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REVIEW

The Risks of miRNA Therapeutics: In a Drug Target Perspective

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Abstract: RNAi therapeutics have been growing. Patisiran and givosiran, two siRNA-based drugs, were approved by the Food and Drug Administration in 2018 and 2019, respectively. However, there is rare news on the advance of miRNA drugs (another therapeutic similar to siRNA drug). Here we report the existing obstacles of miRNA therapeutics by analyses for resources available in a drug target perspective, despite being appreciated when it began. Only 10 obtainable miRNA drugs have been in clinical trials with none undergoing phase III, while over 60 siRNA drugs are in complete clinical trial progression including two approvals. We mechanically compared the two types of drug and found that their major distinction lay in the huge discrepancy of the target number of two RNA molecules, which was caused by different complementary ratios. One miRNA generally targets tens and even hundreds of genes. We named it "too many targets for miRNA effect" (TMTME). Further, two adverse events from the discontinuation of two miRNA therapeutics were exactly answered by TMTME. In summary, TMTME is inevitable because of the special complementary approach between miRNA and its target. It means that miRNA therapeutics would trigger a series of unknown and unpreventable consequences, which makes it a considerable alternative for application.

Keywords: RNAi, miRNA, siRNA, therapeutics

Introduction

In 1993, Lee et al discovered endogenous single-stranded approximately 22-nt lin-4 (a miRNA) could decrease the level of LIN-14 protein in *C. elegans*,[1] and since then, researchers have reported a series of miRNAs that could inhibit the expression of specific proteins. In 2001, Elbashir et al reported double-stranded 21-nucleotide (nt) siRNA could induce RNA interference (RNAi) in different mammalian cell lines,[2] and soon siRNAs were used as useful tools for gene silencing in biomedical research. Both types of RNA molecules could lead to inhibition of gene expression, but miRNA is complementary to the 3′ untranslated region of mRNA[1] and siRNA is complementary to the coding region of mRNA.[2] However, two similar mechanistic RNA molecules were under different progression for biomedical applications. In 2006, Craig Mello and Andrew Fire were awarded the Nobel Prize in Physiology or Medicine for their contributions to RNAi, which, however, excluded work on miRNA. Until 2020, a number of treatments based on siRNA technology have undergone clinical trials, and two such products, patisiran[3] and givosiran[4], have been approved. But nearly 30 years later, miRNA did not benefit treatment of diseases even with none entering phase III clinical trials. In this article, we explore

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the delayed development of miRNA in a drug target perspective, and identify therapeutic risks of miRNA despite its great appreciation by many reports.[5–9]

Comparison of miRNA Drugs and siRNA Drugs

To understand the latest and detailed progression of RNAi, we first obtained clinical trial information of miRNA drug and siRNA drug (including shRNA, a small RNA molecule executing the same function as siRNA) from ClinicalTrials.gov,[10] Drugs@FDA database,[11] a series of literature,[3,4,12,13] and a company report[14] (Tables 1 and 2). The information presented in Table 2 originated from Weng's study[13], and we updated the current phase status of the clinical trials for these drugs. In our statistics, it launched 10 miRNA drugs in clinical trial, with one phase I, four phase II and five terminated/suspended (Table 1 and Figure 1A). However, 57 siRNA drugs (targeting human proteins/genes) were ongoing or completed clinical trials, including 16 phase I, 16 phase II, eight phase III, 23 terminated/suspended therapeutics (Table 2 and Figure 1A). As shown (Figure 1B), no miRNA drugs were in phase III trials, while siRNA drugs seemed to be in a complete clinical trial system without missing any segments. Moreover, we found that miRNA drugs suffered 50% terminated/suspended therapeutics, while 35.38% terminated/suspended therapeutics appeared in siRNA drugs (Figure 1B). Therefore, it may indicate there were potentially unclear obstacles for miRNA therapeutics.

Inherently, miRNA is endogenously produced and siRNA is exogenously designed. Designers can exactly endow siRNA giving them the purpose of gene silencing, while endogenous miRNA seemed more complicated because nobody assigned them specific tasks. We mechanically analyzed the differences between miRNA and siRNA in their complementary modes with the target sequences. As expected, Figure 2A showed a flexible complementary ratio of miRNA with target sequence (within the range 20–90%) and none with complete complementation, but all siRNAs had 100% complementary ratio. The less restricted complementary mode may lead to low specificity of target sequence. Next, we employed miRTarBase[15] to obtain targets of miRNAs for ten miRNA drugs, and compared the number of targets in miRNA drug* (referring to all targets of miRNA including experiment-validated and high-throughput results in miRTarBase), miRNA drug (referring to targets of miRNA only including experiment-validated results) and siRNA drugs. Beyond our expectation, the targets of the miRNA drug ranged from 30 to 250 in number and almost all miRNA drug* were over 500 and even 1000, but the si0052NA drug generally targets 1–3 genes (Figure 2B). The majority of miRNA targets tens and hundreds of genes, and we named it "too many targets for miRNA effect" (TMTME).

Table 1 Clinical Information of miRNA Drugs

Drug Name	Target(s)/Employed miRNA(s)	Current Status	Condition(s)	Company
Lademirsen (SAR339375, RG-012)	miR-21	II	Alport syndrome	Genzyme
MRG-201 (Remlarsen)	miR-29	II	Keloid	miRagen Therapeutics, Inc.
RG-125 (AZD4076)	miR-103/107	II	Nonalcoholic fatty liver disease	AstraZeneca
MRG-106	miR-155	I	Lymphomas; leukemias	miRagen Therapeutics, Inc.
MRG-110	miR-92a	I	Skin excisional wound	miRagen Therapeutics, Inc.
MesomiR 1	miR-16	Suspended	Malignant pleural mesothelioma; non-small -cell lung cancer	Asbestos Diseases Research Foundation
Miravirsen	miR-122	Suspended	Chronic hepatitis C	Santaris Pharma A/S
RG-101	miR-122	Discontinued	Chronic hepatitis C	Regulus Therapeutics Inc.
pSil-miR200c and PMIS miR200a	miR-200a/c	Discontinued	Tooth extraction status nos	University of Iowa
MRX34	miR-34a	Discontinued	Melanoma; primary liver cancer; hematologic malignancies	Mirna Therapeutics, Inc.

Table 2 Clinical Information of siRNA Drugs

Drug Name	Target(s)	Current Status	Indication(s)	Company
ONPATTRO (Patisiran, ALN-TTR02)	TTR	Approved	Transthyretin (TTR)-mediated amyloidosis	Alnylam Pharmaceuticals
Givlaari (Givosiran, ALN-AS1)	ALAS-1	Approved	Acute hepatic porphyrias	Alnylam Pharmaceuticals
Fitusiran (ALN-AT3sc, ALN-APC, SAR439774)	Thrombin	III	Hemophilia A, hemophilia B	Alnylam Pharmaceuticals
Vutrisiran (ALN-TTRsc02)	TTR	III	Transthyretin (TTR)-mediated amyloidosis	Alnylam Pharmaceuticals
Tivanisiran (SYL1001)	TRPVI	III	Ocular pain, dry eye	Sylentis, S.A.
Lumasiran (ALN-GO1)	HAO1	III	Primary hyperoxaluria type 1 (PH1)	Alnylam Pharmaceuticals
Inclisiran (ALN-PCSsc)	PCSK9	III	Hypercholesterolemia	Alnylam Pharmaceuticals
Vigil vaccine (FANG, vigil, vigil EATC)	Furin	III	Breast cancer (III), ovarian cancer (II), colorectal cancer (I), Ewing's sarcoma (II), metastatic melanoma (II), metastatic non-small-cell lung cancer (II), solid tumors (I)	Gradalis, Inc.
QPI-1002 (I5NP)	p53	III	Delayed graft function (III), acute kidney injury (II)	Quark Pharmaceuticals
DCR-PHXC	LDHA	III	Primary hyperoxaluria	Dicerna Pharmaceuticals
ARO-HBV	HBV gene	II	Hepatitis B	Arrowhead Pharmaceuticals
PSCT19 (MiHA-loaded PD-L-silenced DC vaccination)	PD-L1/PD-L2	II	Hematological malignancies	Radboud University
Cemdisiran (ALN-CC5)	C5a Receptor	II	Paroxysmal nocturnal hemoglobinuria (PNH)	Alnylam Pharmaceuticals
STP705 (cotsiranib)	TGF-β1 and COX-2	II	Hypertrophic scar (wound healing)	Sirnaomics
SYL040012 (bamosiran)	ADRB2	II	Open angle glaucoma, ocular hypertension	Sylentis, S.A.
Lentivirus vector CCR5 shRNA	CCR5	II	AIDS-related lymphoma	AIDS Malignancy Consortium
Cal-1 (LVsh5/C46, Cal-1 modified HSPC, Cal-1 modified CD4 + T lymphocytes)	CCR5	II	HIV/AIDS	Calimmune Inc.
PF-655 (PF-04523655)	RTP801	II	Diabetic macular edema (II), age-related macular degeneration (II)	Quark Pharmaceuticals

(*Continued*)

Table 2 (Continued).

Drug Name	Target(s)	Current Status	Indication(s)	Company
Atu027	PKN3	II	Metastatic pancreatic cancer (II), solid tumors (I), head and neck cancer (hold)	Silence Therapeutics GmbH
TKM-080301 (TKM-PLK1)	PLK1	II	Adrenal cortical carcinoma (II), hepatocellular carcinoma (II), neuroendocrine tumor (II), solid tumors (I)	Arbutus Biopharma Corporation
ND-L02-s0201	HSP47	II	Hepatic fibrosis	Bristol Myers Squibb, Nitto Denko Corporation
CEQ508	CTNNB1	II	Familial adenomatous polyposis	Marina Biotech
RXI-109 (sd-rxRNA)	CTGF	II	Scar (II), wet age-related macular degeneration (II), ophthalmology (I/II), retinal neovascularia on (I/II)	Phio Pharmaceuticals Corp
BMT101	CTGF	II	Hypertrophic scar	Hugel
siG12D LODER	KRAS	II	Pancreatic ductal adenocarcinoma, pancreatic cancer	Silenseed Ltd
ALN-AAT02	AAT	II	Antitrypsin Deficiency Liver Disease	Alnylam Pharmaceuticals
DCR-HBVS	HBV	I	Hepatitis B	Dicerna Pharmaceuticals
Lentivirus vector rHIV7-shI-TAR-CCR5RZ-transduced hematopoietic progenitor cells	HIV-1 tat/rev(shI)-trans-active response element (TAR), CCR5 ribozyme	I	Chronic lymphocytic leukemia (CLL) hepatitis C	City of Hope Medical Center
iPsiRNA (Proteasome siRNA and tumor antigen RNA-transfected DC)	LMP2, LMP7, MECL1	I	Metastatic melanoma, absence of CNS metastases	Scott Pruitt, Duke University
pbi-shRNA EWS/FLI1 type 1 LPX	EWS/FLI1	I	AIDS-related lymphoma	Gradalis, Inc.
SXL01	AR V7 variant	I	Metastatic castration-resistant prostate cancer (CRPC)	Institut Claudius Regaud
siRNA-EphA2-DOPC	EphA2	I	Advanced cancers	M.D. Anderson Cancer Center
ARO-ANG3	ANGPTL3	I	Dyslipidemia, hypercholesterolemia	Arrowhead Pharmaceuticals
NU-0129	BCL2L12	I	Gliosarcoma	Northwestern University
ARO-AAT	AAT	I	Alpha-1 antitrypsin deficiency	Arrowhead Pharmaceuticals

APN401 (siRNA-transfected PBMC)	Cbl-b/DC cancer vaccine	I	Solid tumors	Wake forest university health sciences
OLX10010	CTGF	I	Cicatrix, hypertrophic	Olix Pharmaceuticals
pbi-shRNA STMN1 LP	STMN1	I	Ewing's sarcoma	Gradalis, Inc.
AMG 890	Lp(a)	I	Cardiovascular disease	Amgen
ARO-APOC3	ApoC3	I	Hypertriglyceridemia, familial chylomicronemia	Arrowhead Pharmaceuticals
TD101	Keratin 6A N171K mutant	I	Pachyonychia congenita	Pachyonychia Congenita Project
Mesenchymal stromal cells-derived exosomes with KRAS G12D siRNA	KrasG12D mutation	I	Pancreatic cancer	M.D. Anderson Cancer Center
TKM-Ebola-Guinea (TKM-1 30,803)	VP35, Zaire Ebola L-polymerase	Suspended	West African, Ebola virus Infection	Arbutus Biopharma Corporation
ARB-1467 (ARB-001467)	HBV genome	Suspended	Hepatitis B	Arbutus Biopharma Corporation
ALN-HBV	HBV gene	Suspended	Hepatitis B	Alnylam Pharmaceuticals
TKM-Ebola (TKM-Ebola Kikwit, TKM-100,201, TKM-100,802)	VP24, VP35, Zaire Ebola L-polymerase	Suspended	Ebola virus infection	Arbutus Biopharma Corporation
TT-034 (PF-05095808)	HCV gene	Suspended	Hepatitis c	Tacere Therapeutics
ALN-VSP02 (ASC-06)	VEGF, KSP	Suspended	Solid tumors	Alnylam Pharmaceuticals
Bevasiranib (Cand5)	VEGF	Suspended	Diabetic macularedema, macular degeneration, age-related macular degeneration	OPKO Health, Inc.
AGN211745 (AGN-745, Sirna-027)	VEGFR1	Suspended	Age-related macular degeneration, choroidal neovascularization	Allergan, Inc.
SLN124	TMPRSS6	Suspended	Beta-thalassemia, myelodysplastic syndrome	Silence therapeutics
QPI-1007	Caspase 2	Suspended	Nonarteritic anterior ischemic optic neuropathy (III), acute primary angle closure glaucoma (II)	Quark Pharmaceuticals
TKM-ApoB (PRO-040201)	ApoB	Suspended	Hypercholesterolemia	Arbutus Biopharma Corporation

(*Continued*)

Table 2 (Continued).

Drug Name	Target(s)	Current Status	Indication(s)	Company
ALN-RSV01(Asvasiran sodium)	RSV nucleocapsid "N" gene	Discontinued	Respiratory syncytial virus infections	Alnylam Pharmaceuticals
ARC-520	Conserved regions of HBV	Discontinued	Hepatitis B	Arrowhead Pharmaceuticals
ARC-521	cccDNA and integrated DNA regions of HBV	Discontinued	Hepatitis B	Arrowhead Pharmaceuticals
Revusiran (ALN-TTRsc, SAR438714)	TTR	Discontinued	Transthyretin (TTR)-mediated amyloidosis	Alnylam Pharmaceuticals
ALN-TTRO1	TTR	Discontinued	Transthyretin (TTR)-mediated amyloidosis	Alnylam Pharmaceuticals
CALAA-01	RRM2	Discontinued	Cancer, solid tumor	Calando Pharmaceuticals
Excellair (ACU-XSP-001)	SYK	Discontinued	Asthma	ZaBeCor Pharmaceuticals
DCR-PH1	HAO1	Discontinued	Primary hyperoxaluria type 1	Dicerna Pharmaceuticals, Inc.
ALN-PCS02	PCSK9	Discontinued	Elevated LDL-cholesterol (LDL-C)	Alnylam Pharmaceuticals
DCR-MYC (DCR-M1711)	MYC	Discontinued	Solid tumors, hepatocellular carcinoma, multiple myeloma, non-hodgkin 's lymphoma, pancreatic neuroendocrine tumors	Dicerna Pharmaceuticals, Inc.
ARC-AAT	AAT	Discontinued	Alpha-1 antitrypsin deficiency	Arrowhead Pharmaceuticals
ALN-AAT	AAT	Discontinued	Antitrypsin deficiency liver disease	Alnylam Pharmaceuticals
SV40 vectors carrying siRNA	Bcr-Abl	Unknown	Chronic myeloid leukemia (CML)	Hadassah Medical Organization
Bcr-Abl siRNA	Bcr-Abl	Unknown	Chronic myeloid leukemia (CML)	University of Duisburg-Essen
shRNA	XPO1	Unknown	Chronic lymphocytic leukemia (CLL)	Peking University People's Hospital

Note: The information originated from Weng's study[13] and we updated the current phase status of these drugs in clinical trials.

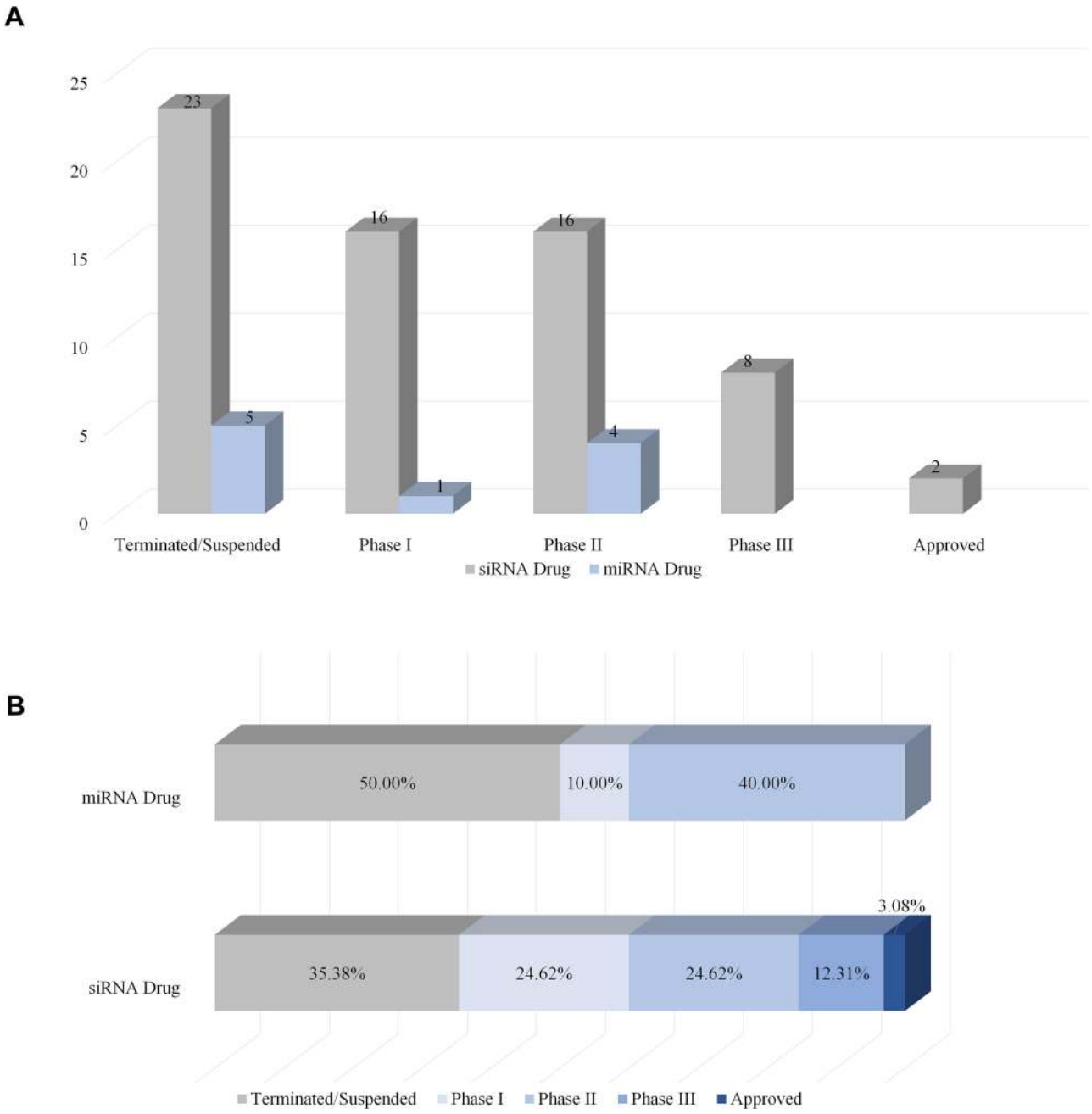

Figure 1 Clinical progression of miRNA drug and siRNA drug. (**A**) The number of miRNA drugs and siRNA drugs in different clinical periods (terminated/suspended, phase I, phase II, phase III, approved). (**B**) The ratio of miRNA drugs and siRNA drugs of different clinical periods.

To further explore the cause for the delayed development of current miRNA therapeutics compared to siRNA drugs, we attempted to examine how many targets were not approved when these miRNA drugs entered into the tested subjects. Therefore, we collected all FDA-approved drugs (targeting human genes/proteins) from 1939 to 2019.[3,4,16–19] Figure 3A showed all ten miRNA drugs had tens and hundreds of unapproved targets, far beyond the siRNA drug. So, how many targets does a recognized drug have? We next profiled the target number of approved drugs (Figure 3B), and found the number of drug targets was no more than five. SiRNA drugs fell within the range, but miRNA drugs did not.

Analyses for Two Discontinued miRNA Therapeutics-based Projects

To further verify this finding, we collected and analyzed two available miRNA clinical projects that suffered

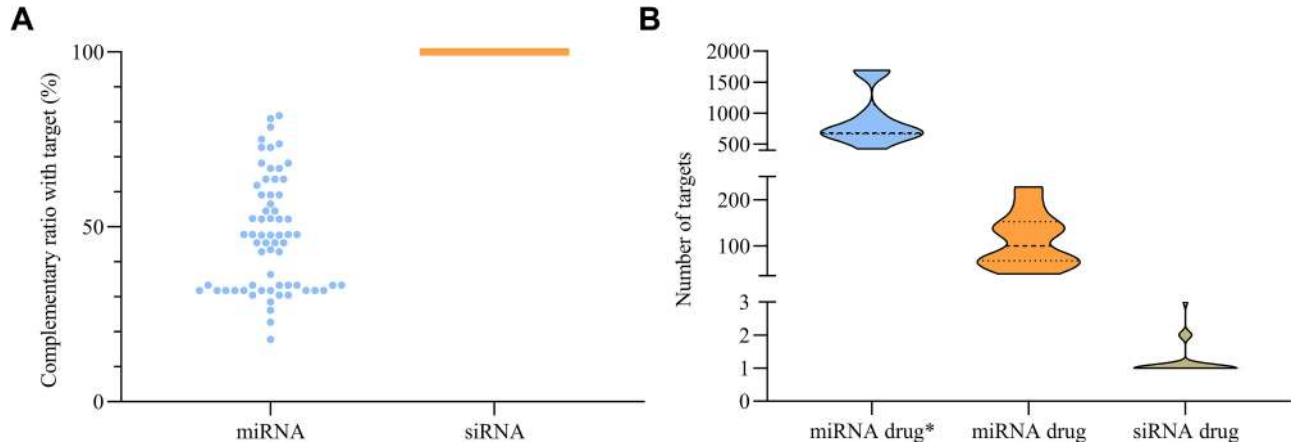

Figure 2 Flexible complementary ratio between miRNA with target sequence led to TMTME. (**A**) Complementary ratios of miRNA and siRNA with target. (**B**) Target number of miRNA drug* (all containing high-throughput results), miRNA drug (validated) and siRNA drug.

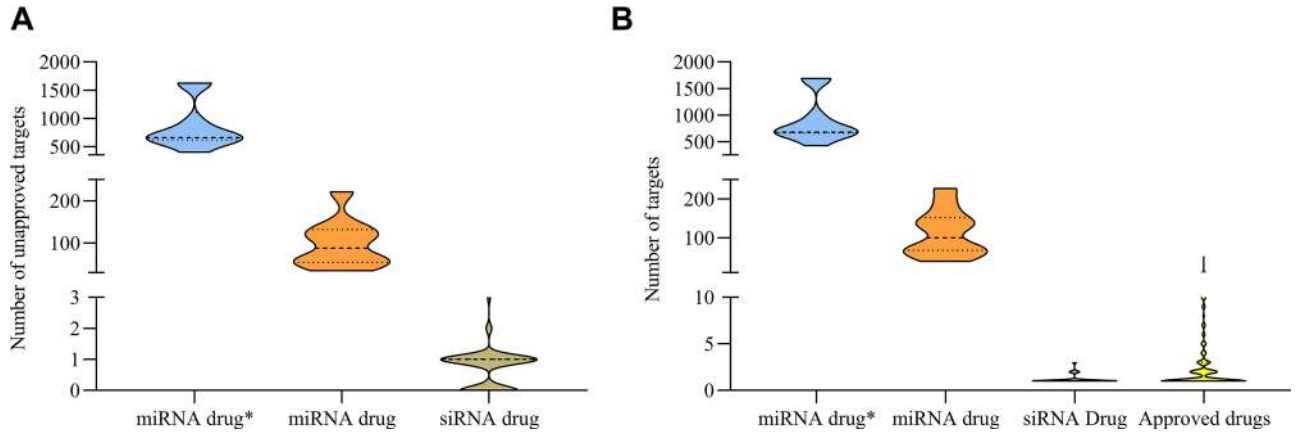

Figure 3 Target number of siRNA drugs obeyed the regular of all approved drug target number. (**A**) Unapproved targets of miRNA drug*, miRNA drug and siRNA drug. (**B**) Target number of miRNA drug*, miRNA drug, siRNA drug and approved drugs.

discontinuation caused by adverse events. According to the ClinialTrials.gov database[10] (NCT01829971) recorded, MRX34 (a miR-34a mimic) in a phase I clinical trial led to tested objects undergoing five serious immune-related adverse events, therefore terminating the project. Using KOBAS (a web server for annotation and identification of enriched pathways and diseases),[20] we enriched 139 experiment-validated target genes of miR-34a in pathway analysis, and selected the top ten pathways, see Figure 4A according to the ranking of enrichment score and Figure 4B according to the ranking of number of genes. It showed that two immune-related pathways (cytokine signaling in the immune system and immune system) were on the list, and some related pathways like signaling by interleukins were also included. We further found that there were 15 approved genes and 25 unapproved genes in the immune system pathway and 13 approved genes and 16 unapproved genes in cytokine signaling in the immune system pathway (Figure 4C). Based on these facts, it is not hard to understand the emergence of five serious immune-related adverse events in phase I. According to the literature[12] and the Regulus Therapeutics Inc. company report,[14] RG-101, an anti-miR-122 drug, succeeded in phase I but was discontinued in phase II for the occurrence of a few cases of hyperbilirubinemia. We next enriched 71 experiment-validated target genes of miR-122 in disease analysis by KOBAS web server. However, no hyperbilirubinemia-related diseases were among the listed top 10 (Figure 4D and E). And hepatocellular carcinoma (HCC) was enriched as it has been reported that miR-122, a most frequent miRNA in the adult liver,[21] played a crucial role in HCC in a series of literature,[6,22,23] indicating the correctness of

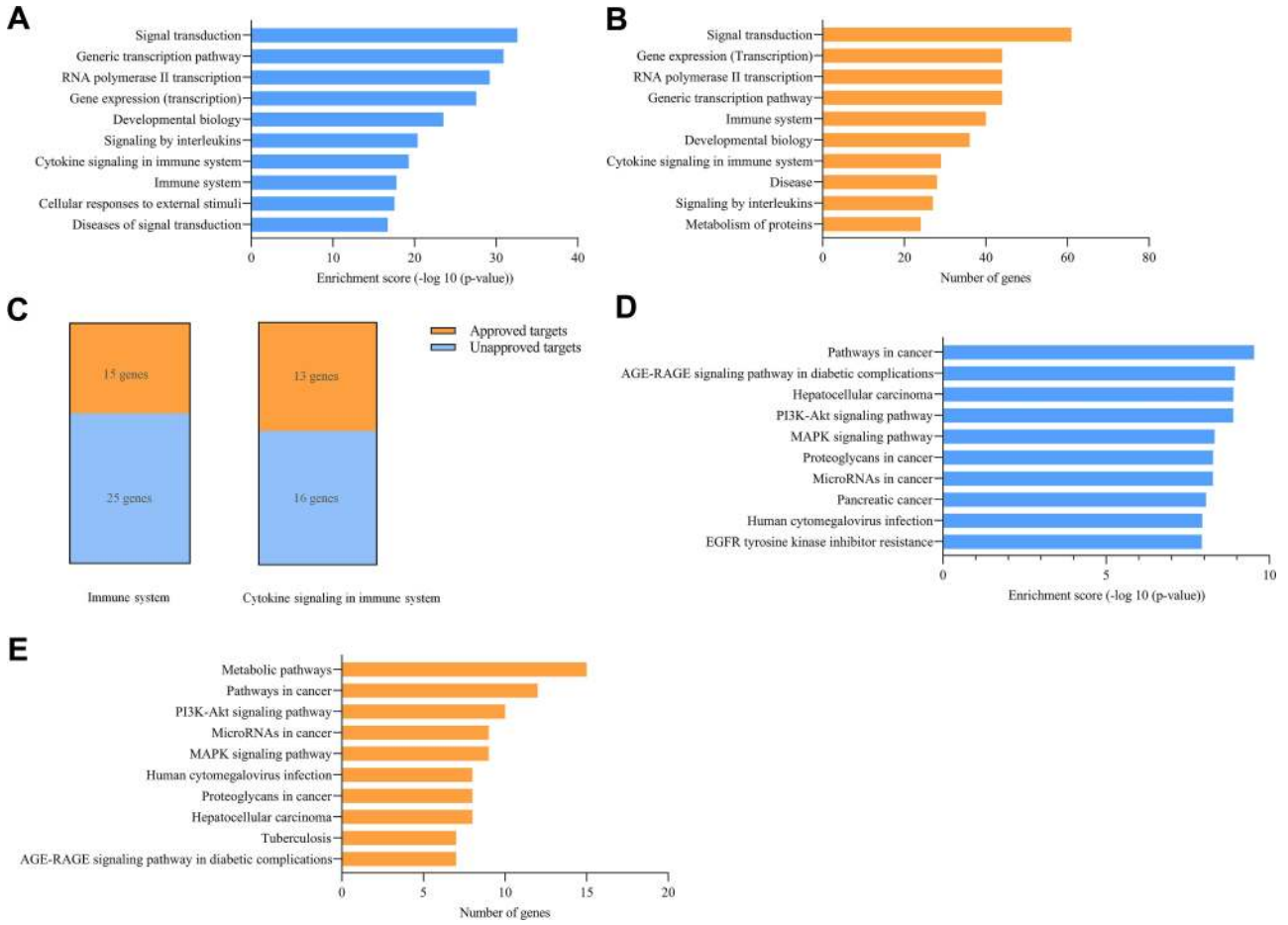

Figure 4 Analyses of discontinuation of two miRNA-based clinical projects. (**A** and **B**) Enrichment for targets of MRX34 in terms of enrichment score and number of genes. (**C**) Unapproved and approved targets number of MRX34 validated targets in the immune system and cytokine signaling in the immune system. (**D** and **E**) Enrichment for targets of miR-122 (drug RG-101) in terms of enrichment score and number of genes.

the enrichment. Given its emergence in phase II not in phase I, we filtrated 71 target genes in the literature and the KEGG database,[24] and got seven hyperbilirubinemia or jaundice (hyperbilirubinemia-related disease) related genes (Figure 5). Only seven related genes were found, and it revealed why hyperbilirubinemia did not occur in most tested objects in phase I but appeared in a few cases in phase II.

Future Directions and Conclusions

TMTME is a typical and inevitable property of miRNA molecules, which is caused by incomplete complementation with the target sequence. TMTME leads to that miRNA could bind to various sequences suitable for the interaction (including protein-coding genes, lncRNA,[25] circRNA,[26] etc), which is different from all approved drugs (including siRNA drugs) with only a few targets. Emerging siRNA-based products, patisiran and givosiran approved by the FDA, have been in clinical application, however, miRNA therapeutics was still in its early clinical stage. Therefore, both introduction and removal of miRNA in humans can lead to changes of a wide series of pathways and some of them are unknown, even unpredictable, probably triggering disorders of physiological function or the occurrence of additional disease.

Targeted drug delivery is a pattern of delivering drugs to specific lesion sites of a patient, largely based on nanomedicine, which can enhance solubility and efficacy of drugs and avoid needless interaction with healthy tissues.[27,28] Targeted drug delivery systems can be designed by recognition of a specific feature in lesion sites.[29] In cancers, the antigens and receptors on cell membranes are considered suitable targets of recognition sites for design of these nanoparticles.[29] Therefore, targeted drug delivery systems may eliminate or weaken the existing obstacles that were caused by TMTME.

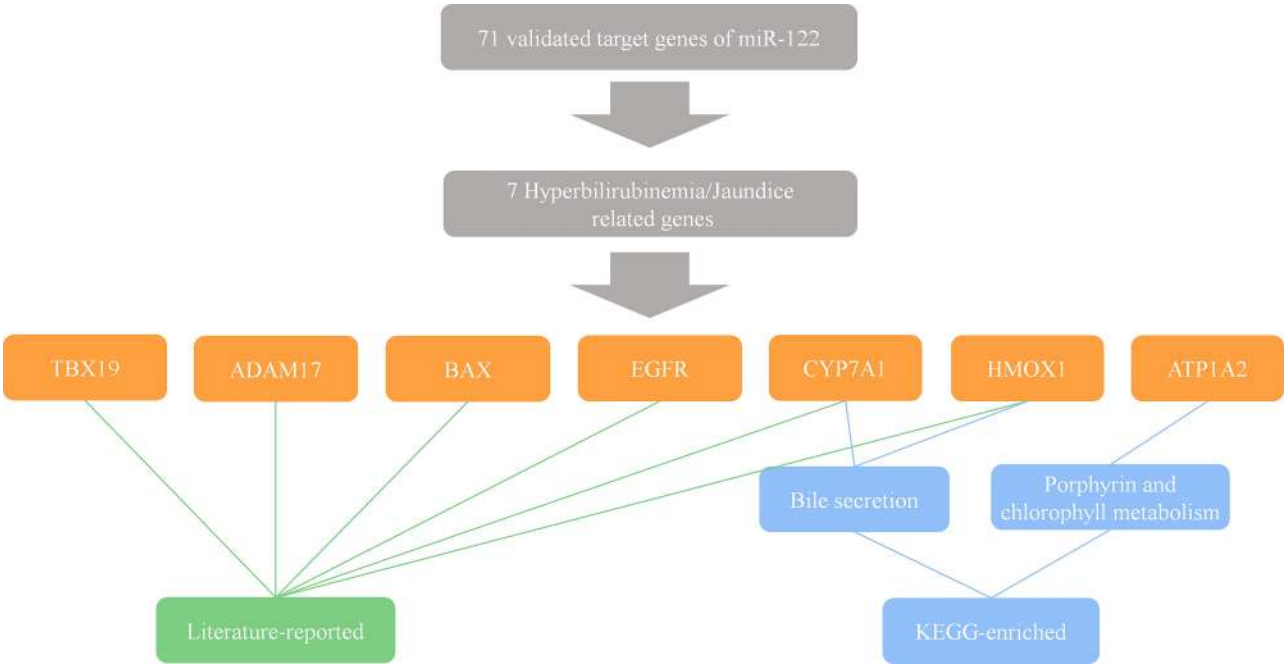

Figure 5 Discontinuation of RG-101 is caused by its hyperbilirubinemia or jaundice-related targets. The hyperbilirubinemia or jaundice-related target genes of miR-122 are identified by literature searching and KEGG enrichment.

Delivering miRNA drugs to pathogenic sites may efficiently avoid excessive toxicity and side effects. However, the defects of the system are high cost, hard to make productivity for delivering nucleotides, and the increased difficulty for adjusting the dosages.[28,30] Moreover, due to instability of unprotected miRNAs, delivering miRNAs required chemical modifications to avoid rapid degradation in serum, which may impair specificity of miRNAs and lead to off-target effects.[31] Besides, parenteral or local injection is the primary approach for delivering miRNA drugs,[32] which reduces the amount of miRNA that is transported to the target tissue. Another challenge is that exogenous artificial miRNAs will trigger competition and saturation effect, a competition among exogenous and the endogenous miRNAs for the intracellular machinery, and thus affecting unexpected gene expression and leading to untoward side effects.[33–35]

Conclusively, in this study, we analyzed the key cause that leads to the slow development of miRNA therapeutics in a drug target perspective and attributed it to TMTME.

Resources, Databases and Servers

We use ClinicalTrials.gov database[10] for obtaining available information of clinical trials, a series of literature[36–89] for obtaining information for complementary ratio of miRNA with target gene, a study[90] for getting all siRNA sequences with target genes, NCBI gene and NCBI blast database for calculating complementary ratio of siRNA with mRNA, KOBAS[20] web server for enriching pathways and diseases for adverse events' condition of miRNA therapeutics, KEGG database[24] for obtaining hyperbilirubinemia or jaundice-related genes and pathways.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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