov: NCT02507583). In this study, patients received IGV-001 and standard care which consists of maximal safe resection, adjuvant radiotherapy and temozolomide and maintenance therapy with temozolomide. Median progression-free survival (PFS) in the intent-to-treat population was 9.8 months, which was significantly better than that of patients who received standard care in published studies (6.5 months; P = 0.0003). Because the promoter methylation status of the *MGMT* gene was previously shown to positively predict the therapeutic efficacy of temozolomide [52, 53] and overall survival (OS) [54], the authors quantified the methylation levels of *MGMT* and revealed that the *MGMT* methylation status is a potent biomarker for PFS and OS. Furthermore, they assessed serum cytokines and identified that some of the pro-inflammatory cytokines such as IFNγ and IL-2 were elevated after IGV-001 treatment (before initiation of standard care). Although these responses were not associated with therapeutic outcomes, these results suggested that IGV-001 treatment induces a local environment at implantation which promotes a proinflammatory innate immune response [42].

3.2 siRNA therapy

Although most clinical trials on siRNA drugs in oncology are currently phase 1, there are some promising results from these trials. In addition, some phase 2 trials have been recently initiated (**Table 2**).

3.2.1 siRNA therapy targeting MYC

MYC is one of the most famous and most commonly activated oncogenes and has thus far been considered one of the major "undruggable" targets in cancers. As described above, a therapeutic approach using RNA interference (siRNA) is a

NCT Number	Phase	RNA therapy	Target	Start date	Status
NCT04844983	Phase 2	cSCC	TGF-β1 and COX2	2021/5/18	Recruiting
NCT02866916	Phase 1	Prostate cancer	AR	2017/9/1	Withdrawn
NCT02166255	Phase 1	Cancers	CBLB	2014/12/1	Completed
NCT03087591	Phase 1	Cancers	CBLB	2017/4/28	Completed
NCT01591356	Phase 1	Cancers	EPHA2	2015/7/1	Recruiting
NCT03819387	Phase 1	Cancers	GST	2019/3/18	Recruiting
NCT01676259	Phase 2	Pancreatic cancer	KRASG12D	2018/3/7	Recruiting
NCT03608631	Phase 1	Pancreatic cancer	KRASG12D	2021/1/27	Recruiting
NCT02110563	Phase 1	Cancers	MYC	2014/4/1	Terminated
NCT02314052	Phase 1/2	Hepatocellular carcinoma	MYC	2015/1/27	Terminated
NCT01808638	Phase 1/2	Pancreatic cancer	PNK3	2013/3/1	Completed
NCT04995536	Phase 1	NHL	STAT3	2022/8/1	Recruiting

Abbreviations: cSCC, cutaneous squamous cell carcinoma; NHL, non-Hodgkin lymphoma.

Table 2.
Recent siRNA therapy in clinical trials.

promising strategy because a number of studies have shown that silencing MYC induces growth inhibition in MYC-activated tumors in multiple cellular and animal models. An anti-MYC siRNA formulated in lipid nanoparticles called DCR-MYC has shown anti-tumor potential *in vivo* across several tumor models [55]. In phase 1 dose-escalation study, 19 patients with a variety of cancers were treated with DCR-MYC. DCR-MYC was well tolerated and demonstrated promising clinical efficacy across various dose levels, including a complete response in one patient and tumor regression in several other patients, validating the hypothesis that siRNA targeting *MYC* is a potential therapeutic strategy to make the "undruggable" target druggable.

Recently, another strategy to pharmacologically target MYC was reported [56]. In this study, the authors performed a pan-cancer transcriptome and splicing analysis of RNA sequence data generated from cancer patients with or without hotspot mutations in *SF3B1*, which is the most frequently mutated splicing factor across cancer [57, 58]. In this study, detailed molecular and biological experiments using isogenic murine models and cancer patient samples revealed that a mis-splicing event in *PPP2R5A* induces MYC activation via post-translational modifications. More specifically, mutant SF3B1 induced 3′ alternative splice site in *PPP2R5A*, which led to a reduced protein expression of PPP2R5A, a regulatory B subunit of PP2A phosphatase complex. PP2A complex containing PPP2R5A was shown to regulate phosphorylation of MYC protein which was critical for the regulation of protein stability. Therefore, loss of PPP2R5A function stabilized MYC protein. Importantly, FDA-approved activator FTY-720 suppressed mutant SF3B1 leukemogenesis *in vivo*, providing a preclinical insight into the use of PP2A activators in SF3B1 mutant cancers [56]. Furthermore, the mis-splicing event in *PPP2R5A* can be potentially targeted by a specific ASO, which will also create a therapeutic opportunity for pharmacological intervention toward activated MYC.

3.2.2 siRNA therapy targeting mutant KRAS

Another "undruggable" target commonly detected across cancers, especially in pancreatic cancers is a hotspot mutation in *KRAS*. Based on the results that siRNA-mediated KRAS silencing resulted in growth inhibition of pancreatic cancer cells *in vitro* and *in vivo*, Silenseed Ltd. has developed a siRNA drug named siG12D-LODER, which is a siRNA targeting KRAS G12D and other additional G12X mutations such as G12C and G12V with a miniature biodegradable polymeric matrix. LODER™ allows slow and prolonged local release of the encapsulated drug. siG12D-LODER was designed to keep releasing the drug for 4 months, which can be inserted into the pancreatic tumor via a standard endoscope ultrasound-guided biopsy procedure.

In the phase 1/2a dose escalation and expansion study, patients with pancreatic cancer received a one-time dose of siG12D-LODER via endoscopic intervention with chemotherapy including gemcitabine or FOLFIRINOX. The combination of chemotherapy and siG12D-LODER was safe and well-tolerated, with five of 15 treated patients experiencing serious adverse events including grade 3–4 neutropenia and cholangitis. Regarding efficacy, the median OS was 15.1 months. Tumor progression was not observed in any patients at 8 weeks after the treatment. In addition, in 10 patients whose tumor marker CA19-9 levels were elevated at enrollment, more than 20% decrease in CA19-9 levels were observed in seven patients [59]. Following these promising results, a phase 2 clinical trial is recruiting patients with both borderline resectable and locally advanced pancreatic cancer [60].

3.3 miRNA therapy

Compared to the ASO and siRNA modalities, the number of clinical trials for evaluating miRNA therapeutics is limited as below (**Table 3**). However, research on miRNA or miRNA therapeutics are being greatly increased in number, according to a survey by Bonneau et al. [61].

Here are some examples of miRNA therapeutics developed or being developed. Therapeutic strategies using miRNA are mainly classified into the following two groups: (1) AntagomiRs to repress overexpressed miRNAs (Example: MRG-106), and (2) miRNA mimetics to restore downregulated miRNAs (Example: MRX34).

NCT number	Phase	RNA therapy	Target	Start date	Status
NCT04675996	Phase 1	Cancers	JNK1	2020/12/18	Recruiting
NCT03713320	Phase 2	CTCL	miR-155	2019/4/2	Terminated
NCT03837457	Phase 2	CTCL	miR-155	2019/10/1	Terminated
NCT01829971	Phase 1	Cancers	miR-34a	2013/4/1	Terminated
NCT02862145	Phase 1/2	Melanoma	miR-34a	2016/8/1	Withdrawn
NCT02369198	Phase 1	Cancers	miR-16	2014/9/1	Completed

Abbreviation: CTCL, cutaneous T-cell lymphoma cutaneous squamous cell carcinoma.

Table 3.
Recent miRNA therapy in clinical trials.

3.3.1 miRNA therapy against miR-155

miR-155 is overexpressed in various malignancies, especially in cutaneous T-cell lymphoma (CTCL) including Mycosis fungoides (MF) [62–64], and is associated with enhanced cell proliferation and survival [65–67] and genomic instability [68, 69]. In addition, in a number of studies, genetically engineered mice with overexpression of miR-155 murine homolog in lymphoid cells had an increased susceptibility to develop lymphomas and leukemias [64, 70–72]. Molecularly, miR-155 directly targets SHIP1 [73], SOCS1 [74] and some other cancer-associated genes. Overexpression of miR-155 is also related to activation of the PI3K-AKT [75], NF-κB [76] and JAK/STAT [77] pathways. Collectively, these observations provided a rationale to target miR-155 in cancer therapy.

Evidenced by these scientific results, miRagen therapeutics has developed cobomarsen (MRG-106), an oligonucleotide inhibitor of miR-155 which is optimized for efficient uptake in $CD4^+$ T-cell and MF cells with lipid nanoparticles. Cobomarsen was shown to de-repress direct miR-155 target genes as well as de-activate multiple survival pathways in MF cell lines *in vitro* [78]. The phase 1 trial of cobomarsen recruited 15 patients with biopsy-proven stage I-III MF [79]. Intratumoral or subcutaneous administration of cobomarsen resulted in almost no clinically significant adverse events. On the other hand, histological examination of pre- and post-treatment tissue revealed a reduction in cell density and depth in most patients. In addition, a gene expression analysis on these specimens demonstrated significant inactivation of PI3K-AKT, NF-κB and JAK/STAT pathways. This led to the Orphan Drug Designation of cobomarsen for MF type CTCL in 2017 and the initiation of phase 2 trials.

3.3.2 miR-34a based therapeutic

Accumulating evidence has demonstrated the presence of a normally small fraction of cancer cells, cancer stem cells (CSCs) which share stem-like properties with normal stem cells such as self-renewal and differentiation capacities. miR-34 is a tumor suppressive miRNA whose expression is frequently downregulated in many cancers [80] and CSCs.

miR-34 family is one of the three major tumor suppressive miRNA families consisting of miR-34a, miR-34b and miR-34c. Among them, miR-34a is known to repress the expression of >200 target genes and loss of miR-34a biologically regulates tumor growth by inhibiting multiple processes such as cell cycle, epithelial-to-mesenchymal transition, metastasis, immune response and stemness [81–83].

In addition, the loss of miR-34a is associated with CSC regulation in multiple cancer types. For example, MET, NOTCH1 and NOTCH2 were identified as direct targets of miR-34a in glioma stem cells [84] and restoration of miR-34a expression induced differentiation of glioma stem cells with increased expression of astrocyte and oligodendrocyte markers [85]. Another example comes from colorectal cancer where miR-34a functions as a cell-fate determinant of CSCs in this malignancy. Bu et al. identified that high miR-34a expression decreased both symmetric and asymmetric division (resulting in decreased CSCs and increased more differentiated daughter cells), while low miR-34a expression enhanced symmetric CSC-CSC division and suppressed asymmetric division [86].

The first-in-human phase 1 study was initiated to evaluate the maximum tolerated dose, safety, pharmacokinetics and clinical activity of MRX34, a liposomal miR-34a

mimic in 47 patients with advanced tumors [87]. Although MRX34 demonstrated some clinical response, including one patient with hepatocellular carcinoma exhibiting a prolonged partial response for 48 weeks and four patients with stable disease for more than 16 weeks, the trial was halted by FDA in 2016 due to severe immune reactions and deaths in four patients in the expansion cohort.

3.4 Aptamer therapy

Although there are only a limited number of clinical trials for Aptamer therapy as is miRNA therapeutics (**Table 4**), there are some promising results, especially from the studies on the aptamer targeting CXCL12.

3.4.1 Aptamer therapy targeting CXCL12

CLL is the most common adult form of leukemia in Western countries which is characterized by the expansion of mature monoclonal B-cells. It has been known that the tissue microenvironment confers survival advantage and drug resistance to the CLL cells via CXC chemokine ligand CXCL12 and other factors such as BAFF, APRIL and CD40 ligand [88–90]. Therefore, drug development has been focused on strategies that interrupt the crosstalk between CCL cells and the stroma such as bone marrow (BM) stroma cells (BMSCs). Importantly, the migration of CLL cells in the tissues is controlled by tissue gradients of chemokines. In the BM, CLL cells are attracted by the CXCL12, which is continuously secreted from BMSCs. The close proximity between CLL cells and BMSCs protects CLL cells from spontaneous- and drug-induced

NCT number	Phase	RNA therapy	Target	Start date	Status
NCT03385148	Early Phase 1	Colorectal cancer	PTK7	2017/1/1	Unknown status
NCT01034410	Phase 2	AML	Nucleolin	2010/1/1	Terminated
NCT00881244	Phase 1	Cancers	Nucleolin	2003/9/1	Completed
NCT00740441	Phase 2	Renal cell carcinoma	Nucleolin	2008/8/1	Unknown status
NCT00512083	Phase 2	Leukemia	Nucleolin	2007/7/1	Completed
NCT01486797	Phase 2	CLL	CXCL12	2012/3/1	Completed
NCT01521533	Phase 2	MM	CXCL12	2012/3/1	Completed
NCT01194934	Phase 1	HSCT	CXCL12	2010/8/1	Completed
NCT00976378	Phase 1	HSCT	CXCL12	2009/10/1	Completed
NCT04121455	Phase 1/2	Glioblastoma	CXCL12	2019/9/12	Recruiting
NCT03168139	Phase 1/2	Cancers	CXCL12	2017/4/18	Completed
NCT04901741	Phase 2	Pancreatic Cancer	CXCL12	2022/12/1	Not yet recruiting

Abbreviations: AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; HSCT, hematopoietic stem cell transplantation.

Table 4.
Recent aptamer therapy in clinical trials.

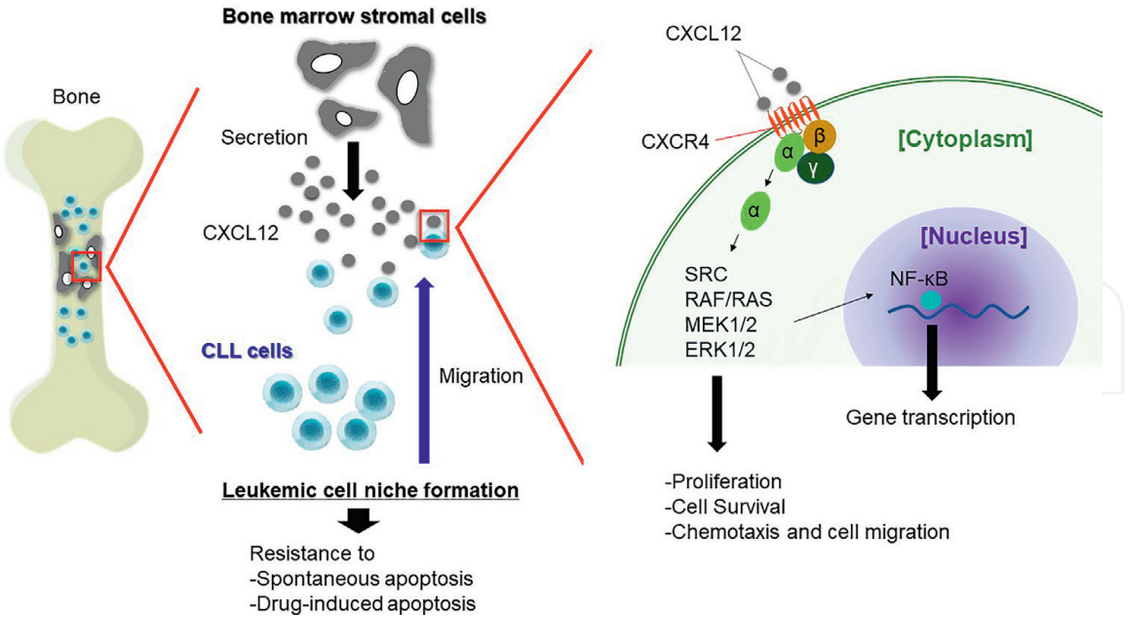

Figure 3.
Schema representing the roles of the CXCL12-CXCR4 axis in CLL. Constitutively secreted CXCL12 from the bone marrow stromal cells attract CLL cells via the chemokine receptor CXCR4, which creates leukemic niche in the bone marrow. Molecularly, CXCR4 activates the downstream multiple cancer-associated pathways such as PII3K/AKT/mTOR, RAS/RAF/MEK/ERK and NF-κB pathways.

apoptosis [90–93]. Besides these protective effects, CXCL12 enhances the expansion of BMSC-dependent pre-B cell clones [94] as well as activates multiple pro-survival pathways associated with ERK1/2, STAT3 and AKT (**Figure 3**) [90, 95, 96].

Because CLL cells are attracted via CXCR4, the chemokine receptor of CXCL12, the first small molecule targeting the CXCL12-CXCR4 axis was developed. A multicenter phase 1 study of plerixafor in combination with the anti-C20 antibody rituximab was performed in 24 patients with relapsed/refractory CLL. In this study, a median 3.3-fold increase of CLL cells in the peripheral blood was observed after the first administration of plerixafor, strongly supporting the mobilizing capacity of the drug on CLL cells or the CXCL12-CXCR4 axis and suggesting that plerixafor would contribute to the sensitization of CLL cells [97].

Another therapeutic approach to target the CXCL12-CXCR4 axis is the blockade of CXCL12. However, CXCL12 is highly evolutionary conserved, which hinders the development of antibody-based drug development for CXCL12. NOX-A12 (Spiegelmer), an RNA oligonucleotide successfully bypassed this issue by using a mirror image configuration of naturally occurring RNA. More specifically, the Spiegelmer technology enables an RNA oligonucleotide to bind target molecules with high affinity and specificity [98, 99]. The major merits of using a mirror-image configuration would be summarized as follows: (i) Spiegelmer is resistant to degradation by nucleases, (ii) Spiegelmer does not hybridize with native nucleic acids, (iii) Spiegelmer is immunologically "cold". NOX-A12 is a Spiegelmer that was designed to bind and antagonize CLCX12.

After a phase 1 trial in healthy volunteers was completed, two clinical trials were initiated. In a phase 1/2 trial (NCT01486797) [100], 28 patients with relapsed/refractory CLL were treated with NOX-A12 (olaptesed pegol) in combination with bendamustine and rituximab (BR). NOX-A12 was well-tolerated and there was no additional toxicity when patients were treated in combination with

chemoimmunotherapy. In addition, an overall response rate of 8%, including a complete response of 11% was obtained, with a median PFS of 15.4 months and a 3-year OS of >80%. These results compare favorably with those reported by BR alone and other recent BR combination trials [100–102], warranting further clinical development.

Similarly, NOX-A12 was evaluated in 28 patients with relapsed/refractory multiple myeloma (MM) in phase 2 clinical trial (NCT01521533) [103]. This was based on the scientific observations that CXCL12 plays an essential role in supporting myeloma cells in the bone marrow microenvironment and in mobilizing myeloma cells to the peripheral [104, 105]. Patients with MM were treated with NOX-A12 alone for 2 weeks in the pilot phase, followed by the combination treatment (NOX-A12 + bortezomib and dexamethasone) for up to 8 cycles. There were no unexpected adverse events. The overall response rate was 68%, including a complete response of 7% and a very good partial response of 18%. The median PFS and OS were 7.2 months and 28.3 months, respectively. Given that overall response rates in the previous MM studies of bortezomib and bortezomib-based combination treatment for relapsed/refractory MM patients were mostly within the range of 43–63% [106–111], the outcome of this phase 2 study is favorable. In addition, the overall response rates of CXCR4 inhibitor ulociplumab or plerixafor with bortezomib + dexamethasone were 40% and 51%, respectively [112, 113], suggesting that NOX-A12 is a promising approach to target the CXCL12-CXCR4 axis in MM. The results of these clinical trials emphasize the importance of further evaluation of NOX-A12 in MM.

4. Conclusion

Numerous efforts to develop RNA therapeutics against cancers have been made as we partly introduced in this chapter. Although there is currently no approval of RNA therapeutics in oncology, some of the phase 2 studies yielded promising results, which greatly encourages investigators in the field. On the other hand, oligonucleotide drug delivery has now almost matured to the position of clinical utility (there are excellent reviews on this topic such as [39, 114]). Therefore, it is possible that the outcome of a previously failed oligonucleotide therapeutic could be improved with the use of next-generation oligonucleotide or with a novel drug delivery system. These developments would provide expectation that RNA therapy for many cancers will be soon available through the use of precision genetic medicine.

Author contributions

A.Y. designed the manuscript. M.K., M.S. and A.Y. wrote the manuscript. M.S. and A.Y. prepared all the figures and tables.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Michiko Kurikawa, Marimu Sakumoto and Akihide Yoshimi*
Division of Cancer RNA Research, National Cancer Center Research Institute, Tokyo, Japan

*Address all correspondence to: ayoshimi@ncc.go.jp

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