Molecular Therapy
Nucleic Acids
Review

25 years of maturation: A systematic review of RNAi in the clinic

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The year 2023 marks the 25th anniversary of the discovery of RNAi. RNAi-based therapeutics enable sequence-specific gene knockdown by eliminating target RNA molecules through complementary base-pairing. A systematic review of published and ongoing clinical trials was performed. Web of Science, PubMed, and Embase were searched from January 1, 1998, to December 30, 2022 for clinical trials using RNAi. Following inclusion, data from the articles were extracted according to a predefined protocol. A total of 90 trials published in 81 articles were included. In addition, ongoing clinical trials were retrieved from ClinicalTrials.gov, resulting in the inclusion of 48 trials. We investigated how maturation of RNAi-based therapeutics and developments in delivery platforms, administration routes, and potential targets shape the current landscape of clinically applied RNAi. Notably, most contemporary clinical trials used either *N*-acetylgalactosamine delivery and subcutaneous administration or lipid nanoparticle delivery and intravenous administration. In conclusion, RNAi therapeutics have gained great momentum during the past decade, resulting in five approved therapeutics targeting the liver for treatment of severe diseases, and the trajectory depicted by the ongoing trials emphasizes that even more RNAi-based medicines also targeting extra-hepatic tissues are likely to be available in the years to come.

INTRODUCTION

In 1998, Andrew Fire and Craig Mello described the pathway of RNAi in the worm *Caenorhabditis elegans*.[1] RNAi induces post-transcriptional gene silencing (PTGS). This conserved and potent gene silencing mechanism may be harnessed for gene therapy and clinical application. PTGS can be induced by short-lived RNAi-based molecules such as small interfering RNA (siRNA), the cells own effectors, microRNAs (miRNAs), or promotor-dependent RNA species such as short hairpin RNA (shRNA).[2] Importantly, siRNA-based PTGS is mediated by cytosolic cleavage of target mRNA and involves the RNA-inducing silencing complex (RISC), including argonaut protein 2 (Ago2) and the target mRNA-specific siRNA guide strand (Figure 1).[3] Ago2 has slicer activity which is used to cleave target mRNA, when the siRNAs are bound to the target mRNA and associated with RISC.[4] siRNAs originate from long double-stranded RNA (dsRNA) sequences, which are cleaved in the cytosol by Dicer.[5] With regard to the outcome of silencing miRNAs induce translational repression, whereas siRNAs, which are fully complementary to target sequences, induce Ago2-mediated degradation.[6]

The cell has its own endogenous PTGS system on the basis of miRNA effectors. Several pathways exist, and the canonical pathway starts in the nucleus (Figure 1).[7] RNA polymerase II (Pol II) or polymerase III (Pol III) transcribes the primary miRNA transcript, which initially is cleaved by Drosha in the nucleus to release the ~70-nt-long stem-loop structure. This pre-miRNA is then transported to the cytosol by exporting 5 (XPO5), and finally cleaved by Dicer.[5,8,9] Next, one of the miRNA duplex strands is incorporated in Ago1–4 inside RISC and here specifies mRNA targets silencing via translation inhibition and by inducing mRNA decay (Figure 1).[10,11] Non-canonical miRNA pathways also exist. One example is the Dicer-independent, Ago2-dependent miRNA, called miR451, where no passenger strand is produced.[12–14] Another example is miRtrons, which bypass Drosha.[15] An alternative to siRNAs are shRNAs, used to express siRNA in the cell.[16] shRNAs are transcribed from DNA *in vivo* by a Pol III promotor and these vector encoded molecules were described in 2002. shRNA bypass Drosha, and XPO5 mediates the nuclear export of the pre-miRNA-like molecules.[16] shRNA can be embedded in a pri-miRNA scaffold, which allows the cell to use the tissue specific Pol II for transcription called miR-shRNA (Figure 1).[16]

RNAi represents an entirely new way to treat disease with an unprecedented potential for specific gene targeting through the endogenous miRNA machinery. Several potential disease targets for RNAi-based therapy currently have unattractive treatment options or no treatment options at all. Hence, the mechanism of RNAi and the emergence of RNAi therapeutics have been studied extensively over the years, and recently reviewed in several prominent papers.[17–20] Selected highlights during this journey include in 2001, three years after Fire and Mello's discovery, the finding that precision duplex silencers consisting of 21 and 22 nt dsRNA sequences with terminal

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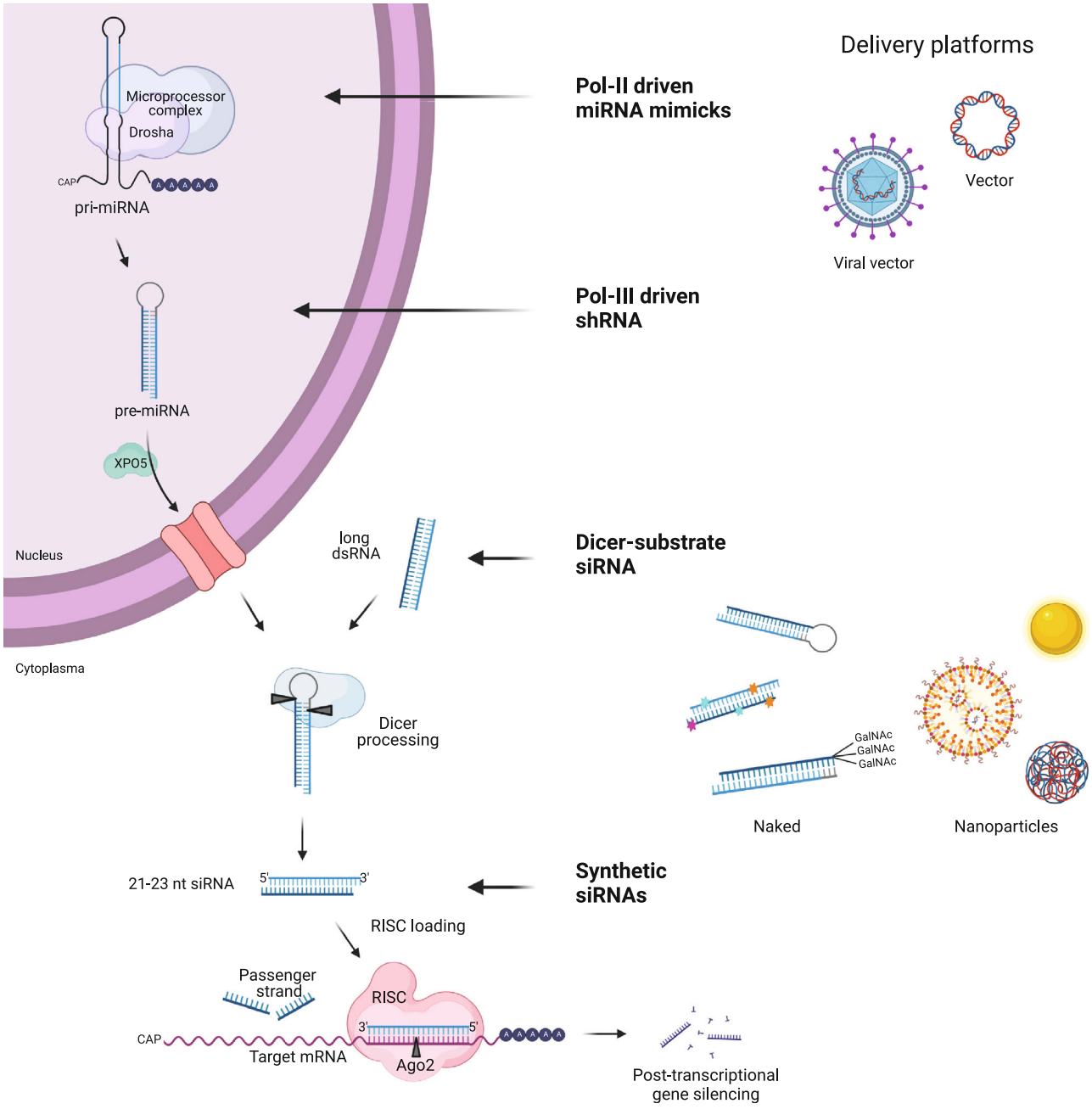


Figure 1. The RNAi mechanism and entry points of RNAi therapeutics

The left-hand side shows the RNAi pathway and formation of siRNAs. The right-hand side shows different entry points for DNA-based or RNA-based RNAi therapeutics that enters the cell and are loaded into RISC to mediate homology-dependent degradation of target mRNA. Delivery platforms for DNA-based therapeutics include viral and non-viral vectors encoding Pol II driven miRNA mimics or Pol III-driven shRNA. RNAi-based therapeutics are delivered naked as GalNAc-conjugated, modified, or unmodified Dicer-substrate siRNA or synthetic siRNAs or complexed in nanoparticles (gold, lipid, or polymer based). Created using BioRender.com.

2 nt 3′ overhangs could elicit RNAi-based PTGS in mammalian cells (Figure 2).[21,22] Another important finding, the demonstration that RNAi holds therapeutic promise to prevent liver injury, was reported in 2003.[23] In this study Song et al. showed that RNAi targeting Fas protects mice from hepatitis (Figure 2). In 2004 the first clinical trial using a siRNA-based treatment called siRNA-027, was initiated for the treatment of age-related macular degeneration (AMD).[24,25] One year later, in 2005, potent nuclear RNAi-mediated knockdown was shown in the nucleus of human cells.[26] In 2006, Fire and Mello were awarded the Nobel Prize in Physiology or Medicine for their

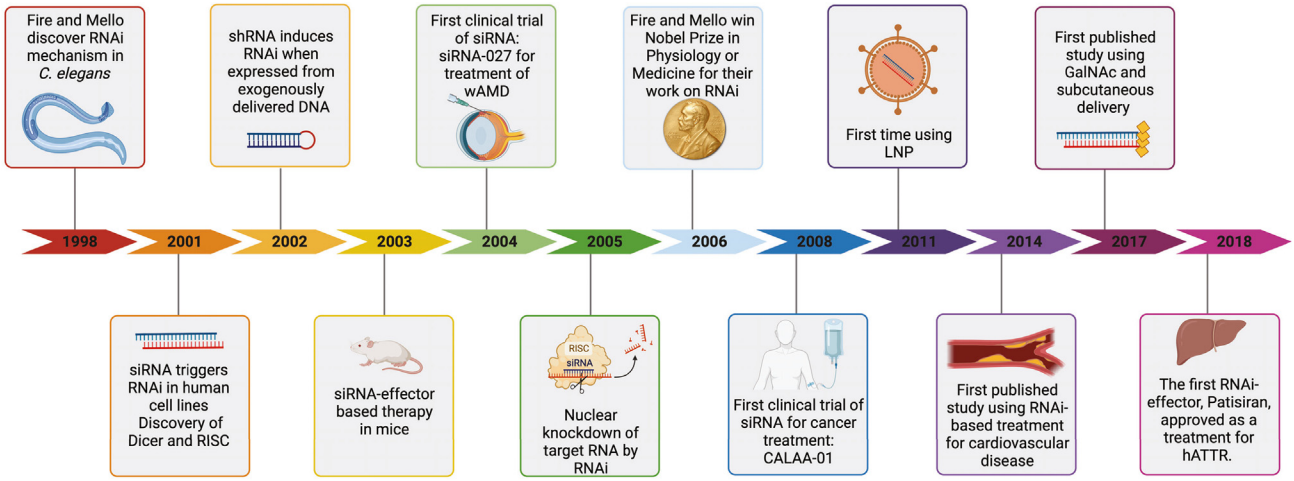


Figure 2. Milestones in the development of RNAi-based therapeutics

Timeline of important milestones since the discovery of the RNAi mechanism in 1998: 1998,[1] 2001,[5,22] 2002,[16] 2003,[23] 2004,*[24,25] 2005,[26] 2006,[27] 2008,*[28,29] 2011,*[30] 2014,*[31] 2017,*[32] 2018.*[33–35] siRNA, short-interfering RNA; RISC, RNA-inducing silencing complex; shRNA, short hairpin RNA; wAMD, wet age-related macular degeneration; LNP, lipid nanoparticle; GalNAc, *N*-acetylgalactosamine. The asterisks before or after numbers indicate that the study mentioned is included in the published clinical trials analyzed in this systematic review. Created using BioRender.com.

groundbreaking discovery (Figure 2).[27] Evidence for RNAi-based nanotherapeutics from systemically administered siRNA via targeted nanoparticles (NPs) for cancer treatment in humans was provided in 2008.[28,29]

A few years later, in 2011, the first study demonstrating delivery of RNAi therapeutics using lipid NPs (LNPs) and its potential applications to treatment was published.[30] Interestingly, Gish and co-workers used LNPs composed of a plasmid encoding RNAi-based molecules complexed with cholesteryl spermine.[30] In 2014, the first RNAi-based treatment for cardiovascular disease using a potent inhibitor of PCSK9 was published.[31] *N*-acetylgalactosamine (GalNAc)-conjugated delivery was introduced in 2017 (Figure 2).[32] It all culminated in 2018, when the first RNAi-based drug, patisiran, was approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) as treatment for hereditary transthyretin amyloidosis (hATTR) (Figure 2).[33–35] Since then, four other RNAi therapeutics (givosiran, lumasiran, inclisiran, and vutrisiran) have been approved for medical use.[34,35]

By its 25th anniversary, RNAi-based therapy has established itself as an important treatment strategy that may benefit patients with otherwise incurable diseases. This systematic review investigates how the maturation of RNAi therapeutics and developments in delivery platforms, administration routes, and potential targets shape the current landscape of clinically applied RNAi. The most remarkable finding was that the approved RNAi drugs successfully but solely target the liver and targeting of extra-hepatic tissues seems to be the next frontier.

RESULTS

During the search period, which was restricted to the publication year of Fire and Mello's seminal paper, 1998, and forward, we retrieved 2,723 articles: 1,016 articles from Web of Science, 1,060 from PubMed, and 647 from Embase; 1,234 duplicates were removed. The two reviewers screened 1,489 titles/abstracts in Covidence; 1,382 articles were found to be irrelevant. The kappa coefficient for agreement between reviewers was 0.92. On the basis of full text screening 118 clinical trials were extracted; 28 not original or duplicate trials were excluded. In total, 90 clinical trials published in 81 articles were included for data extraction (Figure S1A). Four hundred fifty ongoing clinical trials were retrieved from ClinicalTrials.gov. One reviewer screened the entries in the database; 402 were found to be irrelevant, and 48 ongoing clinical trials were included for data extraction (Figure S1B). Ongoing clinical trials were listed by the year of trial initiation. An overview of the published and ongoing clinical trials is shown in Tables S1 and S2. Seven articles presented two or more clinical trials with individual National Clinical Trial (NCT) numbers. A list of these articles is provided as Table S3. Design, inclusion/exclusion criteria, and search strategy are described in materials and methods.

Types of RNAi therapeutics

The first papers describing findings from clinical trials using RNAi therapeutics were published in 2008, ten years after the discovery of the RNAi mechanism. From 2008 to 2022, the annual number of published clinical trials using RNAi-based drugs increased almost 10-fold (Figure 3). Likewise, the number of ongoing clinical trials initiated from 2015 to 2021 increased approximately 7-fold. However, in 2022 the number of ongoing trials using siRNA-based drugs decreased by almost 40%. In each of the investigated years, siRNA was by far the most frequently used type of RNAi-inducing molecule in both the published and ongoing clinical trials, respectively. In 2008 and 2019 siRNA was used in all of the published and ongoing clinical trials. In 2022 siRNAs still dominated; it was used in ~90% of both published and ongoing trials, and in 2023, so far all of the published

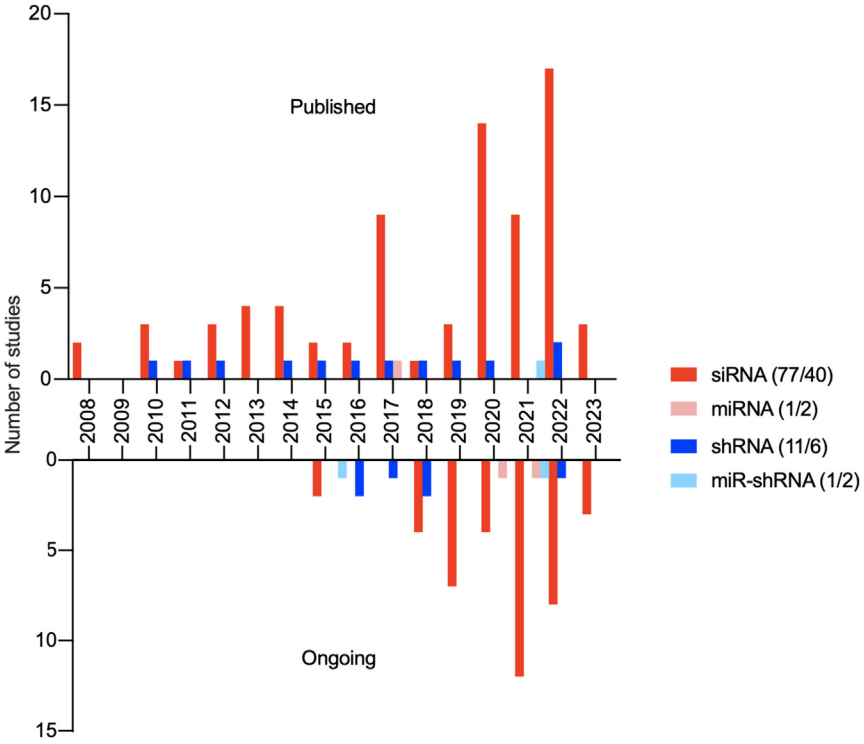


Figure 3. Types of RNAi therapeutics
Frequency distribution of RNAi-based drugs (siRNA, miRNA, shRNA, and miR-shRNA) used in the published and ongoing clinical trials from 2008 to 2023 and 2015 to 2023, respectively. Color-coded annotations for the distribution are included.

and ongoing clinical trials have been siRNA-based. Delivery of expressed shRNAs as a treatment option has a low but almost constant frequency in the published trials through the years. A similar frequency of shRNA-based drugs was observed in the ongoing trials. Only few miRNAs and miR-shRNAs have been investigated in the published studies or are under investigation in the ongoing clinical trials (Figure 3).

A clear tendency from the trials reported during the past 25 years is that synthetic siRNA dominates the field of RNAi therapeutics used in the clinic.

Delivery platforms of RNAi therapeutics

The delivery platform, that is the way to modify or package the RNAi-based molecule, is key to the effectiveness of the RNAi drug. Several factors, including oligonucleotide charge and size, RNase susceptibility, and immunogenicity, as well as interaction with reticuloendothelial system and endocytosis play important roles of the applied delivery platform. From 2008 to 2016 several different delivery platforms were used in the published and ongoing clinical trials, e.g., *ex vivo* methods (see Table S4), LODER technology (Local Deliver EluteR; a cancer drug delivery platform developed by Silenceed to enable insertion of the RNAi therapeutic directly into the core of solid tumors), and NPs (LNPs, polymer NPs, and gold NPs), with NPs being the most frequently used (Figure 4A). From 2017 onward, naked delivery of RNAi-based drugs is the most common method, and in 2022 79% and 63% of the published and ongoing trials used naked delivery. In comparison, in total 11% and 19% of the published and ongoing trials describes NP delivery of RNAi-based molecules, and 11% and 13% of the published and ongoing trial were delivered by *ex vivo* methods (Figure 4A). In 2023 naked delivery is used in all of the published and ongoing trials. LODER technology was used in 1% and 2% of all published and ongoing trials. Degrada BALL technology (a porous NP drug delivery platform developed by Lemonex) and exosomes were both used in 2% of all ongoing clinical trials.

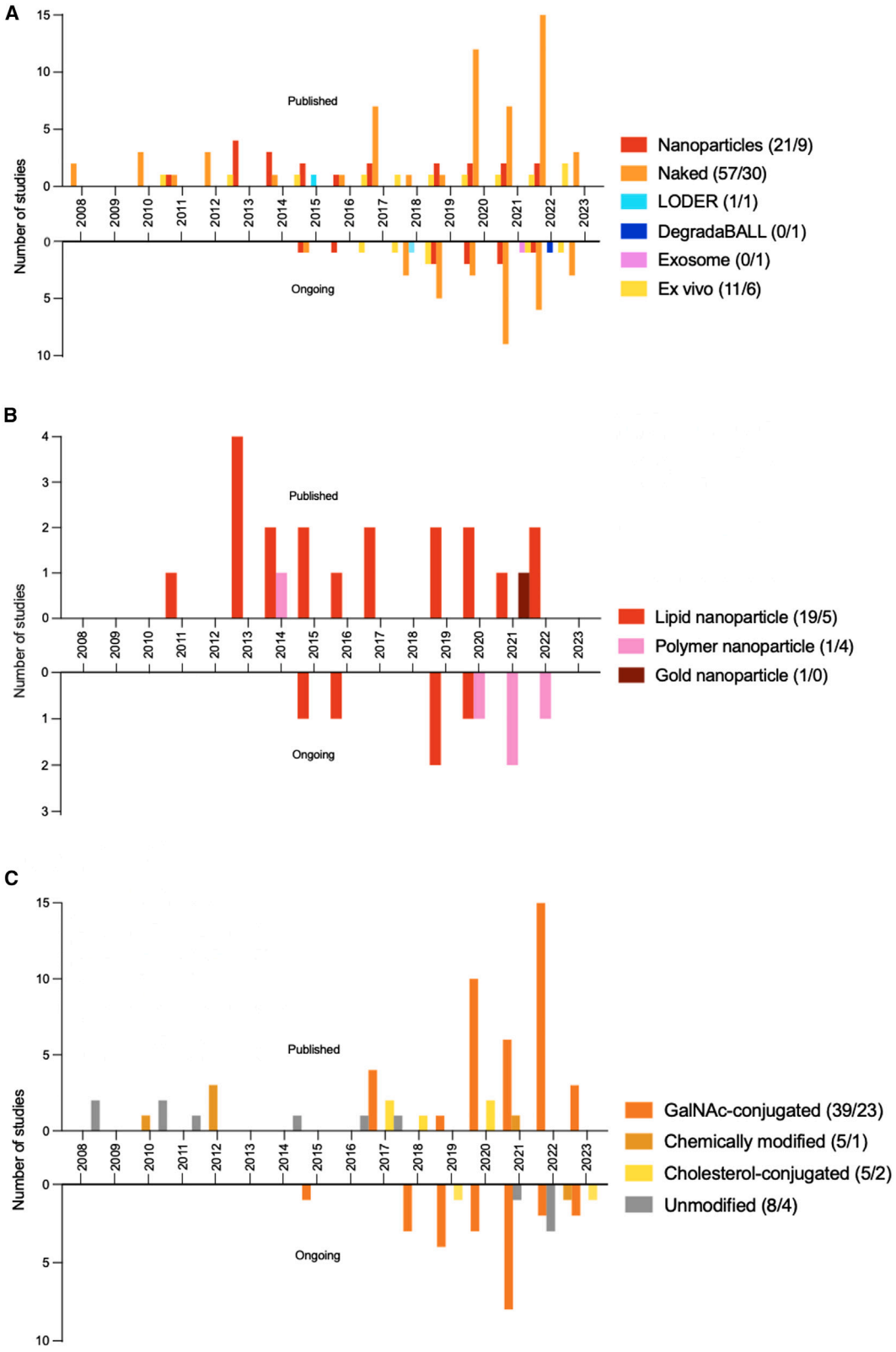
LNPs are most frequently used in the trials for NP delivery. LNPs were used in 91% and 56% of all published and ongoing clinical trials (Figure 4B). Other types of NPs delivery include polymer NPs used in 5% and 44% of all the published and ongoing clinical trials. Gold NP delivery was used in 5% of all the published clinical trials. Notably, the last clinical trial based on LNP delivery was initiated in 2020. From this time forward, polymer NPs seem be the preferred type of NP in the ongoing trials.

GalNAc-conjugated delivery of RNAi-based drugs is the most common form of naked delivery with 68% and 77% in total of the published and ongoing clinical trials (Figure 4C). GalNAc conjugates were not used before 2017, and after its introduction a significant shift in the delivery method unfolded, from LNP based to naked based (Figures 4B and 4C). Other types of naked delivery include chemical modifications (9% and 3%), cholesterol conjugation (9% and 7%), and unmodified delivery (14% and 13%), in the published and ongoing clinical trials, respectively (Figure 4C).

The most remarkable finding in delivery technology since the discovery of RNAi was the replacement of LNP-based delivery by GalNAc conjugation as the predominant method in 2017.

Administration of RNAi therapeutics

A similar shift in administration route was observed after 2017. Hence, before this year various deliver routes had been applied, whereas subcutaneous (s.c.) administration of RNAi therapeutics predominated after 2017. This finding is further substantiated by assessing the accumulated numbers as depicted in the pie charts in Figure 5: 44% and 50% of the published and ongoing trials use s.c. administration, and 36% and 21% use intravenous (i.v.) administration.



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In 2017, i.v. administration was used more frequently than s.c. administration (50% and 40%), and before 2017, s.c. administration was not used at all (Figure 5). However, from 2020 to 2022 s.c. administration was the predominant type of administration, with 79% of the published clinical trials using this type of administration in 2022 alone, compared with 16% of the trials using i.v. administration (Figure 5). In 2023 so far, every published clinical trial used s.c. administration. In the ongoing clinical trials, s.c. administration has in general been the most used form of administration, with 44% of trials being based on this form in 2022.

Other administration routes have also been investigated in the published clinical trials, especially before the emergence of s.c. administration in 2017. These include, e.g., intradermal (6% and 10%), intravitreal (5% and 4%), eye drops (1% and 4%), and inhalation (2% and 2%) in total in the published and ongoing clinical trials (Figure 5). The ongoing clinical trials in 2021–2023 re-investigated a number of administration routes used before 2017, and they also explore transplant and intralesional administration as new administration options, which have not been investigated in the published clinical trials.

Development of the GalNac technology has been the reason for the radical shift toward naked delivery in 2017 and the switch to s.c. administration. As shown in Figure S2A almost all (97% and 96%) of the published and ongoing clinical trials combining naked delivery with s.c. administration, used GalNAc-conjugated RNAi-based drugs. Ninety percent and 83% of the published and ongoing trials used i.v. administration in combination with LNPs (Figure S2B).

In summary, 2017 indicated a shift from the i.v. to the s.c. administration route in the clinical trials, and most clinical trials have been based on GalNAc conjugates combined with s.c. administration, or LNP combined with i.v. administration.

Diseases targeted by RNAi

Diseases were grouped on the basis of etiology (Figure 6) or the tissue targeted by the RNAi therapeutics (Figure 7). Types of disease etiology included broad categories such as cardiovascular, tumor, liver, and viral (Figure 6).

Etiology of the disease

RNAi therapy has been evaluated for several disease categories (Figure 6A). Cardiovascular, liver diseases, and tumor were targeted in about one-fifth of the published and ongoing clinical trials. Viral disease (e.g., HIV, respiratory-syncytial virus [RSV], and Ebola virus) was targeted in 18% and 10% of the published and ongoing clinical trials. Other diseases targeted in the published and ongoing trials include kidney (7% and 10%), eye (6% and 10%), and blood-manifested (3% and 8%) diseases.

Intravenous administration was primarily used to treat tumors (34% and 60%) and liver disease (31% and 10%) in the published and ongoing clinical trials (Figure 6B). The LNP delivery platform was used in more than half (56%) of the published trials with i.v. administration (Figure S2B). On the other hand, 43% and 38% of all s.c. administered RNAi therapeutics in the published and ongoing trials were used to treat cardiovascular-related diseases (Figure 6C).

Tissues and genes targeted by RNAi therapeutics

The number of RNAi therapeutics targeting specific tissues were assessed in the published and ongoing trials (Figure 7). Most RNAi-based drugs from the published and ongoing clinical trials target the liver (52% and 42%), although this may influence disease etiology in other organ systems. Tumors (21% and 23%) and viral infections are the second and third most frequent target, respectively (18% and 10%) of the published and ongoing clinical trials (Figure 7).

Comparison of the results categorized by disease etiology with target of the therapeutics shows that RNAi-based medicines applied in treatment of cardiovascular diseases all target the liver (Tables S5 and S6). These medicines include inclisiran, olpasiran, SLN360, and ALN-AGT01. In principle, the potency of RNAi enables the targeting of every disease-causing gene in the human genome. The gene loci and resulting proteins targeted by RNAi-based drugs are summarized in Figure 8. *PCSK9*, located on chromosome 1, is a prominent example of a targeted gene. The encoded protein, PCSK9, is involved in hypercholesterolemia by regulating hepatic lipoprotein metabolism. The mRNA transcribed by *PCSK9* is the target in 15 and 6 published and ongoing clinical trials for the treatment of atherosclerotic cardiovascular disease (ASCVD) (hypercholesterolemia). Another example is *TTR*, located on chromosome 18. It also encodes a liver-expressed protein, and the involved mRNA is targeted 10 and 2 times in the published and ongoing clinical trials for the treatment of hATTR. Other frequent targets in the published and ongoing trials include *VEGFA* (2 and 0), HBV-antigens (9 and 3), and *ALAS1* (5 and 0). A total of 64 genes (including viral genes) have been targeted in the published and ongoing clinical trials.

Approved RNAi-based drugs

At present, five RNAi-based drugs (patisiran, givosiran, lumasiran, inclisiran, and vutrisiran) have been approved by the FDA and the EMA. Selected characteristics are listed in Table S7. All have been identified in the published clinical trials, and all except for givosiran are applied in the ongoing clinical trials. They are all siRNA-based and target the liver. Except for patisiran, which is delivered i.v. in LNPs, they are administrated s.c. as GalNAc conjugates. In total, 68 different experimental RNAi medicines were identified from the published and ongoing clinical trials (Figure 7). Many of the genes, including *TTR*,

Figure 4. Delivery platforms of RNAi therapeutics

(A) Frequency distribution of methods used to deliver RNAi therapeutics to the target tissue used in the published and ongoing clinical trials from 2008 to 2023 and 2015 to 2023, respectively. See Table S4 for explanation of the "*Ex vivo*" category. (B) Frequency distribution of the types of NP types delivered in the published and ongoing clinical trials from 2008 to 2023 and 2015 to 2023, respectively. (C) Frequency distribution of the type of naked RNAi molecules used in the published and ongoing clinical trials from 2008 to 2023 and 2015 to 2023, respectively. Color-coded annotations are included for every distribution.

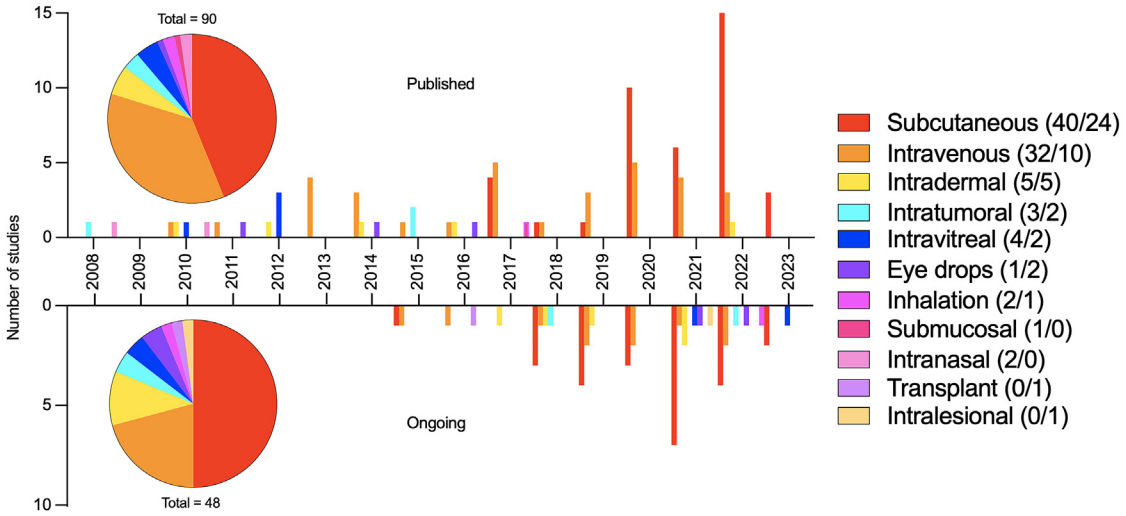

Figure 5. Administration of RNAi therapeutics

Frequency distribution of the types of administration routes used in the published and ongoing clinical trials from 2008 to 2023 and 2015 to 2023, respectively. Pie charts showing the overall distribution of the types of administration routes used in the published and ongoing clinical trials. Color-coded annotations are included for every distribution.

LDHA, *LPA*, *CCR5*, *STMN1*, and a number of oncogenes, are targeted by more than one RNAi therapeutics (Figures 7 and 8).

The world of clinical RNAi

RNAi-based treatment options are investigated all around the world and patients are recruited from 60 different countries, with the vast majority of the published and ongoing clinical trials being recruited from the United States (55 and 40) (Figure S3). A high number of studies were likewise recruited from countries include Germany (27 and 11), United Kingdom (28 and 16), France (19 and 13), the Netherlands (18 and 12), and Italy (10 and 6). Fifty-three percent and 46% of the published and ongoing clinical trials recruited participants from more than one country. Cyprus, Iceland, Serbia, and Sierra Leone published trials with available published data, but for the present there are no ongoing trials. On the other hand, even though ongoing clinical trials are recruited from Chile, Croatia, Estonia, Ireland, Latvia, Lithuania, Mauritius, Puerto Rico, Romania, and Thailand, no studies have yet been published (Figure S3).

DISCUSSION

Since its groundbreaking discovery, RNAi has been used extensively in basic research and clinical trials. The purpose of this systematic review was to provide a detailed overview of RNAi agents applied in the clinic, on the basis of published and ongoing clinical trials from the discovery 25 years ago until present day.

Use of RNAi-based molecules comes with inherent challenges because these molecules are vulnerable to nuclease-mediated degradation, have unfavorable physicochemical features that preclude simple transfer into cells, and may contribute to activation of the immune system. Safe and effective RNAi therapeutics therefore require sophisticated delivery technologies.[36] The success of RNAi drugs critically depends on chemical modifications and/or technologies designed to protect the RNAi effector from degradation and to warrant their systemic stability, to facilitate localization to the target tissue, and finally, to safeguard successful intracellular delivery. On the other hand, RNAi represents an entirely new way to treat disease applicable to a plethora of targets. Indeed, this systematic review clearly demonstrate that the field of clinically applied RNAi is rapidly evolving. Cardiovascular diseases are currently the dominant group of diseases targeted by RNAi. However, treatment for a wide range of tissues from eyes to joints, and genetic to acquired diseases have been and are being investigated.

Recent inclusive narrative reviews, with detailed description of the mechanisms of RNAi[18] and development of novel RNAi therapeutics[6,17,19,37–39] including the chemistry of the RNAi-based drugs,[20] the delivery mechanisms, the cellular uptake mechanisms of antisense oligonucleotides (ASOs) and their endocytotic pathways, should be acknowledged.[40,41]

The current review includes both published and ongoing clinical trials. Thus, the review not only summarizes the historic and contemporary field of clinical RNAi, but also provides a strong indication of its further direction. Notably, most of both published and ongoing clinical trials have recruited or are recruiting patients from the North America, Europe, South America, India, China, Russia, and South Africa, with the United States being the leading country in terms of number of trials. A clear tendency seems to be that regions or countries from which patients have been recruited continue their efforts by participating in the ongoing studies. Importantly, several "newcomers" from Africa, Central and South America, Europe, and Asia actively contribute to the ongoing trials, thereby adding more efforts,

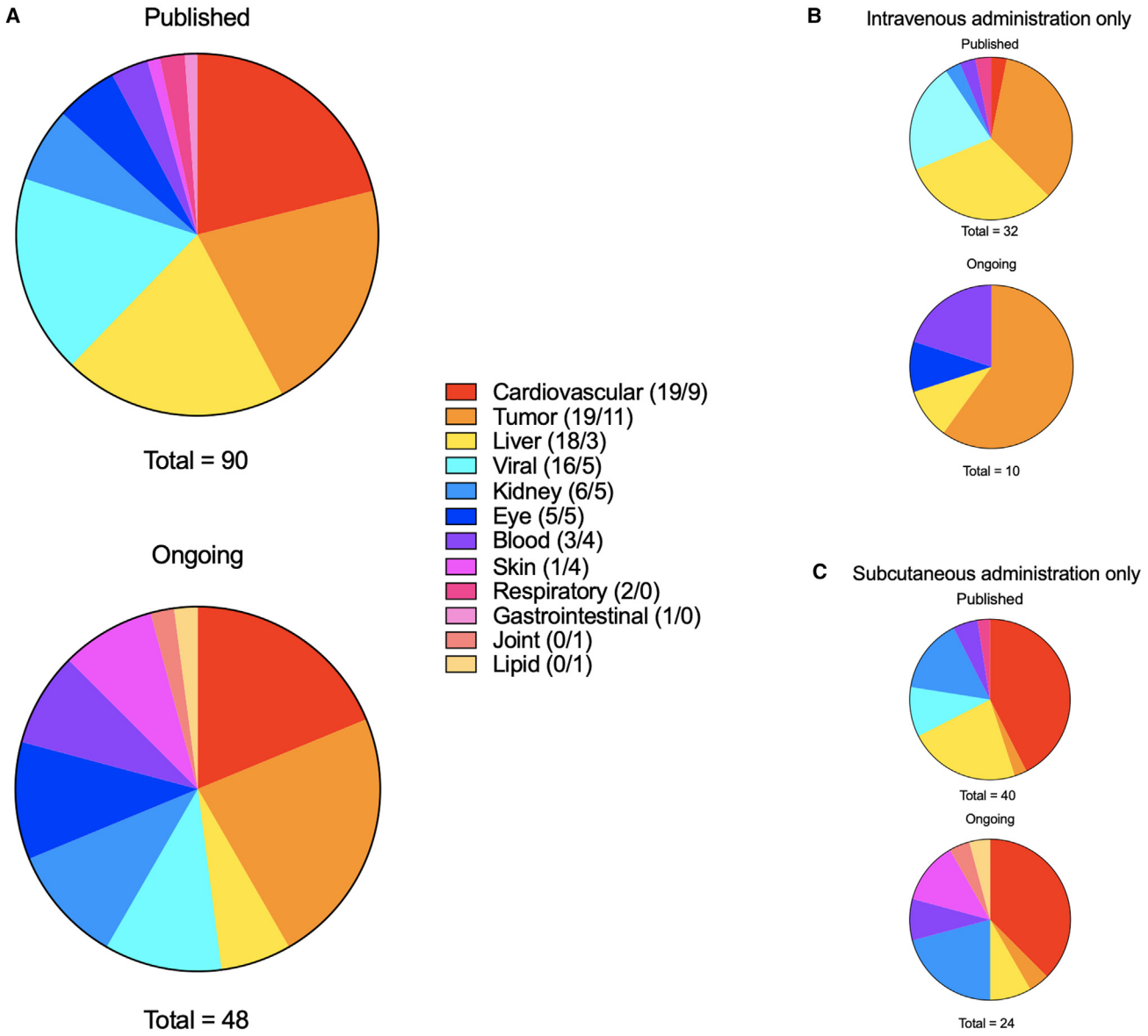

Figure 6. Disease etiology

(A) Overall distribution of the types of RNAi-treated diseases in the published and ongoing clinical trials, from 2008 to 2023 and 2015 to 2023, respectively. (B) Overall distribution of the types of RNAi-treated diseases using i.v. administration in the published and ongoing clinical trials, from 2008 to 2023 and 2015 to 2023, respectively. (C) Overall distribution of the types of RNAi-treated diseases using s.c. administration in the published and ongoing clinical trials from 2008 to 2023 and 2015 to 2023, respectively. Diseases are grouped by etiology. Color-coded annotations are included for every distribution.

capacity, additive partnership, and knowledge into developing RNAi therapeutics. The continuous and increasing interest in RNAi-based treatment, reflected by the increase in the number of published and ongoing clinical trials clearly has been fueled by the successful development of Patisiran for treatment of hATTR spearheaded by Anylam Pharmaceuticals.[33] The approval of the first RNAi therapeutics was a major scientific milestone for the entire field of RNAi and the atmosphere of RNAi optimism has sparked a new flood of interest not only from the scientific society, but importantly also from investors and big pharma. In celebration of the 25th anniversary of RNAi, this important outcome should be highly recognized.

The most remarkable finding was the high number of published clinical trials, amounting to 90 since the discovery of RNAi in 1998. These have paved the way for the five FDA- and EMA-approved RNAi therapeutics for the treatment of severe diseases for which there previously were

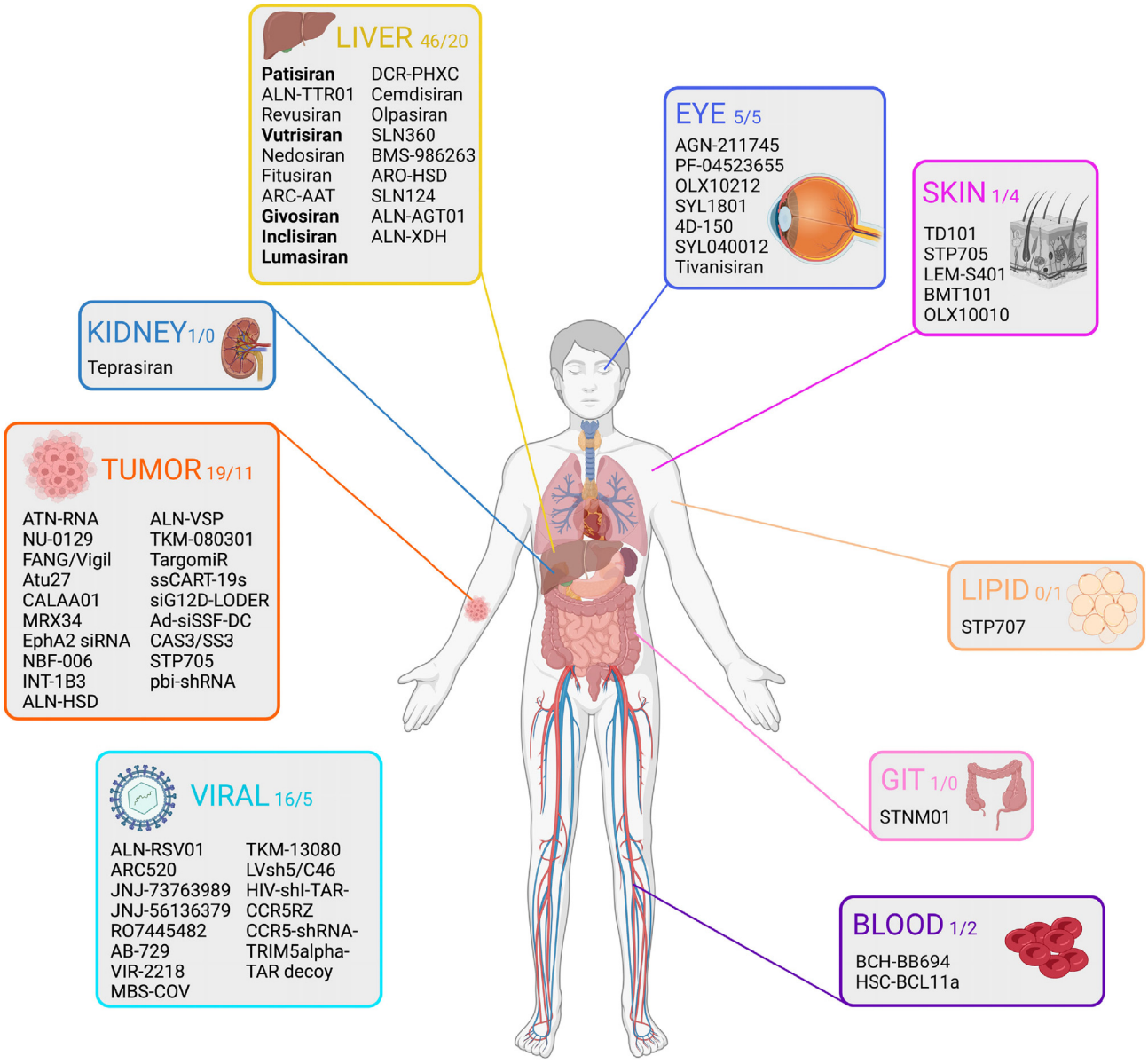

Figure 7. Tissues targeted by the RNAi therapeutics

Overall distribution of tissues targeted by the RNAi therapeutics in the published and ongoing clinical trials, from 2008 to 2023 and 2015 to 2023, respectively. The names of the RNAi therapeutics are included in the organ box together with the number of RNAi therapeutics found to target the specific organ in the published and ongoing clinical trials. The color of the border of the organ boxes correlates with the color-coded annotations in Figure 6. The five FDA- and EMA-approved therapeutical RNAi-based drugs are highlighted in bold. Inspired by Hu et al.[20] Created using BioRender.com.

unattractive or no treatment options at all. The drugs all target the liver which reflects the high accessibility of this organ through hepatic receptors, the specific anatomy of the liver, and the effective transfer of siRNAs by i.v. administrated LNPs or s.c. delivered GalNAc-conjugated siRNAs (see below). A remarkable decrease in the number of ongoing clinical trials targeting the liver was observed, most likely reflecting that the field awaits selection of new targets following the successes obtained by s.c. administration of GalNAc-conjugated siRNAs to hepatic tissue.

Historically, one must acknowledge the variety of types of RNAi therapeutics. From our literature study, it is clear that synthetic siRNAs are used more frequently than vector encoded RNAi-based molecules. An obvious advantage of siRNAs is that they are simpler to manufacture and quality assess, whereas shRNAs needs delivery of large DNA-based constructs to the nucleus. Hence, siRNAs require repeated administration, while shRNA-based therapies are typically more persistent. siRNA duplexes may

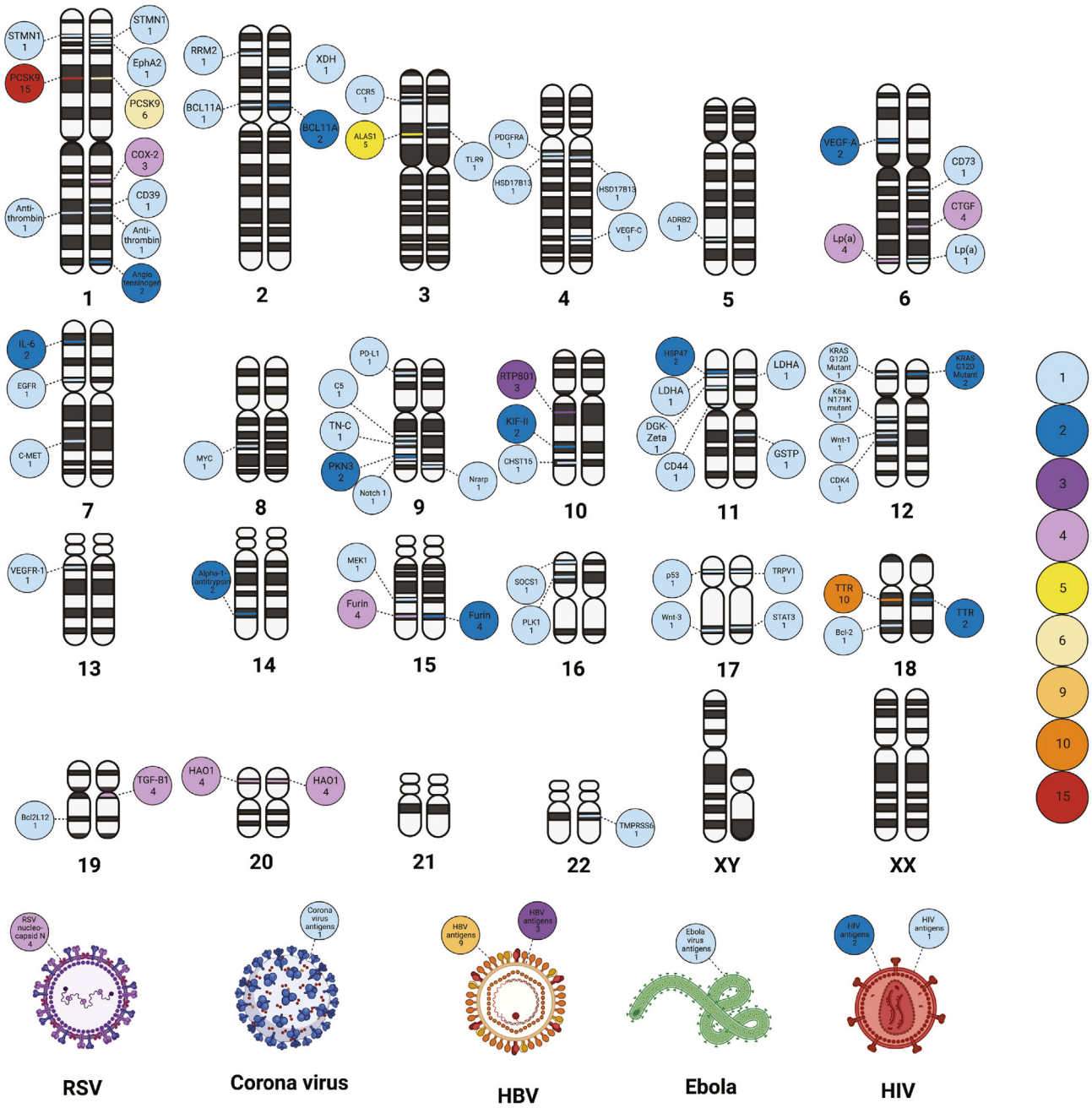

Figure 8. Genome distribution of target loci

Heatmap showing the chromosomal location of genes targeted by RNAi therapeutics in the published (circles on the left chromosome) and ongoing clinical trials (circles on the right chromosome). Viral targets are shown below (circles on the left and right side of viruses depict the target in the published and ongoing clinical trials, respectively). Color-coded annotations are included on the right hand. Red refers to the highest number of studies targeting the mRNA of the given protein, whereas light blue refers to the lowest number of studies targeting the mRNA of the given protein (e.g., *PCSK9* located on chromosome 1 encodes the PCSK9 protein. It is targeted 15 and 6 times in the published and ongoing clinical trials). Created using BioRender.com.

activate the innate immune system via intra- and extracellular RNA sensors.[42,43] shRNAs are less likely to elicit this response. On the other hand, shRNAs are primarily delivered by viral vectors that potentially induce immune and cytotoxicity.[44,45] Additionally, shRNA-based molecules may saturate the processing pathway used by miRNAs, thereby interfering with the function of miRNAs and the endogenous RNAi machinery.[45,46] Repression of off-target mRNAs, primarily mediated by so-called seed site

matches[47,48] is a general risk of RNAi. Both siRNAs and shRNAs can elicit off-target effects, but chemical modifications of the seed site and the co-delivered passenger strand may alleviate this concern.[49]

The biggest challenge of RNAi-based therapy is devising technology that allows efficient, specific, and safe delivery to the target cells. Many of the published and ongoing clinical trials take advantage of the efficient endocytosis of GalNAc-conjugated siRNAs and LNPs by liver cells, where both i.v. and s.c. administration are frequently applied. Hence, the liver can effectively endocytose GalNAc-conjugated siRNAs and LNPs. The first approaches to siRNA administration were focused on LNP-based delivery platforms offering a shielded compartment, isolated from immune components and serum nuclease activity, and a drug-biodistribution profile dictated by the packing agent.[36] The function of LNPs is to protect the siRNA from degradation and renal clearance, thereby prolonging half-life and facilitating easy endosomal escape.[50,51] To deliver siRNAs across barriers, the LNPs also needs to have a neutral surface charge provided by ionizable cationic lipid components, leading to reduced interaction with and binding of non-specific serum proteins.[37,51]

LNPs can associate with blood-circulating apolipoprotein E (ApoE) leading to cell-uptake via the low-density lipoprotein receptor (LDLR) on hepatocytes.[37,41,52] However, dsRNA is recognized as non-self by extracellular and intracellular receptors, thus activating an immune response. An alternative is modifying the siRNA (e.g., by GalNAc conjugation). This receptor-based delivery platform centered on GalNAc conjugation enables the siRNA to bind to the asialoglycoprotein receptor (ASGPR) densely found on hepatocytes, facilitating clathrin-dependent receptor-mediated endocytosis leading to robust RNAi-mediated gene silencing.[53] The unmodified siRNA drug ALN-RSV01 administered intranasally for the treatment of RSV did not make it past phase II, underscoring that competent and discriminating ASGPR-targeting ligands in combination with advantageous administration route have been key factors for clinically translation of GalNAc-siRNA-based molecules.[17,54]

Comparing the published and ongoing trials a clear tendency toward applying RNAi therapeutics in tumor treatment seems to unfold. The enhanced permeability and retention effect, possibly as a consequence of immature blood vessels and reduced lymphatic flow, seems to drive efficient transfer to and accumulation of RNAi drugs in tumor tissue.[55]

Interestingly, both unmodified and chemically modified RNAi therapeutics are investigated for the treatment of diseases using local administration. A prominent example of an unmodified RNAi-based drug is SYL1801 (phase II) which is administered locally by eye drops for treatment of neovascular AMD (NCT05637255). An example of a chemically modified RNAi drug is teprasiran (phase II) used to treat acute kidney injury.[56]

Intravenous administration is attractive as it facilitates quick and widespread distribution of the RNAi therapeutic. Another advantage of i.v. administration is the ability to delivery viral particles encoding shRNA or miR-shRNA. This could allow targeted delivery and safer RNAi treatment. However, i.v. administration is invasive and may result in infection, extravasation, and infiltration. These characteristics may also be challenging if the drug is not well-tolerated by the patient, or if the concentration is too high – resulting in an extensive immune response and cytotoxicity.[57] To avoid these disadvantages the field has attempted to replace i.v. administration by s.c. administration, as this method is less invasive and allows slower uptake through the lymphatic system and capillaries into the circulation.[58] Hence, s.c. administration has dominated the scene since 2017. A similar dominance of s.c. over i.v. administration is also observed in ongoing clinical trials. The fact that i.v. administration is less frequently used for liver-related diseases in the ongoing clinical trials compared with the published trials, further substantiates the prominent efficacy of s.c. administration developed for liver-targeting RNAi therapeutics. Subcutaneous administration has not been used for delivery of LNPs. A plausible explanation for this observation is that LNPs are often retained at the injection site because they are too large to enter capillaries and the lymph system.

Taken together, LNP systems are currently one of the most sophisticated non-viral nucleic acid delivery platforms enabling gene therapies. The recent decades of using lipid-based delivery systems for small molecule therapeutics have driven the efforts of evolving LNP technology for delivery of RNAi-based molecules[38] and profoundly affected the success of, e.g., COVID-19 vaccines.[59,60]

Before 2017 several other administration routes besides s.c. and i.v. were applied. These routes were abandoned because of their unsuccessful application. However, on the basis of the gained knowledge from the success of s.c. and i.v. administrated drugs, the field seems to have shifted toward reimplementation of previously used administration routes, including intradermal, intratumoral, intravitreal, topical eye drops, and inhalations. The idea is to tailor the therapy using local administration of RNAi therapeutics as in the case of intravitreal injections of naked siRNAs to the eye or intertumoral injection of siRNAs. Notably, *ex vivo* delivery of RNAi-based drugs seems to slightly lag behind the rest of the field, and the few examples are based on gene transfer of shRNA- or miR-shRNA-encoding genes to tumor cells, T helper cells, B cells, and hemopoietic stem cells (Table S2).

A notable observation is the relative few gene targets currently exploited for treatment despite the almost unlimited potential of RNAi-based drugs.[61] The trajectory depicted by the ongoing clinical trials shows that future RNAi therapeutics may be based on siRNAs, delivered naked or in a conjugated form (such as GalNAc) using the well-established s.c.- and intravitreal-based administration routes as well as novel, less proven methods. These approaches may hence be based on eye drop, inhalation, submucosal, intranasal, and intralesional administration for the targeting of a wide variety of diseases affecting the eye, CNS, kidney, blood, skin, joints, or lipid tissues (ClinicalTrials.gov; Table S2). This notion is further supported by the massive activity of pharmaceutical corporations including Alnylam, Gradalis, Dicerna, Olix, Novartis, and Sylentis.

Alnylam has pioneered the translation of RNAi medicines for especially liver-related and liver-targeted diseases and has recently also focused their activities on infectious, cardio-metabolic, and CNS disorders. However, the view on RNAi-based drugs looked quite different at the end of 2016 when Alnylam halted their phase III trial of revusiran because of severe adverse events, resulting in several large pharmaceutical companies backing out of the RNAi field. Despite this setback, Alnylam persisted and performed, on the basis of their enhanced stabilization chemistry (ESC), the first clinical trials using GalNAc-siRNA conjugates. The increased stability of ESC-GalNAc-siRNA conjugates resulted in efficacy ten times greater than standard template chemistry because of considerably increased liver exposure and sustained gene silencing.[36] In 2018 Alnylam had six GalNAc-siRNA conjugates in clinical trials and by January 2023 the company has increased its activity to ten active RNAi-therapeutic-based clinical trials.

Gradalis develops shRNA for tumors (*ex vivo* intradermal delivery and i.v. LNP-based delivery, 3 ongoing clinical trials). Olix targets hypertension (s.c. administration of GalNAc-conjugated siRNA, 1 ongoing clinical trial) and AMD (intravitreal administration of cholesterol-conjugated siRNA, 1 ongoing clinical trial). Novartis focuses on treating ASCVD (s.c. administration of GalNAc-conjugated siRNA, 5 ongoing clinical trials). This atmosphere of RNAi optimism has stimulated a new global wave of investment and licensing deals, reinvolving big pharma, such as Novo Nordisk, Eli Lilly, and Janssen.

Only a few studies have focused on diseases of the eye, which otherwise have been at the forefront of gene therapy.[62] Notably, the eye is the target tissue in the first siRNA clinical trial and is especially attractive for RNAi-based treatment, primarily because of its small size and the intraocular immune privilege, which both favor local delivery and thus limiting inflammation and off-target effects.[63] The only approved RNA-based drug for the eye, Macugen, targeting *VEGF* in the eye, has been withdrawn by the request of the marketing holder.[64] The observed discrepancy most likely reflects that retinal gene therapy has aimed at treating loss-of-function diseases rather than gain-of-function diseases such as hATTR. Another reason is unmet endpoint goals, which lead to discontinuation of a phase III trial of bevasiranib (NCT00499590). However, seven different RNAi-based drugs, fighting various eye diseases including wet AMD (wAMD) (AGN-211745 [phase I], PF-04523655 [phase I/II], OLX10212 [phase I], SYL1801 [phase II], and 4D-150 [phase I/II]), diabetic macular edema (DME; PF-04523655 [phase I]), glaucoma (SYL040012 [phase I]), and eye discomfort, particularly dry eye disease (Sjögren's syndrome) (tivanisiran (SYL1001) [phase III]) are currently being investigated in clinical trials. As an alternative to using synthetic siRNAs, novel vector encoded shRNAs may offer a more persistent solution.[16] A number of recent studies using shRNA-, agshRNA-, and miRtron-based therapy have already been investigate in pre-clinical settings,[2,65,66] indicating that such RNAi-based molecules targeting the retina may also find its way into clinical trials.[62,67,68]

In conclusion, the broad spectrum of diseases identified in the ongoing clinical trials is excellent news not only for patients but also the entire field of RNAi. Twenty-five years ago, RNAi was first described in *C. elegans*. After two and a half decades of maturation, a handful of approved RNAi treatments targeting the liver exist and treatment for other tissues are dedicatedly investigated. These exiting findings clearly signal that RNAi-based drugs are here to stay and have almost indefinite potential. As RNAi therapy is making headway in the clinic, the progress of the liver therapeutics discussed will hopefully provide the unmet need for treatment of extra-hepatic disease thereby improving the quality of life for a great spectrum of patients.

MATERIALS AND METHODS

Design and inclusion/exclusion criteria

This systematic review was conducted with adherence to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement guidelines (the PRISMA 2020 statement checklist provided in Table S8). The review was not registered. Inclusion/exclusion criteria, search strategy, and extraction protocol were defined prior to the literature search. For the identification of published clinical trials, we included all original research articles on clinical trials using RNAi. Non-interventional studies were excluded. The search period was restricted to the publication year of Fire and Mello's seminal paper,[1] 1998, and forward. Non-original articles, studies in non-human species, and pure *in vitro*/*ex vivo* studies were excluded. For the identification of ongoing clinical trials, we included active entries in ClinicalTrials.gov using RNAi.

Search strategy

We searched Web of Science (All Databases), PubMed, and Embase from January 1, 1998, to December 30, 2022. The search was performed December 30, 2022, using Google Chrome Version 108.0.5359.124 (Official Build) (arm64). The search string was built from synonyms of RNAi together with the above-mentioned inclusion/exclusion criteria. For a limited number of studies, the online publication date was in the end of 2022 with the consequence that the paper first appeared in the journal the following year. In these cases, the studied are figured in 2023.

Articles were imported to Endnote 20.4 (build 18004) with semi-automated removal of duplicates, and with subsequent manual removal of duplicates not found by Endnote. Articles were imported into Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia) and independently screened by titles/abstracts by two reviewers. Included full text articles were screened by two reviewers, who also extracted clinical trials identified in each article. Clinical trials were excluded according to the above-mentioned criteria and limitations. Furthermore, clinical trials sharing NCT number with a trial published earlier, were also excluded. All conflicts between reviewers during the screening process were resolved by consensus. Data from included clinical trials were extracted according to the extraction protocol (Table S9). If data for a given clinical trial (with NCT number) were missing in the corresponding included article, data were, if possible, retrieved from ClinicalTrials.gov.

We searched ClinicalTrials.gov for ongoing clinical trials using the filters "recruiting," "enrolling by invitation," and "active, not recruiting." The search was performed January 18, 2023, using Google Chrome Version 108.0.5359.124 (Official Build) (arm64). The aforementioned extraction protocol was deployed on these studies.

The full search strategy including inclusion/exclusion criteria for identification of published and ongoing clinical trials via databases and ClinicalTrials.gov is provided as in the supplemental information.

Our assessment of LNPs and chemical modifications of RNAi effectors were limited to general categories of LNP-based and chemical modification delivery platforms. Another limitation was that only one reviewer screened/extracted the ongoing clinical trials. We did not assess the risk for bias in the included published and ongoing studies.

Statistics

Descriptive statistics were performed for study characterization and kappa statistical analysis was performed to determine interrater agreement (title/abstract screening). Statistical analyses and accompanying figures were made in Prism 9.5.1 for Mac OS X (GraphPad Software, San Diego, CA).

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtn.2023.07.018.

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AUTHOR CONTRIBUTIONS

Conceptualization, B.K.F.-J., A.L.A., L.A., and T.J.C.; Methodology, I.J.C., T.S.J., and B.K.F.-J.; Software, B.K.F.-J. and E.G.J.; Article Screening, I.J.C., A.C.J., A.L.A., L.A., and B.K.F.-J.; Data Extraction, I.J.C., B.K.F.-J., and A.C.J.; Writing - Original Draft, I.J.C., L.A., and T.J.C.; Writing - Review and Editing, I.J.C., B.K.F.-J., T.S.J., A.C.J., E.G.J., A.L.A., L.A., and T.J.C.; Visualization, I.J.C., B.K.F.-J., E.G.J., A.L.A., L.A., and T.J.C.; Supervision, A.L.A., L.A., and T.J.C.; Project Administration, L.A. and T.J.C. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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OMTN, Volume *33*

Supplemental information

25 years of maturation:

A systematic review of RNAi in the clinic

Ida Juhl Corydon, Bjørn Kristensen Fabian-Jessing, Thomas Stax Jakobsen, Asbjørn Cortnum Jørgensen, Emilie Grarup Jensen, Anne Louise Askou, Lars Aagaard, and Thomas Juhl Corydon

Supplemental Materials

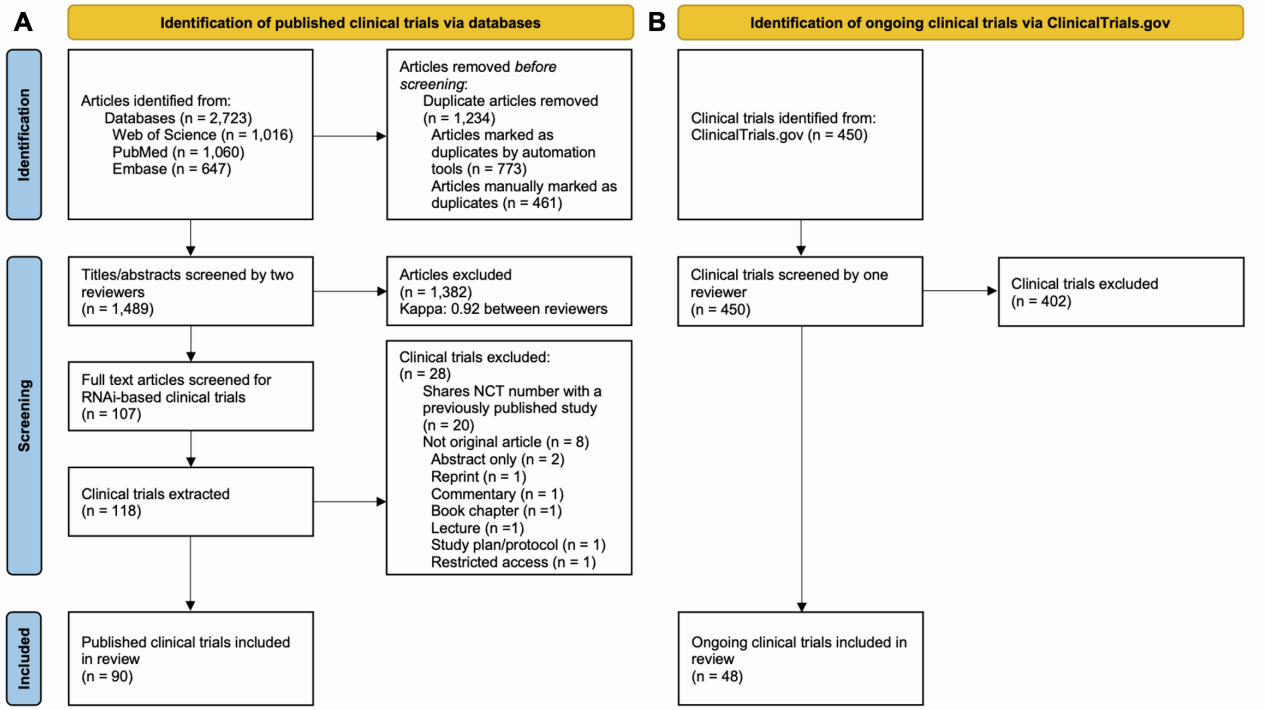

Figure S1: Flow diagram of the selection process. (A) Published clinical trials included in this review. **(B)** Ongoing clinical trials included in this review. *Adapted from:* Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews.[1]

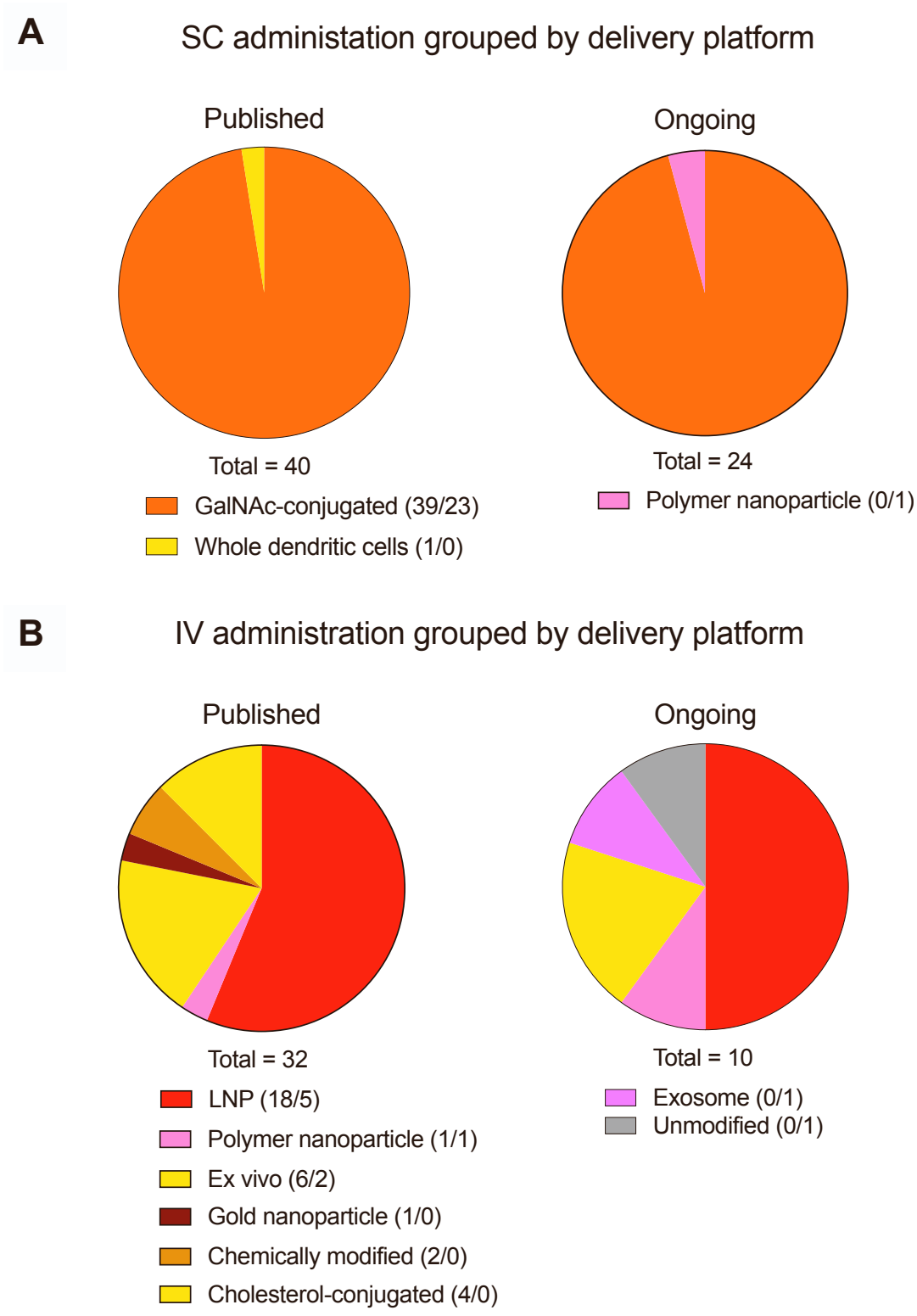

Figure S2: SC or IV administration grouped by delivery platform. (A-B) Overall distribution of the types of delivery platforms used in combination with SC or IV administration in the published/ongoing clinical trials. Color coded annotations are included for every distribution.

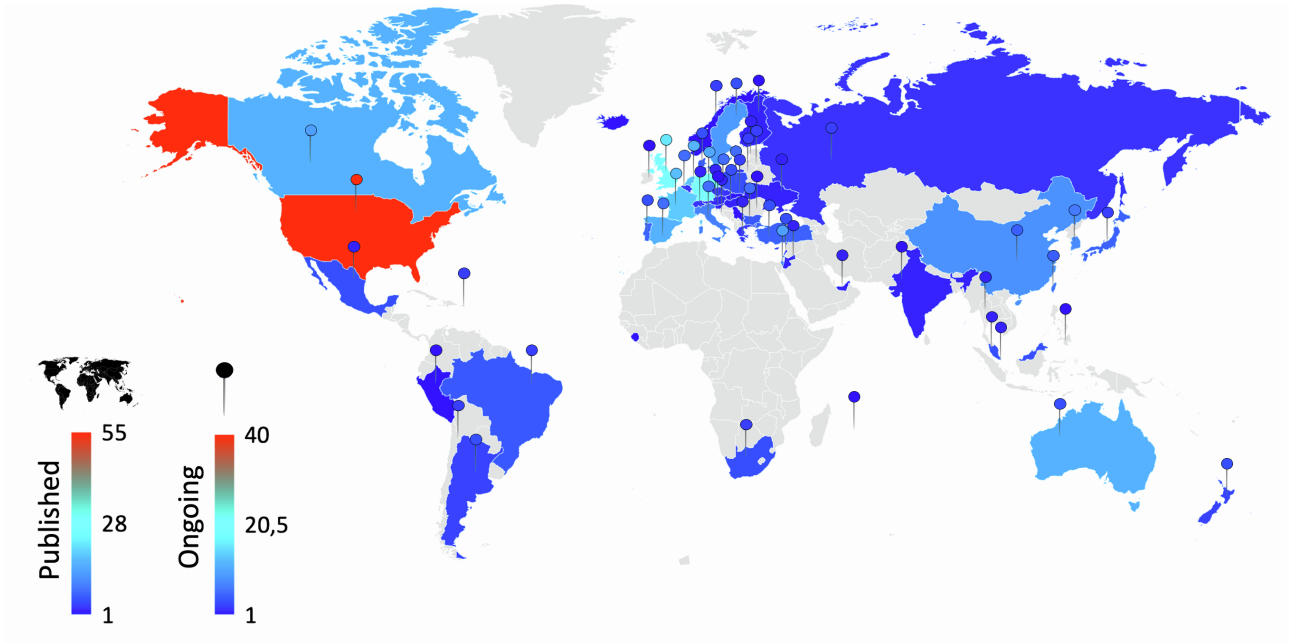

Figure S3: The world of clinical RNAi. Heat map showing the countries from where the patients of the published and ongoing RNAi-based clinical trials have been or are being recruited from. Color-coded annotations are included on the left side. “Red” correlates with the highest number of studies recruiting patients from the involved country, whereas “dark blue” correlates with the lowest number of studies. Grey color indicate that no patients were recruited from the involved country. The countries involved in the published clinical trials are represented by the color on the map, whereas the countries involved in the ongoing clinical trials are represented by the colors on the pins on the map.

Table S1: Overview of the included published cinical trials. Characteristics of the 90 published clinical trials included in this review. The * indicates that the NCT number was not found in the article, but was found manually by the reviewers, who screened the clinical trials. The - indicates that no NCT was found in the article nor manually.

Nr.	Name of first author	Title	Year of publication	NCT
1	DeVincenzo, John	Evaluation of the safety, tolerability and pharmacokinetics of ALN-RSV01, a novel RNAi antiviral therapeutic directed against respiratory syncytial virus (RSV)	2008	-
2	Wyszko, Eliza	A multivariate analysis of patients with brain tumors treated with ATN-RNA	2008	-
3	DeVincenzo, John	A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus	2010	NCT00496821
4	DiGiusto, David L.	RNA-Based Gene Therapy for HIV With Lentiviral Vector-Modified CD34(+) Cells in Patients Undergoing Transplantation for AIDS-Related Lymphoma	2010	-
5	Leachman, Sancy A.	First-in-human Mutation-targeted siRNA Phase Ib Trial of an Inherited Skin Disorder	2010	NCT00716014
6	Kaiser, Peter K.	RNAi-Based Treatment for Neovascular Age-Related Macular Degeneration by Sirna-027	2010	NCT00363714
7	Gish, Robert G	RNA interference and its potential applications to chronic HBV treatment: results of a Phase I safety and tolerability study	2011	-
8	Zamora, Martin R.	RNA Interference Therapy in Lung Transplant Patients Infected with Respiratory Syncytial Virus	2011	NCT00658086
9	Nguyen, Quan Dong	Dose-Ranging Evaluation of Intravitreal siRNA PF-04523655 for Diabetic Macular Edema (the DEGAS Study)	2012	NCT00701181
10	Nguyen, Quan Dong	Evaluation of the siRNA PF-04523655 versus Ranibizumab for the Treatment of Neovascular Age-related Macular Degeneration (MONET Study)	2012	NCT00713518
11	Nguyen, Quan Dong	Phase 1 dose-escalation study of a siRNA targeting the RTP801 gene in age-related macular degeneration patients	2012	NCT00725686
12	Senzer, Neil	Phase I Trial of "bi-shRNAi(furin)/GMCSF DNA/Autologous Tumor Cell" Vaccine (FANG) in Advanced Cancer	2012	-

13	Coelho, Teresa	Safety and Efficacy of RNAi Therapy for Transthyretin Amyloidosis	2013	NCT01148953
14	Coelho, Teresa	Safety and Efficacy of RNAi Therapy for Transthyretin Amyloidosis	2013	NCT01559077
15	Tabernero, Josep	First-in-Humans Trial of an RNA Interference Therapeutic Targeting VEGF and KSP in Cancer Patients with Liver Involvement	2013	NCT00882180
16	Tabernero, Josep	First-in-Humans Trial of an RNA Interference Therapeutic Targeting VEGF and KSP in Cancer Patients with Liver Involvement	2013	NCT01158079
17	Fitzgerald, Kevin	Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial	2014	NCT01437059
18	Moreno-Montanes, Javier	Phase I Clinical Trial of SYL040012, a Small Interfering RNA Targeting beta-Adrenergic Receptor 2, for Lowering Intraocular Pressure	2014	NCT00990743
19	Nemunaitis, John	Summary of bi-shRNA(furin)/GM-CSF Augmented Autologous Tumor Cell Immunotherapy (FANG (TM)) in Advanced Cancer of the Liver	2014	-
20	Schultheis, Beate	First-in-Human Phase I Study of the Liposomal RNA Interference Therapeutic Atu027 in Patients With Advanced Solid Tumors	2014	NCT00938574
21	Zuckerman, Jonathan E.	Correlating animal and human phase Ia/Ib clinical data with CALAA-01, a targeted, polymer-based nanoparticle containing siRNA	2014	NCT00689065*
22	Barve, Minal	Phase 1 Trial of Bi-shRNA STMN1 BIV in Refractory Cancer	2015	NCT01505153*
23	Golan, Talia	RNAi therapy targeting KRAS in combination with chemotherapy for locally advanced pancreatic cancer patients	2015	NCT01188785
24	Suhr, Ole B.	Efficacy and safety of patisiran for familial amyloidotic polyneuropathy: a phase II multi-dose study	2015	NCT01617967
25	Gottlieb, Jens	ALN-RSV01 for prevention of bronchiolitis obliterans syndrome after respiratory syncytial virus. infection in lung transplant recipients	2016	NCT01065935

26	Oh, Jonathan	Phase II study of Vigil DNA engineered immunotherapy as maintenance in advanced stage ovarian cancer	2016	-
27	Dunning, Jake	Experimental Treatment of Ebola Virus Disease with TKM-130803: A Single-Arm Phase 2 Clinical Trial	2016	-
28	Adams, David	Trial design and rationale for APOLLO, a Phase 3, placebo-controlled study of patisiran in patients with hereditary ATTR amyloidosis with polyneuropathy	2017	NCT01960348
29	Beg, Muhammad S.	Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors	2017	NCT01829971
30	Fitzgerald, Kevin	A Highly Durable RNAi Therapeutic Inhibitor of PCSK9	2017	NCT02314442
31	Pasi, K. John	Targeting of Antithrombin in Hemophilia A or B with RNAi Therapy	2017	NCT02035605
32	Ray, Kausik K.	Inclisiran in Patients at High Cardiovascular Risk with Elevated LDL Cholesterol	2017	NCT02597127
33	Schluep, Thomas	Safety, Tolerability, and Pharmacokinetics of ARC-520 Injection, an RNA Interference-Based Therapeutic for the Treatment of Chronic Hepatitis B Virus Infection, in Healthy Volunteers	2017	NCT01872065
34	Suzuki, Kenji	Phase 1 Clinical Study of siRNA Targeting Carbohydrate Sulphotransferase 15 in Crohn's Disease Patients with Active Mucosal Lesions	2017	-
35	van Zandwijk, Nico	Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: a first-in-man, phase 1, open-label, dose-escalation study	2017	NCT02369198
36	Wooddell, Christine	RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg	2017	NCT02065336*
37	Zimmermann, Tracy S.	Clinical Proof of Concept for a Novel Hepatocyte-Targeting GalNAc-siRNA Conjugate	2017	NCT01814839
38	Turner, Alice M.	Hepatic-targeted RNA interference provides robust and persistent knockdown of alpha-1 antitrypsin levels in ZZ patients	2018	NCT02363946
39	Wang, Danhong	Efficacy of intracellular immune checkpoint-silenced DC vaccine	2018	NCT01956630

40	El Dika, Imane	An Open-Label, Multicenter, Phase I, Dose Escalation Study with Phase II Expansion Cohort to Determine the Safety, Pharmacokinetics, and Preliminary Antitumor Activity of Intravenous TKM-080301 in Subjects with Advanced Hepatocellular Carcinoma	2019	NCT02191878
41	Kavita, Uma	A Fit-for-Purpose Method for the Detection of Human Antibodies to Surface-Exposed Components of BMS-986263, a Lipid Nanoparticle-Based Drug Product Containing a siRNA Drug Substance	2019	-
42	Sardh, Eliane	Phase 1 Trial of an RNA Interference Therapy for Acute Intermittent Porphyria	2019	NCT02452372
43	Delville, Marianne	Safety of CD34+ Hematopoietic Stem Cells and CD4+ T Lymphocytes Transduced with LVsh5/C46 in HIV-1 Infected Patients with High-Risk Lymphoma	2019	NCT03593187*
44	Brandts, Julia	Clinical implications and outcomes of the ORION Phase III trials	2020	NCT02963311
45	Brandts, Julia	Clinical implications and outcomes of the ORION Phase III trials	2020	NCT03060577
46	Brandts, Julia	Clinical implications and outcomes of the ORION Phase III trials	2020	NCT03851705
47	Brandts, Julia	Clinical implications and outcomes of the ORION Phase III trials	2020	NCT03060577
48	Coelho, Teresa	A phase II, open-label, extension study of long-term patisiran treatment in patients with hereditary transthyretin-mediated (hATTR) amyloidosis	2020	NCT01961921
49	Ray, Kausik K.	Two Phase 3 Trials of Inclisiran in Patients with Elevated LDL Cholesterol	2020	NCT03399370
50	Ray, Kausik K.	Two Phase 3 Trials of Inclisiran in Patients with Elevated LDL Cholesterol	2020	NCT03400800
51	Balwani, Manisha	Phase 3 Trial of RNAi Therapeutic Givosiran for Acute Intermittent Porphyria	2020	NCT03338816
52	Raal, Frederick J.	Inclisiran for the Treatment of Heterozygous Familial Hypercholesterolemia	2020	NCT03397121
53	Judge, Daniel P.	Phase 3 Multicenter Study of Revusiran in Patients with Hereditary Transthyretin-Mediated (hATTR) Amyloidosis with Cardiomyopathy (ENDEAVOUR)	2020	NCT02319005

54	Wright, R. Scott	Effects of Renal Impairment on the Pharmacokinetics, Efficacy, and Safety of Inclisiran: An Analysis of the ORION-7 and ORION-1 Studies	2020	NCT03159416
55	Yuen, Man-Fung	RNA Interference Therapy With ARC-520 Results in Prolonged Hepatitis B Surface Antigen Response in Patients With Chronic Hepatitis B Infection	2020	NCT02604199
56	Yuen, Man-Fung	RNA Interference Therapy With ARC-520 Results in Prolonged Hepatitis B Surface Antigen Response in Patients With Chronic Hepatitis B Infection	2020	NCT02604212
57	Chen, Li-Yun	Successful application of anti-CD19 CAR-T therapy with IL-6 knocking down to patients with central nervous system B-cell acute lymphocytic leukemia	2020	NCT03064269
58	Schultheis, Beate	Safety, efficacy and pharcacokinetics of targeted therapy with the liposomal RNA interference therapeutic atu027 combined with gemcitabine in patients with pancreatic adenocarcinoma. A randomized phase Ib/IIa study	2020	NCT01808638
59	Garrelfs, Sander F.	Lumasiran, an RNAi Therapeutic for Primary Hyperoxaluria Type 1	2021	NCT03681184
60	Esrick, Erica B.	Post-Transcriptional Genetic Silencing of BCL11A to Treat Sickle Cell Disease	2021	NCT03282656
61	Badri, Prajakta	Pharmacokinetic and Pharmacodynamic Properties of Cemdisiran, an RNAi Therapeutic Targeting Complement Component 5, in Healthy Subjects and Patients with Paroxysmal Nocturnal Hemoglobinuria	2021	NCT02352493
62	Gupta, Sheha V. Gupta	Clinical and Preclinical Single-Dose Pharmacokinetics of VIR-2218, an RNAi Therapeutic Targeting HBV Infection	2021	NCT03672188
63	Kumthekar, Priya	A first-in-human phase 0 clinical study of RNA interference-based spherical nucleic acids in patients with recurrent glioblastoma	2021	NCT03020017
64	Adams, David	Long-term safety and efficacy of patisiran for hereditary transthyretin-mediated amyloidosis with polyneuropathy: 12-month results of an open-label extension study	2021	NCT02510261

65	Frishberg, Yaacov	Phase 1/2 Study of Lumasiran for Treatment of Primary Hyperoxaluria Type 1 A Placebo-Controlled Randomized Clinical Trial	2021	NCT02706886
66	Thielmann, Matthias	Teprasiran, a Small Interfering RNA, for the Prevention of Acute Kidney Injury in High-Risk Patients Undergoing Cardiac Surgery A Randomized Clinical Study	2021	NCT02610283
67	Vassiliou, Daphne	A Drug-Drug Interaction Study Evaluating the Effect of Givosiran, a Small Interfering Ribonucleic Acid, on Cytochrome P450 Activity in the Liver	2021	NCT03505853
68	To-Figueras, Jordi	Dysregulation of homocysteine homeostasis in acute intermittent porphyria patients receiving heme arginate or givosiran	2021	-
69	Gane, Ed	JNJ-73763989 pharmacokinetics and safety: Liver-targeted siRNAs against hepatitis B virus, in Japanese and non-Japanese healthy adults, and combined with JNJ-56136379 and a nucleos(t)ide analogue in patients with chronic hepatitis B	2022	NCT03365947
70	Gane, Ed	JNJ-73763989 pharmacokinetics and safety: Liver-targeted siRNAs against hepatitis B virus, in Japanese and non-Japanese healthy adults, and combined with JNJ-56136379 and a nucleos(t)ide analogue in patients with chronic hepatitis B	2022	NCT04002752
71	Gong, Wen-Jie	Investigation of the risk factors to predict cytokine release syndrome in relapsed or refractory B-cell acute lymphoblastic leukemia patients receiving IL-6 knocking down anti-CD19 chimeric antigen receptor T-cell therapy	2022	NCT03275493
72	Hoppe, Bernd	Safety, pharmacodynamics, and exposure-response modeling results from a first-in-human phase 1 study of nedosiran (PHYOX1) in primary hyperoxaluria	2022	NCT03392896
73	Kallend, David	An evaluation of a supratherapeutic dose of inclisiran on cardiac repolarization in healthy volunteers: A phase I, randomized study	2022	-
74	Koren, Michael J.	Preclinical development and phase 1 trial of a novel siRNA targeting lipoprotein(a)	2022	NCT03626662
75	Lawitz, Eric J.	BMS-986263 in patients with advanced hepatic fibrosis: 36-week results from a randomized, placebo-controlled phase 2 trial	2022	NCT03420768

76	Nissen, Steven E.	Single Ascending Dose Study of a Short Interfering RNA Targeting Lipoprotein(a) Production in Individuals With Elevated Plasma Lipoprotein(a) Levels	2022	NCT04606602
77	Rejman, M. Doortje	Rationale and design of two trials assessing the efficacy, safety, and tolerability of inclisiran in adolescents with homozygous and heterozygous familial hypercholesterolaemia	2022	NCT04659863
78	Rejman, M. Doortje	Rationale and design of two trials assessing the efficacy, safety, and tolerability of inclisiran in adolescents with homozygous and heterozygous familial hypercholesterolaemia	2022	NCT04652726
79	Rocconi, Rodney P.	Proof of principle study of sequential combination atezolizumab and Vigil in relapsed ovarian cancer	2022	NCT03073525
80	Sohn, Winnie	Pharmacokinetics, Pharmacodynamics, and Tolerability of Olpasiran in Healthy Japanese and Non-Japanese Participants: Results from a Phase I, Single-dose, Open-label Study	2022	-
81	Strnad, Pavel	Fazirsiran for Liver Disease Associated with Alpha(1)-Antitrypsin Deficiency	2022	NCT03946449
82	Sas, David J.	Phase 3 trial of lumasiran for primary hyperoxaluria type 1: A new RNAi therapeutic in infants and young children	2022	NCT03905694
83	Adams, David	Efficacy and safety of vutrisiran for patients with hereditary transthyretin-mediated amyloidosis with polyneuropathy: a randomized clinical trial	2022	NCT03759379
84	Kallend, David	Pharmacokinetics and pharmacodynamics of inclisiran, a small interfering RNA therapy, in patients with hepatic impairment	2022	-
85	O'Donoghue, Michelle	Small Interfering RNA to Reduce Lipoprotein(a) in Cardiovascular Disease	2022	NCT04270760
86	Schmidt, Hartmu H.	Patisiran treatment in patients with hereditary transthyretin-mediated amyloidosis with polyneuropathy after liver transplantation	2022	NCT03862807
87	Ventura, Paolo	Efficacy and safety of givosiran for acute hepatic porphyria: 24-month interim analysis of the randomized phase 3 ENVISION study	2022	NCT03338816
88	Li, Haiyan	Pharmacokinetics, Safety, and Tolerability of the siRNA JNJ-73763989 in Healthy Chinese Adult Participants	2023	NCT04586439

89	Mak, Lung-Yi	A phase I/II study of ARO-HSD, an RNA interference therapeutic, for the treatment of non-alcoholic steatohepatitis	2023	NCT04202354
90	Michael, Mini	Lumasiran for Advanced Primary Hyperoxaluria Type 1: Phase 3 ILLUMINATE-C Trial	2023	NCT04152200

Table S2: Overview of the included ongoing clinical trials. Characteristics of the 48 ongoing clinical trials included in this review.

Nr.	Sponsor	Title	Year of clinical start date	NCT
1	Genzyme, a Sanofi Company	An Open-label Extension Study of an Investigational Drug, Fitusiran, in Patients with Moderate or Severe Hemophilia A or B	2015	NCT02554773
2	M.D. Anderson Cancer Center	EphA2 siRNA in Treating Patients With Advanced or Recurrent Solid Tumors	2015	NCT01591356
3	AIDS Malignancy Consortium	Gene Therapy in Treating Patients With Human Immunodeficiency Virus-Related Lymphoma Receiving Stem Cell Transplant	2016	NCT02797470
4	Gradalis, Inc.	Pbi-shRNA™ EWS/FLI1 Type 1 LPX in Subjects With Advanced Ewing's Sarcoma	2016	NCT02736565
5	Gradalis, Inc.	Trial of Atezolizumab and Vigil for Advanced Gynecological Cancers (A Companion Study to CL-PTL-119)	2017	NCT03073525
6	University of Oxford	A Randomized Trial Assessing the Effects of Inclisiran on Clinical Outcomes Among People With Cardiovascular Disease (ORION-4)	2018	NCT03705234
7	Alnylam Pharmaceuticals	A Study to Evaluate Lumasiran in Children and Adults With Primary Hyperoxaluria Type 1 (ILLUMINATE-A)	2018	NCT03681184
8	Gradalis, Inc	Vigil + Irinotecan and Temozolomide in Ewing's Sarcoma (VITA)	2018	NCT03495921
9	Alnylam Pharmaceuticals	An Extension Study of an Investigational Drug, Lumasiran (ALN-GO1), in Patients With Primary Hyperoxaluria Type 1	2018	NCT03350451
10	Boston Children's University	Gene Transfer for Sickle Cell Disease	2018	NCT03282656
11	Silenseed Ltd	A Phase 2 Study of siG12D LODER in Combination With Chemotherapy in Patients With Locally Advanced Pancreatic Cancer (PROTACT)	2018	NCT01676259
12	Alnylam Pharmaceuticals	HELIOS-B: A Study to Evaluate Vutrisiran in Patients With Transthyretin Amyloidosis With Cardiomyopathy	2019	NCT04153149

13	Dicerna Pharmaceuticals, Inc.	Long Term Extension Study in Patients With Primary Hyperoxaluria (PHYOX3)	2019	NCT04042402
14	Hugel	The Hypertrophic Scar Prevention of BMT101	2019	NCT04012099
15	Alnylam Pharmaceuticals	APOLLO-B: A Study to Evaluate Patisiran in Participants With Transthyretin Amyloidosis With Cardiomyopathy (ATTR Amyloidosis With Cardiomyopathy	2019	NCT03997383
16	Alnylam Pharmaceuticals	A Study of Lumasiran in Infants and Young Children With Primary Hyperoxaluria Type 1 (ILLUMINATE-B)	2019	NCT03905694
17	Nitto BioPharma, Inc.	A Study of NBF-006 in Non-Small Cell Lung, Pancreatic, or Colorectal Cancer	2019	NCT03819387
18	Novartis Pharmaceuticals	Trial to Assess the Effect of Long-Term Dosing of Inclisiran in Subjects With High CV Risk and Elevated LDL-C (ORION-8)	2019	NCT03814187
19	Sirnaomics	A Study of STP707 Administered by IV in Healthy Subjects	2020	NCT05309915
20	InteRNA	First-in-Human Study of INT-1B3 in Patients With Advanced Solid Tumors	2020	NCT04675996
21	Alnylam Pharmaceuticals	A Study of ALN-HSD in Healthy Adult Subjects and Adult Patients With Nonalcoholic Steatohepatitis (NASH)	2020	NCT04565717
22	Hoffmann-La Roche	A Trial To Evaluate The Efficacy And Safety Of Multiple Combination Therapies In Participants With Chronic Hepatitis B (Piranga)	2020	NCT04225715
23	Alnylam Pharmaceuticals	A Study to Evaluate Lumasiran in Patients With Advanced Primary Hyperoxaluria Type 1 (ILLUMINATE-C)	2020	NCT04152200
24	4D Molecular Therapeutics	4D-150 in Patients With Neovascular (Wet) Age-Related Macular Degeneration	2021	NCT05197270
25	Alnylam Pharmaceuticals	Zilebesiran as Add-on Therapy in Patients With Hypertension Not Adequately Controlled by a Standard of Care Antihypertensive Medication (KARDIA-2) (KARDIA-2)	2021	NCT05103332
26	Novartis Pharmaceuticals	Study of Inclisiran to Prevent Cardiovascular (CV) Events in Participants With Established Cardiovascular Disease (VICTORION-2P)	2021	NCT05030428

27	Alnylam Pharmaceuticals	A Study to Evaluate Efficacy and Safety of ALN-AGT01 in Patients With Mild To-Moderate Hypertension (KARDIA-1) (KARDIA-1)	2021	NCT04936035
28	Olix Pharmaceuticals, Inc.	Study to Evaluate Efficacy of OLX10010 in Reducing Recurrence of Hypertrophic Scarring After Scar Revision Surgery	2021	NCT04877756
29	Sirnaomics	A Study to Evaluate Safety, Efficacy of Intralesional Injection of STP705 in Patients With isSCC	2021	NCT04844983
30	Sirnaomics	A Study for Safety and Efficacy Evaluation of Various Doses of STP705 in Reducing Keloid Recurrence	2021	NCT04844840
31	Assembly Biosciences	A Study Evaluating Treatment Regimens Containing Vebicorvir (ABI-H0731) in Participants With Chronic Hepatitis B Infection	2021	NCT04820686
32	Sylentis, S.A.	Tivanisiran for Dry Eye in Subjects With Sjögren's Syndrome	2021	NCT04819269
33	Novartis Pharmaceuticals	Study of Efficacy and Safety of Inclisiran in Asian Participants With Atherosclerotic Cardiovascular Disease (ASCVD) or ASCVD High Risk and Elevated Low Density Lipoprotein Cholesterol (LDL-C)	2021	NCT04765657
34	Novartis Pharmaceuticals	Study to Evaluate Efficacy and Safety of Inclisiran in Adolescents With Homozygous Familial Hypercholesterolemia (ORION-13)	2021	NCT04659863
35	Novartis Pharmaceuticals	Study to Evaluate Efficacy and Safety of Inclisiran in Adolescents With Heterozygous Familial Hypercholesterolemia (ORION-16)	2021	NCT04652726
36	M.D. Anderson Cancer Center	iExosomes in Treating Participants With Metastatic Pancreas Cancer With KrasG12D Mutation	2021	NCT03608631
37	Oneness Biotech Co., Ltd.	A Phase I Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of Single and Multiple Ascending Doses of Inhaled MBS-COV (SNS812) in Healthy Participants	2022	NCT05677893
38	Sylentis, S.A.	A Randomized, Double Masked, Parallel Group, Dose-finding Study to Evaluate SYL1801 in Patients With Neovascular Age-related Macular Degeneration (AMD)	2022	NCT05637255
39	Vir Biotechnology, Inc.	Effect of Hepatic Impairment on the Pharmacokinetics and Safety of VIR-2218 and VIR-3434	2022	NCT05484206

40	Sirnaomics	A Study to Evaluate Safety, Tolerability of Subcutaneous Injection in Adult Subjects Undergoing Abdominoplasty	2022	NCT05422378
41	Boston Children's University	A Gene Transfer Study Inducing Fetal Hemoglobin in Sickle Cell Disease (GRASP, BMT CTN 2001) (GRASP)	2022	NCT05353647
42	Sylentis, S.A.	Safety Study of Tivanisiran to Treat Dry Eye (FYDES)	2022	NCT05310422
43	Alnylam Pharmaceuticals	A Study to Evaluate ALN-XDH in Healthy Subjects and Patients With Gout	2022	NCT05256810
44	City of Hope Medical Center	CpG-STAT3 siRNA CAS3/SS3 and Localized Radiation Therapy for the Treatment of Relapsed/Refractory B-Cell NHL	2022	NCT04995536
45	Lemonex Inc.	A Study of Single Ascending Dose of LEM-S401 in Healthy Participants	2022	NCT04707131
46	Olix Pharmaceuticals, Inc.	Evaluation of OLX10212 in Patients With Neovascular Age-related Macular Degeneration	2023	NCT05643118
47	Silence Therapeutics plc	Evaluate SLN360 in Participants With Elevated Lipoprotein(a) at High Risk of Atherosclerotic Cardiovascular Disease Events	2023	NCT05537571
48	Silence Therapeutics plc	Study to Assess SLN124 in Patients With Polycythemia Vera (SLN)	2023	NCT05499013

Table S3: Studies presenting with two or more clinical trials with individual NCT number. Overview of the included studies presenting with two or more individual NCT numbers.

Title	Name of first author	Year of publication	Number of different NCT number	The different NCT numbers
Safety and Efficacy of RNAi Therapy for Transthyretin Amyloidosis	Coelho, Teresa	2013	2	NCT01148953 NCT01559077
First-in-Humans Trial of an RNA Interference Therapeutic Targeting VEGF and KSP in Cancer Patients with Liver Involvement	Tabernero, Josep	2013	2	NCT00882180 NCT01158079
Clinical implications and outcomes of the ORION Phase III trials	Brandts, Julia	2020	4	NCT02963311 NCT03060577 NCT03851705 NCT03060577
Two Phase 3 Trials of Inclisiran in Patients with Elevated LDL Cholesterol	Ray, Kausik K.	2020	2	NCT03399370 NCT03400800
RNA Interference Therapy With ARC-520 Results in Prolonged Hepatitis B Surface Antigen Response in Patients With Chronic Hepatitis B Infection	Yuen, Man-Fung	2020	2	NCT02604199 NCT02604212
JNJ-73763989 pharmacokinetics and safety: Liver-targeted siRNAs against hepatitis B virus, in Japanese and non-Japanese healthy adults, and combined with JNJ-56136379 and a nucleos(t)ide analogue in patients with chronic hepatitis B	Gane, Ed	2022	2	NCT03365947 NCT04002752
Rationale and design of two trials assessing the efficacy, safety, and tolerability of inclisiran in adolescents with homozygous and heterozygous familial hypercholesterolaemia	Reijman, M. Doortje	2022	2	NCT04659863 NCT04652726

Table S4: Types of *ex vivo* delivery. Table summarizing the different types of *ex vivo* administration in the published/ongoing trials, shown in Fig. 4A**.** "Number" indicates the total number of studies utilizing the type of *ex vivo* administration.

Published clinical trials		**Ongoing clinical trials**	
Type	**Number**	**Type**	**Number**
DNA transfection of tumor cells	4	CD34+ cells transduced with lentivirus	3
T lymphocytes or hematopoietic stem cells transduced with lentivirus	3	DNA transfection of tumor cells	2
CAR-T cells transduced with lentivirus	1	Adenovirus transduction	1
Adenovirus transduction	2		
Minicells	1		

Table S5: Disease etiology. Diseases treated with RNAi therapeutics grouped by disease etiology. Information regarding the name of the RNAi-based drug, the number of times the RNAi therapeutic has been used in published/ongoing clinical trials, and total number of diseases treated with the RNAi therapeutics in published/ongoing clinical trials. The color of the boarder of the organ-boxes correlates with the color-coded annotations in Fig. 6. ASCVD, Atherosclerotic cardiovascular disease; hATTR, Hereditary ATTR amyloidosis; RSV, Respiratory Syncytial Virus; HBV, hepatitis B virus; HIV-1, Human Immunodeficiency Virus-1; wAMD, wet Age-dependent Macular Degeneration; DME, Diabetic Macular Edema; IOP, Intraocular Pressure.

Organ	Disease	Name of RNAi-based drug and number included in published/ongoing clinical trials	Total number of diseases treated in published/ongoing clinical trials
Cardiovascular			19/9
	ASCVD	Inclisiran	15/6
	Elevated Apolipoprotein A (cardiovascular disease) and hypercholesterolemia	Olpasiran: 3/0	4/1
		SLN360: 1/1	
	Hypertension	ALALN-AGT01	0/2
Tumor			19/11
	Cancer with CNS involvement	ATN-RNA:1	2/0
		NU-0129: 1	
	Advanced cancer	FANG/VIGIL: 2/0	6/4
		Atu27:1/0	
		CALAA-01: 1/0	
		STMN1: 1/0	
		MRX34: 1/0	
		EphA2 siRNA: 0/1	
		NBF-006: 0/1	
		INT-1B3: 0/1	
		ALN-HSD: 0/1	
	Cancer with liver involvement	ALN-VSP: 2/0	3/0
		TKM-080301: 1/0	
	Recurrent Malignant Pleural Mesothelioma	TargomiR	1/0
	Central nervous system B-cell acute lymphocytic leukemia	ssCART-19s	1/0
	Cancer with pancreas involvement	siG12D-LODER: 1/2	2/2
		ATU027: 1	

	Relapsed or Refractory B-cell acute lymphoblastic leukemia	ssCAR-T19	1/0
	Acute leukemia	Ad-siSSF-DC	1/0
	Recurrent B-Cell Non-Hodgkin Lymphoma	CAS3/SS3	0/1
	Cancer with female reproductive organs involved	FANG/VIGIL: 2/1	2/1
	Squamous Cell Carcinoma	STP705	0/1
	Ewings Sarcoma	pbi-shRNA: 0/1	0/2
		Vigil: 0/1	
Liver			18/3
	hATTR	Patisiran: 6/1	10/2
		ALN-TTR01: 1/0	
		Revusiran: 2/0	
		Vutrisiran: 1/1	
	Acute hepatic porphyrias	Givosiran	5/0
	Advanced hepatic fibrosis	BMS-986263	2/1
	Non-alcoholic steatohepatitis	ARO-HSD	1/0
Viral			16/5
	RSV	ALN-RSV01	4/0
	Chronic HBV	ARC-520: 6/0	9/3
		JNJ-73763989: 2/0	
		JNJ-73763989 and JNJ-56136379: 1/0	
		RO7445482: 0/1	
		AB-729: 0/1	
		VIR-2218: 0/1	
	Ebola virus	TKM-13080	1/0
	HIV-1 infection	LVsh5/C46: 1/0	2/1
		HIV-shI-TAR-CCR5RZ: 1/0	
		CCR5 shRNA/TRIM5alpha/TAR decoy: 0/1	
	Coronavirus	MBS-COV	0/1
Kidney			6/5
	Primary Hyperoxaluria Type 1	Lumasiran: 4/4	5/5
		Nedosiran: 1/0	
		DCR-PHXC: 0/1	

	Acute Kidney Injury	Teprasiran	1/0
Eye			5/5
	wAMD	AGN-211745: 1/0	3/3
		PF-04523655: 2/0	
		OLX10212: 0/1	
		SYL1801: 0/1	
		4D-150: 0/1	
	DME	PF-04523655	1/0
	Elevated IOP associated to glaucoma	SYL040012	1/0
	Dry Eye Disease	Tivanisiran (SYL1001)	0/2
Blood			3/4
	Hemophelia A or B	Nedosiran: 1/0	1/1
		Fitusiran: 0/1	
	Sickle cell disease	BCH-BB694: 1/0	1/2
		CD34+ HSC-BCL11a: 0/2	
	Paroxysmal nocturnal hemoglobinuria	Cemdisiran	1/0
	Polycythemia Vera	SLN124	0/1
Skin			1/4
	Pachyonychia congenita	TD101	1/0
	Keloid	STP705	0/1
	Scar prevention	LEM-S401: 0/1	0/3
		BMT101: 0/1	
		OLX10010: 0/1	
Respiratory			2/0
	Alpha-1 antitrypsin deficiency	ARC-AAT	2/0
Gastrointestinal tract			1/0
	Chrons Disease	STNM01	1/0
Joint			0/1
	Gout	ALN-ZDH	0/1
Lipid-tissue			0/1
	Decrease inflammation to decrease abdominal obesity	STP705	0/1

Table S6: Tissue-targets of RNAi therapeutic. Diseases treated with RNAi therapeutics grouped by tissues targeted by the effector. Information regarding the name of the RNAi-based drug, the number of times the RNAi therapeutic has been used in published/ongoing clinical trials, and total number of diseases treated with RNAi therapeutic in published/ongoing clinical trials. The color of the boarder of the organ-boxes correlates with the color-coded annotations in Fig. 7 ASCVD, Atherosclerotic cardiovascular disease; hATTR, Hereditary ATTR amyloidosis; RSV, Respiratory Syncytial Virus; HBV, hepatitis B virus; HIV-1, Human Immunodeficiency Virus-1; wAMD, wet Age-dependent Macular Degeneration; DME, Diabetic Macular Edema; IOP, Intraocular Pressure.

Organ	Disease	Name of drug and how many (published/ongoing)	Number in total (published/ongoing)
Tumor			19/11
	Cancer with CNS involvement	ATN-RNA:1/0	2/0
		NU-0129: 1/0	
	Advanced cancer	FANG/VIGIL: 2/0	6/4
		Atu27:1/0	
		CALAA-01: 1/0	
		STMN1: 1/0	
		MRX34: 1/0	
		EphA2 siRNA: 0/1	
		NBF-006: 0/1	
		INT-1B3: 0/1	
		ALN-HSD: 0/1	
	Cancer with liver involvement	ALN-VSP: 2/0	3/0
		TKM-080301: 1/0	
	Recurrent Malignant Pleural Mesothelioma	TargomiR	1/0
	Central nervous system B-cell acute lymphocytic leukemia	ssCART-19s	1/0
	Cancer with pancreas involvement	siG12D-LODER: ½	2/2
		ATU027: 1/0	
	Relapsed or Refractory B-cell acute lymphoblastic leukemia	ssCAR-T19	1/0
	Acute leukemia	Ad-siSSF-DC	1/0
	Recurrent B-Cell Non-Hodgkin Lymphoma	CAS3/SS3	0/1

	Cancer with female reproductive organs involved	FANG/VIGIL: 2/1	2/1
	Squamous Cell Carcinoma	STP705	0/1
	Ewings Sarcoma	pbi-shRNA: 1/0	0/2
		Vigil: 1/0	
Liver			46/20
	hATTR	Patisiran: 6/1	10/2
		ALN-TTR01: 1/0	
		Revusiran: 2/0	
		Vutrisiran: 1/1	
	Hemophelia A or B	Nedosiran: 1/0	1/1
		Fitusiran: 0/1	
	Alpha-1 antitrypsin deficiency (AATD)	ARC-AAT	2/0
	Acute hepatic porphyrias	Givosiran	5/0
	Atherosclerotic cardiovascular disease (ASCVD) and hypercholesterolemia	Inclisiran	15/6
	Primary Hyperoxaluria Type 1	Lumasiran: 4/4	5/5
		Nedosiran: 1/0	
		Lumasiran: 4	
		DCR-PHXC: 0/1	
	Paroxysmal nocturnal hemoglobinuria	Cemdisiran	1/0
	Elevated Apolipoprotein A (cardiovascular disease)	Olpasiran: 3/0	4/1
		SLN360: 1/1	
	Advanced hepatic fibrosis	BMS-986263	2/1
	Non-alcoholic steatohepatitis	ARO-HSD	1/0
	Polycythemia Vera	SLN124	0/1
	Hypertension	ALN-AGT01	0/2
	Gout	ALN-ZDH	0/1
Viral			16/5
	RSV	ALN-RSV01	4/0
	Chronic HBV	ARC-520: 6/0	9/3
		JNJ-73763989: 2/0	

		JNJ-73763989 and JNJ-56136379: 1/0	
		RO7445482: 0/1	
		AB-729: 0/1	
		VIR-2218: 0/1	
	Ebola virus	TKM-13080	1/0
	HIV-1 infection	LVsh5/C46: 1/0	2/1
		HIV-shI-TAR-CCR5RZ: 1/0	
		CCR5/shRNA/TRIM5alpha/TAR decoy: 0/1	
	Coronavirus	MBS-COV	0/1
Kidney			1/0
	Acute Kidney Injury	Teprasiran	1/0
Eye			5/5
	Wet Age-dependent Macular Degeneration (wAMD)	AGN-211745: 1/0	3/3
		PF-04523655: 2/0	
		OLX10212: 0/1	
		SYL1801: 0/1	
		4D-150: 0/1	
	Diabetic Macular Edema (DME)	PF-04523655	1/0
	Elevated IOP associated to glaucoma	SYL040012	1/0
	Dry Eye Disease	Tivanisiran (SYL1001)	0/2
Blood			1/2
	Sickle cell disease	BCH-BB694: 1/0	1/2
		CD34+ HSC-BCL11a: 0/2	
Skin			1/4
	Pachyonychia congenita (PC)	TD101	1/0
	Keloid	STP705	0/1
	Scar prevention	LEM-S401: 0/1	0/3
		BMT101: 0/1	
		OLX10010: 0/1	
Gastrointestinal tract			1/0
	Chrons Disease	STNM01	1/0
Lipid-tissue			0/1
	Decrease inflammation to decrease abdominal obesity	STP705	0/1

Table S7: Approved RNAi therapeutics. Characteristics of five FDA and EMA approved RNAi-based drugs: Parisiran, Givosiran, Lumasiran, Inclisiran, Vutrisiran

	Patisiran	**Givosiran**	**Lumasiran**	**Inclisiran**	**Vutrisiran**
Year of approval by FDA and EMA	2018	2019	2020	2021	2022
Disease	Hereditary Transthyretin Amyloidosis (hATTR)	Acute Intermittent Porhyria	Primary Hyperox-aluria Type 1	Hypercholesterolaemia	hATTR
Delivery	Nanoparticle	Naked	Naked	Naked	Naked
Type of delivery	LNP	GalNAc conjugate	GalNAc conjugate	GalNAc conjugate	GalNAc conjugate
Type of RNAi	siRNA	siRNA	siRNA	siRNA	siRNA
Administration	Intravenous	Subcutaneous	Subcutaneous	Subcutaneous	Subcutaneous
Gene target	TTR	ALAS1	HAO1	PCSK9	TTR
Number of trials included in the published clinical trials	5	5	4	15	1
Number of trials included in the ongoing clinical trials	1	0	4	6	1

Table S8: PRISMA 2020 statement checklist.

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title page
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Page 2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page 4
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Page 4
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Page 6 and Supplemental material
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Page 6
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Supplementary material
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Page 6, Supplemental material
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Page 6 and Supplemental material
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Supplemental material
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Supplemental material
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Not relevant
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Page 6
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Page 6 and Supplemental

Section and Topic	Item #	Checklist item	Location where item is reported
			material
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Page 6
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Supplemental material
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Page 6 and Supplemental material
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Not relevant
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Not relevant
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Not relevant
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Not relevant
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Figure S1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Not relevant
Study characteristics	17	Cite each included study and present its characteristics.	Not relevant
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Not relevant
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Not relevant
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Not relevant
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Not relevant
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Not relevant
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Not relevant
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Not relevant
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Not relevant
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Page 10-11
	23b	Discuss any limitations of the evidence included in the review.	Page 14
	23c	Discuss any limitations of the review processes used.	Page 14

Section and Topic	Item #	Checklist item	Location where item is reported
	23d	Discuss implications of the results for practice, policy, and future research.	Page 14
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Page 5
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Page 5 and Supplemental material
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Not relevant
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Title page
Competing interests	26	Declare any competing interests of review authors.	Title page
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Not relevant

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

Table S9: Extraction protocol.

Variable	Categories	Definition of the variable
Article characteristics		
Title, Journal, Authors	**-**	Variables are filled out in spreadsheet automatically. **Important: Check that the variables correspond to the downloaded article to make sure you are extracting from the correct article.** The journal name for a specific journal may differ in the spreadsheet; should just be ignored. May be due to retrieval from different data bases (PubMed and Embase).
Name of last author	-	Enter full name
Name of first author	-	Enter full name
Year of publication	1998-2023	Years corresponding to search period. Choose year of reference publication **not epub** from drop down options.
Study setting (Country)	Categorical variable	Enter the English name of the country where the study was conducted (use this alphabetical list of countries; the spreadsheet should save the name of a country after first entry: https://www.worldometers.info/geography/alphabetical-list-of-countries/). Start with capital letter; make sure spelling is correct. The country should be selected based on the country corresponding to the last author´s affiliation.
Name of drug	-	Enter name

Disease	-	Enter disease
Target	Name of gene	E.g. "*TTR*" (https://www.genecards.org/cgi-bin/carddisp.pl?gene=TTR)
Delivery	Nanoparticle Naked Viral vector Other	
Type of delivery	Description of delivery platform	E.g. Lipid nanoparticle, Lentiviral vector, Cholesterol-conjugated
Type	siRNA miRNA shRNA miR-shRNA	
Administration	Description of administration route	E.g. Intravenous, Subcutaneous, Intranasal, Inhalation

Supplemental Document: The full search strategy. Included are inclusion/exclusion criteria for identification of published/ongoing clinical trials via databases and ClinicalTrials.gov.

Inclusion criteria:
Original research articles on RNA interference published between 1998-2023
Human *in vivo* clinical trials

Exclusion criteria:
Non-original articles
Articles published before Fire and Mello´s 1998-paper[2].
In vitro or *ex vivo* studies
No intervention

Search engines/databases (assessed December 30, 2022 (Web of Science, PubMed and Embase and January 18, 2023 (ClinicalTrials.gov)):
Web of Science (All Databases)
Assessed using Google Chrome Version 108.0.5359.124 (Official version) (arm64)
https://www.webofscience.com/wos/alldb/advanced-search

PubMed (assessed using Google Chrome Version 108.0.5359.124 (Official version) (arm64)
https://pubmed.ncbi.nlm.nih.gov

Embase (assessed using Google Chrome Version 108.0.5359.124 (Official version) (arm64)
https://www.embase.com/search/quick

ClinicalTrials.gov
Assessed using Google Chrome Version 108.0.5359.124 (Official version) (arm64)
https://clinicaltrials.gov

Web of Science search strategy (results: 1,016)
https://www.webofscience.com/wos/alldb/summary/1b1bdc88-e1ea-4f32-a752-b53bcd7631ba-67e7634c/times-cited-descending/1

(TI=("RNA interference" OR RNAi* OR "posttranscriptional gene silencing" OR "post-transcriptional gene silencing" OR "post transcriptional gene silencing" OR PTGS OR "RNA silencing" OR "small interfering RNA*" OR siRNA* OR "short hairpin RNA*" OR "small hairpin RNA*" OR shRNA* OR microRNA* OR miRNA*) OR AB=("RNA interference" OR RNAi* OR "posttranscriptional gene silencing" OR "post-transcriptional gene silencing" OR "post transcriptional gene silencing" OR PTGS OR "RNA silencing" OR "small interfering RNA*" OR siRNA* OR "short hairpin RNA*" OR "small hairpin RNA*" OR shRNA* OR microRNA* OR miRNA*))

Refined by:
Publication Years: 1998-2023

Document Types: Clinical Trial
NOT Document Types: Abstract OR Review Article OR Editorial Material

PubMed search strategy (results: 1,060)
("RNA interference"[Title/Abstract] OR RNAi*[Title/Abstract] OR "posttranscriptional gene silencing"[Title/Abstract] OR "post-transcriptional gene silencing"[Title/Abstract] OR "post transcriptional gene silencing"[Title/Abstract] OR PTGS[Title/Abstract] OR "RNA silencing"[Title/Abstract] OR "small interfering RNA*"[Title/Abstract] OR siRNA*[Title/Abstract] OR "short hairpin RNA*"[Title/Abstract] OR "small hairpin RNA*"[Title/Abstract] OR shRNA*[Title/Abstract] OR microRNA*[Title/Abstract] OR miRNA*[Title/Abstract]) AND ("1998/01/01"[Date - Publication] : "2023/12/31"[Date - Publication]) AND (clinicaltrial[Filter] OR clinicaltrialphasei[Filter] OR clinicaltrialphaseii[Filter] OR clinicaltrialphaseiii[Filter] OR clinicaltrialphaseiv[Filter] OR controlledclinicaltrial[Filter]) NOT ("review"[Publication Type] OR "editorial"[Publication Type])

Embase search strategy (results: 647)
('rna interference':ab,ti OR 'rnai*':ab,ti OR 'posttranscriptional gene silencing':ab,ti OR 'post-transcriptional gene silencing':ab,ti OR 'post transcriptional gene silencing':ab,ti OR 'ptgs':ab,ti OR 'rna silencing':ab,ti OR 'small interfering rna*':ab,ti OR 'sirna*':ab,ti OR 'short hairpin rna*':ab,ti OR 'small hairpin rna*':ab,ti OR 'shrna*':ab,ti OR 'microrna*':ab,ti OR 'mirna*':ab,ti) AND [01-01-1998]/sd NOT [31-12-2023]/sd AND ('clinical trial'/de OR 'controlled clinical trial'/de OR 'phase 1 clinical trial'/de OR 'phase 2 clinical trial'/de OR 'phase 3 clinical trial'/de OR 'phase 4 clinical trial'/de) NOT ('conference abstract'/it OR 'conference paper'/it OR 'conference review'/it OR 'review'/it OR 'editorial'/it OR 'note'/it)

ClinicalTrials.gov search strategy (results: 450):
Other terms (Recruiting, Enrolling by invitation, Active, not recruiting): "RNA interference" OR RNAi OR "small interfering RNA" OR siRNA OR "short hairpin RNA" OR "small hairpin RNA" OR shRNA OR microRNA OR miRNA

References

1. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ *372*: n71.
2. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, and Mello CC (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature *391*: 806-811.